GENETICS IN THE 20TH CENTURY
Gregor Johann Mendel, 1822-1884
GENETICS IN THE 20th CENTURY

ESSAYS ON THE PROGRESS OF GENETICS DURING ITS FIRST 50 YEARS

EDITED FOR
The Genetics Society of America

BY
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THE MACMILLAN COMPANY
NEW YORK
FOREWORD

THIS VOLUME is a compilation of the invitation papers presented at the program of the Golden Jubilee of Genetics at Ohio State University, Columbus, Ohio, September 11-14, 1950. The celebration was in honor of the fiftieth anniversary of the rediscovery of Mendel's work which marked the beginning of the science of genetics.

The meeting was held under the sponsorship of the American Institute of Biological Sciences (AIBS), holding its first general gathering of the member societies of which the Genetics Society of America is one. The first speaker was Richard B. Goldschmidt, whose topic was "The Impact of Genetics Upon Science." He related how genetics in its short life span of fifty years had had a remarkable effect on almost every branch of biology. The unusually high plane on which the meeting was initiated continued throughout the three-day invitation program. It was a notable experience enjoyed by the largest number ever to attend a meeting of the Genetics Society. The enthusiasm of those attending is a tribute to the work of the Golden Jubilee Committee that planned the program.

The Golden Jubilee of Genetics was proposed by the Executive Committee of the Genetics Society of America at its eighteenth annual meeting in New York City. At the business meeting of the Society on December 29, 1949, the motion to celebrate in 1950 a half century of progress in genetics was adopted unanimously. The committee appointed by the President of the Society, Curt Stern, consisted of L. C. Dunn and M. R. Irwin, co-chairmen; C. L. Huskins, I. M. Lerner and P. C. Mangelsdorf. Dr. Dunn was in charge of publications and is editor of this volume. At a meeting of the committee in New York City on March 15, 1950, comprehensive plans were made for the Golden Jubilee Celebration of which the Columbus meeting was the most important part. The Genetics Society is grateful to the Rocke-
feller Foundation for making available funds to finance the meeting. This permitted the participation of several invited speakers from foreign countries.

The Genetics Society of America is happy to be the agent that brings together so many valuable papers into one volume. It represents the thought and study of some of the most distinguished geneticists of our day, persons whose work has had far reaching influence in many branches of science and other fields of human endeavor.

W. R. Singleton
Secretary of the Genetics Society of America
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INTRODUCTION

THE CHARACTER of this collection of essays was determined by several circumstances. The primary purpose was to survey the progress of the first fifty years of genetics and to exemplify the status of some of its problems today. This was to be done not merely for professional gratification, for the study of heredity does not exist by or for itself alone. It is a part of a larger whole and needs to be looked at sometimes in its wider context, which includes not only the biological sciences of which genetics forms a part, but the fields through which it influences the lives of human beings. Moreover, no one in 1950 can be unaware of the fact that the principles upon which genetics rests have been declared politically unacceptable in Russia and that the other communist countries generally have followed suit.

One can discern therefore, in these first fifty years of genetics, two antithetic currents: one, the building up of a body of tested facts upon which rests a theoretical structure and a body of practice which seem both sound and useful to scientists who are free to express their opinions; the other, the attempt to subject the acceptance or rejection of both theory and practice to political decision.

It was clearly the intent of the Genetics Society of America to survey only the constructive events in the development of this science. An examination of the factual foundations and applications of genetics would be of service everywhere regardless of their political palatability.

Genetics is one of the few sciences of which the birth date can be specified. It should have begun, of course, on that February evening in 1865 when Mendel announced to his fellow members of the Brünn Natural Science Society that he had found some laws or principles underlying the process of inheritance in plants. Actually, the investigations which grew together into the field of genetics, although they stemmed directly from Mendel’s discovery, did not take form until
after the dramatic and independent rediscovery of the same principles by three European botanists in 1900.

Fifty years later genetics has become a many-sided body of knowledge and method dealing with questions which are recognized as of central importance in all efforts to understand living matter—how it perpetuates itself through reproduction, how it changes and adapts itself to its environment. Many of its principles have turned out to have a general character, so that not only do the rules apply to plants, as Mendel first found, but to animals of all kinds; to man himself, and to the whole world of microorganisms, bacteria, and viruses, revealed since Mendel's time. What has been learned about heredity and variation has been found useful not only in learning more about biological problems but in practical ways too; and agriculture, medicine, and society in general have begun to share the profits from genetical research.

In spite of its evident diversification, genetics has fortunately retained the essential unity given to it by the discovery of a fundamental element of heredity, the gene, so that varied problems can be stated in a common language which is becoming more generally understood.

This simplified the task of the committee responsible for planning the program. Although it could not hope to treat the development of all fields of genetics and its applications comprehensively, it did attempt to bring together in an extensive symposium representative leaders from the early, the middle, and the late periods of genetical research, who could speak with authority concerning the development of the science, its present content and future promise, and its actual and potential contributions to human welfare. It is a matter of gratification that most of the facets of genetical research of these fifty years were represented, including some discoveries made in 1950. The different forms of life each had some share of attention. The status of most of the central problems of genetics was discussed: the mechanism of heredity, and the physical and chemical constitution of the hereditary material in the nucleus and chromosomes; the behavior of the hereditary particles and their effects upon the life of the cell and the organism; the stability of the genes; and the relation of all those to the great problem of organic evolution.
Introduction

It is noteworthy that the large share of space devoted to genetics in the direct service of man was in no sense separated from the historical and theoretical discussions. Many of the applications of genetics have not developed into separate fields but have arisen from the work of the same investigators who share in the theoretical development of the science.

Through the cooperation of The Macmillan Company it has been possible to publish these papers promptly and in a form designed to make them available to a wide audience. The committee and the editor have not attempted to impose any artificial uniformity upon the various contributions, and have put them between covers with a minimum of editing.

In rereading these papers the editor has been at once impressed and gratified by their lack of uniformity. No two of them are alike, in style or method, or in immediate objective. Nothing could indicate more clearly than this the sources and strength of present day genetics. The experimental, analytical, and theoretical work which provided the foundations for the papers in this volume was carried out in several countries of the world by persons differing in language, in religion, in political and social views. The contributions of many workers have grown together into a great and useful science because they were free to pursue their facts and free to exchange ideas with investigators of other countries in the spirit of friendly cooperation which has marked the development of this science. Genetics, like most other sciences, is supranational in origin and character. It is quite clear that each of the papers included here bears an implicit testimonial to the freedom of inquiry and of communication upon which this fifty years of progress in genetics has rested.

Because the potential contributions of genetics to human welfare in the next fifty years are so great, the continuation of freedom and cooperation among geneticists of all countries of the world may well be the hope which will provide force and direction for the greater effort and devotion which the future will require of all scientists.

L. C. Dunn

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GENETICS IN THE 20TH CENTURY
THE IMPACT OF GENETICS UPON SCIENCE

RICHARD B. GOLDSCHMIDT

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THE student of the humanities as well as the intelligent public looks at the history of human thought as a history of abstract ideas. Even today in the age of the sciences these ideas, which have impressed their stamp upon subsequent centuries and are still continuing to do so, are regarded as religious or philosophical in nature. It is true that minds like those of Plato, Thomas Aquinas, Spinoza, Descartes, Hegel and Kant have exercised a strong influence upon the progress of thinking in all spheres, even upon the actual course of historical events. The scientist who looks beyond his specialized work is as fully aware of these historical facts as the humanist. But he is also aware that abstract thinking, remote from, and even antagonistic to the study of nature, leads easily into dogma, taboos and fettering of free thinking because it does not carry its own corrective, the recourse to factual evidence. The scientist, therefore, with all respect for the many facets of the human mind, is more impressed by the revolutions in thinking brought about by great factual discoveries, which by their very nature lead to generalizations which change at once the outlook of many, if not all, lines of thought. Such events are rare. In modern history three are most conspicuous: the explanation of the movements of the celestial bodies by Kepler, Copernicus and Newton; Galilei's experiments inaugurating the age of inductive science, and Darwin's establishment of the theory of evolution on the basis of an overwhelming body of facts. All of them at once evoked the wrath of the vested interests of the
mind; all conquered within a generation or two all fields of intellectual endeavor and changed the basic aspects of practically every science, natural or humanistic.

If I claim that the rise and development of genetics to mature age is another instance of an all-comprising and all-affecting generalization based upon an overwhelming body of integrated facts, I at the same time realize that a speaker at a jubilee is apt to paint in too brilliant colors. I realize, also, that such a claim requires a certain distance both in personal interests and in time to achieve objective value. But, being convinced that the rise of genetics will rank in the history of science with such other great events as mentioned, I propose to show that the basic tenets of genetics have already influenced decisively all parts of biology after what has been only a short span in the history of science; and further that beyond this, many other fields of science have fallen under the spell and we have every reason to believe that genetics is bound to remain in a pivotal position in the future.

It is obvious that this claim is made as well for the individual and detailed facts of which the science of genetics is built, many of them reaching out into other fields of knowledge, but still more for such general tenets of our science as are likely to be relevant to the broader aspects of other fields. Let us, therefore, try first to characterize those general insights which genetics has added to the store of our knowledge.

THE CHIEF RESULTS OF GENETICS

The first general result of primary importance is the unequivocal separation of hereditary and non-hereditary changes of the organism as first established by Johannsen. Darwin in one of his early drafts, which present his ideas much more clearly than the fact-laden final book, had written about the variations occurring in nature, noting "and some of these tend to become hereditary." Even after Weismann had proven logically the impossibility of the inheritance of acquired characters, variation, the acknowledged basis of evolution, remained a befogged subject. This fog lifted only when the work on pure lines showed the difference between phenotype and genotype,
between the hereditary change of the genotype and the non-hereditary
changes of the phenotype which are frequently not visibly different
from the above, and which are caused by the action of the external
and internal environment. This insight at once excluded any possi-
bility that acquired characters might be inherited and apportioned
clearly the relative roles of heredity and environment in the deter-
minalion of organization. Since then, there has not been per-
formed a single experiment in genetics which does not show those
principles at work and always valid. One hears frequently that the
organism is the product of heredity and environment. I cannot agree
with this. Environment can affect the organism only within the
limits set by its hereditary constitution. Beyond these limits there
is no reaction or no organism. Thus, after all, it is heredity that
makes the organism. This idea, a basic one, I think, is expressed best
in Wolterschek’s definition of the genotype as a norm of reaction.

The second great result of genetics is the establishment of muta-
tion as the only proven means of hereditary change. The theory of
mutation claims that all hereditary changes are based upon sudden
and largely, if not completely, irreversible changes in the chromo-
some set, an individual chromosome, a section of a chromosome or
an ultra-microscopic part of a chromosome, the gene. Whether the
results of this change are large or small, visible or invisible, normal
or pathological, more or less viable, a new heredity is always estab-
lished at once. In 50 years of experimentation no other type of hered-
tary change has come to light in animals or plants.

The third basic result of genetical experimentation is the final
proof of the older idea that the material basis of heredity is localized
in the chromosomes which are in control of all the processes which
result in the production of the typical organization. This proof
is based upon the fact that the statistical distribution of genetic dif-
ferences among the offspring of different parents is, in all cases,
simple or complicated, expected or unexpected, normal or abnormal,
completely parallel to the simple or complicated, normal or abnormal
distribution of the chromosomes in the offspring. As this is also the
essence of Mendelism the entire content of static genetics can be
put into these words: the distribution of hereditary differences,
brought together by cross-fertilization, within an individual, between sibs or between generations is the consequence of the movements and distribution of the chromosomes and their parts.

The fourth general result of genetics is the proof that the chromosomes have a polar organization which is completely constant in each case. This means that the chromosome is different from point to point along its length, as proven by the fact that individual mutants are localized at definite and constant points of the chromosome. Thus, the smallest section known to contain one mutant locus becomes a unit in the structure of the chromosome, which is called a gene. As the majority of the mutants do not seem to affect more than one point in the chromosome, mutants may as a whole be treated as point mutants, that is, changes within that small section of a chromosome called the gene.

The last generalized fact of static genetics is the tendency of a pair of homologous, maternal and paternal, chromosomes to break between two genes and to exchange sections. This “crossing over” permits a recombination of all mutant loci introduced by two different parents, not only if located in different chromosomes, but just as well if located in a pair of homologous chromosomes. Thus, in principle, all mutants may be reshuffled and brought together in new combinations, if interbreeding between different parents occurs. Furthermore, this linkage permits, also, the existence of mechanisms which prevent such exchange and thus keep more or less large sections of the chromosomes together.

**DYNAMIC GENETICS**

To these general facts of static genetics should be added the most generalized results of dynamic genetics. The genetic material in the chromosomes controls the typical series of events which constitute development. This means that an exactly timed and integrated system of reactions is set up, which leads at the proper time, in the proper substratum and under proper threshold conditions, to the production of specific enzymes, formative stuffs, organizers or hormones. These again set in motion the individual steps of differen-
tiation as an orderly, attuned, balanced system of determining processes.

It was the discovery and establishment of these elementary facts and tenets of genetics which rapidly moved our science, which even 40 years ago was looked upon with suspicion and derision, into the center of biological thought. Here, a system of knowledge was erected which had all the features of a quantitative science; predictability of experimental results and description of the facts in simple quantitative terms. One of the basic riddles of life, reproduction by organisms of their own kind, thus was solved, at least in its static aspects, and the way was opened to a dynamic understanding. Thus, it was not long before other fields of biological knowledge were forced to incorporate the new ideas, or at least to look for points of contact. This process, started soon after genetics had left its infancy, is still going on and is bound to expand to more and more scientific fields for the mutual good.

GENETICS AND TAXONOMY

The basis of our study of the living world is the inventory and appraisal of the existing material by the science of taxonomy. Darwinism had lifted taxonomy from a mere matter of cataloging to an inductive science, the aim of which was to show the natural relationship of the forms in the sense of descent by evolution. The method was comparison of structure and study of the distribution of forms, the differences of which were quietly assumed to be hereditary. The main effort made was to distinguish clearly the various species and to ascertain their distribution. By the end of the last century progressive taxonomists already realized that the species is a conglomerate of distinguishable forms which are able to interbreed if not separated geographically or ecologically, and they developed the Rassenkreis concept of the species.

It was in this realm that genetics first began to influence taxonomy. The taxonomist who looked beyond cataloguing his specimens had now to realize that the distinguishable forms of animals and plants in nature might be hereditary, or environmental modifications with-
out evolutionary importance. Furthermore they might be unimportant occasional mutants within an otherwise constant population; or mutants floating in a population as a numerically constant feature, or genetically different subspecies with constant combinations of characters within an area of distribution, or finally, different sympatric or allopatric species. Intermediate types became either hybrid populations between two interfertile groups, or independent intermediate subspecific types based upon quantitative characters forming clines, or again non-hereditary intermediate habitat forms. Homogeneity of a population in a definite area, as well as variation within such a population, became important points of description. Genetic analysis of populations as well as crosses on the subspecific level showed the taxonomist the essence of the form differences he found and thus gave taxonomy a new meaning and impetus and simultaneously ended the period of isolation of taxonomy from modern experimental biology. Genetics has made taxonomy, once in danger of becoming a combination of label writing and philological squabbles, a basic biological discipline.

GENETICS AND ECOLOGY

During the last 60 or 70 years, the fascinating old science of Natural History not only changed its name to ecology, but in addition, developed into a quantitative science, part of which is outright population study, another part actually physiology of specialization. As ecology studies the organism in its natural associations, which resolve themselves more or less into the specific adaptations to inorganic and organic environments, its work was bound to come in contact with genetics and to be influenced by genetical facts and theory. Again, adaptation may be a direct non-hereditary reaction to environmental agencies. The lability of many organismal features is such as to permit an individual response to the surroundings. But the major ingredients of the ecology of an organism must be and are based on heredity. Thus, it becomes a central problem of ecology to know the genetical mechanisms which permit the adaptation of the organism to its ecological niche.
Richard B. Goldschmidt

To illustrate this point let us choose the adaptation of the life cycle of an organism to the seasonal cycle of its habitat. Let us consider a certain moth. The eggs are laid in the fall and the young larva hibernates without hatching from the egg shell. In order to be adapted to the seasonal cycle, the reaction of hatching must not occur before food is again available, but early enough to leave sufficient time for development during the vegetative period of the food plant. If this form is to be distributed over a large geographic area comprising very different seasonal cycles, the population in each region must be genetically adapted to its specific seasonal cycle. This means that, largely independent of local environmental variations, the genetic control of the hatching reaction must be exactly in tune with the specific seasonal cycle. This is actually the case in the most subtle and amazing way, which shows that the ecological niche is available only on the basis of the proper genetically controlled norm of reaction. Mutatis mutandis, the same problem will occur in practically any ecological study, with the consequence that sooner or later every ecologist will be confronted with the necessity of thinking in genetical terms. In plant ecology, of course, the same situation exists. In addition, specific features of plant genetics like the different types of polyploids and their relative fitness for life in definite habitats, have led to still closer collaboration between genetics and ecology.

GENETICS AND PSYCHOLOGY

From the point of view of the biologist, psychology may be considered a part of ecology as well as of cultural anthropology, though it originated historically from philosophy. This origin may be largely responsible for the relative indifference which many psychologists have been showing toward genetical problems. This may also have been responsible for the extreme belief in the power of the environment found in some groups of psychologists. It was partly from the medical side that genetics entered psychological work, namely in the ideas on constitutional types in which somatic and psychic qualities were supposed to be linked genetically. Another breach in the bastion of environmentalism was made when twin studies were extended.
The Impact of Genetics upon Science

to the psychic properties. Animal psychology, of course, was always
cognizant of the genetic side as its work would hardly make much
sense otherwise. Geneticists have already entered the field (with one
laboratory at least specializing in such work) as the simple mention
of wild and tame, clever and dull experimental animals indicates,
and even a less respected aspect of genetics, the inheritance of
acquired characters, has been given much publicity in the field of
psychology. With a little prodding from genetics, both animal and
human psychology will increasingly assimilate genetic notions and
be stimulated by them for mutual benefit.

GENETICS AND MORPHOLOGY

One of the oldest and ever-fascinating fields of biology is com-
parative morphology. Goethe's term morphology means the visible
structure of the organism, independent of the function, and com-
parative morphology is mainly concerned with the analysis of the
manifold expressions of the same structural material in different
groups of organisms. The relation not only between leaf, sepal, petal,
or arm, wing, flipper, but also of the products of transformation of
the hyoid arch in different vertebrates are examples. Comparative
morphology has become a modern science by applying its facts to
the analysis of the evolutionary background of the divergences, simi-
larities and dissimilarities. This analysis can be made and has been
made largely without inquiring into the genetical basis and possi-
bilities of evolutionary transformation. The magnificent rise of com-
parative morphology in pregenetic days testifies to this. But, just as
was the case in ecology, sooner or later in his studies, the compara-
tive anatomist will be faced by the necessity to inquire how his
results may be conceived in terms of genetical control of diversity.

Let us look again at one of many examples in order to realize
where the contact between the two sciences is established. In the
reptile group of Sauropsids, one observes the assumption of snake-like
forms. Many intermediate conditions exist between a lizard-like ani-
mal with normal number of vertebrae and normal extremities and
an animal with complete absence of extremities and snake-like seg-
mentation, by way of an increase in the vertebral number anterior to the sacral vertebra and increasing rudimentation of the extremities. The comparative anatomist who studies such a series comes to the conclusion that the following has happened in evolution; a caudal vertebra behind the sacral one assumed the role of a sacral vertebra while the former sacral vertebra was added to the lumbar series. Thus vertebra by vertebra the spinal column is elongated and the pelvic girdle is shifted backwards. Simultaneously the extremities become more and more rudimentary. The comparative anatomist who tries to account for such evolutionary procedure looks for a principle which controls the direction of possible changes in function. He is, or was, content to conclude that such evolutionary series follow the “law” of arcellaxis, anaboly, aphanesy, etc., whatever these terms may mean. He can go one step further if he studies the development of the different forms. He finds, contrary to his morphological appraisal, that the developmental differences in segmentation in the different types consist of different rates of segmentation, if embryos of the same stage are compared, and not of shifts of individual vertebrae. He finds, further, that the progressive rudimentation of the limbs is first connected with a primary formation of a smaller limb-bud, with the further completely orderly stoppage of differentiation at progressively earlier times parallel to the degree of rudimentation. Thus, the last bone to differentiate in the normal case is the first to disappear in the series of rudimentation.

It is at this point of the study that the comparative morphologist has to turn to genetics and especially to physiological genetics. What kind of mutation is needed to change the rhythm of segmentation, while the differentiation of a pelvis remains unchanged in regard to the position in the whole? Are mutants known, acting upon processes of differentiation into equal parts, which affect the numerical, serial result? Will such mutants have a secondary, pleiotropic effect or are new mutants needed for correlative changes? Are mutants known which affect rates of growth and gradients of differentiation? How do they act? Is an accumulation of many small mutants needed to lead to the known transformations or is a single mutant, affecting a decisive, early embryonic process of material segregation sufficient
to produce one or any of the known grades of transformations? I think that this example can be used as a prototype to show how all work in comparative morphology, as far as it tries to solve a problem in evolution, will finally lead to genetics, including developmental or physiological genetics.

The demonstration that comparative morphology, implemented by embryology, is bound to lead to genetics if special cases are to be understood, may even be extended to the consideration of such a broad generalization as the law of recapitulation. The general facts upon which this law is based are incontestable. But let us consider a specific case, the formation and subsequent resorption of the teeth in the development of the jaw of a whalebone whale. The discussion which has been going on for almost a century tries to prove that such facts permit phylogenetic conclusions or, on the contrary, that the facts are only a consequence of the mechanical features of development without any phylogenetic significance. There is no reason why the arguments pro and contra should not be continued for another century as long as genetics is not consulted, especially that part which deals with the genetic control of development. A mutant or an accumulation of mutants which produce a major departure in type, that is, a phylogenetic change, must act upon definite processes of development by channelling them into a different direction. This requires that the change occur at a definite stage in development. Known genetic changes do not affect the development as a whole, but only produce definite departures from normal after a certain stage has been reached, departures which are the more extreme the earlier they occur. From this it follows that development up to the time at which the mutants act, remains untouched by phylogenetic changes based upon such mutations. In our previous example this means that the mutational step or steps which produce the whalebone type of jaw acted upon the differentiation of the ancestral jaw at a stage following the formation of the teeth-anlagen. Since further mutants have not changed this situation the formation of embryonic teeth remains and the embryologist may speak of recapitulations. However, if the mutants had changed jaw development from an earlier stage on, there would have been no recapitulation
of the ancestral condition. The example shows how genetic thinking may throw new light on age-old problems of morphology.

GENETICS AND EMBRYOLOGY

These discussions have led us repeatedly into one of the most successful fields of modern biology, embryology. Both descriptive and experimental embryology have made their phenomenal rise without being influenced by genetics. But now that the principles governing development are at least visible, if not yet completely understood, it becomes more and more necessary to forge a link to genetics, since the typical unrolling of development must be controlled, in all essential features, by the genetic basis. Actually physiological geneticians, as well as experimental embryologists and students of the biochemistry of development, have tried to link the two fields to the advantage of both. The embryologists have shown (I am speaking now only of animals, as parallel work in plants has not yet progressed correspondingly) that development consists in a general way in a gradual narrowing down of prospective potencies from the equipotentiality of the whole to restricted but still manifold potencies of the parts, to restriction within the parts. This may be described as a series of processes of patterning in time, each subsequent step producing the final determination of a smaller area. The anlage of the extremities of a vertebrate may first be able to produce a fore or hind leg, a little later only a foreleg, a little later it is decided which half will be dorsal or ventral and so on. Whether these decisions are reached locally by spatial separation of potencies within a field, or whether they are induced from the outside by diffusion of an inductor substance is not important for the general interpretation. But all these processes are, of course, genetically controlled, with specific genetical differences sometimes existing between near relatives.

Thus at the basis of all embryology lie these problems: How does the genic material, present equally in all cells, control the occurrence of the proper order in time of steps of differentiation? Does this seriatim "activation of the genes" mean that their action begins
when they find themselves in the proper substrate? Further, how are these steps of determination arranged properly in space so as to produce the correct developmental pattern? Does this mean that a part of the genic material controls the production of the stuffs which characterize the diverse substrates and that another set of gene-controlled reactions leads to their intricate spatial arrangements in the consecutive steps of patterning which prepare the substrate for the next following genetic action? Clearly embryology is transformed at this point, or to express it with a little conceit, is sublimated into physiological genetics. This reminds me of a discussion which I heard about 30 years ago. Valentin Haecker had read a paper in which he proposed the term “phenogenetics” which is more or less synonymous with the more recent term of physiological genetics, and had discussed some of the aspects of such studies. In the discussion Hans Spemann attacked him violently for making believe that he was doing something new by introducing a new term while in fact he was just studying one small fraction of embryology. Certainly both were right; on the level of fundamentals, embryology and phenogenetics or physiological genetics are one and the same thing; but they are different in their immediate aims.

**GENETICS AS A BRIDGE BETWEEN BOTANY AND ZOOLOGY**

We just had to mention the difference of embryological analysis of animals and plants which is partly due to the specific features of plant development and partly to historical reasons. This statement leads us to the great role genetics has played in unifying zoological and botanical research, in some way comparable to the role of atomic physics in making chemistry and physics a single science. I remember well the time when a sometimes bitter antagonism existed between zoologists and botanists who rarely met or collaborated scientifically. There were historic reasons for this. Zoology had developed in the second half of the 19th century mostly under the banner of evolution while botany kept evolutionary thinking in the background, in favor of its physiological aspects. The last 50 years, under the impact of genetics, have changed this picture completely. The differ-
rences inherent in the material still exist. Plants are open systems, animals closed ones. The alternation of haploid and diploid generations in plants sets them apart from animals in many respects. The relations of development and growth to environment are different in both kingdoms. Ecological features, adaptations, and reproductive mechanisms are largely divergent. But if we come to the broad, and even to the most minute genetical and cytological facts, there is no difference between the two kingdoms. All of the basic and most of the specialized facts of genetics may be explained equally well with maize or snapdragon for examples as with Drosophila or guinea pig. Thus, from the very beginning of genetics, plant and animal scientists have been united in a continued give and take and not a few were at home on both sides. Botanists by training have given us Mendel's laws, the pure line concept, multiple factor inheritance, polyploidy, segmental interchange, to mention only a few major contributions; and zoologists by training have reciprocated with interaction of factors, crossing over, linkage groups, the classic theory of the gene, most of physiological genetics and the analysis of sex determination. Many of the geneticists on both sides had to broaden their primarily genetic work by morphological, physiological, embryological or taxonomic studies. By this they not only contributed vastly to the general fields of zoology and botany, but also emphasized the problems common to both and made the two basic biological sciences a continuum on a large and ever-expanding front.

**GENETICS AND CYTOLOGY**

A considerable share in this happy development falls to cytology as one of the pillars of genetics. Cytology of animals and of plants has always been in closer contact than other fields of botany and zoology. It is a characteristic historical fact that after the zoologist Bätschli had discovered and understood mitotic division, the botanist Strasburger called on him, studied his preparations, and realized that what he had seen in plant cells was exactly the same thing. Ever since, cytology has not distinguished between animals and plants. All the basic facts of chromosomal structure and behavior,
mitosis, fertilization, sex-chromosomes and cytoplasmic inclusions and, of course, cell physiology, are identical. Thus, cytology developed into an independent science, drawing its discoveries from both kingdoms. The advent of genetics removed cytology from its position as a specialized field of study and made it actually the most generalized center of biological research. While cell physiology became more and more a branch of colloid chemistry, biochemistry and biophysics, cell morphology became so closely integrated with genetics that some geneticists forget completely that other aspects of cytology may be of greatest importance. This integration presented cytology with problems which formerly would have looked like playing with more or less dull minute details. Counting chiasmata or analyzing chromosomal bridges would not have impressed many as very important in 1900. The entry of genetics raised the most minute studies of the cell to a level of supreme importance so that today morphological as well as biochemical cytology draws one of its main raisons d'être from its linkage with genetics.

GENETICS AND ANTHROPOLOGY

For a long time, zoology, studying structure and behavior of animals, remained separate from human anatomy and anthropology, which did the same for man. This was largely due to the attachment of anatomy and physical anthropology to medicine in spite of the fact that classic comparative anatomy was mostly done by human anatomists. Anthropology, which added to the study of human anatomy the study of human differences and behavior, tended to leave the zoological side to the anatomists and lean more heavily towards the cultural sciences. It is obvious that genetics from its very beginning included man in the group of living beings whose heredity was analyzed. The first case of sex-linked heredity studied was in man. Ever since, human heredity has remained an integral part of genetics, though its study required special techniques. Thus, it was not long until the traits which were of paramount importance to the physical anthropologist as hair, eye, skin color, shape of hair, size, skull indices, form of ear and nose were subjected to genetical study. This
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allowed, or should at least have allowed, the anthropologist to appraise so-called racial differences in more exact terms and to form ideas on physical differentiation in man which are based upon the insight drawn from general genetics. The time has been short and the material too difficult to have permitted the physical anthropology of the age of skull measurements to be replaced by one solidly based upon genetic knowledge. But it is, I think, a fair statement, that the impact of genetics has given to physical anthropology new directions and new hope for a more reliable approach to its problems.

A reason for this optimistic statement may be found, for example, in the remarkable results obtained in the study of the population genetics of blood group distribution. When genetics claims credit for such change in outlook, it realizes the great difficulties which physical anthropology faces by the very nature of its material. This can be brought out by mentioning only one of the many problems. If the much-quoted museum curator from Mars already imbued with knowledge of genetics had landed in a flying saucer on this planet 4000 years ago to study the genus homo, he would have concluded that it consisted of a number of geographically completely isolated species, with considerable physical differences, easily distinguished and without any intergradation between them. Within these ecospecies he would have discovered what looked like subspecies, adapted to different habitats and also largely isolated from each other. Within these subspecies he would have found subgroups, clearly recognizable, but sometimes interbreeding in an intermediate zone. Now, the present day successor to this curator decides that he must check up on the old data and return to the earth in his improved saucer. What he finds does not agree at all with the old data. Instead of the isolated species he finds only a single species, interbreeding successfully on a larger or smaller scale all over the globe. Where formerly largely distinguishable ecospecies had been, much more diversified populations have been formed which at best he may call subspecies, some of them overlapping and connected by zones of unanalyzable mixture. The former rather clear-cut subspecies had more or less been welded into populations which showed only statistical differences or not even these. Let us not continue this picture.
It was meant to show that at the human level it is not so easy for genetics to foster clear and indisputable notions. But it is to be hoped that the stimulus given by genetics to anthropology will in the end lead to making physical anthropology a well-founded science. Cultural anthropology has only begun to consider its results also in the light of genetics. Our knowledge of the genetics of the human mind has not yet progressed sufficiently to permit a deep influence upon the study of cultural problems. Some beginnings have been made and I am sure that a jubilee speaker after another 50 years will be able to report convincingly upon the impact of genetics on cultural anthropology.

**GENETICS AND MEDICINE**

In another realm the science of man has come in contact with genetics, a contact of mutual fruitfulness, namely in the realm of medicine. It would be over-optimistic to say that the impact of genetics upon medicine is in fact already what it could and should be. Except in one special field to which we shall return later, the blood groups, medical science as a whole is not yet sufficiently aware of what genetics means for it. Not many members of the profession realize that the majority of the major eye diseases and abnormalities are hereditary, that the same is true for the non-infectious skin diseases, diseases of the ear and mental diseases. The special genetic features like mono- or polygenic inheritance, variability of expressivity and penetrance, modifiability by genetic modifiers and environmental action, interplay with the residual heredity, genetically controlled time of incidence, occurrence of multiple alleles and also deceptive occurrences of phenocopies, all these raise many difficulties. But the difficulties will in the end be a spur to try, where it is possible, to put the human mind against the inexorable, but nevertheless sometimes controllable effects of the genetic constitution.

We have just mentioned the blood groups, one of the most obvious and also most fortunate examples of the impact of genetics upon a medical field of study, serology, which only 25 years ago seemed to be very remote from genetics. In this case we have the
remarkable circumstance of one serologist making an important discovery in his field, another serologist realizing the genetic nature of the facts, and a mathematician finding the correct genetic interpretation. Only recently the same story has repeated itself with the same kinds of actors. The entrance of genetics into the field of serology has undoubtedly conquered for it a new province, and new discoveries will continue to be made. It is this sphere also in which probably the closest union between genetical theory and medical practice has been accomplished. Every successful blood transfusion and each child saved from Rh-incompatible parents bears witness to this statement.

Coincidentally in the same sphere of work, another very distant science, jurisprudence, has been influenced by genetics to its own advantage. It happened first I think when Norwegian geneticists demonstrated to a law court the overwhelming probability that a certain brachyphalangic man had fathered a disputed child who showed the same abnormality. The same reasoning should be applied today in paternity suits, though strangely enough judges are still found who trust their legal knowledge more than genetic analysis.

GENETICS AND AGRICULTURE

Let us leave man again to turn to the sciences of animal and plant breeding, the most obvious and most complete conquest which genetics has accomplished. All breeding work for the sake of producing better crops or strains adapted to specific conditions is concerned with two main features: the attainment of the best nourishment in the broadest sense of the word and the production of genetically superior and constant breeds. To a certain extent the two methods overlap, as some genetic superiority may consist in the ability to make better use of the environmental factors. Therefore, genetics and physiology of nutrition have been always closely integrated. It is known that for centuries breeders have used unconsciously, but successfully, genetic methods such as crossing and selection. The advent of genetics, therefore, did not introduce completely new methods into agricultural breeding but put the old em-
The Impact of Genetics upon Science

pirical ones upon a quantitative basis. This made it easier for genetic ideas to permeate practical breeding procedures. The pure line concept furnished the basic insight into the problem involved. Combination breeding led to the first conspicuous successes, especially in the field of adaptation to specific environments and of the breeding of disease-resistant varieties. Natural and induced mutation could be used to a certain extent and selection for multiple factor combinations and modifiers could be established on a quantitative, statistical basis. Hybrid luxuriance together with the proper use of inbreeding and outbreeding led to remarkable results. Experimental polyploidy and allotetraploidy assumed an importance for practical breeding which is still increasing. Cytology became as important a tool of breeding as any other. Thus, genetics took over decisive functions for the attainment of practical results in improving farm animals and crop plants. Thus far no end is visible to possible progress on these lines and new discoveries in fundamental genetics may open still further avenues of approach to the practical problems of the science of breeding. It is fair to state that the brilliant results of applied genetics may have led sometimes to an underestimation of the physiological side of the art of breeding. There is not great danger in this, as the soil and fertilizer specialists, the nutritionists, endocrinologists, plant physiologists and pathologists will take care that their side of the story is listened to. What genetics has done for agriculture and certainly will still do is one of the feats of which we are all justly proud.

GENETICS AND BACTERIOLOGY

Practice and theory are not very far apart in the present world of science. Progress in fundamental science is the prerequisite for practical progress, while practical problems may in return stimulate fundamental research. Thus, it is not an anticlimax when we turn from the impact of fundamental genetics upon an applied science to the influence genetics has exercised upon fundamental progress in apparently remote fields, in an ideal interplay of give and take. We may first mention bacteriology, a science which for a long time had made great strides
in the fields of pathology, general physiology, biochemistry, even oceanography and geology. But there was practically stagnation in the basic aspects of the study of bacteria, the inquiry into their life cycle. This has completely changed, thanks to the work of genetics. Geneticists are well acquainted with situations in which an unknown and invisible cytological event can be proven to occur by purely genetic experimentation, which may be confirmed later by cytology, if visible chromosomal features exist, but which are also considered as firmly established when only the genetic attack is possible. If, for example, sex-linked inheritance is encountered, sex-chromosomes must be present, even when cytologically invisible. Exactly such a situation has been created for bacteriology by the work of the geneticists. They showed that mutation in the same sense as used in genetics occurs. Furthermore, genetic recombination, after what must be hybridization, takes place and even the equivalent of linkage and crossing-over is observed. This implies that bacteriology faces new tasks, the unravelling of a life cycle which parallels the sexual cycle of other organisms, or the discovery of hitherto unknown methods of reproduction which permit the genetic consequences of the typical sexual cycle to appear. Practically the same gift has been made by genetics to virology, which faces the facts of mutation and recombination after a kind of crossing with the possibility, even the probability, of unravelling a primordial, prechromosomal genetic mechanism.

Genetics and Biochemistry

At least as important and pregnant with future possibilities is the relation of genetics to biochemistry. For a long time genetics had been only at the receiving end of this association. Whenever geneticists tried to formulate ideas on the nature of the hereditary material and on the mode of its action, they had to think in terms of enzymes, autocatalysts, monomolecular reactions, mass law of reaction velocities, substrates and coenzymes, prosthetic groups and stereoisomers. As early as the first days of Mendelism, definite biochemical notions were used to explain Mendelian results. For example, the collaboration of chromogen and oxidase in pigment
production, and in some special cases the biochemistry of genetically controlled syntheses was studied. The debt of genetics to biochemistry has now begun to be repaid and the geneticists may have become entitled to a modest claim of an impact of genetics upon biochemistry. We all know that biochemistry has been enriched recently by a powerful genetical method of analyzing details of synthesis or degradation which had not been accessible to chemical methods.

Simultaneously, the impact of genetics upon biochemistry is increasing in force and is bound to increase still more the study of the nature of the genic material. The biochemical analysis of the chromosomes and the deliberations on the possible relations between the proteinic and the nucleinic elements, pose definite, still unsolved problems to the biochemist. It is a fact that the demands of genetics have already succeeded in making biochemistry aware not only of the need for more knowledge of the nucleic acids, but also of the steps which make possible the synthesis of long protein molecules and supermolecules as replicas of existing ones. It is quite conceivable that notions derived from intricate genetic facts will help the biochemist to direct his search into definite channels. As a sign that there has begun an era in which genetics will play the role of the giver, we may mention the fact that biochemists are trying to develop ideas on the nature of the gene. If we include immunochemistry within biochemistry, we notice with satisfaction and expectation that the chemist in search of the explanation of immunity reactions is already taking notice of basic facts of genetics which suggest a common denominator.

STATISTICS

Since the early days of genetics, the time when Johannsen wrote that genetics must be studied with mathematics, not as mathematics, statistics has furnished important and indispensable tools to genetic research and the contributions have constantly increased. But I think that I am not mistaken if I state that genetics has given something to statistics. I do not mean the actual contributions which some mathematically-minded geneticists have made to the science of sta-
tistics. What I have in mind is that the rise of genetics has stimulated mathematicians to expand their studies into new directions and to discover new tools and avenues of approach designed for the use and originated by the needs of the geneticist. As some of the best statisticians have devoted themselves to the perfection of the tools of the geneticist, one might well speak of the impact of genetics upon statistical science, the more so as it is known that the impact was so strong as to turn a statistician into a geneticist.

**GENETICS AND HUMAN ECOLOGY**

I wish I could mention also sociology as one of the fields which has felt the impact of genetics. Sociology is, after all, ecology of the human animal, his adaptation to the environment, his association with and reactions to the group and to those outside the group. The basis of this ecology should be found primarily in the genetic constitution of the population, group, family and individual. This implies that the genetical background should be the first object of sociological research. If we state this, however, we forget that man is not a mere animal in so far as he has succeeded in replacing the non-existing influence that the environment might exert upon his hereditary constitution by the pseudo-heredity of tradition, the possibilities of communicating his experiences to posterity. This changes his ecology completely from that of animals. If the famous horse "Kluger Hans," had really learned how to calculate, this ability would have died with him and the horse family would be where it was before Hans learned. But man can transmit what he learns without a change in heredity and thus he circumvents the limitations imposed by the facts of genetics. As all his ecology is influenced by this fact, ecology becomes something different, namely sociology in which genetic constitution has become to a certain extent only a minor partner. If this is true, it deprives sociology of the exactness of a quantitative science in which known causes produce predictable results with only statistical limitations. Therefore, even the most devoted geneticist seems entitled to scepticism regarding a major impact of genetics upon sociology, at least for the time being, even if he does
not agree with Aldous Huxley's definition of sociology as the science of human senselessness.

RELATIONS OF GENETICS AND PHYSICS

No science has accomplished more and been more in the forefront of progress in lifting the veil from nature than physics. Therefore, it would be one of the proudest feats of genetics if it had succeeded in making, not necessarily an impact upon physics, but merely a modest quantum hit. It is a source of gratification to the geneticist that some of the greatest physicists have been led by a study of the fundamental tenets of genetics to consider the possibility that the established ultimate concepts regarding the physical world may not be sufficient to explain both. The physical and the animate world may be mutually exclusive. Or to express it somewhat crudely in the language of one who does not master quantum mechanics, that there might be two physics: one which accounts for the mechanized world of the atom controlled by quantum mechanics and the uncertainty principle, and another one, a future one which accounts for the world of life, for which basic concepts of the order of, but different from, those at work in the physical world will have to be developed.

We come to the conclusion of our proud, maybe too proud, accounting of what genetics thinks it has already accomplished in the short span of its existence, in building its own house and in planting its stamp upon other neighboring or even distant sciences. Years ago, I was once asked by a nonbiologist in which line I worked. When I answered, “Genetics,” he retorted, “Oh yes, I know, three red and one white peas.” Though this statement sounds naive, three red: one white still embodies for the initiated, the quintessence of statistical genetics, while 50 years ago, it meant to many biologists only some amusing, but unimportant playing at the outskirts of the life sciences. We have travelled a long road and what looked like a shack at the periphery of the city of biological knowledge has turned into a towering edifice right in the center. All geneticists, who have contributed their share to the erection of this building may be proud today, and look at their work and say well done. But they will also be aware that
the building is not yet finished and that many a worker, artisan and master architect will be needed to perfect it. Our hope and wish is that in the following five decades there will never be a dearth of bricklayers, builders and architects, and that the successes of the past are only the precursors of future triumphs.
WHEN I was asked to speak about Gregor Mendel’s heritage I immediately saw the difficulties of the task. Mendel’s was a sober mind, his thoughts were concerned mainly with concrete facts and he had little inclination for sentimentalism of any kind. He never kept a diary and the few letters still preserved throw but little light upon the inner man. No philosophical essays are left, no last will, no message to future generations.

The best way to learn what Mendel was like, what he thought and what he wanted, will be to unfold some characteristic pictures, to sketch some historical miniatures from his life and to draw some conclusions from these facts concerning his philosophy of life and his heritage to us. We start with a scene from Mendel’s early boyhood.

1832.—It is a warm evening in April. The air is moist, the gentle breeze smells of spring and soil. In his orchard which slopes down from the house to the road and to the stream, Anton Mendel starts grafting his fruit trees. Johann or “Hansl” as they call him, his only son, a short, sturdy fellow of ten years, watches the work with lively interest.

“Father, why can’t we start earlier? It will be dark in a while and you can’t do much in one hour.” “Don’t ask silly questions, Hansl. A farmer’s life is a hard life. I had to work for the lord of the Odrau manor today and I have to finish my own plowing and sowing tomorrow. I would prefer to work in my orchard early in the morning
instead of now when I am tired and longing for sleep. But such is
the farmer's life." "Why do you have to slave for the lords, father?
Why do we have to work and they take the harvest?" "So it was and
so it is, my boy. Maybe it will be different when you grow up. The
French did away with it, maybe we will too. But you had better
help me now and pass me some of the scions of those fine apples I
got from Father Schreiber today. We shall graft them upon that big
tree that is thriving and growing fast but bearing poor fruit all the
time." "Father, our teacher, Mr. Makitta, taught us today that the
tree upon which you graft does not change the scion. A small scion
from a noble variety will grow into a large branch and will bear fine
fruit even if it gets all its food from a poor stock. I can't understand
how this can be." "I can't either, but it is true, nevertheless. It's na-
ture or what they call heredity that is stronger than the change in
food. It seems to be the same way with men. If one comes of good
parents he will grow up to be a fine fellow, even in poor circum-
stances."

The boy stands silent and meditating. But the experiences and
thoughts of childhood remain firmly anchored in his mind, and, years
later, will shape his ways of thinking and of acting.

1861.—In his modest two room apartment in the second story of
the Augustinian monastery in Brünn, the 39-year-old member of the
order, Gregor Mendel, receives a distinguished guest. Seated opposite
Father Gregor is a tall, slender young man, elegantly but inconspicu-
ously dressed, his dark, intelligent face framed by black whiskers. It
is Gustav von Niessl, the young professor of Geodesy of the newly
established Brünn Polytechnical Institute who came to see Mendel
about the foundation of a Brünn Society of Natural Science. Father
Gregor readily accepts the invitation to join the Society. Then they
start to discuss the theme of the day, the theory of evolution, brought
into focus by the book of Charles Darwin, published just two years
before. "I don't see any evolution when I look upon the wild flowers
and animals," remarks Father Gregor, "maybe because it is a slow
process which needs thousands of years. But I think I can watch evol-
ution among our garden flowers. When the different species and
varieties are crossed new variations arise again and again. Maybe in
the open field, too, hybridization is the force behind evolution."
"But that is not what Darwin teaches," answers Niessl, "and I can't
imagine that evolution and the origin of new species could be ex-
plained just by combination of the old ones." "We shall wait and
see," says Mendel, "maybe my children will give the answer to the
question some day." And while Niessl looks startled at such a remark
from the mouth of a Catholic priest, Father Gregor smilingly takes
his house coat and invites Niessl to follow him. They walk through
the resounding corridors and down the steps to the small, sunny strip
of a garden where, with the permission of Prelate Napp, Father
Gregor grows his peas. "These are my children," Mendel explains
smiling. "If I do my artificial crossings, I only copy what wind and
bees are doing in nature every day. In formation of the sex cells and
in fertilization of the hybrids the differentiating characters are
brought into new combinations again and again. This is the eternal
game of dice which decides the fate of the next generation." Niessl
seems startled again that such bold and newfangled ideas should be
brought forth by a monk and a simple high school instructor at that.
"Does not that sound somewhat strange coming from a Catholic
priest?" Niessl remarks, "the fate of the next generation, the fate of
the child dependent on a game of dice, dependent on mere chance?
Would not that conception, if accepted, mean as great a revolution
in thinking as that brought about by Copernicus who opened the
infinite space and relegated earth to the role of an insignificant
planet?" "You are comparing a great man with a little one. I greatly
admire Copernicus. Do not forget that he, too, was a Catholic priest.
I do not think that it can be a sin to seek the truth. And don't forget
there is a law behind chance, too. But you are right, I shall have to
be cautious." Closing the discussion, Mendel asks his guest to wait
for him. After a while, he returns, dressed now in a black coat, top
boots, and a silk hat. They both wander up the Bäckergasse and
down through the vegetable market, to the tall building of the Real-
schule. Here Niessl says farewell and Mendel walks up to the class-
room where the boys are happily expecting their most beloved
teacher. It is the happiest time in Gregor Mendel's life. Those were
the years when he went through life full of enthusiasm for his pro-
fission and for his great idea, a secret emperor in his newly discovered realm.

1865.—In the classroom of the then new building of the Brünn Realschule, a school very similar to an American high school, about 40 persons are gathered on an evening in February, 1865. They are members of the Brünn Society of Natural Science and they are expecting a lecture by Father Gregor Mendel, instructor at the same school, who is to read a paper on “Experiments in Plant Hybridization.” The lecturer is welcomed by the secretary of the society, Gustav von Niessl, astronomer and botanist, a brilliant mind, though rather proud and autocratic. There are present Professor Makowsky, the botanist and geologist; Nave, the algologist; Kalmus, an expert on mosses; Czermak, the chemist; and many others—a small group of people, but most of them able scientists closely united by the same interests, a respectable intelligent audience. For about one hour Mendel reads from his manuscript an account of his experiments in hybridization of the edible pea which had occupied him during the preceding eight years.

He expresses the view that the failure of his predecessors to discover the laws that govern heredity must be mainly ascribed to the fact that they used for their crossings species and varieties which differed in too many characteristics. The new thing in Mendel’s method is that he confined his attention to the behavior of only one or two or three pairs of characters, and also that he examined not only each generation but also each individual of the hybrids separately instead of taking only a summary view as his predecessors had done. By his method he had been able to show a regular mathematical ratio in which the characters, united by crossing in the hybrid, segregated in the following generation. The hearers, who like the lecturer for his pleasant personality and respect him as well for his original observations in various fields of natural science, listen with considerable astonishment to his account of the seemingly regular algebraic ratio in which the characters segregate and combine among the hybrids. At the close of this address Mendel declares that the explanation he had formulated for this regular segregation would be given at the next meeting.
There is a goodly audience once more at the March meeting. They again listen respectfully. It must be admitted, however, that the attention of most of the hearers is inclined to wander when the lecturer becomes engaged in unusual mathematical deductions. And there is probably not a soul among them who really understands what Mendel is driving at. His main idea is that what is handed down by heredity is not a picture of the individual as a whole but rather single, discontinuous characteristics; and that these characteristics—or, as we say today, their carriers, the genes—compose the image of the species, just as many little stones compose a mosaic. This is indeed a strange notion! Mendel’s method by which the elementary quantitative relationship between hereditary phenomena could be tested and re-tested is new and strange to the audience.

It may be that there is some prejudice among some of the members who look ironically upon a speaker who wants to reform a science in which they know he had failed in his examination. “Nemo propheta in patria!” From the oral report of some of those who listened to Mendel’s lecture, I know that it was received with respect but with not too lively an interest. There was no discussion; no questions were asked. The audience dispersed and the matter was soon forgotten. The time was not ripe for the understanding of Mendel’s discovery, either in Brünn or elsewhere.

1884.—A crowd of people dressed in black fill the square in front of the Augustinian monastery in Altrünn. The church bells toll, the funeral procession is arranged. They carry to his tomb the prelate and abbot of the Augustinians who died after a life which had been rich in work and in strife. The dignitaries of the church and government stride behind the coffin. They whisper and exchange remarks, for the churchman whom they bury had been a “nonconformist,” a querulous person who kept the authorities busy during the last ten years of his life. In all Austria the stubborn prelate had been known for the nonsensical fight against government and law which he had conducted quite alone, deserted finally even by his conventuals to whom the struggle for justice had become too expensive. Then come the members of the Catholic clergy, but also the Protestant pastor and the Jewish rabbi, the professors and the teachers who had known
him in his teaching days, representatives of the many societies which he had supported, deputies from his home village of Heinaendorf and from the fire brigade there which he had founded. But behind all these names and representatives come the unknown crowd, come the hundreds of the poor, and here one could hear a different obituary. Here the goodhearted man is praised, who did not preach much but acted as a true disciple of Christ, who knew how to give alms and not to humiliate the receiver, he who has been for many years their only refuge in time of need. But of all these, of the great and of the little ones, of the rich and of the poor, not one knows that they buried a genius. That Gregor Mendel had wrested from nature a secret which would become a revelation to future generations had never been recognized during his lifetime, and, at the time of his death, his work as a scientist had been almost forgotten.

We have put a searchlight upon some important and characteristic scenes of Mendel's life. We know the effect of his work upon our time. What is the scientific heritage contained in the forty pages of his monograph, what the personal and moral heritage shown by the facts and tendencies of his actions?

Mendel accepted natural evolution and was suspected as a Dar- winist not without reason. But to him evolution resulted from the combination and recombination of given elements. He tried to explain the mystery of heredity by giving us a glimpse of what is today called its mechanism. What seemed to be a mystical and unanswerable fate was resolved into a mathematical pattern directed by chance and by the laws of probability. By his simple yet fundamental hypothesis Mendel became one in the group of those few great thinkers and discoverers who changed our Weltanschauung, our ideas about the world and about the life of man: Copernicus—Newton—Darwin—Mendel.

Copernicus, Newton, and their followers destroyed the old and narrow picture of the world and opened to view the unlimited sparkling universe of millions of stars followed by their planets, governed and directed by the same laws of gravity which act on the earth. The universe became a gigantic mechanism, the earth in comparison a
grain of dust. The infinite smallness and unimportance of earth and
man was demonstrated.

Darwin, 300 years later, destroyed the fable of the isolation and
uniqueness of man, made man a part of nature related with all other
living beings. Moreover, Darwin solved the mystery of the wonderful
fitness and useful adaptation of all organisms for their tasks and
functions by his theory of natural selection. Before Darwin, the mar-
velous design of all living beings could not be understood except as
the result of a mystical, teleological force. Darwin, however, suc-
cceeded in explaining that fitness scientifically. According to his the-
ory, both the fit and the unfit, the useful and the useless organs and
organisms are born and appear on the stage of life again and again.
But the unfit and the useless are eliminated continually by the strug-
gle for life and thus only the useful organs and the fit individuals
survive. Here, too, mystery was replaced by law, and wonder by a
scientifically explainable mechanism.

And into this scheme of evolution from myth to science Mendel’s
discovery and theory fit well. The mystery of heredity and variation is
seen as a result of the chance combination of given elements, a com-
bination which is governed and directed by the laws of probability.
What seemed to be a mystic fate dependent upon an arbitrary deci-
sion of a magic power became, too, the outcome of a mechanism.
The discovery of the chromosome mechanism many years after Men-
del’s death was the greatest triumph a scientific hypothesis could
achieve. The mechanism conceived by analysis and imagination was
found to exist as invented by Mendel. We are reminded of the hy-
pothetical construction of the course of an unknown planet outside
the orbit of Uranus by Leverrier and the subsequent actual discovery
of Neptune by Galle who directed the telescope upon the calculated
spot and discovered the unknown planet. But how small seems the
importance of that discovery when compared with the great concep-
tion of Mendel.

Like Copernicus, Mendel was a Catholic priest. But like that great
astronomer he helped to change our traditional, mythical picture of
the world into a modern scientific system. Of course, that does not
mean that Mendel believed that he had solved the riddle, that he had
found the whole truth. Like all great scientists, he was modest. He knew well that we are limited beings confronted by the unlimited and that what we can achieve is not more than just a glimpse into the workshop of nature. Mendel did not preach modesty, but he practiced it. He probably would not agree with those modern scientists who proclaim that the riddle is solved, that the mechanism of heredity is known to us. He knew that science is always starting, never finishing its work.

That Mendel accepted evolution and that by his investigations he only wanted to show a new and better explanation of how evolution takes place could be proved by numerous quotations from his papers and letters. But he also accepted the theory of natural selection as can be proved by a sentence from the end of his last letter to Naegeli of November 18, 1873: “If that were the situation,” he writes, “we would have to attribute the spontaneous hybridization in Hieracium to temporary disturbances which, if often repeated and becoming permanent, would result finally in the disappearance of that particular species; whereas one or another of the more favorably organized hybrids, which might be better adapted to the existing conditions, might succeed in maintaining itself in the struggle for existence, and in continuing for long periods of time until ultimately it, too, would suffer the same fate.” Mendel was a Darwinist not only in accepting evolution but also the theory of selection of the fittest by the struggle for life. His additional contribution was the discovery of the most important material for evolution. To him, the variations from which the struggle for life selected the fittest were the result of hybridization.

Mendel, of course, never drew any philosophical consequences from his theory. But neither did any of the other great pathfinders, neither did any of the following generations of scientists and philosophers. We are still living with our philosophy and our morals adapted to a stage which was long ago outstripped and replaced by science. A solution of that controversy will be one of the greatest tasks for future generations.

From early youth Mendel had wanted to become a scientist. He knew that as a scientist he had to be impartial, that his only task was...
to search for truth, however painful the result might be, however difficult to harmonize with traditional opinions and tendencies. I am sure that Mendel, though he probably often went through periods of doubt and inner struggle, remained faithful to his creed and oath. But he probably was convinced that the search for scientific truth cannot be sinful.

Both his experiments and his explanations seemed from the beginning to be purely theoretical and of no direct practical value. Nowhere in his monograph or in his letters to Naegeli do we find any attempt to look for practical consequences and applications. But as so often happens, what started as a theory became the foundation for the greatest practical progress. Everywhere in the world of today new varieties of plants and animals combining the desired characters are bred by application of Mendelian methods. The production of hybrid corn opened new avenues of improvement. The harvest of the earth was increased and it became possible to feed those that were hungry before.

Only impartial, pure science which adapts its theory to the facts will have great practical progress as reward. If the facts are distorted for political purposes, if science is misused to please those in power, the result can only be failure and finally collapse. We saw that when German science worked in the service of the Nazis, and we see it again in Russia today. What Lysenko and his group prepare will become disastrous for Russian science. An agriculture, an economy based upon distortion of facts will become a failure. Science means adaptation to facts. Those who try to neglect the hard facts will gain nothing but bloody heads.

Mendel was the son of a poor farmer. In the days of his boyhood he still saw his father slaving under the corvée for the lord of the manor. Mendel never forgot this impression. To his nephew, Ferdinand Schindler, Mendel often spoke of the Bauernbefreiung, the liberation of the peasant. He was proud that a Silesian like himself, Hans Kudlich, as a member of the revolutionary Parliament, proposed the bill for the abolition of the corvée which a short time later became a law. Mendel never forgot his humble origin, never forgot his sympathy with the poor.
My father, who was a physician in Brün during the last years of Mendel's life, had as patients many of the poor to whom Mendel had been a friend and helper. From what they told him he did not learn that Mendel had been a scientist, but that although a high churchman and dignitary at that time, he had remained a friend of the people, a man of the people. All those little fellows told stories about how often he had helped the poor with advice and with money, never haughty or highhanded, as a friend rather than an almsgiver. He did not preach Christianity, he practiced it!

As his nephew, Dr. Alois Schindler, mentioned in his commemorative speech, Mendel was a follower of the liberal party and voted with his party at the elections. The liberal party of those days was, of course, not very radical. But it was unusual for a Catholic priest and a prelate to be called a liberal, and Mendel, who was already suspected as a Darwinist, had to suffer for his convictions. How completely absurd and ridiculous in the face of these facts sound the assertions of Lysenko and his crowd who call Mendel a reactionary!

I know that these few glimpses into Mendel's life and heritage will give you a picture that is necessarily incomplete and probably not faultless. But it is the best I could do under the circumstances and I hope that you will accept it kindly.
3

THE KNOWLEDGE OF HEREDITY
BEFORE 1900

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NOTHING recently has illustrated Mendel’s overwhelming importance to the science of genetics so well as the fact that the Genetics Society of America is celebrating an anniversary not of Mendel’s own discoveries but of our discovery of Mendel. Fifty years ago three biologists found and read Mendel’s classic paper of 1865 and added greatly to their standing as scientists by understanding what it was all about. This event properly marks the advent of genetics as an independent discipline. Prior to 1900 there existed a great mass of data on heredity, indeed it had been accumulating for a very long time, but it had never been organized into a unified and coherent system. Actually, the basic principles which would insure its ultimate systematization were unknown, although, as we can see from the vantage point of 1950, all of the facts Mendel had were available to the pre-Mendelian biologists so that they could have constructed a real science of genetics had they viewed their data as Mendel viewed his. These facts had been properly discovered and accurately recorded, but they seem never to have come together in the mind of any one individual. Certainly no one ever understood their basic connections.

Today it would be very easy for a historian of science to call attention to these overlooked records of the past and to show that every single discovery of Mendel’s was, in reality, a rediscovery. Long before 1865 such phenomena as dominance and recessiveness and segregation had been described and described frequently; a definite and
accurate segregation ratio had been recorded eleven years earlier, but, needless to say, what we call Mendelism was unknown, nor was there any basic theory of heredity which could stand the test of well-designed and critical experimentation. The accurately recorded data were diluted with numerous errors, sound theory and fantastic notions were so mixed and jumbled that they could not be separated until post-Mendelian times. Any true picture of the status of the knowledge of heredity during the two or three millennia which precede our present century must include the grotesque as well as the valid, the superstitious as well as the scientific.

Practical knowledge of heredity precedes the dawn of history. The science and art of agriculture had to develop to a point where populations could be relatively concentrated before civilizations could emerge from simpler cultures. Practically all of our domestic animals and plants were tamed before writing was invented, and not only tamed but selected, improved, and so altered that in some cases their wild ancestors cannot be recognized. Many of them had to depend on man for their very existence. The great prehistoric improvements which occurred in our domestic flora and fauna could not have taken place in the absence of good breeding techniques, although the sound practices were generally imbedded in much irrelevant nonsense. The prehistoric farmer was like the character satirized by Voltaire who claimed that he could destroy a herd of swine very effectively by means of a proper combination of prayers, incantations and arsenic. Crops would be planted in the proper phase of the moon, sacrifices would be made to the appropriate deities, and the seed would be selected with the greatest care. After a few thousand years this resulted in real progress and sometimes our modern plant breeders find it very difficult to improve further the varieties which have been bred and selected for thousands of years.

Animal breeding was also pursued with skill, and the end results were good. We even have evidence of prehistoric hybridization. Our modern cattle, Bos taurus, are descended from crosses between B. primigenius and B. longifrons (Watson, 1910). However, we find that in classical times inbreeding was the standard practice and was considered desirable both in human beings and in farm animals. In
fact, it supposedly had great eugenic virtues. The Pharaohs married their own sisters when possible; in Greece, uncle-niece marriages were common, and the offspring of mother and son (Oedipus and Jocasta) and father and daughter (Cyniras and Myrrha) were depicted as exceptional specimens, not at all degenerate. Aristotle even stated, “Horses will cover mares from which they have been foaled and mares which they have begotten; and indeed a troupe of horses is only considered perfect when such promiscuity of intercourse occurs,” and Ovid, years later, describes the intensive inbreeding of all domestic animals as a perfectly “natural” routine. In this connection we might cite the fact that more than 2000 years after Aristotle, in 1763, the importation of all cattle was forbidden into the islands of Jersey and Guernsey so that the development of two famous pure bred stocks would not be hindered by foreign contamination (Walter, 1938). We may safely assume that by classical times domestic bred horses had been inbred for so long a period that continued inbreeding had no measurable ill effects.

We can also find an ancient prejudice against hybridization. The practice was forbidden to the Hebrews. “Thou shalt not let thy cattle gender with a diverse kind; thou shalt not sow thy field with mingled seed . . .” (Leviticus 19:19). But in spite of this prejudice the mule was a valued animal. It is mentioned in Genesis (36:24) and by Homer, Hesiod, and Plato. The sterility of the mule was discussed briefly by Empedocles and Democritus, and in great detail by Aristotle. Rare instances of fertile mules were recorded by Herodotus and Varro. Mules were described by Columella, Pliny, and later by practically every medieval writer on natural history. The mule, apparently, had come to stay.

The ancients were not so fortunate, however, in their concepts of other hybrids. They believed in the most fantastic creatures. Aristotle stated, in describing Libya, “… in that country animals of diverse species meet, on account of the rainless climate, at the watering places and there pair together, and such pairs will often breed if they be nearly of the same size and have periods of gestation of the same length.” (History of Animals 606 b 20). Pliny repeated this story and it remained in vogue for over 2000 years. A complete ac-
count of these hybrids is out of the question here. The grotesque explanations of the origin of the more unusual types of animals persisted into the sixteenth and seventeenth centuries, and are to be found in the works of such zoologists as Conrad Gesner and Johannes Jonston. Only a few illustrative examples can be mentioned. The giraffe was an oddity. It was clearly not the kind of animal any serious-minded deity would create, so its origin was explained more reasonably by assuming that it was a cross between a camel and a leopard. Indeed the camel had the reputation of being especially prone to experimentation. In Bactria the female camel mated with the wild boar and thus produced the two-humped variety. When the camel mated with the sparrow, we are not told how this cross was made but the result, according to Oppian, was the ostrich. The lioness was supposedly very lecherous and mated with the panther whenever she had the opportunity. After coition she would try to conceal her affair by washing herself in a river. If, however, the lion caught the scent of the panther on her she could expect the worst. Dogs were supposed to mate with wolves, foxes, goats (this produced the wild boar), lions and tigers. Aristotle says (H.A.607a5), “They take the bitch to a lonely spot and tie her up; if the tiger be in an amorous mood he will pair with her; if not he will eat her up, and this casualty is of frequent occurrence.”

One of the best known and most frequently described hybrid matings was that of the eel and the viper. This took place on the beaches. The viper came from the land and deposited his poison in a hollow rock, the eel came from the water; they paired, then went their separate ways. The viper retrieved his poison and continued to be a viper. This is described by Pliny and the early Christian fathers. It is recorded innumerable times in medieval natural history, and survived even the Renaissance to appear in the writings of the early zoologists. Still another hybrid, although one which came on the scene somewhat late, was the jumar. This beast, a cross between the horse and the cow, appeared in the middle of the sixteenth century and was not disposed of finally until the first decade of the nineteenth. During the 250 years that it existed in zoological literature it was described by such scholars as Gesner, Cardan, Scaliger,
Porta, Aldrovandus, Nieremberg, Jonston, Reaumur, Voltaire, Locke, Buffon, Bonnet, von Haller, and Spallanzani. (Zirkle, 1941)

It would be well to emphasize here that the animal hybrids I have mentioned are typical of those in the literature and that the number of such hybrids which could be cited could be increased indefinitely. Belief in such hybrids was a part of the mental equipment of zoologists for well over 2000 years and was an integral part of the atmosphere in which early genetics developed. More time cannot be devoted to this phase of the subject except to list, and that as briefly as possible, some of the animal crosses in which human beings were involved.

Stories of human beings impregnating animals and of being impregnated by animals tell us a great deal about what our ancestors thought concerning the actual mechanism of reproduction. There was, of course, much actual experimentation. The early Hebrews disapproved of such research, however, and among them the crime of bestiality was punishable by death. Elsewhere an occasional instance of human intercourse with animals was a part of religious ceremony so the Hebrew objection to bestiality may have been basically theological. Pindar (500 B.C.), Herodotus (484-425 B.C.), Strabo (7 B.C.), all record that in a city of Egypt where Pan was worshipped, women had intercourse with goats in public. The wife of Minos mated with a bull and produced the minotaur. Pliny records cases of women giving birth to a hippocentaur, to an elephant and to a serpent. That women bred with pet dogs occasionally was believed well into the seventeenth century, when Bartholini records an instance of a woman and new-born daughter being burned at the stake for the supposed crime of bestiality. The reciprocal crosses, however, were generally thought to produce human beings. Plutarch (46-125 A.D.) describes one girl born from a mare, and another from a she-ass, and Aelian cites the birth of a boy from a she-goat, but in this case the hybrid had goat legs. Sometimes human beings were born supposedly from the semen of wild animals. In Scandinavia and Russia it was the bear who kidnapped women and begat on them human offspring, who developed into men of great strength, heroes who married back into the human stock (Saxo Grammaticus and others). In southern and
western Europe the stories flourished which described how apes abducted women and begat semi-human offspring. These stories are numerous and detailed. Indeed the apes themselves were supposedly derived from the cross of a human being with some unknown quadruped. The height of absurdity seems to have been reached by Bur- zurgh-ibn-Shahrizar (ca. 954) who described the manatee as a hybrid between an Arab and a fish.

Stories of plant hybrids are much more prosaic though they are equally imaginary. In spite of the fact that the existence of sex had been recognized in the date palm since about 2400 B.C., and that the practice of caprification had a known sexual significance, the role of pollen in reproduction in general was not realized. Grafting, however, had supposedly a sexual symbolism and, through some sort of sympathetic magic, the ability to increase the yield. The description of the ceremony accompanying the insertion of the scion into the stock as described in the Nabatean Agriculture makes this clear. While this book is an attempted 10th century fraud, it undoubtedly describes very ancient customs. Theophrastos describes grafting and budding very accurately and records that the seedlings from grafted trees do not breed true for variety. Like statements can be found in all of the references to the subject in the works on classical agriculture. Cross grafting closely related plants (such as pears on apples) was a common practice. As time passed, however, more spectacular combinations were reported and the mutual interaction of stock and scion as described go well beyond any of the modern claims of Michurin or Lysenko. Florentinus (ca. 220 A.D.) gave directions for making citrons black by grafting them on apples and red by grafting them on mulberries and pomegranates. The reported graft hybrid, produced by the citron on the pomegranate, was the orange, and this origin of the orange was described by Diophanes (ca. 350 A.D.), Palladius (ca. 375 A.D.), and in the middle of the sixteenth century by the Spanish botanist, Monardes (Tolkowsky, 1938). On the other hand Al-Haj (ca. 1160) reported that all his attempts to produce this hybrid failed. Other Arabian botanists were not so careful however. Ibn Wahshya told how kumquats could be produced by grafting the orange on the olive. Abt-al-Latif (1162-1231) stated that when one planted the dif-
ferent citrus fruits near one another one could obtain from them innumerable varieties, perhaps a true record of spontaneous hybrids, but he described also the banana as a hybrid produced by inserting the seed of the date palm into the corn of the colocasia. This story lasted in Egypt until the eighteenth century when it was recorded by Hasselquist (1765), one of the pupils of Linnaeus. Other fantastic graft hybrids were believed in until well into the seventeenth century. Even Francis Bacon (1626) gave directions for producing them but at about this time a real sectorial chimera was recorded (1644). We will have to await the early eighteenth century when the role of pollen was at last recognized generally before we encounter accounts of real plant hybrids.

Turning from the fantastic to the reasonable should, by this time, be a relief. To begin with, the Greeks had some very rational ideas on heredity itself. They knew that heredity was not a simple matter, and this knowledge of heredity permeates both their poetic and scientific literature. Samples from a single tragedy of Euripides, Electra, will be used to illustrate their knowledge as shown in their drama. Orestes, a fugitive, returns to his native country in disguise. He makes a sacrifice at the tomb of his father Agamemnon and leaves there a lock of his hair. An old servant, suspecting his identity, takes the lock of hair to compare it with that of his sister, Electra. Obviously, family resemblance in hair types were known.

**Servant:** But view these locks, compare them with thine own
Whether like thine the color: nature loves,
In those, who from one father draw their blood,
In many points a likeness to preserve.

But Electra’s reply showed that the inheritance of such characters was not as simple as the servant thought.

**Electra:** Nothing may be inferred; besides, old man,
Tresses like-colored often may’st thou find
Where not one drop of kindred blood is shared.

One more example from the same tragedy! The usurper, Aegisthus, knowing that the blood feud would extend to any male offspring that Electra might bear, mated her to a low born fellow.
Orestes: Why did Aegisthus offer this base wrong?
Electra: Thus placing me he wished my children weak.
Orestes: That from thee no avenger might arise.

But Electra's husband shows himself to be a man of modesty, self control, and strength and Orestes declaims,

I oft have seen,
One of no worth a noble father shame,
And from vile parents worthy children spring,
Meanness oft grovelling in the rich man's mind.
And oft exalted spirits in the poor.

This was written in the fifth century B.C. and we would have to await a knowledge of the Mendelian segregation of polygenes and a knowledge of the irregularities of gene frequencies within a whole population before we would have a genetic explanation of Euripides' observations.

In Greece and Rome it was common knowledge that a child would sometimes resemble its male parent, sometimes its female and sometimes both parents. Plutarch and Lucretius even noted that heredity often skipped a generation and that the child would resemble one of its grandparents. The story of the "black baby" was also current and was supposed to show how a hereditary factor would appear in alternate generations. That is, the appearance of a black infant in a white family indicated that one of the parents had an Ethiopian ancestor. Instances of this happening are recorded by Aristotle, Antigonus, Pliny, and Plutarch and, of course, with such sponsors the story persisted and can be found in medieval literature. It has even come down to the present.

The peculiarities of heredity, the seemingly erratic nature of the appearance and reappearance of recognizable traits led, of course, to much speculation. Aristotle, in his attempt to explain its observed vagaries, cites, but does not endorse, perhaps the first account of a particular mechanism for the transmission of characteristics. It is worth quoting.

There are some who hold that the semen, though a unity, is as it were a "seed-aggregate" consisting of a large number of ingredients; it is as though someone were to mix and blend together a large number of juices
into one fluid, and then take off some of this mixture; in doing so he could take off not always an equal amount of each juice, but sometimes more of this and sometimes more of that, and sometimes he might take some of one and nothing at all of another: So they say, it is with the semen, which is the mixture of a large number of ingredients; and in appearance the offspring take after that parent from whom the largest amount is derived. (Generation of Animals 769 a 25)

For the fourth century B.C. this is not a bad attempt. We might compare it with a passage which Francis Galton published in 1889.

We appear, then, to be severally built up of a vast host of minute particles, of whose nature we know nothing, anyone of which may be derived from any one progenitor but which are usually transmitted in aggregates, considerable groups being derived from the same progenitor.

Homologies between animal and human inheritance were known in classical times and a rather drastic eugenics was practiced in Sparta and in Rome. The technique used was a socially approved infanticide. Deformed infants or those with recognizable defects were exposed. The Spartans in particular had rigid moral standards for they knew precisely the type they wished to breed. In the rest of Greece, however, eugenic ideals often yielded to practical considerations as described by the sixth century (B.C.) poet Theognis.

We seek well-bred rams and sheep and horses and one wishes to breed from these. Yet a good man is willing to marry an evil wife, if she bring him wealth; nor does a woman refuse to marry an evil husband who is rich. For men reverence money and the good marry the evil, and the evil the good. Wealth has confounded the race. (Roper, 1913)

While wealth could be secured by a dysgenic marriage, it could be obtained in animal breeding only by sound biological practices. The Roman agricultural writers gave very precise directions for selecting and breeding all types of domestic stock, and little or no improvement over their methods occurred until after the discovery of Mendel’s paper. Even the great eighteenth and early nineteenth century breeds, which are still our thoroughbreds, were created by an extension and systematization of the Roman practices. There is not time for illustrating this with many examples, but it is obvious that the Roman animal breeders knew some of the principles of heredity. In his de-
The Knowledge of Heredity Before 1900

The description of the desirable qualities in a ram which was to be used for breeding, Varro included the statement, "you must see that he has not a black or parti-colored tongue, for those which have beget as a rule either black or parti-colored lambs." He stated also that rams whose mothers had given birth to twins were valuable breeders and that in the breeding of mules both parents should be selected with care as the offspring took after both.

It might be well to include here a number of miscellaneous observations, some accurate, some fanciful, which illustrate both the virtues and the shortcomings of the ancient and medieval knowledge. In no case, however, could a reasonable explanation be offered for even sound observations before Mendelism was discovered. To begin the miscellany, Aristotle noted that, "In human beings, more males are born deformed than females; in other animals there is no preponderance either way." (G.A.775a5) The first half of this statement conforms with our knowledge of the higher prenatal death rate of males. In the pseudo-Aristotelian Problems there is the statement that while blondes have their good points and are courageous, they are not very well supplied with brains—an idea now paying very well on Broadway. Also we can infer from this that Aristotle was himself a brunette. Plato in the Politicus held that courage bred to courage through many generations culminates in insanity and that the soul full of an excessive modesty mated to a similar soul becomes in the end useless and paralyzed, therefore to achieve a proper mean, opposites should marry (Roper, 1913). Jumping 1500 years we find the statement in the Summa theologica (ca. 1256) of St. Thomas Aquinas, where he treats of hereditary disease, "that even some defects of soul are transmitted in consequence of a defect in the bodily habit, as in the case of idiots begetting idiots. . . ." Certainly this is a very early recognition of the inheritance of a mental trait. Skipping another 500 years we find Maupertuis in 1745 describing the inheritance of a sixth finger. This character was transmitted by both male and female parents and was used to refute both the spermatists and ovacists. Glass (1947) has called attention to the fact that Maupertuis described a particulate mechanism for heredity, and that his hypothetical particles had some of the properties of Mendelian genes.
These are but instances of numerous observations and deductions which should of course be collected, systematized and incorporated in any proper history of genetics.

From the vantage point of 1950 we can easily evaluate these early records and judge their worth very accurately. Many of the actual facts can be accepted as reported, and we know now that they must actually have been as they were described. The explanations, however, are another matter. This contrast between sound fact and impossible theory can be shown very clearly by comparing the early and even later pre-Mendelian descriptions of sex-limited and sex-linked heredity with the preposterous guesses as to the mechanism of sex determination itself. Sex-limited inheritance was recognized from the earliest times. Castration as a practice antedates history and its use lay in its known effects upon secondary sexual characters. That certain abnormal characteristics appeared in only one of the sexes was also known. The fact that pattern baldness (alopecia) is a sex-limited character was first recorded 400 years before Christ. It is well to contrast this accurate classification of baldness with the erroneous statement in some of our modern textbooks of genetics that baldness is sex-influenced.

Hippocrates reported (ca. 400 B.C.), "Eunuchs neither get gout nor grow bald," and Aristotle stated, "For no boy ever gets bald, no woman and no castrated man." In the sixteenth century, Rhodiginus and Donatus noted that eunuchs were never bald and as late as the nineteenth century Darwin held baldness to be an "incipient secondary sexual character." In 1942, Hamilton finally showed that alopecia depended for its expression on the male sex hormone. Another sex-limited character, triorchidism, was known in classical times and in the sixteenth century, Fernel, Montanus, Forest, and Zwinger and in the seventeenth century, Sinibaldus showed that it was hereditary, but, as we would infer, it appeared only in the males. In 1731 the first member of the famous porcupine family appeared before the Royal Society of London. The pedigree of the family was followed until 1851 and it shows that this character was transmitted in the Y chromosome. Again, the peculiar inheritance of the tortoise shell coat
color of cats was noted in the nineteenth century by Layard (1854), Darwin (1868), (1871), and Mivart (1881).

Knowledge of sex-linked inheritance dates from the end of the eighteenth century. The sex-linked inheritance of color-blindness was recorded by Priestley (1777), Lort (1778) and Dalton (1798). In 1793 the sex-linked inheritance of hemophilia was recorded in the *Medicinische Ephemeriden* of Chemnitz and ten years later, in 1803, by Otto in Philadelphia. By 1820, enough cases of hemophilia were known to justify a review paper by Nasse. In 1878, Pagenstechter found a type of night blindness which was sex-linked and in 1882 Owen described sex-linked nystagmus. In 1893, Samuel Cushman published a partial description of sex-linkage in poultry. In fact, sex-linkage was a well known but not understood phenomenon long before the discovery of Mendelism. (Zirkle, 1946b)

There is also pre-Mendelian evidence of attached X-chromosomes in women. This is probably a very rare occurrence and for it to happen under conditions where it could be recognized is rarer still. The attached X's would have to carry a recognizable dominant gene or be homozygous for a recessive. This gene or these genes would be transmitted to all of the female descendants and to none of the males, nor would it or they be transmitted through the males. Two pedigrees which meet these conditions have been published. In 1645, Sir Kenelm Digby described the inheritance of two thumbs on the left hand. In five generations, as far as the pedigree tells, all the women but none of the men had the extra digit. In 1838, Cunier published a very complete pedigree of a color-blindness which was transmitted from mother to daughter and which met all of the requirements of attached X's.

In the genetics of sex determination itself, needless to say, no progress whatever was made until the role of the chromosomes was understood. But lack of the basic information did not in any way inhibit speculation. Indeed we find three widely known and used hypotheses for the explanation of the remarkable fact that some babies were born male, others female. Two of the hypotheses were of such a nature that they could be tested, but in spite of this they persisted a very long time. The first seems rather naive. The fact that there were two
sexes and that males had two testicles seemed more than a coincidence. What more reasonable than to assume that the semen from one begat males and from the other females? The dexter testis, of course, produced the dominant sex, the sinister one females. This hypothesis was prevalent among the ancient Hebrews and it was the one preferred by Anaxagoras. Leophases claimed that males who copulated with the right or left testis tied off produced female or male offspring respectively. Some of his contemporaries even alleged that the same effects occurred when the father had one testicle removed. Actually attempts were made to alter the sex ratios in the flocks and herds, and the custom of tying off a testis before copulation lasted a long time with the sheep and cattle breeders. Instances when the technique did not work were apparently ignored. Aristotle, however, condemned the practice and said that the statements of the reports of controlling sex by inactivating a testis were untrue and mere guesswork.

The second hypothesis was not subject to experimental testing and consequently lasted much longer than the above. Stated in most general terms it is that the sex of the offspring is determined by whether the male or female parent contributes the most semen to its formation or by which one of the parents was the more heavily sexed. There are countless variations of this basic theme occurring from the time of Aristotle to the twentieth century. An effeminate man could supposedly beget only daughters, and this notion has given rise to an allegedly humorous situation which is still exploited by comedians. To show how little progress was made during the centuries we may note that this hypothesis was still respectable at the time of the discovery of the X-chromosome. Even Darwin held that the embryo did not develop from a single egg impregnated by a single spermatozoan but was influenced by the whole mass of semen.

A third hypothesis, to us the most fantastic, was that the sex of the offspring was determined by the direction the wind was blowing at the time of coition; when the north wind blew males were engendered; when the south wind, females. Shepherds tried to take advantage of this supposed fact to increase the number of females in their flocks by letting the rams in to the ewes only when the south wind
was blowing. The copulating animals should also face south to get
the full benefit of the wind. Aristotle treated this notion with respect
and it was further cited, also with respect, in the third century B.C.
by Antigonus, in the first century of our era by Columella and Pliny,
in the third by Aelian, in the fourth by Palladius, in the seventh by
Bassus, in the thirteenth by St. Thomas Aquinas, St. Albertus Magnus
and Vincent of Beauvais. In the fourteenth century it was cited by
Joannes de Sancto Gemmianus and Pierre Bersuire. In the fifteenth
century it was noted by Volateranus, in the sixteenth by Wotton
and in the seventeenth by Jonston and DuPleix. I have found no later
reference to it but I believe that we should never forget so remarkable
and so lasting an hypothesis.

Two more subjects will have to be considered briefly before we come
finally to the pre-Mendelian ideas of the mechanism of heredity.
These topics are mutation and segregation. A belief in sudden, large
mutations, actual species changes, was universally held up to the
time of Linnaeus. This fact has been obscured somewhat by an acci-
dent of language. The term used to describe these mutations was
degeneration, which in the earlier days did not necessarily connote
deterioration but had its literal meaning of generating away from the
normal. Such degenerations were very close to the DeVriesian muta-
tions in concept even when they did not actually happen. Theop-
hrastos described how wild plants were changed when they were
cultivated and how one species changed to another when it was
grown in a new country. Nicolaus of Damascus also reported the
transmutation of many species. Virgil and Pliny told how the farmers
planted wheat and barley only to discover that some of the seed
suddenly produced wild oats. A grain of oats was reported occurring
in an ear of wheat by Thomas Johnson in 1633 and a grain of rye
in an ear of barley by Ole Worm in 1655. These records are offered
here only for comparison with Lysenko's supposed discovery, as re-
ported this year, of grains of rye occurring in an ear of wheat.

Peter of Crescentius, the great fourteenth century agriculturist,
devoted three chapters to sudden species changes. Levinus Lemnius
in the sixteenth century also discussed the matter in detail and for the
next 200 years the sudden mutation of species was recorded in prac-
tically every work on natural history. We should remember that the idea that species are immutable units was dominant for only a little over 100 years, 1750-1859.

Many of the supposed instances of degeneration, particularly in flower color, were really instances of Mendelian segregation. The herbals describe these cases faithfully. Tulips and marigolds were particularly aggravating for when the seed was sown no one could ever foretell what the color of the flowers would be. James Garrett, according to John Gerard (1597), worked for twenty years on tulips but he could never get them to breed true and could never discover why they did not. It was not until the early-eighteenth century, after plant hybridization was recognized, that any sort of explanation was possible.

In 1716 Cotton Mather described spontaneous hybridization in Zea mays, which was a year before our earliest record of Fairchild’s male, a cross between a carnation and a sweet william. The next year (1717) Bradley described hybrid auriculas, and in 1721 Marchant recorded a hybrid in Mercurialis and Miller a hybrid cabbage. Some thirty workers had written on plant hybrids when Koelreuter’s epoch-making contribution appeared (1761-6). From this point on the sudden appearance of an unsuspected type was generally ascribed to its hybrid ancestry. (Zirkle, 1935)

The discovery of the reappearance of ancestral types in the second hybrid generation was only a matter of time. Goss and Seton in 1822 and Sageret in 1826 made a good beginning. In crossing a musk melon with a cantaloupe Sageret listed the contrasting characters and found that the hybrid was not a blend but took some of its characteristics from one parent and some from the other. In 1849 Gartner published his prize essay. In this he noted the uniformity of the first generation hybrid and the extreme diversity of the second and succeeding generations. Both the types of the parents and in addition entirely new types appeared. The variability in the later generations was not confined to flower color but affected the entire habit of the progeny. Naudin in papers published in 1863 and 1864 contrasted the uniformity of the F₁ with the “extreme medley of forms” in the F₂, “some approaching the specific type of the father,
In 1865 Verlot noted the fact that in a hybrid progeny certain individuals bred true but others produced numerous atavisms. Today we would call the two types with which he worked homozygous and heterozygous. (Roberts, 1929)

We have now come in our discussion of segregation to the year in which Mendel published. Any further listing of segregation in the F₂ generation of plant hybrids would be post-Mendelian, although as long as Mendel's own work was unknown it would belong with that of his predecessors. Darwin in 1868 recorded the “prepotency” of a character which appeared in the F₁ hybrid snapdragon and the existence of both types in the F₂. He even counted the progeny and got a ratio of 88 to 37. Such a ratio would mean little to anyone in 1868 who had not read Mendel's paper, and Darwin made nothing of it. In 1879, Vilmorin's experiments with Lupinus were reported. Here in the segregation of red and blue flower color certain 3 to 1 ratios were recorded. In different strains the ratios were reversed but again no fundamental inferences were drawn. Singleton (1935) has called attention to the work of three American corn breeders, W. A. Kellerman, W. T. Swingle, and Willet M. Hays. These men in the 1880's counted the different types of grain on segregating ears and reported several 1 to 1 and 3 to 1 ratios. McCluer in 1892 recognized the reappearance of the parental types in the F₂ generation of corn, and Spilman in 1901 noted the reappearance of such types in the F₂ generation of wheat and recorded their relative abundance.

We now come to the pre-Mendelian experiments on Mendel's own genus, Pisum. In 1729, Henchman had noted the occurrence of peas of different colors in a single pod on a hybrid plant. In 1822, the famous experiments of Goss and of Seton were published. Goss had reported dominance in the first hybrid generation and segregation in the second, but he did not count the progeny and he published no ratios. Seton also noted that the hybrids were not intermediate between the parents and that in the F₂ generation “they were all completely either of one color or of the other, none of them having an intermediate tint.” In 1823 Thomas Andrew Knight verified the work of Goss and Seton and in addition he back-crossed a first generation hybrid to the recessive parent and he got in consequence two types
of peas. Again he did not count the progeny. In 1872, Laxton got results similar to the above and he actually published some evidence of linkage in peas between height of plant and flower color. He did not call attention to the evidence of linkage, however, and he made no counts. In 1889, Vilmorin reported different colored peas in the same pod. (Roberts, 1929)

We have only to read Mendel’s paper to see how much further he saw into the nature of heredity than did any of his predecessors or contemporaries. Indeed no one caught up with him until 16 years after his death and 35 years after his work was published. A problem which faces us now and which will face us for some time is, did Mendelism spring fully matured and fully armored from Mendel’s own brow without there having been any previous insemination? How did Mendel know what to look for and why did he count the F2 progeny? Did he get no hint at all from the previous work of other investigators? I, for one, believe that he did and that the predecessor who helped him most was Johann Dzierzon.

Dzierzon bred honey bees. He reported that drones were hatched from unfertilized eggs but that the workers and queens came from the eggs that had been impregnated. This idea was so novel at the time that it gave rise to a violent controversy. In the 1850’s Dzierzon published a number of papers and he was well known at least to those who were interested in honey bees. In one of his experiments he crossed German with Italian bees and found that the unmated hybrid queens produced German and Italian drones in equal numbers, a definite one to one ratio (Whiting, 1935). I believe that Mendel knew of this work for three reasons, (1) Dzierzon was a well known bee breeder, (2) Mendel also raised bees, and (3) Dzierzon was a fellow cleric who lived in nearby Silesia.

If a hybrid female would produce two types of eggs in equal numbers, the idea that a hybrid male would likewise produce two types of sperm, also in equal numbers, was indicated by the internal logic of the situation. In the absence of a selective fusion of gametes the ratio of 1:2:1 was inevitable. Where dominance existed this would be seen as a three to one ratio. It seems to me that Dzierzon’s work at least alerted Mendel to the importance of definite ratios in inter-
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preting his own results and in describing a possible mechanism of heredity.

If we omit, as we must, any description of the great contributions to genetics made by Francis Galton and Karl Pearson, only one more topic remains to be considered, the pre-Mendelian concept of the physical basis of heredity. This can be treated briefly, for, before Mendel published there was but one general picture of the hereditary mechanism, although before his work was discovered a theory extraordinarily like our own was in vogue. If we omit such related problems as that of embryo formation and such vagaries as preformationism and concentrate directly upon the supposed means of the transmission of characters from one generation to the next, we find that all of the speculations lead to some form of what Darwin called "pangenesis." Darwin thought that every part of the body produced a germ of itself which he called a "gemma." These gemmules were supposedly discharged into the bloodstream and were concentrated ultimately in the semen. In the next generation they supposedly reproduced that part of the body which had produced them. Buffon had described an hypothesis very close to Darwin's and the earlier views were also like Darwin's only much simpler, merely consisting of the notion that the semen came from all parts of the body. This doctrine of pangenesis in all its forms was always associated with the long prevailing view that acquired characters were inherited. Indeed one of its chief functions was to explain how such characters could be transmitted.

The idea of acquired characters being inherited is extremely old and had become enshrined in prehistoric myths. In classical times it was described in detail by Hippocrates, Aristotle, Antigonus, Strabo, Pliny, Galen, Solinus, Justinus, and others. Fourteen descriptions of it were published during Lamarck's own lifetime before Lamarck used it to explain evolution. It had been the generally accepted view for over 2000 years. Lamarck, however, did not help the belief along. He seems to have been a man with no trace of a sense of humor and hence he showed a real talent for making himself ridiculous. After he had finished his account of the inheritance of acquired characters it had become so laughable that a real scepticism developed. The doctrine had persisted two millennia but it could not stand Lamarck's
endorsement. After Darwin had proven the fact of evolution, however, it had a brief revival until it again lost standing as a result of Weismann.

The earliest record that we have of pangenesis is a fragment of Anaxagoras. A half century later Hippocrates stated, in explaining the inheritance of an acquired head shape, “The seed comes from all parts of the body, healthy seed from healthy parts, diseased seed from diseased parts.” Some ninety records of pangenesis down to the time of Darwin have been listed elsewhere and need not be repeated here (Zirkle, 1946a). One point should be emphasized, however. Pangenesis was the accepted doctrine when Weismann began his great series of contributions to biology. It was the doctrine he replaced. This fact helps to explain the emphasis Weismann placed on several aspects of his theories.

Today Weismann is definitely in need of a re-evaluation. He has been a historical figure so long that we have become accustomed simply to labelling him with the phrase “the continuity of the germ plasm,” to bowing politely in his direction and immediately directing our attention elsewhere. Doubtless many biologists were surprised when he was recently resurrected in Russia to serve as a target for the scorn of official Soviet biology. Weismann was one of the most remarkable of all biologists. When we re-read his books today we get the definite impression that he could foresee the experiments which were to be performed in the twentieth century but, of course, could not foresee what the results of the experiments would be. He raised great hypotheses on slender factual bases but his hypotheses were so specific that today they have practically all been either proved or disproved. We now have no excuse for not evaluating Weismann accurately.

Weismann’s detailed picture of a germ plasm composed of undifferentiated cells, separated from the soma, was doubtless influenced by the prevailing ideas of the time. We can see why he would emphasize the separation of the germ plasm because of the widespread but erroneous belief in pangenesis, and why he had to consider the germ plasm completely undifferentiated for he believed that cellular differentiation was accompanied by what we would now call a loss
of genetic material. Philosophically, we can simplify his concept somewhat. We no longer have to disprove pangensis, and modern research has not substantiated Weismann's concept of the causes of cellular differentiation. All we have to hold to now is that all cells come from the division of pre-existing cells and that the eggs and sperms are no exception in this respect. The immortality of the germ plasm implies only that dead cells never divided to produce living eggs and sperms and probably never will. The emphasis placed on the separation of the germ plasm from the soma has, in the past, confused some botanists but what misunderstanding exists today seems purely verbal. If the plant meristems had simply been named germ plasm the matter would have been settled long ago.

We can bring this discussion of pre-Mendelian concepts of heredity to a close by summarizing some of Weismann's later ideas as he published them in 1892. It will be clearer if we express his thoughts in our own modern language and avoid the specialized terms which he used and which have long since been discarded. A number of Weismann's contemporaries, particularly cytologists, held like views.

The scientist who seems to have influenced Weismann's later views more than any other was Wilhelm Roux. In 1883, Roux had described the behavior of the nucleus in mitosis and inferred from the activities of the chromatin that (1) heredity was particulate and (2) that the hereditary particles were in the chromatin. Weismann followed Roux and held that heredity was controlled by a special hereditary material which was particulate and not distributed throughout the entire cell. This hereditary material, the real germ plasm, was carried in the nucleus but not in the whole nucleus. The nucleolus was not composed of hereditary material nor was the centrosome. Only the nuclear rods or chromosomes bore the hereditary particles. The heredity carriers were actually in the chromatin granules which were qualitatively different. They were divided equally whenever the chromosomes split longitudinally. The number of hereditary determiners had to be halved in the formation of egg and sperm but their number was restored by the fusion of the nuclei of these two cells. The halving of the hereditary particles was secured in one of the peculiar
cell divisions which preceded gametogenesis where the chromosomes did not split longitudinally but the cell itself divided.

In regard to the actual transmission of characters he noted that sometimes a child would be intermediate between his parents, sometimes he would resemble only one of them and sometimes be like the father in some characteristics and like the mother in others. In spite of this, however, children were equally related to their parents, but were not necessarily equally related to their grandparents, hence a child would often resemble one grandparent more than the others. "I am convinced," wrote Weismann, "that the two forms of amphimixis—namely the conjugation of unicellular, and the sexual reproduction of multicellular organisms—are means of producing variation. The process furnishes an inexhaustible supply of fresh combinations of individual variations which are indispensable to the process of selection."

With the prevalence in 1892 of such views as we have quoted from Weismann, we can easily understand why Mendel received such a welcome in 1900.

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THE BEGINNINGS OF MENDELISM
IN AMERICA

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BECAUSE of limitations of time, space, and my own knowledge, I
shall confine this discussion of the beginnings of Mendelism in
America to the first decade of the century.

As is well known, Mendel's law was rediscovered and verified in
1900 by three botanists independently, de Vries in Holland, Correns
in Germany, and Tschermak in Austria.

What Mendel had shown and they verified may be summarized
thus:

1. When parents differ in a character, the offspring resemble one
parent but not the other. This is the principle of dominance.

2. But when the hybrid reproduces, it transmits with equal fre-
quency either the dominant character of one parent or the recessive
character of the other, but not both. This is the principle of segrega-
tion.

3. When parents differ in two or more pairs of characters, each
pair shows dominance and segregation independently of the other.
As a consequence all possible combinations of the two or more pairs
occur in the reproductive cells of the hybrid, and in their chance
frequencies. This is the principle of recombination.

De Vries called Mendel's law the law of the splitting of hybrids.
He seems to have regarded it as of less importance in evolution than
the mutations which he observed occurring in the evening primrose,
in which new species seemed to be originating suddenly, rather than by gradual changes.

Bateson in England had long been looking for evidence that discontinuous variations, sports, may be of importance in evolution. He saw in Mendel's law a means by which different, discontinuous variations may be combined in all possible ways, thus making progressive evolution possible. Hence his immediate enthusiasm for Mendelism which he regarded as a master key in the study of evolution. He later christened the subject genetics, and the experiments conducted by him and his associates contributed much to its early development. He was the real founder of the science of genetics, as well as the one who gave it that name.

Mendelism found a hearty welcome in America from two groups of biologists, those who were interested in the study of evolution from a pure science viewpoint, and those who sought more efficient ways to produce improved varieties of plants and breeds of animals.

Members of the first group had been electrified by the revolutionary ideas of Weismann and de Vries and had set out to give those ideas experimental tests. Outstanding in this group were C. B. Davenport, who became the founder and first director of the Station for Experimental Evolution, later known as the Division of Genetics of the Carnegie Institution of Washington; Geo. H. Shull, pupil and colleague of Davenport at Cold Spring Harbor; and D. T. MacDougal, director of the New York botanical garden, champion of de Vriesian mutation as exemplified in the evening primrose. Later he founded and became director of the Department of Botanical Research of the Carnegie Institution.

Outstanding in the second group were several plant breeders connected with the United States Department of Agriculture, or state agricultural experiment stations, namely, W. M. Hays, H. J. Webber, W. J. Spillman, R. A. Emerson and E. M. East. They saw in Mendelism a new tool for the production of new and improved varieties of plants and animals.

W. M. Hays was professor of plant breeding at the University of Minnesota. He founded and became permanent secretary of the American Breeders' Association in 1903. Shortly after this he became
Assistant Secretary of Agriculture, with headquarters at Washington, D. C., where he spent the all too short remainder of his life. H. J. Webber was in 1900 a plant breeder in the Department of Agriculture engaged in hybridization experiments with cotton and citrus fruits, which gave results recognized by him as Mendelian, as soon as he became acquainted with Mendel's law. W. J. Spillman had a similar experience in hybridizing wheat in the experiment station of Washington State College. Under the sponsorship of Hays, he was presently transferred to Washington, D. C, to become advisor in animal breeding projects in the Department of Agriculture. R. A. Emerson was a young and wide-awake professor of horticulture at the University of Nebraska who had for some years been studying crosses between different varieties of beans. In 1904 he published a report on his results interpreting them in the Mendelian terms used by Batson and Saunders. Early in the second decade of Mendelism, in 1912, he went to Harvard to study with East, although East was several years his junior in age and experience in teaching. They cooperated in an epoch-making study of the inheritance of quantitative characters in maize which earned for Emerson a doctor's degree. Later Emerson went to Cornell, in succession to H. J. Webber, where he made maize a tool in the study of genetics second in importance only to Drosophila. E. M. East, a graduate of the University of Illinois, was agronomist at the Connecticut Agricultural Experiment Station in the years 1905-1909. In 1907 he published a paper entitled "The relation of certain biological principles to plant breeding." It was of such outstanding excellence as a discussion of mutation, Mendelism, selection and evolution that Harvard University in 1909 invited him to come to the newly reorganized Bussey Institution, to develop the field of plant genetics in coordination with the field of animal genetics in which I was working. My pupils and I over the succeeding years profited greatly from this association.

THE AMERICAN BREEDERS' ASSOCIATION

Something more should be said about the American Breeders' Association, which probably most present day geneticists have never
heard of. As already indicated the moving spirit in its formation was W. M. Hays of Minnesota.

In an opening address as chairman of the organization committee at St. Louis on December 29th, 1903, he said:

Gentlemen: We have assembled at the suggestion of the American Association of Agricultural Colleges and Experiment Stations to consider the improvement of animals and plants. That association for three years has had under favorable consideration the formation of an American Association of Plant and Animal Breeders and those interested in the problems of heredity. That association, which is in touch with scientific and experimental agriculture as is no other organization in the world, has suggested that the scientists in biological lines turn for a time from the interesting problems of historical evolution to the needs of artificial evolution. It asks practical breeders, while seeking financial returns from breeding living things, to occasionally pause and study the laws of breeding. It has invited the breeders and the students of heredity to associate themselves together for their mutual benefit and for the common good of the country and the world. It has thus recognized that the wonderful potencies in what we are wont to call heredity may in greater part be placed under the control and direction of man, as are the great physical forces of nature. It has suggested that we meet together that each may get the point of view of the other and that each may appreciate the problems of the other. It suggests that the scientists should know the conditions of the living organisms under improvement, and the practices and the problems of the practical breeder, that they may apply their scientific methods to the solution of these problems. It suggests to the practical breeders that they, in turn, study the facilities, methods, facts, and theories of the scientists, that they may not only take advantage of the accumulating facts, but may place in the crucible of practical test any promising facts and theories which may be brought out by the scientists.

With these eloquent words was launched an organizational experiment "noble in purpose" but doomed to a comparatively short life because of its too great inclusiveness. Mendel's law received at this meeting only a passing mention. Its bearing on the problems of practical plant and animal improvement was still problematical.

At the second meeting of the association in February, 1905, Mendel's law began to receive more attention. A paper by H. J. Webber explained the operation of Mendel's law in cotton breeding as illustrated in the inheritance of two character pairs, smooth versus hairy
stem, and fuzzy versus smooth seed. W. J. Spillman gave a paper on
Mendel's law in relation to animal breeding, showing that in cattle
breeding the hornless (polled) character is dominant to the normal
horned condition, and how it is thus possible permanently to dehorn
a breed by controlled matings. I also, having recently joined the
association, gave a paper on recent discoveries in heredity and their
bearing on animal breeding, using as illustrations experiments with
guinea-pigs and rabbits. At this meeting Professor C. G. Hopkins of
Illinois presented a paper on inbreeding in corn and methods of
preventing its ill effects.

The fourth meeting of the association was held in Lincoln, Ne-
braska, in January 1906. Its proceedings contain little of present day
interest, but resulted in a characteristic American organizational
spree, leading to the formation of special committees on breeding of
nearly every different crop or breed of farm animals.

The fifth meeting of the association was held in Columbus, Ohio
in January 1907, and for the first time, papers on theoretical prob-
lems of breeding began to outnumber those on purely practical as-
pects of the subject. C. B. Davenport gave papers on inheritance in
pedigree breeding of poultry and on recent advances in the theory
of breeding. I gave a paper on the production and fixation of new
breeds. G. H. Shull gave a paper on the importance of the mutation
theory in practical breeding. W. J. Spillman gave a paper on the
chromosomes in the transmission of hereditary characters. In this
paper Spillman endorsed the idea that the segregation of maternal
and paternal chromosomes at the reduction division afforded a basis
for explaining Mendel's law, as had been suggested by E. B. Wilson
in 1902, and strongly supported by Sutton in 1903. From this list it
will be seen that Mendelism was really getting a firm hold in the
thinking of the American Breeders' Association. It is fitting that the
city where these papers were presented should be chosen as the place
in which to celebrate the semi-centennial of Mendelism.

The sixth meeting of the association was held in Washington,
D. C. in January 1908. It climaxed the development of the associa-
tion from an original mixed assemblage of scientists interested in the
study of heredity, agricultural experts guided by scientific knowledge,
and rule of thumb horticulturalists, and animal breeders governed by old wives' tales about heredity. It had now largely eliminated the third unprofitable constituent of its membership and become a full-fledged American society of genetics, in fact though not yet in name.

The program at this meeting in 1908 included papers by David Starr Jordan and Alexander Graham Bell on eugenics; by W. A. Orton on the theory and practice of breeding disease resistant plants; by H. J. Webber on cotton breeding; by W. J. Spillman on color factors of mammals in which he postulates the transmission of factors influencing coat color as being located in at least four different chromosomes, Spillman being now fully committed to the chromosome hypothesis.

I gave a paper on color variation among domestic animals, and Davenport gave a report on recent advances in the theory of heredity. E. M. East presented a paper on organic correlations in the course of which he discussed the reduplication hypothesis of Bateson and Punnett as an explanation of what later came to be called genetic linkage.

But the most memorable paper presented at this meeting was given by C. H. Shull under the title, "The composition of a field of maize." He showed that under enforced self-fertilization a strain of maize becomes resolved into a number of distinct biotypes with greatly diminished productiveness, but recombination of these types by intercrossing them restores the lost productiveness. He concluded that "continuous hybridization, instead of the isolation of pure strains, is perhaps the proper aim of the corn breeder."

In this paper Shull outlined the theoretical basis for "hybrid corn." The correct method for giving it practical application was outlined in detail by Shull at the next meeting of the association held in Columbia, Missouri, in January 1909, the title of that paper being "A pure line method in corn breeding."

This 1909 meeting place of the association at Columbia, Missouri was too remote from the geographic center of geneticists, for those with academic connections to attend in the midst of their busy winter semester. Nevertheless a large number of important papers were presented, either in person or by title, and later published in Volume
of the Proceedings. They included an important paper by Lawrence Balls, one of Bateson’s pupils who had been carrying on experiments in hybridization with cotton in Egypt. After extensive study he concluded that “Mendel’s law applies to all characters of the cotton plant which have so far been investigated.”

Papers on recent advances in knowledge of heredity were presented by Spillman, Davenport, Webber and others including (notably) Luther Burbank who under the title “another mode of species formation,” stressed the importance in hybridization experiments of producing large populations, going beyond the first hybrid generation, and ruthlessly selecting the progeny. Possibly this paper was ghost-written by G. H. Shull. It bears his fingerprints.

Papers on eugenics were presented by Alexander Graham Bell, David Starr Jordan, V. L. Kellogg, F. A. Woods, and others. Besides the important paper of Shull already mentioned an important paper was presented by Emerson on factors for mottling in beans.

A sour note was injected into the proceedings by T. H. Morgan, then a member of the opposition, who under the title “What are ‘factors’ in Mendelian explanations?” questioned the reality of the existence in the chromosomes, or elsewhere in the germ cells, of the supposed material bodies responsible for the production of Mendelian characters. He suggested instead that “the condition of two alternative characters may equally well be imagined as the outcome of alternative states of stability (or of conditions) that stand for the characters that make up the individual.” Of course it was only a short time later that Morgan, having begun breeding experiments with Drosophila and discovered sex-linked inheritance of white eye, changed sides completely and became the most thorough and orthodox of Mendelians, but that was in 1911 and is part of a story which it is the province of later speakers to tell. Morgan was too good a scientist to hold to a conclusion after he believed it had been clearly disproved.

AMERICAN BREEDERS’ MAGAZINE

After 1909 formal annual meetings of the Breeders’ Association were discontinued, and instead a monthly American Breeders’ Maga-
zine was started under the editorship of Hays. This continued for four years but was discontinued with the death of Hays in 1913.

Hays literally worked himself to death in his joint role of Assistant Secretary of Agriculture and Secretary of the American Breeders' Association. Suffering from a nervous breakdown which he did not long survive, he entrusted the welfare of his beloved association and magazine to an emergency committee, of which David Fairchild was named president and I vice president. We held a meeting in Washington at which it was decided to attempt salvage of the magazine, but abandonment of the more ambitious association, except as a ghost organization which might be helpful in securing support for the magazine, just as "membership" in the National Geographic Society supports the National Geographic Magazine.

The ghost organization was named the American Genetic Association, with Fairchild as president and myself as vice president, as we still are, at least by title, "honorary" officers. The vital function of this organization as planned was to make a success of a monthly journal devoted to the publication of short articles, clearly written in language understandable to the non-technical reader, and always with good photographic illustrations, if obtainable. This was Fairchild's idea and it was a good one, which he admittedly borrowed from his brother-in-law, Gilbert Grosvenor, founder and editor of the National Geographic Magazine.

For the magazine we adopted the name Journal of Heredity, to avoid confusion with the Journal of Genetics published in England. The carrying out of Fairchild's idea, with a measure of success, has made the Journal of Heredity perhaps the most valuable of existing media for the wide dissemination of knowledge about genetics. Great credit for this belongs to the present managing editor, Robert C. Cook.

And now may I be permitted, with no show of false modesty, to say something about my own part in the beginnings of Mendelism in America, since I know more about it than anyone else, and few of my contemporaries in that first decade survive.

I came under the inspiring influence of C. B. Davenport in 1893 when he was a young instructor in zoology at Harvard University,
and I, a graduate student, served as his laboratory assistant in an
elementary course. I also took as minors the courses which he gave in
the field of experimental morphology and related physiological as-
pects of biology. These interested me greatly. Fellow students in
these courses were H. S. Jennings and H. V. Neal. A few years later I
in turn became an instructor in zoology at Harvard and shortly there-
after Davenport went to Chicago University, leaving the department
with no one to give his advanced courses. I asked Professor Mark to
let me undertake the job, and he, I suspect with some reluctance,
consented.

At that time all graduate students in the department of zoology
working for a Ph.D were expected to do their thesis work under the
direction of Professor E. L. Mark, while we of subordinate rank, G.
H. Parker and myself, gave advanced courses in the subjects in which
we were ourselves working, which might be taken as minors by
candidates for the higher degrees. So it came about that in the
decade 1900-1910, I supervised the thesis work of only two candidates
for the doctorate, but had several cooperators in research, limited in
scope and duration, whose names appear as junior authors in several
publications.

The rediscovery of Mendel’s law left many related problems unsol-
solved. Our work in this decade was largely directed toward their
solution.

1. First came the problem, how extensive is the applicability of
Mendel’s law? Does it apply to all discontinuous variations? Does it
apply to cases of intermediate or blending inheritance?

2. The assumed purity of the gametes produced by a hybrid after
the association of contrasted characters in the same zygote for many
cell generations. Is it true?

3. The assumption that a character segregating as a unit in hybrid-
ization cannot be modified by selection however long and persist-
ently continued. Is it true?

4. The conclusion of Weismann that germ cells and body cells
are distinct, germ cells alone being the vehicle of heredity, and
consequently that acquired characters are not inherited. Is it true?

As material with which to work toward the answer to these ques-
tions laboratory rodents were employed—mice, rats, guinea-pigs and rabbits. Our work with these was complementary to work done simultaneously by Cuénot in France, and Doncaster, Hurst, and Punnett in England. Mice and guinea-pigs I had already been using before 1900 in a study of the possibility of influencing the sex ratio by selection. At my suggestion, a student had studied the sex ratio of successive generations in the record books of pedigreed Berkshire pigs, but had found no indications of inheritance of deviations from equality of the sexes in litters. I undertook an experimental investigation of the matter in mice and guinea-pigs, but had not got far when Mendelism was rediscovered. This gave the question a new aspect: Mendel's law applied clearly to discontinuous variation. Sex in mammals is sharply discontinuous, so perhaps its inheritance accords with Mendel's law. I published a paper advocating this view in 1903, under the title "The heredity of sex." Later I learned from the letters of Mendel, published by Correns, that Mendel himself had so regarded sex and with this in mind had experimented with bees.

This same year, as an outcome of earlier breeding experiments which were continued by G. M. Allen, we jointly published a paper showing that albinism is a Mendelian recessive. A paper entitled "Mendel's law of heredity," with examples based on the work of Allen and myself was published in January 1903, and reprinted later in the same year in Science. Other publications of 1903 showed that the long-haired angora coat of mammals is a recessive character, and that albinism in man as well as in rodents is a Mendelian recessive. A paper on the laws of heredity of Galton and Mendel showed that color inheritance in successive generations of mice fails in numerous instances to conform to "the law of ancestral heredity" of Galton but accords perfectly with the law of Mendel. This paper drew a sharp and critical comment from Karl Pearson in Biometrika, but was welcomed by Bateson in his battle for Mendel in opposition to Pearson and Weldon.

EXPERIMENTS WITH GUINEA-PIGS

The first extensive publication on our breeding experiments with guinea-pigs was made in 1905. I showed that three pairs of characters
in the guinea-pig are inherited independently and recombine in $F_2$ in typical Mendelian fashion. They are color versus albinism, short versus long hair, and rough versus smooth coat. An article in which this discovery was described, published in the Popular Science Monthly, was republished in a German translation in *Die Umschau*, and on the basis of this Baur constructed a wall chart which became widely used as a demonstration of the independent inheritance of characters in mammals.

In 1908 I described the production of a new color variety of guinea-pig, cinnamon agouti, obtained in $F_2$ from a cross of wild colored agouti with chocolate. This color variety was previously unknown, but its production had been confidently foreseen the previous year (1907) when I reported a case of reversion induced by cross-breeding and its fixation. In this case the wild agouti color pattern had been synthesized by crossing black with red, the factor for agouti pattern being carried unobserved in the red variety until the introduction of extended black pigmentation from the black parent made it visible as agouti.

The production of cinnamon agouti reported in 1908 involved only a reversal of the process, substituting brown for black pigmentation in the cross of agouti with chocolate.

A further important use of the guinea-pig in our work was reported in 1909, the successful transplantation of ovaries from an immature black guinea-pig to an albino foster mother which was then mated to a male albino. In the subsequent year this pair produced three litters of young, all black pigmented. This showed that the black ovary had survived unmodified and functional in an albino body, and that Weismann was right in maintaining that germ cells are distinct from body cells and are merely symbionts with them. Consequently inheritance of acquired characters is unlikely.

My indispensable cooperator in this investigation was Dr. John C. Phillips, who also assisted me in the long-continued selection experiment with rats presently to be described. He was an amateur of the sort, rare in America, but exemplified in England by men like Charles Darwin and Francis Galton; a man of independent means so that he was able to exploit his zeal for scientific discovery and exploration. As
an undergraduate at Harvard he had been in my elementary courses in zoology, had later entered the medical school from which he graduated as a well qualified surgeon, but he did not enter medical practice, being more interested in natural history. So it happened that he joined me at the Bussey Institution as a voluntary assistant without candidacy for a degree. It was his surgical skill that made our transplantation experiments successful.

EXPERIMENTS WITH RABBITS

Our work with rabbits during this decade was to a considerable extent paralleled by the work of Hurst and Punnett in England. The basic color varieties and color patterns were analyzed into Mendelian factors, segregating and recombining in orthodox fashion. Hurst and I independently discovered the explanation for the production of atavistic gray coat on crossing black with yellow rabbits, a matter which had been erroneously thought by Darwin to be a possible effect of the environment. The correct explanation is the same (genetic recombination) as in the case of agouti in guinea-pigs resulting from the cross of black with red.

My earliest investigations with rabbits showed that the ordinary albino character is recessive to all colored types, and that it is also recessive to the Himalayan type of albino which has colored ears, nose, and feet. It remained however for Sturtevant to point out later (1911) the logical consequence of these relations in establishing the principle of plural alleles, a disproof of Bateson’s presence-absence hypothesis.

An investigation with rabbits of a sort which was not undertaken elsewhere was the study of intermediate or blending inheritance of body weight and ear length to see if these were or were not Mendelian. We concluded (1909) that they were not, since the blend obtained in F1 persisted into F2 and later generations. But Lang (1910) reviewing our observations concluded correctly that they might be interpreted in terms of multiple factors devoid of dominance, a phenomenon newly discovered by Nilsson-Ehle. However I was not at that time satisfied that this explanation was correct, so I continued
to investigate size inheritance in rabbits for many years longer, until
the interpretation of Lang was fully corroborated.

EXPERIMENTS WITH RATS

Investigations with rats were made by us primarily to ascertain
whether Mendelian characters are, as generally assumed, incapable
of modification by selection, whether or not attended by outcrossing.
My own early observations indicated that they were modifiable, and
to this view I stubbornly adhered, like Morgan in his early opposition
to Mendelism, until the contrary view was established by a crucial
experiment.

From pre-Mendelian breeding experiments with rats made by
Crampe in the years 1877-1884, which were analyzed by Bateson in
1903, it was known that color variation in rats involved three inde-
pendent character mutations. (1) from colored to albino (2) from
wild gray to black, and (3) from uniformly colored to hooded. I had
been able to verify this analysis in crosses of tame albinos both with
tame black hooded and with wild gray rats, but I had observed great
variation in the hooded pattern, involving differences in the length
of the pigmented hood and the width of the back stripe. I undertook
to see if such differences were subject to heredity and capable of
further increase by selection. The first report on this study was made
in 1907 by MacCurdy and Castle. We showed that selection for
decrease in the extent of the pigmentation for three successive gen-
erations was clearly effective, and that crossing with wild rats in-
creased the extent of the hooded pattern in the hooded individuals
extracted from the cross in F9. But such effects seemed to contradict
the current doctrine of purity of the gametes, so a continuation and
extension of the selection experiment seemed desirable.

This was undertaken by Castle and Phillips who continued the
investigation, in the face of increasing adverse criticism, well into the
second decade of Mendelism, producing some 50,000 rats, in selec-
tions in opposite plus and minus directions through sixteen succes-
sive generations. The fact of modifiability of the hooded pattern was
thus firmly established, but its interpretation was still doubtful. Two
interpretations were possible: (1) that the unit character, or unit factor, or gene for the hooded pattern, as it had successively been designated, was itself fluctuating, or (2) that the observed modification had been effected by a modifying influence of other genes than the gene for hooded pattern. Such hypothetical genes might be designated modifying genes. Their reality in other genetic material became increasingly clear from 1911 on.

The crucial test was reported by Castle in 1919. It was shown that when the divergent plus and minus races were crossed with the same wild race, all the F₁ young were non-hooded, but produced the usual 25 percent of hooded in F₂. The F₂ hooded extracted from the two crosses now showed less divergence. A second similar cross to wild reduced the divergence still more, and a third cross nearly extinguished it. The fact that the hooded gene itself had not been changed as a result of long continued selection was thus demonstrated, but incidentally mutation had been observed to occur in the hooded gene itself in a single instance. Thus we now knew that in mammals color patterns may be modified by selection (1) through cumulation of modifying genes, and (2) much more rarely, by isolation of mutations in the particular pattern gene itself.

I should like to say a few additional words about those who cooperated with me in Mendelian studies on rodents begun in the decade 1900-1910. The work on mice was shared or extended independently by G. M. Allen and C. C. Little; in later decades by L. C. Dunn, C. E. Keeler, Geo. Snell, W. H. Gates and Sheldon Reed. The work on rats was shared or extended by H. MacCurdy and John C. Phillips; in later decades by L. C. Dunn, W. L. Wachter, Sewall Wright, Gregory Pincus, and E. W. Livesay. The work with guinea-pigs was shared in the first decade by Alexander Forbes and John C. Phillips, later by J. A. Detlefsen, and Sewall Wright. The work with rabbits was shared late in the first decade by R. C. Mullenix and H. E. Walter, later by E. C. MacDowell, C. E. Keeler, P. W. Gregory and P. B. Sawin.

Through the work done then or since by these twenty men a large share of the world’s knowledge of mammalian genetics has been accumulated.
FIRST EXPERIMENTS WITH DROSOPHILA

In conclusion I should like to speak of just one other investigation begun in 1901 and terminated in 1905, which was not based on studies made on mammals. One of the baffling problems of breeders in pre-Mendelian days had been the effects of inbreeding and cross-breeding. What these were was a much debated question. We set out to give it an experimental test and found ready to hand a rapidly breeding little fly, Drosophila, being cultured in the laboratory by a graduate student as embryological material. This, he told us, would complete a generation within a fortnight. Our informant was C. W. Woodworth, later professor of entomology in the University of California.

We began culturing the fly on pulped Concord grapes as Woodworth was doing, but this gave us poor results, as many of the larvae would get drowned and then our population statistics were no good. As grapes became out of season we tried other fruits, and finally hit the jack-pot, as we thought, in bananas. With banana for food, brother-sister mated pairs produced cultures reared in tumblers covered with a square of glass.

The results of this work, in which five students had in successive years cooperated, were brought together and published in May 1906. The conclusion drawn was that inbreeding reduces very slightly the productiveness of Drosophila but that productiveness may be fully maintained under constant inbreeding (brother with sister) if selection is made from the more productive families. This was not a conclusion of world-shaking importance.

The important outcome of this investigation was that it called to Morgan's attention a new source of material for experimental study, not subject to the limitations of slow-breeding laboratory mammals. So Morgan began breeding Drosophila and you know the rest, or will know it before this program is completed.
The Beginnings of Mendelism in America

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The Beginnings of Mendelism in America


THE DEVELOPMENT OF THE GENE THEORY

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QUITE independently of Mendel, the more progressive group of biologists had in the last quarter of the nineteenth century arrived at the following viewpoint, as stated by Wilson in 1896, in the first edition of *The Cell* (p. 326-7)¹:

...normal development is in a greater or less degree the response of the developing organism to normal conditions; ...we cannot hope to solve the problems of development without reckoning with these conditions. But neither can we regard specific forms of development as directly caused by the external conditions; for the egg of a fish and that of a polyp develop side by side, in the same drop of water, under identical conditions, each into its predestined form. Every step of development is a physiological reaction, involving a long and complex chain of cause and effect between the stimulus and the response.

In its physiological aspect, therefore, inheritance is the recurrence, in successive generations, of like forms of metabolism; and this is effected through the transmission from generation to generation of a specific substance or idioplasm which we have seen reason to identify with chromatin. This remains true however we may conceive the morphological nature of the idioplasm—whether as a microcosm of invisible germs or pangens, as conceived by de Vries, Weismann, and Hertwig, as a storehouse of specific ferment as Driesch suggests, or as a complex molecular substance grouped in micellae as in Nägeli's hypothesis. It is true, as Verworn insists, that the cytoplasm is essential to inheritance; for without a specifically organized cytoplasm the nucleus is unable to set up specific forms of synthesis.

¹Wilson: *The Cell in Development and Inheritance*, copyright 1896 by The Macmillan Company. All the italics in the passages quoted from Wilson are his own.
This objection, which has already been considered from different points of view, both by de Vries and Driesch, disappears as soon as we regard the egg-cytoplasm as itself a product of the nuclear activity; and it is just here that the general role of the nucleus in metabolism is of such vital importance to the theory of inheritance. If the nucleus be the formative centre of the cell, if nutritive substances be elaborated by or under the influence of the nucleus while they are built into the living fabric, then the specific character of the cytoplasm is determined by that of the nucleus, and the contradiction vanishes. In accepting this view we admit that the cytoplasm of the egg is, in a measure, the substratum of inheritance, but it is so only by virtue of its relation to the nucleus, which is, so to speak, the ultimate court of appeal. The nucleus cannot operate without a cytoplasmic field in which its peculiar powers may come into play; but this field is created and molded by itself. Both are necessary to development; the nucleus alone suffices for the inheritance of specific possibilities of development.

Although we would today admit, for a limited group of cases, inheritance through some cytoplasmic inclusions as well as through nuclear materials, we should agree with the essential thesis above that the distinction between the hereditary and non-hereditary materials is a cardinal one, and that the main seat, at any rate, of the hereditary material is the chromatin of the nucleus, in organisms in which nuclei exist. And although the words genotype and phenotype had not yet been invented, the core of the concepts for which they stand is present in the above passages, the genotype here being represented by the words idioplasm or chromatin and the phenotype by the idea of the organism's being produced as a complicated resultant of chains of biochemical processes of development, the nature of which is determined both by the hereditary material and by environmental influences. The recognition of this antithesis between the germinal and the somatic had received its main impetus from Weismann, as Wilson had acknowledged earlier in his book.

However, as Wilson, following the above quoted passage, proceeds to point out, the question of how the hereditary material itself is caused to vary was still entirely unsettled. He might also have added that the manner of distribution of the hereditary material from one generation to the next was similarly unknown, but very few men realized that there was a field here of which they were grossly igno-
H. J. Muller

Mendel was almost unique for his day in realizing the existence of this problem of how hereditary characters are distributed. He was still more unique in realizing how this problem might be attacked, and finally in being able to interpret correctly the results of that attack. So unique was he as to be given the brush-off. But the rise of the particulate and chromosomal conception of heredity, in the preliminary form indicated in the above quotation from Wilson, paved the way for the rediscovery of Mendel's principles and for their eventual recognition.

As knowledge of the behavior of the chromosomes, especially at meiosis, grew rapidly just after the rediscovery of Mendel's principles, it soon became evident that the elements studied by the microscopist on the one hand and by the experimental breeder on the other hand were in fact to be identified with one another. Thus in a sense we may say that the east and west coasts of Mendelism had been discovered independently and that the explorers from both sides finally met. They later found, quite literally, that their "maps" of the intervening terrain, made from their respective vantage points, agreed to a remarkable degree.

As we can see today, the main principle established by Mendel is that of recombination (for which, however, the principle of segregation is prerequisite), but the randomness of recombination found by him represents a special case. Only gradually has the significance of recombination, whether random or otherwise, for the organism been realized to lie in its furtherance of the organism's evolution, although the principle was early taken advantage of by breeders for purposes of artificial evolution, in the work of practical plant and animal breeding. But even more significant for biological theory, and at once realized, was the demonstration which this principle provided of the particulate nature, the atomicity, or the mosaicism, as one may choose to call it, of the hereditary material, that is, the fact that it is compounded of numerous separately inheritable "factors" or "genes." To this extent then the believers in self-reproducing determiners—whether under the name of pangens, micellae, Altmann's granules or chromonemes—were at last vindicated. And since these units were on
the whole transmitted as much by the male as by the female germ cell, as Mendel among others had pointed out, it became evident, even quite aside from the tying up of Mendelian units with chromosomes, that the great bulk of the cytoplasm, of the egg at any rate, must carry little, if any, of this hereditary material.

It was of course mainly through the exploration of the departures from random recombination—that is, through the study of linkage and crossing over, which were unknown to Mendel—that the tie-up of hereditary processes with chromosome structure and behavior was at last made so complete. Thus the hereditary elements were found not to be a crowd of independently inherited units, but to fall into definite determinable arrangements, corresponding with their linear positions in the chromosome threads. This great extension of Mendelism is, however, too well known a story to be further reviewed here and we shall consider mainly the other features of the development of gene theory.

THE EXTENT OF MENDELLIAN HEREDITY

Immediately after the rediscovery of Mendelism and for the first two decades thereafter one of the most burning questions of biology was: how far does the Mendelian type of heredity extend? Or, as the question was soon phrased alternatively, how far does chromosomal heredity extend? It was soon obvious that such heredity occurs among sexually reproducing organisms in general, but for a long time it was not at all clear how much of the heredity of an organism is of this type. A few of the early cases of seemingly non-Mendelian heredity, notably those of Correns and of Baur concerned with plastid color, have stood the test of time as examples of determination by hereditary materials lying in the cytoplasm. However, it became increasingly clear that the cases of inheritance which showed more or less blending, and those showing so-called aberrant ratios, were illustrations of the interaction of more or fewer essentially Mendelian units and/or environmental influences, which affected either the appearance or the viability of the different genetic classes or both.

Not only was such interaction to be expected as a result of that
complicated network of developmental processes which, as we have
seen, Wilson and other well-balanced students of embryology had
long recognized, but their existence had been noted even by Mendel
himself. He had interpreted his results on flower and seed color in
crosses between two widely-differing species of Phaseolus—multiflorus
and nanus—as probably brought about through multiple factors act-
ing on the same set of characters. Moreover he had concluded that
the less definitely recognizable or overlapping characters in his crosses
of Pisum, although not so valuable in actual proof of Mendelian
heredity as the definite ones, were probably inherited according to
the scheme of segregating units, which however varied in their ex-
pression. In addition, he recognized that a given unit could have
effects on different parts of the organism which differed to some
degree; effects of this kind are included in what is now called
"pleiotropy."
After numerous multiple factor cases had come to light in various
organisms, it was even found possible in the Drosophila work by the
use of completely linked marker genes in the heterozygous male, to
demonstrate that the whole of the inheritance affecting the quantita-
tive character differences studied depended upon pairs of regularly
Mendelizing units, which often were very numerous, and very minute
in their individual effects on the character. That the same explana-
tion usually applied in plants, even in the case of the whole of the
wide differences between species, was later shown—less directly but
no less surely—by the evidence from tetraploids. I refer here to the
striking contrast between the relative invariability, equalling that of
a pure species, shown by the offspring of allotetraploid hybrids, in
which the chromosome situation prevents Mendelian segregation,
and the variability of the offspring of the diploid hybrids, when the
latter are able to breed. Federley had previously obtained similar
results in moths. In these cases, it can only be concluded that all of
the hereditary differences under observation, differentiating the
species from one another, must depend upon those elements, the
chromosomes, which are known normally to undergo Mendelian
segregation and recombination, but which in the tetraploids undergo
what amounts to an equational partition. Here, then, the difficulties
of analysis in species crosses are brushed aside, and even the widest
differences between barely crossable species are at one sweep shown
to reside in elements of an essentially Mendelian nature. The same
conclusion has been reached wherever, as in some Drosophila and
other species crosses (e.g. Drosophila pseudo-obscura by persimilis
and melanogaster by simulans), actual analysis through the obtaining
of recombinational forms has been possible, if only we except the
part played in some plant species crosses by cytoplasmically located
genes. At the same time, one after another of the very numerous
intra-specific hereditary differences found in such forms as Drosophila,
maize and Datura, in which the tools for analysis had been
perfected, were also proved to have their place in the chromosomes,
and therefore to be Mendelian.

**CYTOPLASMIC HEREDITY**

We see then that, except for the relatively much rarer differences
due to inheritance through plastids and other cytoplasmically con-
tained materials, all inheritance concerning which evidence could be
obtained proved to be in essence Mendelian. However, even the
genuinely cytoplasmic inheritance proved itself, in the case of
chloroplasts, to share with the nuclear determinen the most funda-
mental characteristic of Mendelian inheritance—that of being partic-
ulate and resident in special bodies, which appeared to be transmitted
seemingly unchanged except for individually very rare mutations. And
the same conclusion is being reached in recent years with regard to
various cases of transmission through other cytoplasmic materials:
that of carbon dioxide susceptibility in Drosophila, the
determiners of certain enzymatic reactions in yeast, and the
apparently particulate entities causing a type of male sterility in maize.3

In mitigation of the current conception that cytoplasmically lo-
cated genes or gene-complexes form an essential part of the genetic
constitution of animals, the following points should be noted: (1)

1 We do not refer above to the remarkable cases of antigen transmission in
Paramecia, since these do not appear to represent plasmagenes.
the extreme rarity with which illustrations of such inheritance have
been found in animal material, in contrast to the thousands of Men-
delian differences found in them; (2) the dispensability of the cyto-
plasmically located particles in the cases studied and the absence of
evidence of the existence of normal alternative forms of them; (3)
the fact that, in these same cases, the agents have been proved to be
able to pass as infections from one cell to another; and (4) the lack
of a fundamental basis for distinguishing between these and cases of
undoubtedly parasitic or symbiotic microorganisms or viruses of
exogenous derivation. These are points which, taken together, would
appear to argue for most or all of these agents in animals having at
one time arrived as invaders; for their still constituting, in a sense, an
adventitious part of the inheritance, and for their tenure usually
being insecure, as compared with that of the native chromosomes.
This conclusion is, moreover, reënforced by a consideration of their
mode of distribution and aggregation, since it is not only rather
precarious but apparently far less suitable than that of the chromo-
somal genes for the simultaneous retention and the accumulation of
numerous different types within the same germ plasm.

In plants, on the other hand, there seems little doubt that several
different kinds of cytoplasmically located genes are regularly present
and that they have persisted tenaciously throughout plant evolution.
It seems likely, especially in consideration of the blue-green algae,
that they were derived from some of the genes which were originally
present in the protoplasm before the major portion of the chromatin
had become sequestrated within a nuclear membrane. Their persist-
ence in plants only has probably been occasioned by their monopoly
of certain biochemical operations which are peculiar to plants and
important for them. This point is well illustrated in the case of the
genes of chloroplastids, which are indispensable for the production
of chlorophyll and therefore for carbohydrate synthesis. It is not
uncommon, however, for chloroplastids to mutate to the white form,
and to become smaller and less conspicuous. But these small mito-
chondria-like granules still retain their power of self-reproduction and
perhaps continue to carry on other biochemical functions than that
of carbohydrate synthesis. Thus it may well be that some or all of
the plasmagenes normal to plants, other than those in green chloroplasts, have been derived either from the chloroplasts themselves or from earlier primordia which were common ancestors of both the chloroplasts and themselves. This would explain their relative prevalence in plants as contrasted with animals. During all this period, moreover, it is to be expected that they would have assumed, by mutation and selection, various accessory functions, although the total amount of such accretion would tend to be kept down by the competition of the (from an evolutionary point of view) more efficient nuclear gene organization.

THE PRECISION OF MENDELIAN SEGREGATION

Equally as important as the question of the extent or universality of Mendelian inheritance in the early decades of this century was that of the degree of precision with which it operates. In this connection it is of interest to note that Mendel with great astuteness purposely chose, for his main work, to cross rather closely related types which were, both separately and in the crosses, fully viable and fertile, and which differed in few but distinct characters. One gets the impression that he did this with the conscious intention of avoiding the complications of low and differential viability, of uncertain classification, and of what we now call multiple factors. In consequence, Mendel found ratios which indicated no bias towards the production of germ cells with one allele versus its alternate. Moreover, each allele appeared to have been unaffected in its nature by its sojourn in an individual containing the other allele as well. Neither was there any indication of its having been changed by association with any of the other differentiating genes that had been present. In these respects then the units did seem to act like atoms.

As it is manifestly impossible to prove with complete certainty any universal negative, we cannot assert that "contamination" of one allele by the other may not be the cause of some unanalyzed cases where there appears to be some "blending," just as we cannot completely deny the possibility that there may be a lion waiting outside the door of our room. We can only say that the possibility is grossly
inconsistent with what has been found in similar cases, when these were of a kind that allowed analysis. As previously mentioned, such cases of apparent contamination or blending have, when genetically dissected, always boiled down to situations in which there were multiple factors and/or the influence of environmental differences on the expression of traits, but in which the individual hereditary elements were transmitted unchanged. Moreover, there are plenty of cases now known of the transmission of genes in heterozygous condition for scores or hundreds of generations without evident diminution of their distinctiveness. For example, the genes for the characters called scute bristles, tan-2 body, vermilion eye, small wing and Bar eye present in the combination called “CIB” in Drosophila have been continually heterozygous since 1919, some 800 generations ago, but show no noticeable effect of this. In fact, there are many cases, such as those of certain blood groups in some populations of man, or of striping in some snails, where the character in question must have been heterozygous throughout the large majority of thousands of generations, without its having been noticeably weakened, in comparison with its condition in populations where it was much oftener homozygous. And there are analogous cases where, although the genes were kept in homozygous condition, their characteristic action had been sidetracked or prevented from occurring by the presence of other genes, as when so-called genes for “black” are kept in albinos. In such cases too, when the interfering genes are at last replaced by those of appropriate type, the original characteristic effect is seen to return in full force.

In cases of both the above kinds, if a great number of generations has elapsed, this can serve in lieu of exact measurements of the given character. For even if the effect were too small to be detected without measurement after one generation the repetition of the influence for, say, 75 generations should multiply it by about 75. In this way then the bogey of “contamination” has been retired behind the door, to join the supposititious lion. And in a similar way the bogey of cases of supposed failure of a pair of alleles to conform to a 1:1 ratio in the germ cells has been sent to keep them company also.
THE EFFECTS OF THE ENVIRONMENT

The fact that each Mendelian unit preserves its individuality regardless of what other units, even of what contrasting alleles, it may be associated with, and regardless of how "epistatic" or of how dominant they are, is at the same time an excellent illustration of the independence of the genotype from the phenotype. In other words, it shows the sharpness of that distinction between heredity and development which had already been made by 19th century biologists. For in all these cases given hereditary materials emerge unaffected in the descendants even after generations of sojourn within organisms which, at the instigation of certain other hereditary materials, had developed characteristics different from those which the first hereditary materials would ordinarily have given rise to. Clearly, the type of development failed to affect the heredity. But if this was true even in these cases where the influence was such a very intimate one, and where the organism itself was engaged so actively in producing the developmental result, can we well expect that, when development is shunted in this or that direction by environmental influences, the hereditary material will be any more vulnerable?

But we have no need to rely on such arguments alone in drawing conclusions concerning this crucial matter. For there is plenty of entirely direct evidence of the lack of reflection in the hereditary material of effects produced by the environment on the development of the organism containing it. If we consider only characters known to be dependent upon given Mendelizing pairs of alleles, the genetic literature is full of cases in which a given allele produces a certain effect only under particular conditions, such as abnormal abdomen in a given strain of Drosophila in old dry cultures or reduplicated legs or longer vestigial wings in other strains at high temperatures, or, in maize, the red color of given strains when exposed to sunlight during development. In these cases return after an indefinite number of generations to the alternative environmental condition results in an immediate resumption of the type of development which previously took place under this condition, in as typical a form as before, the
genes having therefore preserved their nature unchanged. Similar results are of course obtained when we use characters the inheritance of which has not been analyzed. We must of course except here those rare cases in which the character depends upon an infectious or protoplastic agent, unstable in numbers, the multiplication of which can be prevented or promoted in body and germ alike. Aside from this, we must also be careful to rule out or to allow for the effects of selection, acting on material that was genetically heterogeneous to start with, for in that way too the appearance of an inheritance of acquired characters can be imitated.

Genotype and Phenotype

It was the avoidance of this last source of error that made Johannsen's work on pure lines so decisive. Using material that was self-fertilizing and therefore, if genes have a high stability, homozygous, he was able to show by exact measurements that the developmental differences between individuals of the same line, which in that case must have been caused by environmental influences, were not reflected in the characters of the succeeding generations. This was true even when he chose cases in which the same environmentally caused deviation had been repeated in successive generations: that is, the genes even of the last generation proved unaffected. And since these were quantitative characters which must have been dependent on many genes, the stability of all these genes, and their immunity from the effects produced in development, was thereby proved for all of them at once. It is no wonder that the clarity of this result led to the coining, in connection with it, of the words "phenotype" and "genotype," nor that Johannsen also originated the word "gene."

To criticize this magnificent result by saying that, had crossing of different genotypes been allowed, the mixed hereditary material might have been more receptive to environmental influences, would be to call a base retreat, by means of an absurd ad hoc objection, into the highly improbable unknown. For a long time, however, even this dubious path of escape for objectors has been cut off. For in the Drosophila work, several similar careful selection experiments have been carried out with quantitatively varying characters, in material
in which, however, segregation and recombination of heterozygous genes was allowed. This was the work in which, by the method of using completely linked "markers" in the male, it was possible to dissect the genetic basis of the character differences into groups of multiple factors, each one of which was stably inherited. For this work at the same time showed that, whenever a given combination of parental chromosomes was inherited, a given mean and spread of variation of the character in question was exhibited by the group of offspring having this combination. And this remained true regardless of the grade of development which fluctuating environmental influences had caused the character to have in the parents and ancestors. That is, as with Johannsen's beans, the genotype held constant despite selection of parents showing a given type of environmental effect throughout a succession of generations. It followed that the pure line principle could in its essence be generalized for crossbreeding as well as selfing forms. The observed apparently continuous differences found in crossing populations thus resolved themselves into an intricate combination of Mendelian differences, on the one hand, and environmental effects, on the other hand. And, while the former were inherited regularly, the latter were definitely not inherited.

Inheritance of Acquired Characters

Thus genetics has come a long way beyond having to use such evidence as that of circumcision or Chinese foot-binding or Weismann's pioneer amputation of mouse tails to discredit the ancient doctrine which now goes by the name of Lamarck. We cannot do justice here to all the other evidence against this doctrine, and will content ourselves with pointing, as a final and in itself crucial argument, to those experiments in which gonads, transplanted at an early age, nevertheless bred true to the types from which they had been taken. This was successfully accomplished first in rodents, and later in both intra- and interspecific crosses of Drosophila. But it is of course easy for those who still in 1950 persist in giving even Mendel the brush-off to brush all these laboriously attained results aside even more lightly.

All the work cited above, showing that the gene is ordinarily im-
mune to having permanent alterations caused in it which reflect the
effect of other genes or of environmental conditions upon develop-
ment, demonstrates at the same time the high stability of the gene
in general, the fact that it changes very infrequently either as a result
of internal or external disequilibrating influences. In what manner,
then, have the changes come about which resulted in evolution, and
how did the existing allele differences within each species arise?

**MUTATION**

As maintained early by Bateson, and pointed out also by Lock
in his classical survey of genetics in 1906, the mere existence of these
very stable discontinuities of Mendelian heredity raised the pre-
sumption that they had originated by sudden steps. A number of
instances of the occurrence of such steps, leading to almost immedi-
ately stable strains, were already known, as in the Ancon ram. In
addition, there were the so-called mutations observed by de Vries in
the evening primrose, first described in the epochal year 1900. Al-
though we now know that these changes in the evening primrose
mainly represent complicated and unusual types of recombination,
their finding at that time lent plausibility to the view that ordinary
allele differences arise by similar jumps. We have long since come to
recognize them as special cases, and yet time has proved to the hilt
that the ordinary allele differences do arise by sudden steps, gene
mutations. Moreover, there are few geneticists who would still dis-
pute the inference that these gene mutations provide the main build-
ing blocks of evolution. They had seldom been observed with
certainty in the experiments on Mendelism and selection already re-
ferred to, however, because of the fact that mutations distinctly af-
flecting any given character are usually so rare.

There is no use in attempting to review here the development of
modern mutation theory, nor to list all of the principles established
under it. Concerning the types of effects produced by gene muta-
tions it should, however, be recalled that these effects are of all de-
grees, but that they are oftener small and even invisible than large.
They are oftener "physiological" than externally morphological. They
occur in many directions, but are preponderantly detrimental, as expected for changes induced at random in a complicated organization, and the smaller changes are on the whole less detrimental. Changes causing a decrease of gene effectiveness occur oftener than the reverse. Both this observation and the preceding one are to be expected in accordance with the general statistical principle of increasing disorder, from which the second law of thermodynamics also is derived. On the other hand, changes of the opposite type do occur too, and there are even some changes that give effects called "neomorphs," which are different in kind from those produced by the old gene. Moreover, as was first pointed out in connection with the truncate series of alleles in Drosophila, different changes of a given gene may even affect quite different characters, although far more often the same character or characters are affected. Finally, we may be reminded that gene mutations of all kinds tend to be recessive, but are usually incompletely so.

All the above points are important in the modern genetic conception of evolution. They show that a relaxation of selection leads to degeneration and disorganization. Thus the direction of evolution is decided by selection, not by some tendency of the organism to vary preferentially, in a given direction, either as a result of some direct germinal adaptability, or by the inheritance of acquired characters, or just through a predetermined inclination to vary that way. Most of the selected changes will, however, be small steps, as Darwin thought, so that evolution will usually proceed along a slow and apparently continuous gradient, and these steps will usually be expressed as increases or decreases in the quantity or proportion of an already existing character. Occasionally, however, a greater or lesser degree of novelty can enter the picture, though oftener through a combination of mutants which together transgress some qualitative dividing line than by the change of a single gene.

In regard to the manner of occurrence of mutations in the genes, the point of greatest importance is their high degree of randomness. Under very diverse conditions one still gets the most heterogeneous collection of mutations, sporadically scattered, and this is also true under the best and most constant living conditions attainable. That
this indetermination exists even on the smallest microscopic scale and probably on a submicroscopic one is shown by the finding that usually only one of two identical alleles, lying almost in contact within a Drosophila cell, undergoes mutation on a given occasion. Obviously the cause of the mutation lies buried in the vicissitudes of the “molecular chaos” (to use a term proposed by Troland even before this most telling bit of evidence was known).

The above results are in no wise inconsistent with the fact that given conditions can greatly alter the overall frequency of these mutational accidents, just as conditions en a highway can be such as to result in more or fewer collisions. Among the conditions, as yet not understood in chemical terms, which predispose to the occurrence of mutations, are those existing in the cell during certain stages of development, and others that obtain when given “high mutation rate” genes are present. Also conducive to raising the overall mutation frequency are higher temperature, and energetic radiations of all kinds, just as we might expect from a consideration of their effects in heightening the “molecular chaos.” Finally, certain specific types of chemicals, including especially the mustard gas group, peroxides, and ethyl urathane have been shown to have a strong influence in increasing the total frequency of mutations.

Although the path of chemical action in the production of a mutation is still very much of a mystery, these facts indicate that the types of chemical alterations involved in gene mutations in general are very similar to one another and require a similar amount of energy. The determination of just which gene shall mutate and in which way must usually depend, not upon the type of condition to which the cell is subjected, but upon the ultramicroscopic topographical accidents of the thermal disorder.

In this light, we see how utterly naïve is the view that changes of a given kind may be produced in the hereditary material through the influence of given changes in the body containing it, and more particularly naïve when it is postulated that this supposed change in the hereditary material is of just such a nature that, when it finds expression in a subsequent generation, it will mirror that change in the body which supposedly induced it. Even more magical would it be,
however, if the hereditary alteration occurred as a direct adaptation of the germ to a given outer condition, as though the germ knew how to alter its own constitution in such a way as to cause it to have appropriate effects on development, effects which would help the resultant body to meet the difficulties arising from the given new conditions.

The origin of adaptability in organisms is the thing to be explained, not the premise to start with. The modern theory of gene mutation, coupled with Darwin's basic concept of natural selection, provides the basis for a process of evolution in which such adaptability will come into existence by the operation of natural processes. To assume the existence of adaptability first, and then to have evolution result from it, is to return to animistic superstitions as unscientific as the doctrine of special creation of species, and to cast into the discard the most significant findings of biology.

In this discussion I am attempting, in the interests of that better understanding of fundamentals which again seems to be needed, to lay emphasis upon first things first. This does not give us time to touch upon those more recent refinements of gene theory whereby the principles of Mendelian and chromosomal heredity and gene mutation have been made use of in the investigation and calculation of their effects on the composition of populations, on the manner in which that composition will change in the course of short or long periods of time under different conditions of crossing, selection, migration, population size and subdivision, and in the presence of different breeding systems and genetic configurations. However, all this, as well as its practical applications, is rightly to be considered as an outgrowth of gene theory. Neither can we enter here into such fascinating modern studies, promising to give us a deeper insight into the nature of the genetic material, as those on the size and number of genes, their types of allelism, the position effects, the nature and influence of heterochromatin, the special genes that produce centromeres, telomeres and blocks, unstable genes, the behavior of the genes or their prototypes in bacteria and viruses, and the special characteristics of plastid and plasmagenes. Problems of gene dominance and of heterosis cannot be entered into. The whole field of
radiation genetics must be left untouched. The relating of genes to the biochemical processes which they initiate in the cells containing them, and to development and physiology in general, must likewise be neglected. Fortunately, however, we still have, in the Western world, numerous scientists actively at work on these frontier problems, and some of them have reported at this meeting.

CHANGES IN CHROMOSOME STRUCTURE

The subject of structural changes in chromosomes, whereby the genes acquire a different arrangement in line, is, however, so important for basic gene theory as to require special mention, even here. After accidental breakage of chromosomes, as by mechanical stress, radiation and/or chemical weakening, the broken ends, being mutually adhesive, can happen to rejoin in a new linear arrangement. Such rearranged chromosomes provide admirable tools for investigation of chromosome properties and of the evolutionary relations of some groups. Occasionally they fill a need of the species for a change in chromosome number, size or shape, since certain configurations are meiotically or mitotically optimal for given conditions. But they usually fail to present the organism with genetic material that is superior in immediate phenotypic effects, unless by chance the rearranged chromatins happened to contain, locked within it (that is, unable to escape by crossing over with the old arrangement), genes whose mutations had been more advantageous, or less disadvantageous, than the average, in relation to certain existing conditions of life.

There is, however, a rare class of structural changes which are of far greater ultimate significance. These are the cases in which a small section of chromatins from one chromosome set—not so large as to cause serious phenotypic disturbances—has become inserted somewhere into a previously complete chromosome set, which thereby has come to possess the given section in duplicate. Thus the genotype

1 It is not commonly known that valid evidence for the existence of duplications, and interpretations, substantially identical with what is given in the above paragraph, of their significance in evolution, were first published by Bridges and by the present writer, independently, in 1935. It may be recalled that Bridges had believed in 1919 that he had obtained small duplications, but that these were later
has acquired more genes. And although these genes are at first, in the main at least, duplicates of others that are present, they and the others must in further evolution diverge more and more from each other through the establishment of mutations which occurred independently in the two locations, so as finally to gain quite different properties. By a succession of such duplications, followed inevitably by the differentiations, the genotype can gradually become not only more compound but more complex, with a complexity that applies not only to each individual gene but to the genes as a group. This then opens to the genotype the evolutionary opportunity of producing and maintaining a more highly organized body than would otherwise be possible.

found to be "specific suppressors." Apparently the first person to suggest the repeated incorporation of duplications in the genotype, as an important evolutionary process, was E. C. Anderson, in informal discussions held in the Columbia laboratory in 1920. A reference to this is given in the present writer's paper (1935a). This paper also brought forward the findings concerning the adjacent but separate positions of the functionally related genes "achaete" and "scute" as evidence of duplication, followed by differential mutation, having occurred in the evolution of the normal genotype of Drosophila. A case of origination of a duplication in a position remote from its original site was given by the present writer in a simultaneous paper (1935b). This paper described a duplication which originated from the normal type and which was visible in the homozygote, as an illustration of how duplications may gain a foothold. It was a curious coincidence that Bridges' paper (1935) which clearly showed two "repeats," as he called them, in the second chromosome of Drosophila salivary glands, and which interpreted their general evolutionary significance in the same way as in the above two papers of the present writer, appeared in the same year, and that all three papers had been submitted for publication in 1934. A further discussion of the subject was given by the present writer (1936). It may be added that previous to Anderson's suggestion, polyplody of whole chromosome sets and of individual chromosome pairs (heteroploidy) had been thought of as a method of increase in gene number in evolution, and that it had been suggested (Muller 1918) that this would be followed by the evolutionary differentiation of the duplicated genes.

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H. J. Muller

GENE REPRODUCTION

All the topics discussed above form an essential part of gene theory; nevertheless, the real core of gene theory still appears to lie in the deep unknown. That is, we have as yet no actual knowledge of the mechanism underlying that unique property which makes a gene a gene—its ability to cause the synthesis of another structure like itself, a daughter gene in which even the mutations of the original gene are copied. It is true that certain provisional interpretations have been proposed for this, for instance, by the physicist Jechle and the biochemist Friedrich-Freksa, but these suggestions have not yet received the serious attention and discussion which will be necessary before their respective merits and demerits can be clearly apprehended. On account of the importance and difficulty of the subject, however, it is to be hoped that, despite features that might at first arouse objections, they will be given a wide hearing among those equipped to consider them, and not dismissed without careful and open consideration. It must be remembered that the truth is seldom discovered all in one piece. At the same time, there should be an earnest search for methods of attacking the problem experimentally, along physiochemical lines.

This is a case in which the mere recognition of the existence of the problem constitutes in itself a distinct step. Those who postulated “pangens,” “determinants,” or other self-reproducing particles in the old days did not seem to realize what a monster they had by the tail. They were still, subconsciously, so close to the ancient lore of animism, in which practically all things were living and even the rocks had spirits and the spirits often had offspring, that to attribute reproduction to a particle, especially when this formed a component of a larger thing, itself living anyway, seemed to present no problem.

Later, taking Driesch’s notion that the hereditary material consisted of what were originally called “ferments,” that is, enzymes, Hagedoorn in 1911 appeared to solve their reproduction by calling them “autocatalytic enzymes.” But this is only a descriptive term, and insufficiently descriptive at that, which hides the real difficulty. For gene reproduction is not just a matter of some reaction being
accelerated which might readily occur anyway. What must happen is that just that precise reaction is selectively caused to occur, out of a virtually infinite series of possible reactions, whereby materials taken from a common medium become synthesized into a pattern just like that of the structure which itself guides the reaction. We do not know of such things yet in chemistry. And it may be added, incidentally, that Hagedoorn's scheme did not even try to confer upon these enzymes the attribute most essential for them as the basis of evolution, that is, the ability to change in such a way as to be able to reproduce themselves in their new form. Instead, all the enzymes for future evolution were providentially supplied full-fledged to the ancestral form of all life, and evolution proceeded by the successive loss of one enzyme after another, much as on the later views of Lotsy and of E. E. Just.

Troland, who did not seem to know of Hagedoorn's proposals, did not make this last mistake with his "autocatalytic enzymes" in his papers of 1914 to 1917. However, he too failed to realize the uniqueness of the problem presented by them, for he thought that the "autocatalysis" could be explained by the ordinary processes of crystallization. Even Haeckel and others had long before compared growth to crystallization. But again it is not evident where the necessary precise selectivity is to come from, in a case where so many alternative possibilities of reaction are open. It is easy enough to imagine more molecules of a substance being deposited, in virtually the only orderly way possible, upon a crystal of it, out of a supersaturated solution in which there is already plenty of it present in a not too different form, and little else, just as a cloud when seeded with a little snow may be caused to snow much more. But dry ice will do the trick even better than snow, showing that there is nothing fundamentally autocatalytic in this crystallization. Moreover, snow crystals in a given shape will not give you more of just that shape, and, passing to more complicated things, I am inclined to doubt that, even with appropriate seeding operations, it would ever rain cats and dogs.

No, the answer to gene reproduction cannot be as easy as crystallization. Life would arise too readily. Something in the process has
eluded us here—some feature or features which hold the clue to that
amazing self-patterned selectivity of the gene's autosynthetic behavior which, once it arose, made all or practically all subsequent life
and evolution the outgrowth of this original event.

At least a part of the answer must somehow be buried in what is
already known of the gene's chemical constitution. I refer to the fact
that all genes—that is, all particles capable, in unlimited measure, of
reproducing their variations, whether they be chromosomal genes,
plasmagene, kappa particles, viruses or the "transforming agents" of
bacteria—have been found, whenever their composition could be approximate determined, to consist of or to contain polymerized
nucleic acid, and usually, if not always, some protein as well. But as
yet no one has been able to correlate these features of chemical struc-
ture with the gene's peculiar property of self-reproduction. It seems
evident also, as I mentioned some quarter century ago (1922), that
these features of each gene's pattern which differentiate it from that
of other genes, those features presenting differences which arose by
mutation, must be arranged in only one or two dimensions. For
otherwise the parts of a daughter gene could hardly become arranged
to match the distinctive pattern present in the old gene.

Moreover, as was noted at the same time, there is another known
peculiarity of the gene—known at least for chromosomal genes—
which parallels the self-selectivity of its synthetic process, and which
probably exhibits another facet of that same problem. This peculiarity
is the self-selectivity with which a gene tends to conjugate with an-
other gene having a structure identical with its own. It is necessary,
moreover, to infer that this selectivity, like that of its reproduction,
automatically changes in an appropriate manner when it mutates.
And no matter to how great or small a distance this attractive force
may extend—a matter under dispute—the self-selectivity of it appears
to be unique for such forces, at least in anything like the degree here
exhibited. One seems practically driven to conclude from this that
the ability of the gene to reproduce its pattern depends upon an
ability to fix like components, derived from the heterogeneous me-
dium, against its own corresponding parts, thus conferring upon those
formerly disorderly components an arrangement identical with its
own. From this point of view, this synaptic property of genes and chromosomes deserves more intensive study. I still believe it probable that the interpretation of it must involve electrical periodicities of complex form, with distinctive time as well as space characteristics that differentiate the genes from each other. The position effects operating between nearby genes so as to influence their functioning may well represent still another aspect of this same phenomenon, and may thus afford another handle for the investigation of it.

But whatever the secret of the gene's ability to reproduce itself and its mutations may consist in, it seems today clearer than ever, especially in the light of modern knowledge of microorganisms and viruses, that this is also the most fundamental secret of life itself. This would mean that the gene, in some primitive form, had come first, utilizing for its reproduction floating components like those out of which it had been accidentally built. All the rest of living matter, its protoplasm and its higher integrations in general, was then gradually evolved through the operation of natural selection upon the varied mutant forms of the descendant genes.

This view is in essentials different from that of Haeckel and the others who long ago speculated that life originated in the form of a primitive kind of protoplasm, in which the anabolism managed to exceed the catabolism and so resulted in growth. For it was not realized that this so-called anabolism would require an amazing self-selectivity in synthesis, the like of which is not known in ordinary biochemistry and physiology, until we come to the gene itself. The same mistake is made even today by the Lysenkoist Oparin, in whose book, The Origin of Life, the concept of the gene does not appear.

It is of great interest to observe that, once the complexities of protoplasm have been developed, with its rich streams of substances and processes provided by its gene-initiated reactions, it then becomes possible, on an upper level, so to speak, to have operating in the protoplasm mechanisms of an autocatalytic character, if we may now use this term in its more ordinary and non-committal descriptive sense, as chemists use it. Once started, or "primed," such a self-promoting reaction would tend to continue, but it could be switched off in favor of another one. The remarkable findings in Paramecia on
the production of alternative mating types and of mutually exclusive antigens give the clearest and most elaborate demonstration of such phenomena. Their investigation may well prove fundamental for the understanding of somatic development in higher forms as well. Here then we have a kind of epigenic process which in externals seems to resemble the reproduction and even the mutation of a gene. However, if our distinction is a valid one, such a process would have open to it when given genes were present, only certain fixed alternatives, numerous though these might sometimes be, and it would not be capable, through its own changes alone and without help from actual gene mutations, of following an unlimited evolutionary course. This view of a matter still so in flux must, of course, be regarded with caution, but it seems at present the one which best harmonizes these findings with the accumulated genetic evidence, gathered from many organisms, of the overwhelmingly predominant importance of the dimensional genes themselves in the origin of evolutionary differences.

The speculations of these last pages have been indulged in to show what important problems still lie hidden both within the gene and within the cellular processes which it controls. But let those unfamiliar with the subject not gain the impression from this that the gene is still only a concept, a way of thinking, as even Johannsen and East in their time maintained. Still less should it be supposed that there are yet today valid grounds for dispute concerning the main features of the genes' mode of behavior in heredity, variation and evolution. It is true that there are ever richer realms of inquiry open to genetic science today, but they can be exploited only if we make use, as our vey tools, of those solid achievements which have already been gained. Furthermore, even the ultimate destiny of man himself must in time to come depend upon his taking into the reckoning, and adjusting his practices to, these same hard, inescapable and far-reaching facts which the gene science of the past 30 years has established.
THE RELATION OF GENES AND CHROMOSOMES

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The year 1866, in which Mendel's paper was published, also saw the appearance of Haeckel's "Generelle Morphologie"; and in that work Haeckel stated very clearly the conclusion that the nucleus is the part of the cell that is responsible for heredity. In the years between 1866 and the rescuing of Mendel's work from oblivion in 1900, cytology underwent great changes. The chromosomes and their behavior in mitosis, meiosis, and fertilization were described; and Roux, Weismann, Hertwig, Strasburger and others developed ideas on the relation of chromosomes to heredity. It so happens that the year 1900 saw the publication of the second edition of Wilson's The Cell—for years the standard work in cytology. If one reads this book it is at once evident that the understanding of mitosis and fertilization was well enough developed to serve as a basis for the interpretation of genetics, but that knowledge of the critical stage of meiosis was inadequate. The precise pairing of homologues, the occurrence of reduction in a longitudinal rather than a transverse plane, the independence of the separate chromosome pairs, even the qualitative differences between non-homologous chromosomes, and in fact the very existence of definite distinct pairs of chromosomes—these were all disputed or discredited views. Accordingly, it is not surprising that the early attempts at correlating genetics and cytology were rather disappointing. It is clear that Correns, Cannon, and Guyer were thrown off the track by the inadequacy of the existing cytological
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background. In the years 1901 and 1902 some of the essential gaps in cytology were filled by the work of Montgomery, Boveri, and Sutton, and the first clear and satisfactory account of the relation between genes and chromosomes was made by Sutton in 1903. In that paper we are given a cytological interpretation of segregation and independent assortment—that is, of genetics as Mendel knew it—that still stands today.

The first attempt to associate a particular inherited character with a particular chromosome was made by McClung (1901, 1902), in his suggestion of the relation of the X-chromosome to sex-determination. It happens that in the Orthoptera that he was studying the X is strongly heterochromatic at the meiotic divisions in the male and not in the female; McClung was thus led to suppose that the X-bearing sperm are male-producing—an inversion of the true relations first demonstrated by Stevens in 1905. Following this paper, the work of Stevens, Wilson and others soon established the widespread occurrence of this mechanism of sex-determination; the work of Morgan (1908-1912) on the complex life cycle of the Phylloxera showed so detailed an agreement between the cytology and the sex of the individual as to leave no doubt of the sex-determining role of this chromosome. Nevertheless, there developed a paradox that delayed the general acceptance of the chromosome interpretation of Mendelian heredity. Sex linkage was discovered by Doncaster and Raynor in 1906 in moths, and by Durham and Marryat in 1909 in birds; but in both cases the female was the heterozygous sex, whereas the cytological evidence indicated the male as the heterozygous sex in several groups of insects, in mammals, and in several other groups of animals. There was thus evidence indicating that both relations were of very general occurrence—and they were contradictory.

It was at this point in the story that the work on Drosophila first became important, for it was in 1910 that Morgan reported sex-linkage of the type with heterozygous males, in a form in which Stevens had already described sex chromosomes of the same type. It was almost immediately recognized that the same type of sex-linkage accounted for previously puzzling cases in man. Thus began the resolution of the paradox mentioned above, with the recognition that
the two types of sex-linkage characterized the birds and Lepidoptera on the one hand, and the mammals, Diptera, and other groups on the other hand.

Additional sex-linked mutant types rapidly accumulated in Drosophila, and the stage was thus set for the next great advances. In 1910 Morgan reported that he had made crosses involving two different pairs of sex-linked genes, and had found recombination between them. Thus was found the answer to what had seemed a serious objection to the chromosome interpretation, for it had long been probable that there were more pairs of genes than pairs of chromosomes in many organisms. It had been very generally assumed that the doctrine of the individuality of the chromosomes was inconsistent with any interchange of materials between homologues, and Punnett (1905) has recently stated that this was the reason he and Bateson were reluctant to accept the chromosome interpretation. It is true that Correns, de Vries, and Lock had all suggested something very much like crossing over—but as a means of accounting for independent segregation, not linkage—though Lock had suggested the possible relation to linkage. But the real bases for the development of the modern interpretation of linkage came from two sources: Janssens' (1909) cytological study of chiasmata, which gave possible observational support to the idea of interchange of parts between homologues; and Morgan's 1911 paper, which showed that recombination between different pairs of sex-linked genes might be much less than random—that is, that sex-linked genes are linked to each other. Morgan specifically correlated these conclusions, and stated the present view that closeness of linkage implies nearness together in the linear dimension of a chromosome—or at least that distance apart, as a factor influencing frequency of crossing over, is an important element in the situation. From this it was but a short step to the construction of chromosome maps by Sturtevant (1913).

More and more mutant types were found in Drosophila, and Morgan's laboratory at Columbia became a very exciting place, as those of us who were there can testify; for there were new discoveries to be made by the dozen, and there was an air of excitement, enthusiasm, and friendly and very vocal rivalry that can rarely have been equalled
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in a scientific laboratory. By 1915 the Columbia group—Morgan, Sturtevant, Muller, and Bridges—published *The Mechanism of Mendelian Heredity*, which was the first attempt to present a discussion of the whole field of genetics in terms of the chromosome interpretation. There were still many geneticists and other biologists who were unconvinced or even actively opposed to this interpretation; but with the publication—as the first article in volume I of *Genetics* (1916)—of Bridges' detailed genetic and cytological study of non-disjunction, that phase of the history was closed, for since that time no informed geneticist has taken seriously any of the attempts that have been made to discredit the chromosome interpretation.

I shall not continue with a discussion of purely historical questions beyond this point, for to do so would be to undertake the writing of the history of the whole of genetics for the past 35 years. All I shall attempt is to discuss a few of the general problems that are now under active study.

THE PROCESS OF MEIOSIS

Darlington has developed a detailed theory of the process of meiosis and its relation to ordinary mitosis. This is a beautifully simple and satisfying scheme, which has been very generally adopted by geneticists; it has almost come to be considered the very core of the chromosomal interpretation of genetics. It is with great reluctance, therefore, that I have found myself forced to discard large portions of this scheme—a reluctance made even greater by the fact that I see no wholly satisfactory substitute.

I should like to discuss some of the reasons for feeling that Darlington's scheme is inadequate. On that scheme the conjugating chromosomes are undivided, and conjugate because of that fact. Here we find that competent cytologists are not in agreement on the facts. It seems quite clear that direct microscopic examination shows, in different organisms, all gradations between what appear to be wholly unsplit chromosomes and wholly separate ones. One is reminded of the controversy as to the time of splitting of mitotic chromosomes—a controversy that seems to me to have lost much of its point with the demonstration by McClintock (1942) that the
split occurs at different times in the embryonic tissue and in the endosperm of the maize seed. Is it perhaps the case that there is also no fixed and invariable time of splitting of meiotic chromosomes? In any case, it seems definitely established that, at least in some organisms, they are split before conjugation.

A related difficulty is that, on the Darlington scheme, in triploids or trisomics, conjugation is by twos at any given level, never by threes. Observations in general agree with this, which is taken as further evidence that chromosome pairing is a two-by-two process always and everywhere. But it is quite clear that there is no such relation in the salivary gland cells of Drosophila, where triploids commonly show complete conjugation of all three elements throughout—not to mention the fact that even in diploids it is probable that each paired structure is really made up of a large number of conjugated elements. These relations make it difficult to avoid the conclusion of Cooper (1938) that there is a definite pairing surface of meiotic chromosomes.

Another difficulty lies in the nature and function of chiasmata. It is usual for geneticists to equate chiasmata and chromatid exchanges—I have often done so myself. But a chiasma is a cytologically observable change of partners between two pairs of chromatids, whereas an exchange is the process of breaking of two chromatids and their reunion in a new way. That there is a one-to-one correspondence between these two things is no longer a permissible assumption. It has long been clear that, even on the straight Darlington theory, chiasmata may terminalize and disappear, so that there may have been more exchanges than are visible when counts are made. It is now clear also, from the work of Cooper (1949), that the initial separation of chromatids need not be in the reductional plane at every level. It follows that there may be visible chiasmata that do not represent exchanges at all. Thus vanishes the hope that counts of chiasmata may even give us limiting values for numbers of exchanges.

As indicated above, I know of no wholly satisfactory alternative hypothesis. In many respects the suggestion made by Sax and Sax (1935) is attractive, namely, that conjugation occurs when the chromo-
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Chromosomes reach their maximum extension, and is a consequence of a tendency of like parts to lie closely apposed—a tendency that may be supposed to be always present, but that is geometrically weakened by the usual coiled condition of the chromonemata. This scheme will account for most of the available facts, but is not in agreement with cytological observations indicating that conjugation occurs in some forms at a stage when the chromosomes are strongly contracted [see especially McClintock (1945) on Neurospora].

I am afraid that we shall have to wait for the cytologists to produce a more coherent and satisfactory picture before we can hope for an inclusive description of the process of meiosis, and until that is available it hardly seems possible to plan any experimental analysis of the physical forces at work. Meanwhile there remains much for the geneticist to do in studying the mechanics of segregation and crossing-over by his own precise methods. Such studies have not recently been as widely prosecuted as formerly, but I want to insist here that the law of diminishing returns has not set in in this field.

Genes and Differentiation

If genes are responsible for the inherited characteristics of an organism, and if the genes are contained in the chromosomes, how does it happen that different parts of the same organism show different characteristics? Or, to put the problem in a different way, if the facts of heredity are to be explained in terms of the distribution of qualitatively different genes, may not the facts of differentiation be explained in some similar way?

As long ago as 1883 Roux postulated that differentiation is dependent on somatic segregation, that is, that the genetic determiners necessary for a particular developmental process are segregated to the particular part of the developing organism where that process is to occur. The remarkable cytological behavior of Ascaris seemed to confirm this view, since here there is a large portion of the chromosomal material that is present in the germ line only, and is eliminated from those cells destined to give rise to the somatic tissues. The danger of arguing from this kind of evidence has become obvious.
in the case of the higher plants, where the sporophyte is normally
diploid and the gametophyte is haploid; yet it can be shown that this
difference in chromosome number is not responsible for the phenotypic
differences between the two generations, since diploid gametophytes
and haploid sporophytes both differentiate quite normally.
Roux’s view has been very generally abandoned, and it has come to
be assumed that, as a rule, all the cells of a given individual receive
the same genes. In recent years there has been a tendency to revive
what are essentially somewhat modified forms of Roux’s interpreta-
tion. I should like here to record my opinion that these attempts
are not promising. To cite only two of the numerous reasons for
such an opinion: first, in many organisms, such as birds and many
insects, the egg itself is a highly differentiated cell, and some of these
highly differentiated eggs are capable of developing parthenogeneti-
cally. Here then a differentiated cell must contain all the genes that
are necessary for all the kinds of differentiation characteristic for the
species. Secondly, differentiation is by no means limited to multicel-
lar organisms. It seems clear that no kind of chromosomal seg-
gregation can be involved in the very elaborate differentiation of
such unicellular organisms as Dinoflagellates, Trichonymphids, or
many other such types.

There can be no escape from the conclusion that particular types
of differentiation occur in cells that carry the genes on which depend
a whole series of wholly different kinds of differentiation. This ap-
pears as a paradox only if one thinks of the genes as somehow de-
veloping into the characters concerned, a naive view that no physi-
ological geneticist now entertains. Whether or not one adopts the
“one-gene one-enzyme” view of gene action, it is clear that genes
often act through enzymes, and further that enzymes are very specific
both as to the substrates on which they will act and as to the exact
external conditions under which they will act. This specificity of
enzyme activity is all that is needed to give us a model of differenti-
tation without somatic segregation, though I do not wish to be
understood as implying that the problem of differentiation is solved;
the only implication intended is that it does not seem mysterious.
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POSITION EFFECT AND THE REALITY OF THE GENES

We are sometimes told that the facts that have led to the recognition of the position effect require such a thorough overhauling of the classical concept of genes as to lead to a denial of the very existence of genes. This statement always reminds me of the serious-minded little girl who once asked: “Mother, do you believe in the alphabet?” It is not possible to discuss the questions at issue without using the word “gene,” or inventing another term that has the same meaning. There is no escape from the conclusions that chromosomes are regionally differentiated, physiologically as well as visibly under the microscope; that particular and identifiable regions are necessary for particular reactions in the organism; and finally that these particular regions behave as units in heredity—specifically, in crossing-over. These propositions are established, and taken together they prove the existence of genes. Arguments to the contrary rest either on failure to appreciate the nature of such proofs, or on failure to understand what is meant by the term gene. As an example of the failure to understand the nature of the proof, let me cite Goldschmidt’s view that the phenotypic effects of sectional deficiencies are best considered as due to position effects rather than to a loss of genes. He has here failed to recognize the simple fact that in many cases such sectional deficiencies have been produced by crossing over between inversions, neither of which has such a position effect; all the sequences of loci present in the deficient chromosome can be shown to have no such effect, and the missing sections can be shown on independent grounds to have exactly the properties missing in the deficient chromosome.

It is quite true that in no single case is it possible to specify that one is dealing with a single gene rather than with a nest of two or more genes; it is also not possible to be quite certain that any given mutation is due to a change in the composition of one or more genes rather than in the arrangement of unchanged genes with respect to each other. But neither of these uncertainties in any way weakens the gene theory. For if activity depends on arrangement,
which is still very far from proven as a general proposition, it is still necessary to postulate specific units that are arranged, that have an activity, and that can be shuffled or removed from the system. Those units are genes, and to insist on calling them something else is only to introduce useless confusion.

I should like, in closing, to point out that I do not wish to be understood as implying that the position effect is of no significance in connection with the problem of gene action; the fact is that I am convinced that studies of it now under way are likely to tell us more about how genes act than are any other approaches I know of.

REFERENCES


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IN 1914 William Bateson told the British Association in Melbourne that “... we feel justified in the expectation that ... they (Mendelian factors) will be proved to be responsible for most if not all of the differences by which the varying individuals of any species are distinguished from each other,” and that further, “The question is often asked whether there are not also in operation systems of descent quite other than those contemplated by the Mendelian rules ... none have been plainly demonstrated.” In other words, Mendelian inheritance is virtually universal and virtually exclusive.

It had already been clear from his book *Mendel’s Principles of Heredity*, published five years earlier, that this great generalisation was in his mind—a generalisation which is now taken so much for granted that we are in danger of overlooking the greatness of the man to whom, more than any other, we owe it. Bateson marshalled in his book the wealth of experimental evidence from which his conviction sprang; yet, if we look more closely, we can see that in 1909, the evidence was weak, almost non-existent in one connection. The method of experiment invented by Mendel and extended by Bateson and his fellows, could not be applied directly to the understanding of continuous variation, and in consequence continuous variation had not been shown unambiguously to be a manifestation of Mendelian inheritance. Bateson was, of course, correct in ascribing to it a Men-
delian basis; but a technical fusion of which he was incapable, and a theoretical fusion to which he seemed antipathetic were needed before the properties of continuous variation could be adequately understood and its importance fully realized.

Most of genetics springs directly from Mendel alone; we need go back no further than his paper to trace its origin. Biometrical genetics, the study of continuous variation, has, however, a dual origin. On the one side it sprang, like the rest, from Mendel; but on the other it traces back to Galton and through him to his cousin Charles Darwin. Darwin had recognised the occurrence of "sports," of unitary genetic changes having major effects. His chief emphasis was nevertheless laid on small differences by whose accumulation and mutual supplementation the greater distinctions between varieties and species could come about. To Darwin the important variation was continuous. Galton endeavoured to give quantitative precision to the notions of variation and heredity which Darwin had used, and it was natural that he should take continuous variation as the material for his experimental and biometrical work. His attempts to arrive at the mechanism of heredity were thereby foredoomed, but he did achieve success in arriving at a method of expressing variation and likenesses between relatives in respect of continuously varying characters—methods which were used and extended by Pearson and Weldon. This work was in full swing in 1900, when genetics as we now know it was born.

Now Mendel's method of analysis, like Galton's, was essentially statistical; but, unlike Galton's, it rested entirely on the consideration of frequencies. Correlation methods had no part in it; they were not needed and they were not used. The choice of discontinuous variation for study reduced the statistical treatment to the extreme of simplicity (a simplicity from which we have since found it necessary to depart) and this must have held a great appeal to the less mathematically minded biologist, who as a student of heredity and variation before 1900 had doubtless suffered under a biometrical tyranny. At the same time the often unbridled speculation of the morphological Darwinians (for not all the followers of Darwin were as careful in their work as Galton) was bringing about a revulsion against continuous variation. To Bateson, as to others, Darwinism
was overplayed and suspect. The study of heredity to which it had
given rise was mathematically tortuous and theoretically unsatisfying.
In contrast, Mendel offered an arithmetically simple and biologically
successful approach. So, as commonly happens, the baby was thrown
away with the bath water. Darwinism, biometry, and continuous vari-
ation were out; Mendelism, simple arithmetic, and discontinuous vari-
ation were in.

There was a difficulty of course. Galton had shown continuous
variation to be hereditary. But how could continuous variation of the
phenotype arise from quantal changes of the genotype? And anyway,
Galton had done it only by the most tortuous arithmetic, by using as
Bateson said “the imposing Correlation Table into which the bio-
metrical Procrustean fits his array of unanalysed data.” So need one
worry too much?—especially as Pearson and Weldon chose at first
to fight Mendelism as a trivial exception to biometrical heredity, with
which it could not be made to square. Integration could not be ex-
pected from Bateson and Pearson: it must await another generation of
biometricians and geneticists, with a different perspective of biology
and a keener appreciation of statistics.

The first step (and one which would hardly have surprised Mendel)
was taken by Yule when he suggested that, provided one was pre-
pared to postulate a swarm of genes having small, similar and supple-
mentary effects, there need be no conflict between Mendelian hered-
ity and the properties of continuous variation. Johannsen and Nilsson-
Ehle showed that genes could have effects both small in relation to
non-heritable differences, from which they could not be distinguished
except by a breeding test, and like those of each other. Genes could
in fact have the properties necessary to provide a Mendelian explana-
tion for continuous variation. But must continuous variation be ex-
plained in this way?

The conclusive evidence took time to accumulate. East and his
collaborators proceeded to show by controlled crossing that continu-
ous variation in Nicotiana and maize behaved exactly as would be ex-
pected on this view, particularly that segregation which was the hall
mark of Mendelian inheritance, could be detected by the wider spread
of variation in F2 as compared with parents and F1. Fisher in 1918
carried, as one might say, the war into the enemy's camp by demonstrating that the biometrician's own results must follow from Mendelian inheritance, and that their own methods could be used to partition continuous variation in such a way that Mendel's own phenomenon of dominance could be recognised at work.

The new genetical tool of linkage was used next. If determinants of continuous variation are inherited in the Mendelian fashion, that is as we should now say, carried on the chromosomes, they should show linkage with genes recognised by Mendelian analysis. Such an association was recognised by Sax in 1923 though the evidence for linkage was not final. That linkage was in fact at work was later proved by Rasmussen and others. It has also been shown that the hereditary determinants of continuous variation show linkage with one another. Finally a linkage technique has been used to show that differences, which lead to continuous variation in Drosophila, must be referred to determinants carried by the chromosomes. Though the technique would not permit the action of the entire chromosome set to be followed, some 90 per cent of the variation was in fact unambiguously referrable to nuclear genes. It has been said that if enough genes are invented any result can be explained: that the polygenic theory of continuous variation is like Ptolemy's theory of epicycles, untested and untestable ('Espinasse 1942). Such a criticism can stand no longer. Continuous variation is mediated by genes similar in transmission, though dissimilar in action, to those recognisable by Mendelian analysis.

The Mendelian inheritance of continuous variation gives us a sure basis for the development of methods of analysis and prediction, and it is here that the fusion of Mendel's and Galton's approaches becomes clear. We must measure and analyse continuous variation by means of biometrical quantities and methods, but we must interpret these quantities in a Mendelian fashion. Segregation and linkage must be characterised by components of variation and co-variation for this purpose, just as they are characterised by frequency ratios in Mendelian analysis. This work has been pioneered by Fisher and Wright and is commanding increasingly wide attention in both Britain and the United States. Its progress has not been rapid, partly because of
the need for developing the special statistical analyses required by the
genetical interpretation, and also partly because of the need for cor-
responding and coordinated developments in experimental design.
Without experimental data, analytical methods cannot be tested, and
without a knowledge of the analysis experiments cannot be designed.
We can have little doubt, however, that given the will these technical
troubles will be overcome.

A more serious obstacle, more serious because it is less obvious,
to the rapid development of biometrical genetics arises from a natural
but often crippling reluctance to allow the subject to develop in its
own way, to provide its own justification for its own processes and
concepts. Instead, the notions and processes of Mendelian genetics
are taken over lock, stock and barrel, and unless they are explicitly
accommodated, the biometrical analysis is regarded as suspect and
unjustified. Biometrical genetics is founded on Mendelism. It could
not, as we have seen, have begun to develop until this foundation
had been supplied, and we must equally expect that every phenom-
enon and concept of Mendelian genetics—segregation and linkage,
dominance and interaction, gene and selection—will appear in bio-
metrical genetics. But we must equally be prepared for it to appear
in a new way, for it to demand new methods of recognition, measure-
ment and analysis. Indeed if this were not the case, if continuous
variation had required nothing more than Mendelian analysis, it
would never have posed a problem. Its history is of a search for ways
of recognising and proving that hereditary determinants have the prop-
erties of nuclear genes in circumstances where the classical methods
of observation are denied to us.

A biometrical analysis differs essentially from a Mendelian analysis
in two ways. It is more comprehensive in covering the whole of the
variation in the character, and it is less specific in that the individual
units are not and cannot be considered separately. It can show us the
average behavior of all the genes, but it cannot display the individual
idiosyncrasies of any one of them. We must expect, for example, that
the average behavior will reflect the properties in dominance and in-
teraction of each gene; but it will display these as aggregated over all
the genes, and in this process of aggregating or averaging some special
peculiarity of an individual gene may be swamped and lost in the
mass of more common behavior. Nor need this cause concern, for bio-
metrical predictions must obviously be average predictions. The spe-
cial case is accommodated in the measure of uncertainty, the standard
to each quantity and prediction.
error, which any good biometrical analysis should provide and attach
Where each gene can be considered separately we can investigate
and list its properties, its linkage and dominance relations, and its inter-
to consider. To list all the possibilities of a system of genes of un-
actions with such other genes and external agencies as we may choose
known complexity in a range of environments of unknown structure
is manifestly impossible. Instead we can recognise that in plants (some
is more than we have yet learned to handle. We must therefore
classes of animal may require more complex treatment) there are six
seektoreduceit—knowing, however, that we may be oversimplifying.
great components of variation: (1) the additive, fixable or, as Fisher
even an Olympic runner presumably learned first to walk.
calls it, the genetic component, (2) the component due to domi-
The cytoplasmic component can generally be ruled out, or at least
nance, (3) the component due to interaction between non-allelo-
adjusted, by comparison of reciprocal F1’s. The interaction com-
nomorphic genes, (4) the component due to differences in cytoplasm,
ponents can often be reduced, perhaps eliminated, by adjustment of
(5) the component due to environment and (6) the component due
to interaction of genes and environment. Now this level of complex-
to the scale of measurement of the character—a tool not so readily avail-
ity is more than we have yet learned to handle. We must therefore
able in Mendelian analysis. This leaves us with three components,
seek to reduce it—knowing, however, that we may be oversimplifying.
(1), (2) and (5) to consider. These can be measured, though with
Even an Olympic runner presumably learned first to walk.
very different precisions, by techniques already available. Further-
The cytoplasmic component can generally be ruled out, or at least
more, and this is important, these techniques can be used so as to
adjusted, by comparison of reciprocal F1’s. The interaction com-
reveal the importance of the interactions, by their disturbing effects
ponents can often be reduced, perhaps eliminated, by adjustment of
on the three items specifically recognised. A correspondingly increased
the scale of measurement of the character—a tool not so readily avail-
measure of uncertainty can then be attached to predictions based on
able in Mendelian analysis. This leaves us with three components,
these components. Having recognised the disturbance we can seek to
d(1), (2) and (5) to consider. These can be measured, though with
devise experiments which will bring it explicitly into the analysis.
very different precisions, by techniques already available. Furthermore,
But even this may be unnecessary or at least unprofitable. To take an
example, the variation of flowering time and plant height was followed in a cross between two true breeding lines of Nicotiana rustica (Mather and Vines, 1951). Data were collected from two duplicate sets of experimental plants (or blocks) in each of two years. It was analysed without the explicit recognition of a component for gene-environment interaction, yet the analysis revealed an unambiguous interaction of this kind over the two years. It went still further in suggesting that the genotypic variation changed from block to block within a year, that in fact the particular piece of ground used out of a small experimental field, affected the genetic components of variation. The analysis, however, enabled the standard error to be adjusted to allow for uncertainty due to these interactions with soil and season. No more is necessary, for except in the trivial case of using exactly the same plots of ground or the unlikely case of exact repetition of one year by another, no explicit adjustment can be made or component extracted for such interactions. We cannot reduce the uncertainty engendered in our genetical analysis by soil and season, but we can and must measure this uncertainty.

To proceed in this way may seem at first glance to be closing our eyes to the lessons of Mendelian genetics. We know that genetic interactions occur, so why not make them explicit in our analysis from the first? True we do know that they occur. We know that we may have symmetrical and asymmetrical interactions, complementary and epistatic; but we do not know how these will appear in the analysis of continuous variation. The possibilities are too complex for synthetic prediction. Instead we proceed exactly as the early Mendelians proceeded. We recognise interaction and linkages by departure from the simple expectations which assume their absence. We classify them as is convenient for our purpose by the type of departure produced, and we accommodate them in so far as we must and can in later analyses. We are following the same basic methodology, but we are doing so in our own way. Mendelian genetics warns us of the possible complexities, but because of its different technique it cannot tell us how to cope with them. That biometrical genetics must learn, and I believe is learning, for itself.

The relation between gene and character is complex in continuous
variation. If it were not, the task of analysis would be immeasurably easier. It has often been suggested that instead of accepting the complexity as intrinsic in the problem, an attempt should be made to break down the character into sub-characters whose inheritance would be easier to follow and analyse. Thus yield in a cereal might be broken down into number of ears, number of grains per ear and weight per grain, attention then being directed to the supposedly simpler inheritance of these sub-characters. Such simplification must of course be used wherever possible; but Mendelian genetics warns us not to be over-sanguine about its efficacy. The complex genetic relations of, for example, the various pigments in flower color show us that while some genes may affect the production of only one or other pigment, other genes have more far reaching effects. Thus the only successful analyses can be into genes, each having a recognisable effect on one or more sub-characters, not into sub-characters each affected by different and physiologically independent genes. The somatic analysis always requires genetical justification and this cannot generally be given with the complex inheritance of continuous variation.

If Beadle's "one gene—one enzyme" hypothesis is valid, a more successful approach would be by analysis into processes rather than characters. Strong evidence for this view has, however, yet to come to hand, and indeed, especially with genes of less drastic effect, it seems a priori to be no more likely a hypothesis than the "one factor—one character" view with which the early geneticists toyed. Throughout the history of genetics there have been attempts to relate the mechanical unit of inheritance, of segregation and crossing-over, to the physiological unit of action in development, and to relate the cytologically visible to the genetically inferable. Some successes have been recorded. In general the chromosome set is the minimal unit of development: addition as well as subtraction has disastrous results. The chromosome is equatable to the linkage group, and the chiasma to crossing-over. But not all the cross-relations are so simple. In particular there is little ground for believing that chromomere or salivary band or any physical structure visible in the chromosome is equatable to the gene inferable from its action and change of action. Physical
adjacency of structure may result in special physiological interaction, as we know from the position effect, and Goldschmidt points out that apparently physiologically distinct genes such as yellow and scute may indeed overlap physically. We are sometimes at a loss even to say what we mean by a gene, with for example certain determinants of anthocyanin production in cotton (Silow and Yu, 1941), or the Rhesus gene in man (Mollison et al, 1948). This latter, for example, may be taken as a single unit of varying antigen production, but equally it seems at least triple if we consider the properties in specificity of the antigens produced. It is a trinity or a unity according to point of view, and like other such aggregates it has a high capacity for generating sterile discussion as to whether it should properly be regarded as one or three.

Whatever clarity of definition may ultimately be possible, at present the unit that we take as our gene depends on the means we have for recognising it and the purpose for which we define it. These purposes and means are not the same in biometrical as in Mendelian genetics. In particular we have not in biometrical genetics the test of genic separation afforded in Mendelian genetics by the directly observable recombination of distinct effects unambiguously referable to the determinants. The effects of members of a polygenic system are not unambiguously referable to particular determinants, so that our units are definable only in statistical terms. We must therefore expect to recognise and be content to use units of a nature at least potentially different from genes. These effective factors are likely in general to be linked blocks of genes and they will have properties relatable to, but transcending, those of the individual genes which compose them. They may well show what has been called super-dominance or over-dominance, even though the individual genes have no such effect. It is useful to recognise, measure and utilise such over-dominance, but it affords no secure basis for imputing special properties of dominance to the genes themselves, and we may well doubt the wisdom of denoting it by a term which suggests properties in dominance.

Composite units of this kind will also have compound properties of change. This may obviously come about by change in the individ-
ual genes of the kind which we should call mutation, or by the reassociation of unchanged genes which we should call recombination. Even in Mendelian genetics the ultimate distinction between mutation and recombination of parts may be difficult: in biometrical genetics it must in general be impossible. We can show that changes of both kinds occur; but except in special cases we cannot assign any given change to one or the other class, or even, very often, apportion change in specific fractions to one or the other. All that we can say is that in most cases recombination will release much more variation than mutation will produce de novo.

This difficulty of distinguishing between the effect of mutation and recombination, though only one of several of the same type, epitomises the problems which continuous variation poses. We can show all the phenomena of genetics to be a work in continuous variation, segregation, linkage, dominance, interaction, mutation and selection; and we do it by the familiar means of constructing an experimental situation in which particular results can have only one interpretation. Biometrical genetics, however, must, as we have seen, seek to cope with all the variation which a character is showing. It must seek to give an account of this variation in such a way as to permit predictions of its future behavior. It is not, therefore, enough to be able to show, one at a time by suitably restrictive experimental techniques, that certain phenomena occur: we must seek to be able in the general case to draw up a balance sheet of the variation in terms of such of these phenomena as we can usefully employ. And if two or more of them have indistinguishable, or not readily distinguishable, effects we may have to be content with pooling them, at any rate provisionally, in the balance sheet. Our first task is, of course, that of seeing just how each phenomenon can appear in continuous variation, of seeing just what linkage, selection and the rest can do. Genetic models enable us to do this. They help us to see what can happen, but for all their appealing clarity they do not necessarily tell us what is happening. The methods for discovering what the variation is in fact doing are even now poor and imperfect. Our great task is to perfect them.

To take an example, the effects of linkage and selection on variation are in principle clear and distinct. If we denote the additive
component of variation by D, we can indicate its contribution to any second degree statistic describing variation or covariation by a suitable coefficient which can be calculated from any given set of assumptions. Selection in favor of heterozygotes disturbs these coefficients but does not alter the value of D itself. Now linkage of the members of the polygenic system does not affect the coefficients of D, but does affect the value of D itself, which is in fact no longer fixed from generation to generation. It is not difficult to show that either D or its coefficient is failing to agree with the simple expectations based on the assumptions of no linkage and no selection. But it is difficult to distinguish departures due to progressively changing values of D, which would mean linkage, from progressive departures of the coefficients from expectation, which would mean selection (Bateman and Mather, 1951). There is no reason to doubt that we shall learn how to make the distinction; but the genetic models, clear as they are, do not in themselves tell us how to do so. There is a joint statistical and genetical problem which in practice we have not yet solved.

In devising our methods of analysis and developing the working theory to which they lead we must be prepared to sacrifice some of the notions and distinctions which genetics has taught us and which we have come to respect. They guide us but they must not bind us. We are working at a new level where the system of genes is fixed for us. We cannot choose genes which are particularly convenient for the investigation, genes which have simple relations with phenotypic differences, which do not interact in uncomfortable ways and whose linkage relations are just what we would wish. Instead we must take into account all the genes affecting the particular character, whatever their properties, because they will all contribute to the variation whose analysis and understanding is our aim. Our genetic classification must be correspondingly less detailed and we must expect to learn less about the individual genes. But what is lost in detailed knowledge will be gained in breadth of conclusion, for all the genes affecting variation of the character in the population or family in question will be covered. Whatever items fail to be separated in the balance sheet, the total should be right. Mendelian and
biometrical analysis are thus complementary in the information they yield. From the one we obtain a detailed knowledge of the genotype, its organisation and properties: from the other we obtain less individual detail but a comprehensive average.

In its first thirty years of life genetics concentrated on solving its own internal problems, on learning what it could about the way the genotype was made up and did its work. Indeed, biometrical genetics is still struggling with its own internal problems of analysis. But in general, genetics has of recent years begun to link up with other sciences. To mention but two examples, we are beginning to help in learning something of the way development and differentiation proceed, and the Neurospora experiments have established a close liaison with biochemistry. Genetics is becoming a meeting place for a wide range of other sciences. Biometrical genetics must play its part in this movement, for it can provide the link with the study of populations and their evolution, and with the artificial evolution that we call plant and animal improvement, in a way impossible to other branches of genetics. It can give us that overall picture of variation which is impossible of achievement from the isolated study of particular genes.

Now in its early days genetics was identifiable with an anti-Darwinian point of view. This has gradually changed and the Darwinian principles of random variation and natural selection are now based firmly on genetic evidence. The particulate theory of heredity provides the solution to Darwin’s problem of the conservation of variation, and genetical recombination shows us the special virtue of sexual reproduction. At the same time the principle of natural selection shows us how and why genetical systems have come to be organised as they are. Darwinism and Mendelism are now mutually dependent, each helping to interpret the other. All this has been achieved by the study of Mendelian genetics, without consideration of continuous variation.

We are now moving to a new stage where the study of continuous variation must play its part. We wish to know not merely how in broad terms these things can work, but how they do work in actual fact. Selection operates directly on the phenotype and only indirectly
through it on the genotype. Thus all the variation of the phenotype will be operated on by selection; the results will be overall results. A loss in one direction can be offset by a greater gain in another. The fact that a total phenotype and the total genotype that produced it are favored by a given total of selective forces does not mean that every character or every gene will react in the most favorable way. We must consider the totality of variation including that produced by polygenic systems.

When for these reasons we turn to the general theory of variability, certain remarkable features emerge which go far to aid us in the understanding of behavior in populations (Mather, 1943). The selective advantage or disadvantage of any allelomorph of one of a polygenic system is not unconditional, in fact it will depend very largely on the other members of the system present. This can, of course, be true of a major gene if its effects are sufficiently modifiable, but it must always be true in polygenic systems. Thus it is the balance of the operative polygenic system which is important in selection, not the individual gene; and since the same balance can obviously be achieved in many ways, a wealth of possibilities are open. Combinations of genes giving individually the same balance may produce unbalance when put together. In fact there must be a general tendency for balance to deteriorate as a result of segregation and reassortment except in so far as natural selection is constantly weeding out unbalanced combinations. Combinations which occur naturally will be balanced, those which do not will in general be unbalanced, (Mather and Edwardes, 1943). This expectation is strikingly verified in a number of ways, of which the inbreeding depression found in naturally cross-breeding species has long been known. More recently Dobzhansky's experiments (1948) with the third chromosome of Drosophila pseudoobscura has provided a different and elegant demonstration of this principle. He has confirmed that the balance of combinations in isolated populations drift away from one another within a species. This we must also suppose to happen in the course of speciation, with the result that hybrid incapacity follows on the rise of bars to crossing, whatever their nature (Darlington and Mather, 1950).
Insofar as a given balance or narrow range of balances can be achieved by a wide variety of polygenic combinations, a near uniformity of phenotype in a population will cloak a wealth of genic variability. The notion of latent or potential variability is a familiar one in genetics, for as Fisher (1930) has pointed out, variability is latent in any genic heterozygote. The potential variability of a polygenic system, however, will go far beyond this, for it is hidden not merely in heterozygosis but also in the balance of non-allelic combinations. Again experiment has confirmed this expectation, and shows indeed that the variability potential within a species is almost certainly sufficient, when exposed by selection, to produce differences as great as those between species without any recourse to mutation (Mather and Harrison, 1949). The dynamics of the relations between the various states of potential and free variability involve considerations of breeding systems and linkage relations. Cross breeding encourages the free flow of variability from one state to another, while inbreeding freezes it. Linkage slows the release of variability, while maintaining the possibility of its release, even under cross-breeding. We can thus see the importance of strict control of breeding system (which Darwin himself recognised and investigated) and of recombination, and form an idea of the selective forces which favor and govern the various devices achieving this control (Mather 1943, Darlington and Mather, 1950).

We can see too, that linkage of the members of unlike polygenic systems must mean that deliberate selection for one character may, and generally will, result in alterations of other characters on which no selective force is operating. Indeed these subordinate characters may even change against selection. These correlated responses to selection have been seen in many selection experiments (Mather and Harrison, 1949), fertility being nearly always adversely affected by selection for other characters. Correlated responses and the relation of balance to natural selection also reveal to us a type of inertia in genetic systems. The genetical status quo always has the advantage, other things being equal or nearly equal, so that only long continued selective forces can bring about a permanent adjustment in nature, or for that matter in experiment. The species is protected at least in
considerable measure from responses to temporary changes of environment which could only have the unfortunate effect of depleting variability without improving adjustment.

These results and conclusions, borne out though they are by experiment, are still only capable of qualitative statement. There is need of a quantitative theory of variability. This must in part be a sort of biological thermo-dynamics concerned with the mathematical rules governing the relations and changes between the different states of variability, and the consequent responses to selection. It must, however, also include the improved statistical-genetical technique which, as we have seen, is needed for the measurement and analysis of variation and variability as it occurs in nature and experiment. Both of these quantitative developments are demanding attention, for without them the genetical theory of evolution and of plant and animal breeding are clearly inadequate. To take but one example, we have long known how the genetic constitution of a population will change under inbreeding provided that no other agencies intervene, and we can calculate inbreeding coefficients on these simple assumptions. But we still do not know whether these expectations are realised in practice. There are good general grounds and even some experimental evidence (Düxgunes 1950), that in cross-breeding species heterozygotes will be at an advantage compared with homozygotes. The progress of inbreeding would thus be slowed down. Whether it is generally so slowed, and to what extent, are questions which cannot be answered till we have the biometric means of measuring and analysing variation, especially continuous variation.

The study of continuous variation may help in other ways. The special relation of polygenic systems to heterochromatin suggests that it may, for example, help us to understand the organisation and evolution of genes themselves. It is, however, in the generalisation of the consequences and concepts of Mendelian inheritance (or of other systems of variation such as heterokaryosis in fungi), in the theory of variation and selection and in the understanding and analysis of populations, both natural and experimental, that we can expect the main interest of biometrical genetics to lie, not merely for the geneticist but also for the evolutionist and the breeder of plants and ani-
The Progress and Prospect of Biometrical Genetics

mals. This interest is testified by the activities of plant and animal breeders and of evolutionists in this field. Darwin's small variations, which made so small an appeal to the early geneticists, are coming once more to the center. The combination of Darwinian and Mendelian theory, and of Mendelian and Galtonian techniques, is aiding toward an understanding of these small variations, their importance and their behavior. We have still a long way to go both in genetical theory and statistical practice, but the need is becoming more widely realised. Continuous variation is, in a special sense, the variation of populations and species in nature, and biometrical genetics is especially the study of continuous variation.

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SOME CHEMICAL ASPECTS OF
THE CELL NUCLEUS

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There is going on at the present time a lively chemical investigation of the constituents of the cell nucleus. This investigation can be traced back to Miescher, a contemporary of Mendel. Miescher's successors, such men as Kossel, Stendel and Levene, were aware of Mendelism but it was not the primary impetus for their work. In recent years this field of inquiry has attracted many new workers and for them the great achievements of cytogenetics are surely a powerful impetus. Investigation of the giant salivary chromosomes aroused the interest of some biochemists. Many more were fascinated by the work on Neurospora, which showed how genetics can provide a tool for the solution of a great variety of biochemical problems. At this time too came the discovery that viruses are nucleoproteins, a discovery that drew the attention of biochemists to chromosomes and genes as well as to viruses, for both are self-duplicating bodies.

Before coming to a discussion of some of the more recent chemical studies of the cell nucleus some of the older achievements will be mentioned briefly. Concerning the basic proteins, prolaminates and histones, there was until recently little to add to what is given in Kossel's monograph (1928). A detailed account of much of the older work in which nucleic acids were shown to be polynucleotides is given in Levene's monograph (1931). Quite early came the dis-
covery that there are two types of nucleic acid, for one of which yeast was the usual source, the other usually being obtained from fish sperm or calf thymus. Levene's great contribution was to show that the sugar of the former is d-ribose and that of the latter desoxyribose, so that it has become customary to refer to one type as ribonucleic acid (RNA) and to the other as desoxyribonucleic acid (DNA). There is practically nothing in Levene's book concerning the cytological distribution of the two nucleic acids, a matter of primary concern to all investigators today.

THE SIGNIFICANCE OF DNA

An important step for future cytological work was Feulgen's discovery of a simple, sensitive and fairly specific color reaction for DNA. An indication of the limited point of view of investigators at that period is that there was an interval of 10 years before this test was applied cytologically. The application was made by Feulgen (1924) himself and, although extensively used by cytologists, has been but slightly changed by them. Another important cytological contribution from Feulgen's laboratory was the isolation of cell nuclei by Behrens (1938). This is the most elegant procedure yet devised for the isolation of formed cell components. Application of the Feulgen reaction for DNA and of Behrens' isolation of nuclei led to the demonstration that in practically all cells, those of both plants and animals, DNA exists only in the nucleus, being confined to the chromosomes (Feulgen, 1937). RNA appeared to be primarily a cytoplasmic constituent. It has become clear that although nucleic acids, despite their name, are present in both cytoplasm and nucleus, one of them, DNA, must have a special significance for chromosomes since it is always present in them and in no other part of the cell. The restriction in general of Feulgen-positive material to chromosomes means that when occasionally it is observed in the cytoplasm, as for example in Paramecium where Kappa is now known to be Feulgen-positive (Preer, 1948), such material can be regarded as an intruder in the cytoplasm, rather than as a normal cytoplasmic constituent.
A. E. Mirsky

In addition to the chemical and cytochemical distinction between the two types of nucleic acid another important early contribution was the knowledge that when DNA is isolated from the thymus at low temperatures and without the use of strong reagents the product obtained is highly polymerized. In precisely what state DNA exists in the chromosomes is not yet known, but the highly polymerized DNA prepared from thymus by the Bang-Hammarsten procedure (Hammarsten, 1924) is surely a much closer approximation to unmodified DNA than is that prepared by any other of the early methods. More recently a method has been found for preparing polymerized DNA from a variety of cells and tissues, as well as from thymus (Mirsky and Pollister, 1946).

An interesting approach to the chemistry of chromosomes has come from investigations of the transformation of pneumococcal types. The basic discovery in this field was made by Griffith (1928) when he found that under certain conditions the type specificity of a living, non-encapsulated pneumococcus could be determined by mixing with it heat-killed cells of a given type. The Griffith phenomenon may perhaps be regarded as a hybridization in which only one of the parent strains is alive. As first achieved by Griffith this bacterial transformation was carried out in a mouse. Dawson (1931) found that it could be accomplished in a test tube. Alloway (1933) carried the analysis of the phenomenon much further still by showing that an extract in deoxycholate of the heat-killed organisms would suffice. He also found that the active principle could be precipitated in alcohol and redissolved to form a clear solution. By this time the Griffith phenomenon had become a hybridization in which one parent was no more than an active principle.

Coming now to the more recent developments in the chemistry of chromosomes, let us consider first those which indicate some special significance for DNA in the chromosome. One concerns the rate at which $^{32}P$ is incorporated into DNA. Compared with RNA, whether that of the nucleus or cytoplasm, the turnover of DNA phosphorus in non-dividing cells is exceedingly slow. In the liver, phosphorus of DNA turns over only $1/33$ as fast as does the phosphorus of RNA. Also when $^{32}N$ is used as a tracer, the turnover of
DNA is lower than that of RNA. If $N^{15}$ is given as glycine the turnover in DNA is $1/4$ that in RNA and when $N^{15}$ is given as adenine it is only $1/73$ as much. Concerning these turnover rates (summarized in Furst et al., 1950), all that can be said at present is that they show that the role of DNA in the cell is quite different from that of RNA.

The special significance of DNA is shown when the amount of it per cell is measured in different cells of the same organism. It was discovered independently by two groups of investigators that in the different cells of an organism the quantity of DNA for each haploid set of chromosomes is constant (Boivin and Vendrely, 1948; Vendrely, 1948; Mirsky and Ris, 1949). In Table 1 the values for various nuclei of the domestic fowl are given. It can be seen that in the sperm there is just one-half the quantity found in diploid cells. In Ascaris it has been shown that the DNA contents of egg and sperm nuclei are the same (Mirsky and Ris, in the press). Constancy per cell is certainly an unusual characteristic for a chemical component of cells. It is, of course, common to find the contrary: a substance present in abundance in some cells of an organism and not detectable in most other cells. Even a substance, such as RNA, present in all cells varies greatly in amount in different cells. The quantity of RNA varies also in the nuclei of different cells and even varies in nuclei of the same cell type at different times. In the course of starvation, for example, the quantity of RNA per hepatic nucleus drops considerably, but the quantity of DNA per nucleus remains unchanged (Mirsky and Kurnick, unpublished).

Table 1 - DNA Content of Various Nuclei of the Fowl Expressed as mg. x $10^{-9}$ per Nucleus

<table>
<thead>
<tr>
<th>Determinations by</th>
<th>Erythrocyte</th>
<th>Liver</th>
<th>Kidney</th>
<th>Spleen</th>
<th>Heart</th>
<th>Pancreas</th>
<th>Sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirsky and Ris,</td>
<td>2.34</td>
<td>2.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.26</td>
</tr>
<tr>
<td>1949</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determinations by</td>
<td>2.49</td>
<td>2.56</td>
<td>2.20</td>
<td>2.54</td>
<td>2.45</td>
<td>2.61</td>
<td></td>
</tr>
<tr>
<td>Davidson, Leslie,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smellie and Thom-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>son, 1950</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
In the past DNA has been considered to be a highly variable cell component and there has been, accordingly, much speculation concerning the so-called nucleic acid charge and the nucleic acid cycle. These ideas are based on observations of differences in intensity of staining. Intensity of staining is, however, an unreliable indication of the total DNA present in a nucleus unless the volume of the nucleus is considered, and this has usually not been done.

On the basis of staining intensity Strasburger (1909) came to the conclusion that chromatin (or what we would now call DNA) could not be the material of which the hereditary factors are composed because the quantity of chromatin per cell seemed to be so highly variable. Strasburger's opinion may be found in his contribution to the volume celebrating the 100th birthday of Charles Darwin.

In the stages preliminary to their division, the chromosomes become denser and take up a substance which increases their staining capacity; this is called chromatin. This substance collects in the chromosomes and may form the nutritive material for the carriers of heredity units which we now believe to be enclosed in them. The chromatin cannot itself be the hereditary substance, as it afterwards leaves the chromosomes, and the amount of it is subject to considerable variation in the nucleus, according to its stage of development. Conjointly with the materials which take part in the formation of the nuclear spindle and other processes in the cell, the chromatin accumulates in the resting nucleus to form the nucleoli.

If the measurements of DNA content per cell are correct—if this component of the chromosomes is indeed present in constant amount in the different somatic cells of an organism and in one-half this amount in the germ cells—then it may be said that DNA is part of the gene substance.

DNA content per cell is found by determining the quantity of DNA in a suspension containing a counted number of cells or isolated nuclei and dividing this quantity by the number of cells or nuclei. For sperm, erythrocytes and sponge cells there is no difficulty in counting cells. For tissue cells it is necessary to isolate nuclei by the citric acid procedure and count them. DNA contents of many different animal cells have been determined. The values range from $0.12 \times 10^{-8}$ mg. for sponge cells to $160 \times 10^{-8}$ mg. for the cells of
Amphiuma, a urodele. A comparison of values for different species shows that in the higher animals DNA content cannot be taken as an indication of the number of different genes present. DNA content per cell is related to cell size and there is a significant correlation between DNA per cell and cell size in the homologous cells of different animals (Mirsky and Ris, in the press).

When the DNA contents of a number of different cells had been found by the procedure that has just been described it was possible to show that under certain conditions the Feulgen reaction can be used to determine the relative DNA contents of nuclei (Ris and Mirsky, 1949). In this procedure a microspectrophotometric measurement is made on single nuclei either in isolated cells and nuclei or in tissue sections. That the relative values so obtained are correct is known because determinations by chemical methods on counted numbers of cells were made on the same materials. It should be emphasized that such microspectrophotometric measurements have a quantitative validity only when some standard of reference has been provided. The advantage of the quantitative Feulgen procedure is that measurements on single cells are possible. In this way it has been shown that in mammalian liver the nuclei having different diameters so that their volumes are in the ratio 1:2:4, and which have been regarded as diploid, tetraploid and octoploid nuclei, have DNA contents in the ratio 1:2:4.

The importance of DNA in genetic processes has also been shown by further investigation of the active principle in transformation of pneumococcal types by Avery and his colleagues (1944). The most highly purified preparations of the active principle contain DNA and no other detectable material. That DNA is necessary for activity has been shown by the correlation between loss of activity and loss of viscosity when the DNA in an active preparation is treated with crystalline, protease-free desoxyribonuclease (McCarty and Avery, 1946).

It is quite possible that DNA, and nothing else, is responsible for the transforming activity, but this has not been demonstrated conclusively. In purification of the active principle more and more of the protein attached to DNA is removed, as indeed in the preparation of DNA from any other source. It is difficult to eliminate the possibil-
ity that the minute quantities of protein that probably remain attached to DNA, though undetectable by the tests applied, are necessary for activity—itself an exceedingly sensitive test. Transformation of pneumococci is a self-duplication phenomenon, for the effect finally observed is not due immediately to the active principle added, but to the enormously greater quantity of active principle generated in the course of the experiment. It is, therefore, possible that no more than a few particles of active principle are required; and if no more than a few particles of DNA were effective, there would be no doubt that the active principle consisted of nothing but DNA. In his work on preparation of the bacteriophage Northrop (1937) recognized that he was dealing with a self-duplication phenomenon and was able to demonstrate that the material which he isolated was actually the bacteriophage because only a few particles were required for activity. Actually a very large number of DNA particles (more than $10^6$, if a molecular weight of a million is assumed for DNA) are required for pneumococcus transformation, and the minimal mass of material is about 2,000,000 times that of the minimal mass of nucleoprotein required for phage activity. There is, accordingly, some doubt whether DNA is itself the transforming agent, although it can be regarded as established that DNA is at least part of the active principle. It should be mentioned that even if DNA itself is the active principle, much more than several particles may be needed.

Since it is now known that the material derived from the heat-killed cells that is effective in pneumococcus transformation contains DNA, this is in itself evidence for considering the process to be essentially a hybridization. In those cells which can be studied cytologically, all the DNA is localized in chromosomes and the essential role of chromosomal material in hybridization is well-known. It is remarkable in the pneumococcus transformation that part of the DNA-containing material is derived from heat-killed cells, and that before being used for "hybridization" it can be examined chemically.
Recognition of the biological significance of DNA has stimulated chemical investigation of it along many lines. One active field of investigation concerns the purines and pyrimidines of DNA. The earlier investigators found that the four bases—adenine, guanine, thymine and cytosine—are present in approximately equimolar proportions and on this basis formulated the tetranucleotide theory, according to which a polynucleotide containing one of each of the four bases was considered to be a fundamental unit in DNA. Using chromatographic procedures for analysis Chargaff and his colleagues have shown that the four bases are not present in equimolar proportions and this has removed whatever experimental evidence there was for the tetranucleotide theory.

Chargaff (1950) and his colleagues have analyzed the DNAs prepared from a number of different sources and have obtained results showing that in preparations from different organisms the ratios of the bases are different, although they are the same in preparations from different tissues of the same organism. It is highly probable, therefore, that the proportions of the four bases differ in DNAs of various organisms. An inconclusive feature, however, of these analyses is that only between 76 and 90 per cent of the material used in an analysis is accounted for and it is well known that in the course of hydrolysis different amounts of the purine and pyrimidine bases may be destroyed. It is possible, therefore, that differences in the bases destroyed influence the ratios of those recovered. Analyses carried out by Daly and her colleagues show that when close to 100 per cent of the material is recovered the results for preparations of DNA from different organisms are rather similar, although it is not claimed that they are the same. (Daly et al., 1950)

Some recent analyses by Wyatt (1950) show conclusively that there are differences in the purine and pyrimidine composition of DNA prepared from different organisms. He has found considerable amounts of 5-methyl-cytosine in the DNA isolated from wheat germ, far more than in that from calf thymus. There was a chromatographic
fraction in the hydrolysate of wheat germ DNA that Daly did not identify. Since Wyatt's work Daly has identified it as 5-methylcytosine, thus confirming Wyatt's observation. This addition (Daly, unpublished) brings the recovery of material in Daly's analysis of wheat germ DNA close to 100 per cent and when the analyses of this DNA is compared with that of thymus DNA, where recovery is close to 100 per cent, the difference in composition is striking, as shown in Table 2.

Table 2 · Purine and Pyrimidine Contents of the Desoxypentose Nucleic Acids of Calf Thymus and Wheat Germ. Before the identification of 5-methyl cytosine, the differences in the distribution of the other bases could have been attributed to selective destruction in the course of hydrolysis of the wheat germ nucleic acid.

<table>
<thead>
<tr>
<th>NUCLEIC ACID</th>
<th>ADENINE</th>
<th>GUANINE</th>
<th>THYMINE</th>
<th>CYTOSINE</th>
<th>5-METHYL ACCOUNTED FOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus</td>
<td>27.6</td>
<td>23.5</td>
<td>28.0</td>
<td>19.7</td>
<td>1.2*</td>
</tr>
<tr>
<td>Wheat germ</td>
<td>27.4</td>
<td>21.6</td>
<td>27.0</td>
<td>16.4</td>
<td>5.4*</td>
</tr>
</tbody>
</table>

* These values were calculated from the data given by Wyatt (1950b).

DISTRIBUTION OF RIBONUCLEIC ACID

DNA is present in chromosomes only, but RNA is not limited to the cytoplasm; it is also present in the nucleus. Presence of RNA in the nucleolus was recognized by Caspersson and Schultz (1940) and by Brachet (1940) and its presence in chromosomes was detected by Brachet (1944) and by Minsky and Ris (1947b). There is at present no evidence that DNA is formed from RNA or vice versa. Changes in the RNA content of chromosomes during mitosis have been observed by Jacobson (1950). Caspersson and his colleagues have shown that the appearance of RNA in the cytoplasm is correlated with its appearance in the nucleus, especially in the nucleolus, and their observations have led them to suppose that RNA-containing proteins are synthesized in the nucleus and then pass out into the cytoplasm. In recent experiments with $p^{12}$ as a tracer it has been found that the turnover of nuclear is much greater than that of cytoplasmic RNA;
and, furthermore, that within the cytoplasm the turnover of some RNA fractions is faster than others. Jeener (1950) has pointed out that these differences in turnover rates may represent a sequence beginning in the nucleus where RNA may be supposed to be synthesized. When RNA passes out into the cytoplasm it is supposed that its turnover drops and as this recently emerged RNA is built into cytoplasmic structures its turnover rate drops still further. Such mobility of RNA offers a sharp contrast to the relative fixity of DNA.

**PROTEINS OF CHROMOSOMES**

In Kossel’s monograph there was summarized the information available at that time concerning the proteins of the cell nucleus. The nuclear proteins then known were histones and protamines. Histones had been found in nucleated erythrocytes and in the calf thymus; protamines had been isolated from the sperm of many species of fish. Since then histones have been found to be a nuclear constituent of the generality of tissues, plant as well as animal (Minsky and Polliet, 1946). A protamine has been isolated from fowl sperm (Daly, Minsky and Ris, in the press).

Protamines are combined with the DNA of sperm and are therefore chromosomal constituents. Histones are also combined with DNA. This has been demonstrated by studying how a basic dye, crystal violet, combines with chromosomes (Minsky and Ris, in the press). The dye combines with the phosphoric acid groups of DNA and in doing so, it displaces the histone attached to them. Basic groups of histone and crystal violet compete for the acid groups of DNA. In experiments with isolated nuclei and chromosomes almost all their histone can be displaced by addition of protamine in high concentration which goes to the phosphoric acid groups of DNA just as crystal violet does when it stains the chromosomes.

When the sperm and somatic cells of the same animal are studied, as has been done for the salmon and fowl, it is found that the sperm contain protamine and no histone, while the somatic cells on the contrary contain histone and no protamine (Daly, Minsky and Ris, in the press). Histones and protamines differ from each other in
many respects. Histone molecules, for example, are much bigger than those of protamine. A thorough analysis of the amino acid contents of fowl erythrocyte histone and fowl sperm protamine (gallin) shows that the compositions of these two proteins are entirely different. Since both proteins are constituents of chromosomes, it may be concluded that in different cells of the same animal there may be large differences in the composition of chromosomes. Other differences in composition of chromosomes will soon be mentioned.

It has been known for a long time that trypsin destroys the structure of chromosomes, and for this reason it has been supposed that there must be a fibrous protein in chromosomes. At the time when no other proteins than histones and protamines were known to be present in chromosomes the question arose as to whether these proteins might exist in a fibrous form in chromosomes. Other proteins, fibrous ones, are in fact present in chromosomes. Knowledge of these proteins has come from the study of isolated chromosomes.

ANALYSES OF ISOLATED CHROMOSOMES

Chromosomes have been isolated from certain non-dividing cells—from the nucleated erythrocytes of the fish and turtle and from mammalian thymus, liver, pancreas and kidney (Minsky and Ris, 1947; Minsky and Ris, in the press). These chromatin-containing bodies are prepared from nuclei that have been subjected to shearing forces in the course of which the nuclei are fragmented. The following reasons have been given for considering the isolated bodies to be chromosomes, rather than drawn out nuclei: they are differentiated along their axis in much the way that chromosomes are; many of them are distinctly double so that there can be no doubt that they are threads of chromatin and not simply stretched nuclei; many of the bodies have a characteristic configuration so that a number of individual types can be seen repeatedly. Individuality has ever since Boveri's investigations on chromosomes (1904) been recognized as one of their most important characteristics, and individuality can be observed in isolated chromosomes. In preparations from four different tissues of cattle, chromosomes with the same individual chara-
characteristics are seen. A good example of this is the chromosome that can be identified by its attachment to the nucleolus. The nuclear chromosomes of beef liver and pancreas can be seen to be of the same type.

Isolated chromosomes contain DNA and histone. DNA constitutes 41 per cent of the mass of fish erythrocyte chromosomes, 39 per cent of thymus chromosomes and 25 to 29 per cent of liver, kidney and pancreas chromosomes. In addition to the histone fraction in these chromosomes there is a protein of an entirely different kind. Non-histone protein was first found in thymus chromosomes. When these chromosomes are suspended in 1 M NaCl they disperse to form a viscous, slightly opalescent fluid. After high speed centrifugation a clear, viscous supernatant and a scanty residue are obtained. The supernatant consists of histone and DNA. The residue, observed under the microscope, consists of a mass of minute coiled threads, much smaller than chromosomes. These threads are not stained by basic dyes. No histone or DNA are to be found in the residue, which consists of protein and about 10 per cent of RNA. This protein contains tryptophane, which distinguishes it at once from histones, which contain no more than traces of tryptophane. Since this protein fraction is obtained as an insoluble residue after the DNA and histone have passed into solution, it has been referred to as the residual protein fraction of the chromosome. A complete amino acid analysis of residual protein has been made (Daly and Mirsky, unpublished), and since well over 90 per cent of its nitrogen can be identified as various amino acids, there can be no doubt that it is in fact protein. Its amino acid composition is distinctly different from that of the histones which have so far been analyzed. Non-histone, or residual protein fractions have been prepared from isolated chromosomes of liver, pancreas and kidney, as well as from thymus.

**EFFECTS OF ENZYMES ON ISOLATED CHROMOSOMES**

When a suspension of isolated chromosomes is treated with crystalline trypsin the fluid soon becomes viscous, indicating that DNA is dissolving, and when a drop is observed microscopically nothing at
all can be seen, for the chromosomes have disintegrated. This is in accord with the older observation that in microscopic preparations of chromosomes, their structure is destroyed by the action of trypsin. The effect of trypsin demonstrates that protein is required for the maintenance of chromosome structure but it does not indicate whether the structural protein is histone or residual protein because both of these protein fractions are disintegrated by trypsin.

When isolated chromosomes are treated with crystalline pepsin, they shrink considerably, but they are not disintegrated. This is in accord with observations of the effect of pepsin on the structure of microscopic preparations of chromosomes. Several investigators have supposed that by the action of pepsin on chromosomes they were able to distinguish between histone and non-histone protein because, so they believed, histone is not digested by pepsin whereas non-histone protein is (Mazia, 1950; Kaufmann et al., 1950). The conclusion these investigators came to was that histone forms part of the fibrous structure of a chromosome. A solution of purified histone, however, is digested by crystalline pepsin and so also is histone while it is embedded in isolated chromosomes (Daly, Mirsky and Ris, in the press). Pepsin, therefore, cannot be used to distinguish between the histone and non-histone protein fractions of chromosomes.

In experiments with isolated chromosomes it can be shown that histone is not an essential component of their fibrous structure, for under certain conditions all the histone can be removed without affecting the microscopic appearance of the chromosomes (Mirsky and Ris, in the press). When thymus chromosomes are suspended in neutral M NaCl all their histone is removed but also all their DNA. As the pH of the M NaCl is decreased, all the histone continues to be removed but not all of the DNA is extracted. At pH 2.8 much DNA is still extracted. If the experiment is done not with thymus chromosomes, but with those containing a much higher proportion of residual protein—with chromosomes isolated from liver, kidney or pancreas—at pH 2.8 all the histone and very little of the DNA is extracted. And under these conditions the microscopic appearance of the chromosomes remains unchanged.

From such histone-free chromosomes DNA can be removed by
the action of deoxyribonuclease and at the same time the microscopic appearance of the chromosomes is drastically changed. If a crystalline, protease-free preparation of this enzyme (kindly provided by Dr. Kunitz) is used, fragmented DNA and nothing else passes into solution. In the insoluble protein residue there is no histone and no more than traces of DNA. When the residue is examined microscopically no chromosomes can be seen. What can be seen are the minute coiled threads of residual protein. They are not stained by basic dyes. The absence of histone or other protein in solution after the action of deoxyribonuclease, and the absence of histone in the protein residue are known by treating both the solution and the residue with a mercuric sulfate-sulfuric acid mixture and subsequently with nitrite (the Milon reaction). After such treatment, histones form a red solution and other proteins form red precipitates. In the solution after the action of deoxyribonuclease neither dissolved nor insoluble red pigment appeared (neither histone nor other protein) and in the residue a red precipitate but no soluble pigment appeared (non-histone protein only). Further evidence that nothing but DNA is removed by deoxyribonuclease is that the mass of the residue is equal to the mass of the histone-free chromosomes minus their DNA.

It may be concluded from the experiments that have just been described that the microscopic appearance of chromosomes is due to an association of DNA with residual protein. DNA by itself is soluble, and residual protein itself consists of minute threads distinctly different from chromosomes. The fundamental thread of a chromosome is provided by residual protein, but the configuration observed in the chromosome depends upon the association of this protein with DNA.

In the slightly acid medium in which histone-free chromosomes are prepared the phosphoric acid groups of DNA are combined with residual protein, as can be shown by experiments in which the number of free acid groups is determined by the quantity of crystal violet bound. This is what would be expected, for the pH of the medium is on the acid side of the isoelectric point of most proteins. When a suspension of such chromosomes is neutralized, DNA and residual protein still remain combined, and at least part of this union is through the phosphoric acid groups of the DNA. In a chromosome
there are, therefore, two DNA-containing nucleoproteins, in one of which DNA is combined with histone and in the other with residual protein. Just how the phosphoric acid groups are distributed between the two protein fractions probably depends upon the relative amounts of the two fractions, the pH of the medium and other circumstances. When histone-free chromosomes are in a neutral medium they are swollen and ill-formed, probably because at neutrality the residual protein is unable to combine with all the acid groups of DNA.

The relative amounts of DNA and residual protein vary considerably in the isolated chromosomes that have been studied. Since it is known that the quantity of DNA per nucleus tends to remain constant, it follows that the quantity of residual protein present is highly variable. If the quantity of DNA is considered to be constant; there may be four times as much residual protein in the chromosomes of a liver nucleus as in those of a thymus nucleus. Liver cells also contain far more cytoplasm than do thymus lymphocytes. In Table 3 are given

<table>
<thead>
<tr>
<th>CHROMOSOMES OF:</th>
<th>DNA per cent</th>
<th>RESIDUAL PROTEIN per cent</th>
<th>PART OF TOTAL CELL MASS FORMED BY NUCLEI per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf thymus</td>
<td>39</td>
<td>8.5</td>
<td>61</td>
</tr>
<tr>
<td>Calf liver</td>
<td>26</td>
<td>39</td>
<td>19</td>
</tr>
<tr>
<td>Calf kidney</td>
<td>28</td>
<td>33</td>
<td>20</td>
</tr>
<tr>
<td>Beef pancreas</td>
<td>28</td>
<td>29</td>
<td>9</td>
</tr>
</tbody>
</table>

the compositions of some isolated chromosomes and the part of the total cell mass formed by the nuclei in the tissues from which the chromosomes were isolated. (The procedure for determining the nuclear mass of these tissues will be described later in this paper.) It can be seen that in a cell there is a correlation between quantity of residual protein in the chromosomes and quantity of cytoplasm. An extreme instance of the correlation is offered by the amphibian oöcyte in which the lamp-brush chromosomes appear to contain an exceedingly large quantity of residual protein and in which a great bulk of cytoplasm is present. If the opposite extreme is considered, chromosomes in which exceedingly little residual protein is present,
Some Chemical Aspects of the Cell Nucleus

It seems that there must be a minimum amount of this material in a chromosome, an amount required for the structural integrity of the chromosome. A minimal quantity of residual protein may also be required for the genetic integrity of a chromosome.

Three components of chromosomes have been considered in this work: DNA, the histone protein fraction, and the residual protein fraction. Of the three, DNA alone has been found to be constant in different cells of the same organism. The histone fraction can undergo drastic change, for histone may be replaced by protamine. The quantity of residual protein varies considerably. Whether this is merely a quantitative change is not known. The amino acid composition of the relatively scanty residual protein of the thymus is rather similar to that of the relatively abundant residual protein of liver, kidney and pancreas. This, however, does not necessarily mean that the various residual proteins are qualitatively alike. Attempts to study possible differences in the various residual proteins by immunological methods have failed because these proteins are not antigenic. A possible reason for the non-antigenicity is that the residual protein fraction may be a mixture of such an exceedingly large number of configurations, that not enough of any one is present to be antigenic.

Nuclear Composition

Investigation of nuclear composition is to a considerable extent dependent upon the availability of satisfactory preparations of isolated nuclei. For certain limited purposes, such as determination of the quantity of DNA in the nucleus, preparations made with aqueous media, with citric acid for example, are of great value. Such preparations are, however, of little value for study of the enzymatic composition of nuclei because enzymes may be extracted while the nuclei are being isolated. It should also be recognized that in this process enzymes present in the cytoplasm may become attached to the nucleus. These criticisms apply, of course, to chromosomes isolated in aqueous media. In this case it may be said that the substances removed were only loosely attached to the chromosomes, and much may be learned about chromosomes even if such losses have occurred.
In the procedure developed by Behrens for isolation of nuclei organic solvents are used in which proteins are insoluble. These solvents, however, denature some proteins, so that certain enzymes are inactivated. Some lipids are extracted by the solvents used and not only are they lost, but they may carry with them other substances. The Behrens' procedure, along with its great advantages, obviously has limitations of its own. It has been used but little; one reason being that isolation of nuclei by this method is far more laborious than by others.

In the isolation of nuclei with non-aqueous solvents the material to be used is first dried while frozen and then disintegrated mechanically, cell walls being fragmented far more readily than are nuclei. The disintegrated material is suspended in mixtures of benzene and carbon tetrachloride of varying specific gravity fractionated by centrifugation. In some solvent mixtures the nuclei sediment, in others they float; gradually the nuclei are separated from cell debris. In a satisfactory preparation careful cytological inspection, with staining and counter-staining, shows either no non-nuclear material or no more than traces of it. Evidence that the nuclei isolated are clean is given in some cases by determinations of enzymatic activity. Nuclei isolated from kidneys of beef and fowl are free of certain enzymes which are highly active in the whole tissue and the activity of which remain even after the tissues have been treated just as the nuclei are in the course of isolation. (Much of the information on nuclear composition is taken from unpublished work by Allfrey, Mirsky and Stern.)

When nuclei isolated in non-aqueous medium are compared with those prepared in saline or citric acid it can be seen that much protein material is removed from nuclei by saline and citric acid. Loss of nuclear substance in saline is shown most clearly when nuclei of erythrocytes are isolated. Nuclei of turtle erythrocytes isolated by the use of saline and saponin are colorless, but those isolated with non-aqueous solvents are deeply pigmented, and about 30 per cent of their dry weight consists of hemoglobin. When nuclei isolated by non-aqueous solvents are treated with saline much material is extracted; liver nuclei, for example, lose some 60 per cent of their weight. Much protein material is also extracted with citric acid. Nuclei iso-
lated in non-aqueous solvents have a higher protein content than do those isolated with citric acid, but when the former are subsequently treated with citric acid their composition closely approximates that of nuclei isolated with citric acid. Loss of nuclear material when isolation occurs in citric acid or saline was clearly recognized by Pollister and Leuchtenberger (1949). They attempted to measure the relative amounts of DNA and protein in fixed tissue sections by microphotometric procedures. The protein: DNA ratios for thymus and liver obtained by them are very much higher than those found by direct chemical determinations in nuclei isolated with non-aqueous fluids. Photometric procedures without adequate standards are of little quantitative value.

**THE NUCLEUS AND CELL-DIFFERENTIATION**

The enzymatic composition of the nucleus can be studied only with reference to the enzymatic composition of the whole cell. This means that the differentiation of the cell must be considered, for it is well recognized that cells are differentiated in their chemical constitution as well as in their morphological characteristics. A study of the enzymatic composition of the cell nucleus therefore becomes also a study of the relation of the nucleus to cell differentiation.

It is known from the work of experimental embryologists that differentiation has its origin in the cytoplasm. As development proceeds the influence of the nucleus is manifested. How this occurs is one of the most discussed problems of biology today. It is usually supposed that nuclear composition is fairly constant and that the action of constant nuclear factors on a variable cytoplasmic substratum results in differentiation. Many of the prevalent views go back to those expressed by Goldschmidt in 1927. Goldschmidt (1927) considered that each gene is ready for action, and that “activation of the gene” occurs when the proper substratum in the cytoplasm has been provided. More recently there has been added to this scheme the assumption of plasmagenes, and excellent critical discussions have come from Wright (1945), Sonneborn (1950), Mather (1948), and Spiegelman (1948). In Mather’s discussion there is the statement, of special inter-
est to those engaged in a study of nuclear composition, that "there is
this no evidence that differentiation is characteristically accompanied
by alteration in the nucleus."

A different approach to the role of the nucleus in differentiation
was suggested by T. H. Morgan in 1934, when he said, "The initial
differences in the protoplasmic regions may be supposed to affect the
activity of the genes." Such "variable gene activity," to quote Sonne-
born, has been mentioned as a possibility by Kimball (1947) and
has been considered with favor by Brachet (1949). There are well-
known variations in nuclear constitution which may perhaps be asso-
ciated with variable gene activity. There are, for example, conspicuous
variations in sizes of nucleoli; and even more impressive are the vari-
tions in nuclear size, from the enormous, hardly Feulgen-positive
amphibian oöcyte nucleus to the relatively small, intensely Feulgen-
positive nucleus of the amphibian erythrocyte. Such nuclei obviously
have great differences in chemical composition. These variations in
nuclear composition are surely associated with differences in nuclear
activity, and probably with differences in overall gene activity, but it
is not known that they are associated with variable gene activity in
the sense that different sets of genes come into activity at different
times; and it was in this sense that Morgan supposed that variable
gene activity could account for differentiation.

A study of the chemical composition of nuclei isolated in a non-
aqueous medium from a number of different cell types shows that at
least in some cases the nucleus is differentiated. In these instances
nuclear differences of the same order are found as those known in the
cytoplasms of various cell types. In other cells such nuclear differ-
ences are not found, although cytoplasmic differentiation is marked.

Nuclei of liver and erythrocytes are examples of nuclear differen-
tiation. Considering first the liver, one of the outstanding functions
of this organ in mammals is the formation of urea, and in this process
the enzyme arginase plays an important part. Arginase activity of
mammalian liver is exceedingly high while that of other organs, such
as thymus, pancreas and the heart, is negligibly small. The same
differences are found in the nuclei isolated from these organs. Liver
nuclei show intense arginase activity, whereas in nuclei of the other
organs arginase activity is insignificant. Catalase is another enzyme the activity of which in the liver is high whereas the activity in thymus, pancreas and heart is hardly detectable; and in the nuclei of these tissues it is only in those of the liver that considerable activity is found. There would be no difficulty identifying the liver among other mammalian organs by means of arginase and catalase activities. The same may be said of liver nuclei; if unlabelled samples of nuclei from mammalian tissues were examined with respect to their enzymatic activities, nuclei of the liver could be identified without any difficulty.

The erythrocyte is an example of an exceedingly high degree of differentiation, in which one kind of protein molecule, hemoglobin, forms well over one-half of the dry weight of the cell. It is a striking fact that the erythrocyte nuclei of the fowl, goose and turtle are deeply pigmented and have a high percentage of hemoglobin, about one-half that found in the cytoplasm. No hemoglobin is found in the nuclei of other tissues of the fowl. In the erythrocyte nucleus the non-heme iron forms a much larger fraction of the total iron than it does in the cytoplasm. It may be noted that the iron content varies considerably in nuclei of different tissues.

In differentiated nuclei such as those of hepatic cells and erythrocytes some of the main cytoplasmic components are in the nuclei. These substances form the immediate environment of the chromosomes and may even be loosely attached to them. It would be very unlikely indeed if there were no interaction between chromosomes and these cytoplasmic components enclosed within the nuclei. Some of these cytoplasmic components are enzymes and it may be supposed that their main function in the nucleus, as in the cytoplasm, is to catalyze certain specific reactions which are part of the metabolic activities of the nucleus. This point of view encounters a difficulty when the erythrocyte is considered, for hemoglobin is not an enzyme and cannot be supposed to participate in nuclear metabolism simply as an enzyme. Presence of hemoglobin in the erythrocyte nucleus probably has fundamentally the same significance as has the presence of other cytoplasmic components in other nuclei, and among such substances should be included arginase of the liver nucleus, even though in the cytoplasm this functions as an enzyme. We would sug-
suggest that the significance of the presence of these cytoplasmic components within nuclei is that they there interact directly with chromosomes.

There are, of course, in nuclei certain enzymes whose function is simply enzymatic. An example of such an enzyme is desoxyribodepolymerase. The substrate for this enzyme is within the nucleus and it has recently been reported that in certain cells this enzyme is confined to the nucleus.

Nuclear composition of the kidney does not reflect cytoplasmic composition to anything like the same extent as in the liver and erythrocyte. Most of the enzymes which were studied in liver are also present in kidney, but on the whole, it may be said that these enzymes are not present in the kidney nuclei. There is, for example, four times as much uricase in calf kidney as in calf liver and yet none at all is detectable in kidney nuclei. Both nucleus and cytoplasm of calf and horse liver are rich in arginase; neither nucleus nor cytoplasm of fowl liver contains arginase; and yet in fowl kidney, whereas the cytoplasm contains much arginase, practically none is found in the nuclei. Both liver and kidney contain much catalase but only in the nuclei of the liver is catalase found. Acid and alkaline phosphatase activity are both higher in calf kidney than in calf liver; some liver nuclei show moderate activity but kidney nuclei practically none.

Since kidney nuclei do not contain the components characteristic of kidney cytoplasm, direct interaction between cytoplasmic components and chromosomes does not apparently occur in kidney nuclei. There would seem to be a fundamental contrast between such cells as those of the kidney, on the one hand, and liver cells and erythrocytes on the other.

INTERACTION OF CYTOPLASM AND CHROMOSOMES

The direct interaction in the nucleus between cytoplasmic components and chromosomes is possibly an important factor in all differentiation. The impetus for differentiation arises in the cytoplasm, although the nucleus ultimately plays an important role in the
process. This could happen if cytoplasmic components were to penetrate into the nucleus, there interact with the chromosomes and stimulate them to specific activities which would finally have their effects in the cytoplasm. Cytoplasmic differentiation would accordingly induce nuclear differentiation which would in turn promote the change already under way in the cytoplasm. Cytoplasmic differentiation would thus have the characteristics of an autocatalytic process, which it does in fact have.

It can hardly be supposed that the behavior of chromosomes is not influenced by their immediate intranuclear environment. The environment in which chromosomes function is not constant. The intranuclear environment may be changed in a highly specific way under the influence of the surrounding cytoplasm, and thereby indirectly under the influence of other cells and body fluids, and ultimately changes may have their origin in the environment outside the organism. An example of a change in intranuclear environment produced by an alteration in food supply of an animal is the decline that has been observed in the arginase activity of hepatic nuclei in a fasting horse. Under these conditions it is known that the DNA content of these nuclei remains unchanged, and yet the functioning of the chromosomes is in all probability affected by such specific changes within the nucleus as those that have just been mentioned. This point of view differs from the conception of the nucleus in which interaction between nucleus and cytoplasm, apart from the movement of nutrient metabolites into the nucleus, takes place only when "gene products" come out into the cytoplasm. There is now evidence that interaction between nuclear and cytoplasmic components may also occur within the nucleus. "Gene products" may continue to influence the cytoplasm after the genes that produced them have left the cell. Examples of such lag effects in many different organisms are given by Mather (1948). The length of this lag ranges from a time shorter than one cell generation to a time longer than a whole life cycle. This phenomenon should be considered when such different nuclei as those of liver cells and erythrocytes are compared with those of kidney cells. If presence of cytoplasmic components within a nucleus is taken as evi-
dence of interactions between the components and chromosomes, then it may be that in those cells (of the kidney, for example) with little interaction the lag effects of nuclear genes are pronounced. In such cells gene products may have ceased coming from the nucleus at some period in development—at a time when perhaps there still was interaction between cytoplasmic components and chromosomes. At the later stage, when interaction is not apparent, other types of nuclear activity, such as those of the nucleolus and heterochromatin, will continue.

When the influence of the nucleus on cytoplasm is considered, a question that arises is whether such cytoplasmic components as hemoglobin and arginase are the actual products of nuclear activity or whether they are synthesized in the cytoplasm, partly under the influence of nuclear activity. The most direct evidence bearing on this question comes from studies on the synthesis of hemoglobin. Hemoglobin synthesis is now known from studies on sickle cell anemia to be genetically determined (Pauling et al., 1949; Neel, 1949). It is not this work, however, that has a direct bearing on the role of the nucleus in hemoglobin synthesis. Experiments on the incorporation of $^{15}N$ into hemoglobin show that synthesis of hemoglobin can take place in the mammalian reticulocyte, but not in the mature, non-nucleated erythrocyte (London et al., 1950). The reticulocyte is the erythrocyte immediately after extrusion of its nucleus. If hemoglobin can be synthesized in the reticulocyte, this would seem to show that synthesis occurs in the cytoplasm. Nuclear extrusion, however, is not a fully understood process. Feulgen-positive material is extruded but it is possible that other nuclear material remains in the reticulocyte and that this material may take part in hemoglobin synthesis. Perhaps some definite answer concerning the site of hemoglobin synthesis could be had by carrying out the experiments on the incorporation of $^{15}N$ with nucleated erythrocytes. It would then be possible to separate nucleus from cytoplasm and examine the $^{15}N$ content of the hemoglobin of both fractions to determine in which fraction synthesis had occurred. Such experiments are now being done in our laboratory.

In addition to the experiments concerning hemoglobin synthesis,
there is other evidence that such cytoplasmic components are not synthesized in the nucleus. The presence of arginase, for example, in the calf liver nucleus may at first appear to be an indication that it is synthesized there, and then passes out into the cytoplasm. Arginase, however, is also present in the cytoplasm of the fowl kidney, but in the nuclei of this tissue no arginase is found.

In the different nuclei of an organism there is a constant quantity of DNA for each set of chromosomes. Other nuclear components of a definitely variable character have been recognized. One of these is the basic protein fraction of the chromosomes which in somatic cells is of the histone type and which in the sperm is protamine, an entirely different type of basic protein. Variable components are also those cytoplasmic substances which are represented in nuclei. They are variable in two senses: they are present in the nuclei of some types of cell and not in others; and in a given nucleus the quantity present may vary considerably dependent upon the condition of the organism as a whole. It is supposed that there is interaction between these components and the chromosomes. Finally, there is the residual protein fraction of the chromosomes which is variable in the sense that the quantity of it varies in different nuclei. It is not known whether it varies qualitatively. Both constant and variable nuclear components are important for an organism, the former insuring its genetic continuity and the latter providing for its adaptability.

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Note added to page proof. In the foregoing chapter experiments with isotopes were described which indicated a far greater biochemical stability within the living cell for desoxyribonucleic acid than for ribonucleic acid. Recent experiments show that this idea must be revised. It has been shown by G. A. LePage and Charles Heidelberger (J. Biol. Chem., Vol. 188, p. 593, 1951) and by P. Reichard and B. Estborn (J. Biol. Chem., Vol. 188, p. 839, 1951) that the incorporation of isotopic material into DNA happens far more readily than had previously been supposed if other metabolic pathways are investigated.
CYTOCHEMICAL MEASUREMENTS IN
THE STUDY OF THE GENE

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THE step from the hypothesis that the nucleus is the seat of the
hereditary factors to the precise statement that the genes occupy
definite loci in the chromosomes, required the combined efforts of
two disciplines, cytology and genetics. Separately, either the breeding
technique or the cytological demonstration of chromosomal differ-
cences gave a picture that was ambiguous in an area vital to the de-
development of the gene theory. Their combination, at a time when
each technique was passing through a stage of rapid improvement,
proved to be mutually stimulating: the mutant characters were more
and more precisely localized in breeding experiments, cytological
techniques were refined to permit more accurate analysis of chromo-
some detail. The pachytene maps of maize, the salivary gland chromo-
some analysis of Drosophila, are the monuments to this effort.

In the discussion of the chemistry of the gene, also, a similar step
must be made to the actual localization of the effect. The macro-
chemical studies of chromosomes, or parts of chromosomes, take us
only part of the way. To go farther, the chemical analysis of the loci
in the chromosomes must be correlated with the changes that are
observed by genetic means.
The point of view that we take and shall proceed to discuss, is that the combination of the newer methods of studying the chemistry of cell details with the techniques of genetics, does in fact provide the means for such an attack. The examples we will use are taken from work that we carried out together, beginning in 1937 when Dr. Schultz came to Stockholm with the intention of attempting such an approach.

METHODS

We consider first the prerequisite cytochemical methods. It is clear that the central problem in this approach is the accurate analysis of objects of small dimensions. The measurement of the absorption of light by the structures of the cell could obviously provide a method whereby the composition of these structures and their metabolic changes could be studied (Fig. 1). But if spectrophotometric methods of analysis were to be used, the main question of theory...
was to determine whether measurements could be made on these smallest objects. And indeed it turned out, with the investigation of the optics of the situation, that trustworthy measurements of nucleic acids and proteins could be made of objects three times the dimensions of the wave length of the light by which they were measured (Caspersson, 1936; general review, 1950).

It is now well known that the region of the spectrum that is of most interest to students of chromosomes is the middle ultra-violet, since it is here that the nucleic acids and the proteins have their absorption bands. The development of ultra-violet microspectrophotometry made it possible to carry out measurements of the absorption spectra of parts of the cell, since the lenses designed about 1900 by Köhler and v. Rohr for ultra-violet microscopy also fulfilled the optical requirements for spectrophotometric measurements (Caspersson, loc. cit.). Since World War II, considerable advance in the design of achromatic reflecting lenses has been made in England and in this country (Burch, 1943; Grey, 1950; Seeds and Wilkins, 1949). As yet, however, the refracting lenses are the only ones that meet the requirements of the genetic material for measurements on very small objects.

The kind of measurement necessary for the study of fine structure in cells is not easy. There are a number of pitfalls, some of which can be avoided by the design of the spectrophotometer itself (Fig. 2), but the majority are due to the conditions set by the material to be measured.

The problem of the loss of light by scattering in the object, which varies with the type of fixation, is a serious one. The shape of the absorption curve can be changed completely by the superposition upon the true absorption of the object, of an additional light loss due to scattering. An arbitrary correction can be made for this, or it is possible, as is being done in Stockholm, to construct an apparatus for the direct measurement of scatter. The errors due to lack of homogeneity are also considerable. Where a photographic technique is used they can be avoided completely, since the measurements are made directly on the photograph of the area to be measured. The methods in which the image is projected on a photoelectric cell impose severer restrictions; under these circumstances the area
Fig. 2. Diagram of “universal microspectrophotometer” in use at the Institute for Cell Research (Stockholm). The instruments are so designed as to permit the use of the optimal range of illumination and sensitivity for the particular material being used. A, instrument panel; B, automatic recording panel; C, control panel; D, connections from panels to instruments; E, cooling tubes and housing for mercury lamp (F); G, monochromator; H, prism; I, movable 90° quartz prism; K, beam splitter (transmitted beam goes through microscope to prism P, reflected beam for comparison through prism Q); L, prism; M, condenser; N, object; O, objective and ocular; P, prism for sector and photocell taking beam from microscope; Q, prism for sector and photocell taking beam from lamp; R, rotating sectors; S, photocell array, on rotating table, allowing selection of suitable unit; T, Kohler’s rotating spark-gap arrangement, for high intensity illumination, with apparatus for photographic method; U, rotating sector to reduce intensity as required; V, Backstrom filter; X, microscope; Y, camera.

measured must be restricted to a minimum (Fig. 3). This has led, in recent years, to the development of scanning techniques, in which the absorption of many individual points in the cell is recorded rapidly, so that the total absorbing material can be calculated accurately. There are a number of other complications in the cytochemical work if its aim is quantitative; this is not the place for detail, however. Perhaps the factor of dichroism, or the effect of molecular orien-
tation on the amount of light absorbed, should be mentioned, in connection with the recent discussion by Commoner (1949). The measurement of dichroism, particularly for the nucleic acids in the ultra-violet region, is a very good tool for the study of molecular orientation as was pointed out ten years ago (Caspesson, 1940b). Work of this kind is going on at the Cell Research Institute in Stockholm. In average biological specimens it introduces an error of the order of magnitude some tenths of a per cent in routine absorption measurements and that in only very rare cases. Commoner's strange
overestimation of the effect is conditioned by his using an arbitrarily chosen value of the dichroism coefficients of the nucleic acids without any experimental foundation, instead of using the experimental data from experiments available in the ten-year-old publication just referred to.

It may seem odd to begin the discussion of the uses of a method for gene study by pointing out its difficulties. Yet the point to be emphasized is that the chief value of optical methods is the measurement of quantities. And unless the sources of error are clearly understood, the numbers transcribed from the measuring scale will only serve as sources of confusion.

With the optical methods on a sound basis, the potentialities of cytochemical measurements can be extended by the use of other cytochemical procedures: enzymatic digestion, staining procedures, specific reactions for individual substances, and so on. An important step forward, not yet developed to the point where it can be applied to the chromosomes, is the measurement of the mass of cell structures by their x-ray absorption, a method developed by Engstrom and Lindstrom (1950). Its goal is to carry out determinations of mass and elementary composition on cell structures.

These in outline are the methods of spectrophotometric analysis that can be applied to the study of cell structures. It is clear that they can be manipulated, according to the biological material, to study a variety of problems in cell biology. They are particularly advantageous in the study of the nucleic acids and the proteins (Fig. 4), the substances of major interest at present in the study of gene reproduction and gene function. And here, in the application of these methods to the actual problems, the selection of the biological material comes to the fore.

Two approaches have been useful. The quantitative study of the basic phenomena of chromosome behavior—for example, measurements of transformations of substances in mitosis—is a necessary basis. For the study of the gene, a second approach must be added, involving the use of genetic material, of mutants most advantageously studied for special types of problem, of rearrangements of chromo-
Let us first consider the problem of mitosis. Because of the technical difficulties we still have only a partial description of mitosis in terms of the quantities of nucleic acids and proteins at the various stages facilitating definite types of measurement and so on. We shall now proceed to examine the beginnings that have been made in these fields.
The early measurements were made on the meiotic stages of the grasshopper testis (Caspersson, 1939) and the change in nucleic acid content was measured by the change in absorption spectrum. Certain more recent efforts suffer from the drawback of measurements of inhomogeneous objects by an inaccurate technique. The early measurements indicated that the rise in quantity of nucleic acid came during the earliest stages of the meiotic prophase. As the pro-phases advanced, the nucleic acid remained constant but the ratio of nucleic acid to protein in the nucleus increased, indicating a decrease in the protein content as meiosis proceeded. These studies can still not be regarded as final: reexamination of these stages, with recent improvements in techniques is now under way. The problem is the relation of nucleic acid synthesis to the time of chromosome reproduction, which gives some evidence of the stage of the cycle at which gene reproduction occurs. On the present showing, this is to be placed during the interphases.

The increase in desoxyribosenucleic acid in the nuclei during normal mitosis is only one of the situations in which the process of normal gene reproduction may be studied. The growth of cells without mitosis, correlated as it is in many cases with the reproduction of the chromosomes within the nuclear membrane (the process of endomitosis) gives another opportunity, of which full use has not yet been made. The growth of the salivary glands in Drosophila has become a classical example of this kind of chromosome growth. Here the evidence shows that the dimensions of the chromosomes increase with the size of the cell. This process of growth in the polytene chromosomes was correlated, in measurements by optical methods, with an increase in the nucleic acids (Schultz and Caspersson, 1939), just as the more recent macrochemical analyses have shown.

The usefulness of the genetic material for the analysis of the process of gene reproduction becomes apparent at this point. What is required is a situation in which the products of reproduction can be distinguished from each other, and their compositions compared. Under ordinary circumstances this is scarcely possible. But as Demerec pointed out many years ago, there are cases among the so-called
mutable genes, where the two products of a division differ: one of the daughter cells shows the mutant character, the other is of the original type. If one had representatives of such a process magnified in the giant chromosomes, it might be possible to make measurements of significance for the discussion of the relation of the nucleic acids to the genes. In fact, such a possibility was offered by the class of variegational rearrangements in Drosophila discovered by Muller in his x-ray experiments.

CHRYSOSOMAL REARRANGEMENTS

The variegational rearrangements became of interest to the cytochemical analysis in two ways. In the first place, it was discovered that they were associated with rearrangements of regions of the chromosome (the now notorious heterochromatic regions) distinguished by their high content of nucleic acid during the intermitotic stages of the chromosomes (Schultz, 1936, 1939, 1947). Then it became evident on closer study that there was a correlation between the genetic effects and cytologically visible changes in the structure of the chromosomes, in precisely the regions where the genetic changes were located (Schultz, 1936, 1939; Prokofyeva, 1939, 1947).

The regions in which these changes occur appear to assume the characteristics of the heterochromatic regions. The difference can be interpreted as a difference in the characteristics of the regions between the dense bands, with the high nucleic acid content. Even though the original cell division had taken place in the embryo, a quantitative analysis of the changes in the bands might make it possible to understand what really was going on.

Such an analysis was undertaken, for one especially favorable case, which can equally well serve as an example of the methodological requirements for a result to be reached (Caspersson and Schultz, 1938, Schultz and Caspersson, 1939). These should be emphasized, since the work of Cole and Sutton, undertaken to test the conclusion of our measurements, confused the issue by disregarding these requirements. In this rearrangement, it was possible to compare the normal and the rearranged chromosome on the same microscopic
FIG. 5. Diagram of region of X chromosome, from an individual heterozygous for a rearrangement of the chromosomes X and IV in Drosophila melanogaster. The numbered lines show the path traversed by the microphotometer measurements. Lines 1-9 are over the rearranged chromosome, 9-16 the normal homologue with which it has synapsed. The letters correspond to the bands of the chromosome.

FIG. 6. Microphotometer tracings of lines 2-5 on the chromosome diagrammed in Figure 5. The wedge curve gives the calibration in terms of the transmission of a wedge exposed to a standard illumination, and for which a calibration in terms of ultra-violet light absorbed has been made. Each of the bumps on the tracing corresponds to absorption by the band traversed, and the area beneath is used to give the total extinction.
Fig. 7. Comparison of total extinction in band 3F on Figure 5. The extinction along each measuring line is summed; then those of the area across the band are added, to give the total extinction for the normal and rearranged homologue.

field, in such a way that both the optical and biological conditions were comparable. Figures 5-7 show the procedure, from the initial photograph to the final data of extinction. They also show that the analysis is time consuming, and that a single set of measurements at one place on a band, such as those that Cole and Sutton made, is not sufficient. It is the total amount of substance that is important.

The quantitative results put the cytological analysis on a new basis and added the important point that the closer to the zone of rear-
rangement, the greater the difference between normal and rearranged chromosome. The same type of relation had already appeared in the analysis of the patterns of the genetic changes in the variegated individuals. Thus by the cytochemical measurements, the opportunity is presented to make a detailed study of the mechanism of this instance of genetic position effects. In order to do this, a closer study of the details of the chromosome structure is required. At the time these studies were carried out, the methods were not sufficiently refined to permit such a study. It may be instructive to see, again from the point of view of method, the type of difference within the structures of the normal salivary gland chromosome which can be detected, by the optical methods.

In Figures 8 and 9 the absorption spectra of characteristic parts of the giant chromosomes are shown (Caspersson, 1940a). The bands

![Figure 8](image)

**Fig. 8.** Components of the absorption curves of a chromosome. 1, nucleic acid; 2A, tryptophane; 2B, tyrosine; 2C, non-specific absorption; 3, light loss due to scattering.
Fig. 9. Absorption spectra of different parts of a salivary gland nucleus in *Drosophila melanogaster*. 1, heterochromatic regions of chromocenter; 2, region of chromosome close to chromocenter; 3 and 4, euchromatic bands; 5, nucleolus; 6, interband space.

show the characteristic curve given by a nucleoprotein, the spaces between have a dominance of the protein absorption band. In heterochromatic regions, a characteristically different curve is seen. The analysis of the changes in the variegated chromosomes in these terms, and in this detail is essential for the fuller understanding of what is really going on. We are at the beginning of this work.
THE FUNCTION OF HETEROCHROMATIN

The central problem that we approach here extends farther than the individual case cited. It is the problem of the function of these heterochromatic regions, a problem common to both animals and plants. In fact the recent spectacular analysis by McClintock (1950) of the variegation in maize has presented the problem in that material in terms similar in principle to those found useful in the Drosophila discussion. In cytochemical terms, the problem is the role of the heterochromatic regions in the general nucleic acid metabolism of the cell. The basic concept in this approach is the fundamental importance of the nucleic acids in biological synthesis and particularly in the replication of elementary biological structures. The specific form that these problems assume in the analysis of the nature of the variegation process is not yet clear, due especially to just this involvement of the nucleic acids in so many different kinds of biological synthesis. It is outside the scope that has been set for this discussion to enter into details. As an example, however, we may discuss one possibility.

The association of heterochromatic regions with the formation of nucleoli opens the way to a consideration of the process as having to do with changes in the function of the genes. With the large amount of evidence relating the nucleolus to the synthetic processes of the cell, to permit consideration of the nucleolus as a sort of primary product of the genes, it remained only to demonstrate specific effects of the heterochromatic regions on the composition of the nucleoli. This was in fact shown to be the case (Fig. 10), in an exploratory series of measurements some time ago, of material which remains to be fully exploited (Schultz, Caspersson and Aquilonius, 1940). The direct connection of specific composition with specific region remains to be established. The extraordinarily precise detail of nucleolar formation in Chironomus gives striking material for such work. But it is evident that it would be easy to construct a hypothesis of the variegation process on the basis of a change of function induced by a heterochromatic transformation of the translocated genes. One possible mechanism for this, a speculation of
T. Caspersson and Jack Schultz

Fig. 10. Typical absorption curves of nucleoli from different stocks of Drosophila melanogaster. The differences show changes in the proportion of nucleic acid and protein.

Schultz, might be not dissimilar in principle to what is supposed to happen in the transformation process in Pneumococcus.

There is another field of usefulness of cytochemical measurements which should be discussed briefly. This is the study of nucleo-cytoplasmic interrelations in the function of the genes. Here again a beginning was made in the study of heterochromatin effects, of the
Cytochemical Measurements in the Study of the Gene

Y chromosome on the cytoplasm of the oocyte. But with the survey of methods given, it is easy to see that detailed studies of gradients within the cell, such as those made on nerve cells, should lead to a closer understanding of the precise site of the cellular differentiations consequent on gene function.

It has been our effort in this discussion to emphasize the necessity of the integration of genetic and cytochemical techniques for the advance of direct understanding of the nature and function of the gene. By using these techniques separately, progress can of course be made. But each supplies what the other lacks. Our own experience emphasizes this. The cytochemical work at Stockholm, since the period of our collaboration, has been devoted to problems of nuclear function, without the specific help of the genetic material; and the genetic work in the United States has been partly in the classical cytogenetic tradition, partly in that of the chemical genetics; giving necessary information about genes and their properties but always leading to ambiguities when it is asked where in the cell specific processes occur. We are planning the reunion of the two approaches, and would like to conclude with what is almost a tautology, that the problem of the nature of the gene is basically a problem in cytochemistry applied to the proper genetic material.

REFERENCES


COINCIDENTAL with the birth pains which produced the lusty infant called genetics, whose fiftieth birthday is being celebrated this year, there appeared a paper (Landsteiner, 1900) in another field of biology which at that time seemed to bear only a distant relationship, if any at all, to our infant prodigy. This paper was followed the next year by another (Landsteiner, 1901), and these announced to the world that human blood could be classified in three groups. Shortly thereafter, the number of groups was increased to four (von Decastello and Sturii, 1902), the well known groups O, A, B, and AB. In those days, it is unlikely that anyone would have had the temerity to suggest that the consequences of this original finding would turn out to have extremely important ramifications both in medicine and in the study of heredity in humans.

For some years prior to 1900, many research workers in the field of bacteriology had been engaged in an extension of the knowledge that the animal body would react with, or show an immune response to, a wide variety of foreign materials and agents, called “antigens.” This response was manifested, and could be studied experimentally, by virtue of the occurrence or development of mysterious properties or substances in the blood-stream and fluids of the body which would react in one way or another with the antigens, and to which the name “antibodies” was given. These antibodies in many cases had a power and a specificity which was almost fantastic, in that sometimes their presence in the animal body would denote a calamity
instead of success when in contact with the foreign material. A few workers even in 1900 were beginning to be aware of a fact which has since become widely accepted, namely, that the immune reactions which had previously been noted as directed against bacteria were in reality only individual expressions of very general laws regarding the ability of the animal body to react specifically against material foreign to it, that is, to antigenic substances.

Although the genetic implications of the human blood groups were probably not realized at the time of their discovery, one can now say that their discovery was the first example of a fruitful union of the two branches of biology, immunology and genetics. Each of these subdivisions of biology deals with its own kind of specificities. Thus, genetics is concerned with the specificities of gene action, since it is only because of the differential specificities of allelic genes that their presence can be recognized. And, in immunology, the specificity of the reactions between related antigens and their respective antibodies is also extremely precise, although the reasons underlying the specificities are still a matter of debate.

Until relatively recently, it was generally believed that only proteins were antigenic, that is, only proteins would engender the production of antibodies in an animal. Therefore, the finding by Heidelberger and Avery (1923, 1924) that the immunological specificities of the different types of the pneumococcus (Diplococcus pneumoniae) depended upon the makeup of the carbohydrates of the capsule of each type, represented an important milestone in our understanding of the chemical nature of biological specificity. It is now recognized that in order to be antigenic, that is, to produce antibodies, most substances other than proteins need to be attached to proteins, and a great deal of progress has already been made in studying the antigenic specificities of small molecules of known constitution which have been attached to proteins.

For example, Avery and Goebel (1929) found that derivations of two simple monosaccharides—glucose and galactose—which differ only in the spatial configuration of a single carbon atom, exhibited distinct immunological specificity when combined with chemically distinct proteins derived from widely remote biological species, as
serum globulin of the horse and crystalline egg albumin. (These derivations followed the synthesis of p-aminophenol glucosides of glucose and galactose and coupling these to the different proteins.) In contrast, cross-reactivity was noted between the antibodies against the glucosides to p-aminophenol α- and β-glucosides of glucose (Avery, Goebel and Babers, 1932). (The spatial relations of these are different only at the terminal carbon atom in each sugar component.) These and many other results in laboratories in various parts of the world obtained from the use of conjugated antigens allow the conclusion that cross-reactivity of antibodies between closely related substances may often be obtained, but are not always obtained. A critical review of most of the pertinent papers in this field of experimentation is given by Landsteiner (1945). Also, a recent review by Haurowitz (1949) presents many fundamental findings in immunochemistry which bear on problems in biology.

Thus it is to be expected that specificities of some of the genetic characters which are detectable by the techniques of immunology may be protein in nature, but others may owe their specificities to substances simpler than proteins. Just as our concepts of the specificities of proteins rest largely upon findings in immunology, we may well anticipate that immunology will be of assistance in adding to our understanding of biological specificities which depend on substances other than proteins. In fact, the basic question in genetics of how the gene reproduces itself parallels the question in immunology of how the proteins of the serum, particularly the gamma globulins which contain all or nearly all the antibodies, can be changed specifically during immunization and how this specific change can be reproduced for varying periods of time. In each of these phenomena the concept of a template or master molecule is generally the core of the explanations of the duplication of the molecules, whether gene or antibody.

CELLULAR ANTIGENS OF MAN

It is not the intent in this paper to give a detailed account of the genetic and immunological studies on the antigenic substances of
the erythrocytes of humans. Such an account would require much more space than is available. There are other reviews which have dealt adequately with the subject, by Boyd (1939, 1945), Wiener (1943a) and many others. Rather is it proposed to stress certain aspects of this topic which are of interest to those who are not specialists in the field, and to call attention to various lines of investigation which have followed the original discovery of these substances of man.

As stated above, the blood group substances (blood factors, antigenic factors or antigenic substances, cellular antigens or cellular characters) O, A, B and AB represent the first genetic characters which were detected by immunological methods. According to Rous (1917), Landsteiner had suggested shortly after their detection that these substances were inherited, but it was not until some years later that von Dungern and Hirschfeld (1910) produced evidence of their hereditary nature. As is well known, the original proposal of their method of inheritance was that A and B, respectively, were simple dominants in comparison to their absence in the O group, and that the causative genes were located on independent chromosomes. Bernstein (1924) later proposed, from a statistical analysis of the available data, that there were three allelic genes involved in the production of the substances O, A and B. Bernstein's theory is now widely accepted, although certain exceptions have been noted which do not fit in the scheme. These exceptions involve families in which an O child was born to an AB mother, according to Haselhorst and Lauer (1931), and to a parallel finding by Kossovitch (1929). The infrequency of such exceptions might make plausible the hypothesis that a mutation had taken place, or that a non-disjunction had occurred, as was suggested verbally by Dr. Philip Levine when the first exception was reported. Also, instead of considering the O group as a recessive, there is now a growing belief that this is not necessarily the case, but that a reagent for the detection of the O substance is difficult to obtain (Boyd, 1939; Wiener, 1943a). Recently Boorman, Dodd and Gilbey (1948) have reported evidence for a co-dominance of the factors O, A and B.
cells was made by virtue of the fact that the serum of an individual belonging to group O has antibodies in his serum against both A and B, that of an A individual has anti-B, that of a B individual has anti-A, while the serum of an AB individual has neither of these antibodies. One might conclude from the reciprocal relationship of the presence of a cellular antigen and the antibody for the contrasting substance that a single gene has an effect on both antigen and antibody. Other explanations have been proposed, among which one currently in favor is that the antibodies to both A and B substances are normally present in human serum, but that in A, B and AB individuals they are continually being absorbed from the serum by the antigenic substance of the blood cells and other tissues. (See Wiener, 1943a, for further details and references.) Thus the antibody to A would be absorbed in an individual of group A, leaving anti-B in the serum, that to B in an individual with B, leaving anti-A, and both antibodies would be absorbed in an AB individual.

In passing, it should be stated that, as one of the results of the large scale fractionations of human plasma carried on at Harvard University during the war (1941-45), it was possible to separate and concentrate the iso-hemagglutinins anti-A and anti-B. They are composed mainly of gamma and beta globulins, but no chemical or physical difference between them has been noted (Cohn, et al, 1944; Pillemer, et al, 1944). At present, then, their recognized specificities are detectable only by their interaction with particular antigenic substances.

One of the chief points of interest concerning these antigenic substances of the blood cells is that they, or very closely related substances, have been found in nearly all tissues of the body, as would be expected if the causative genes are present in all these tissues. Further, in the presence of a "secretor" gene, they are found in nearly all secretions, such as saliva, tears, urine, semen, gastric juice and milk. The agglutinogen has been demonstrated by Kemp (1930) in the blood cells of a fetus at 37 days of age. Landsteiner and Levine (1926) reported that the blood group substances of man were properties also of the sperm cells. Thus it would seem that the antigenic substance has been demonstrated in most of the cells in which the
causative gene is present. If additional evidence can be obtained regarding antigenic substances shared by the sperm and other body cells or tissues, these findings would substantiate the hypothesis that the cellular antigens may be primary products of their causative genes (Irwin and Cole, 1936a; Haldane, 1938).

It is a general observation in genetics that allelic genes effect only slight differences in their end products. Assuming the correctness of this generality, it would be expected that the antigenic substances O, A and B should have an antigenic similarity, although major emphasis has nearly always been placed upon the differences, not the similarities, of these cellular characters. Evidence which makes probable the belief that there is an antigenic similarity of the A and B substances was first provided by the observations of Hooker and Anderson (1921), later by Landsteiner and Witt (1926), by Boyd and Warshaver (1945) and others. Hooker and Anderson noticed that when certain anti-B sera, produced in rabbits or chickens, were absorbed by O cells, antibodies were left in the serum for A cells as well as for B cells. If it is assumed that the O, A and B antigens are single antigenic substances, rather than that they possess a separate common antigenic component to account for the above observations, such results would indicate that the A and B factors have similarities in their chemical structure.

Another line of evidence that argues for an antigenic similarity of the O, A and B substances is that it has not yet been possible to differentiate these substances chemically (Kabat, 1949). They can be recognized at present only by the technics of immunology, and the structural relationships which determine their activity and specificity are fundamental problems for the future.

It is hardly necessary to call attention to the benefits to the human race which a knowledge of these blood groups has provided. One area of benefit has been in markedly decreasing the accidents accompanying transfusion of blood, thereby allowing the increased use of transfusions. For instance, Rous (1947) cites that in a large general hospital in New York, no more than 50 transfusions per year were carried out in 1913, as compared with nearly 3000 in 1941. It might be well to emphasize at this point that, for purposes of transfusion,
human blood of the same antigenic group is compatible for transfusions, irrespective of the color of skin, of religious and even of political beliefs. Also, in many countries and in the majority of the states of the United States, the heredity of these blood groups, and of certain others since demonstrated, is admitted as evidence by the courts in criminal and civil cases, particularly for exclusion of parentage in disputed cases.

For the first quarter-century these four groups were the only antigenic factors known in man. An early observation of von Dungern and Hirschfeld (1911) revealed two kinds of A blood, now called subgroups A₁ and A₂. It is still not entirely clear whether the factors A₁ and A₂ are qualitatively or only quantitatively different from each other, although the writer believes that the evidence is on the side of a qualitative difference. In either case four causative allelic genes were proposed by Thomsen, Friedenreich and Worsaae (1930)—for O, A₁, A₂ and B respectively—at a single locus, instead of the triple allelic series. Three exceptions to this hypothesis have been noted. One (Dahr and Bussman, 1938) was a case in which the mother was A₃, the father was A₁B, and the child was A₂B, as shown by repeated tests. The second exception was a case (Haselhorst and Lauer, 1931) in which the mother was A₂B and the child was group O. In a third case (Thomsen, Friedenreich and Worsaae, 1930), parents belonging to groups A₂ and O had three children of subgroup A₁. Mutation or non-disjunction, preferably the latter, might be invoked in explanation of the first two cases, but it is highly improbable that the third exception could be attributed to successive and therefore independent mutations from A₂ to A₁. The authors proposed that the A₂ cells of the parent were relatively insensitive to agglutination because of the advanced age of the individual, and might actually be A₁ instead of A₂. There have been other reports of further subdivisions of both the A and B substances, as cited by Wiener (1943), but to date these have been of doubtful validity because of their infrequent occurrence and the uncertainties of the ability to recognize them.

In 1927, Landsteiner and Levine (1927) announced the discovery in human cells of a new pair of contrasting antigens, called M and N. These were detectable only by the use of immune sera produced
in rabbits, as was another antigenic factor called P. The heritability of the M and N substances was adequately explained by the assumption of a single pair of allelic genes, and the substance P appears to be dominant to its absence.

THE RH FACTOR

Another antigenic factor in human blood which has aroused wide interest is the recently discovered Rh substance, or antigenic complex, as it might be termed. In 1940, Landsteiner and Wiener (1940) reported that a new antibody, derived from a rabbit immunized with the erythrocytes of a rhesus monkey, was reactive with the cells of about 85 per cent of the white population of New York. They gave the name Rh (a contraction of rhesus) to this agglutinable property of human cells. However, as Boyd (1943) aptly states, "The technique of testing for the new factor was difficult, the best available sera were weak, and had it not been for a remarkable series of discoveries which followed in the next few months, the Rho factor might have aroused no more interest than its practically stillborn brethren. . . ."

The Rh factor was shown to be involved in previously unexplained complications following transfusions (Wiener and Peters, 1940), but is most widely known for its role as the etiologic agent in the majority of cases of hemolytic disease of the newborn. The proposal was first made by Levine and Stetson (1939) that an antigen in the fetus, foreign to the mother and presumably transmitted by the father, could pass through the placenta and immunize the mother. A subsequent report (Levine and Katz in, 1940) implicated the Rh factor as the foreign antigen, and gave further evidence that the antibodies developed in the mother may pass back through the placenta and affect the red blood cells of the fetus, before or following birth. The finding that hemolytic disease of the newborn, or a record of unexplained stillbirths, could be attributed to an incompatibility of certain cellular antigens between the mother and the fetus marked a highly significant advance in what had previously been a complete puzzle to the medical profession. In the ten years which have elapsed
since the significance of the mother-fetus incompatibility has been
known, the ramifications of this finding have resembled the rapid
growth of a giant mushroom. The benefits to be derived from these
studies are potentially applicable to every nation in whose popula-
tions two or more of the causative genes for these particular cellular
substances are present in heterozygous form. As might well be ex-
pected, however, there are still many unanswered questions about
this biological phenomenon. For example, although the majority of
cases of hemolytic disease of the newborn may be justly ascribed to
an Rh incompatibility between the father and mother, there is no
satisfactory explanation as to why only about one in forty or so of
such potentially dangerous combinations leads to morbidity.

There would of course be considerable advantage if the many
aspects of this phenomenon could be studied experimentally. It
would seem that any species in which the placental tissues would
allow the passage of antibodies, and in which differences in the blood
cells could be demonstrated, would be excellent material for such
studies. For example, it is well known that there is maternal trans-
mission of antibodies to the young in the rabbit, and the cellular
antigens first reported by Levine and Landsteiner (1929), and called
H1 and H2 by Castle and Keeler (1933), provide the individual dif-
ferences of the blood cells. Peculiarly enough, Keeler and Castle
(1934) reported that female rabbits lacking both H1 and H2, and in
whose sera antibodies to both antigens had been induced, could
produce young with either antigen. More pertinent still, if such a
female had been mated to a heterozygous male, from the young with
agglutinogen H1 on their cells the antibodies to H2 were recovered,
and from those with H2 the antibodies to H1 could be obtained.
Parallel findings in rabbits have been reported by Heard, Hinde and
Mynors (1949). These workers, in experiments parallel to those of
Keeler and Castle but perhaps not using the same cellular antigen,
stated that the red blood cells of the fetuses were coated with anti-
body but that the rabbits were clinically normal. No information is
yet available concerning the manner in which the developing fetuses
in either experiment were protected from the antibody antagonistic
to their cells.
The first example of hemolytic disease in an animal other than man was noted in a species in which the placental membranes seemingly are not permeable to antibodies. In this species, the horse, (Bessis, 1947; Coombs, et al, 1948; Bruner, et al, 1948) the antibodies are transmitted to the young through the milk. Parallel observations have been made in dogs (Young, et al, 1949; Young, Ervin and Yuile, 1949) in that the young from immunized mothers may be afflicted with hemolytic disease, and it is probable that the antibody is transmitted through the milk. Hemolytic disease has also been induced in a species in which there is no contact of maternal and fetal membranes. Briles (1948) noted that those chicks, which carried cellular antigens against which antibodies were circulating in the serum of their parent hen, had hemolytic disease. In this species the antibodies were transmitted through the yolk. With more study along this line in these species, and possibly in others, it may confidently be expected that much more information will be obtained in the future concerning various phases of hemolytic disease which at present are baffling.

To return to the blood groups of man, there exist several subgroups, or subtypes, of the Rh complex, and investigations as to their respective specificities occupy the center of interest of many workers at the present writing. There are two schools of thought as to the mode of inheritance of these subgroups, which also involves the terminology to be used in their identification (see Strandskov 1948, 1949, for leading references). One explanation (by Wiener) is that the various subtypes are manifestations of a series of multiple allelic genes; the other (by English workers) is that they are the result of the action of respective genes at three independent but closely linked loci. It is not within the province of this paper to discuss the arguments for and against these two proposals. However, as was pointed out verbally by Professor J. F. Crow, the genetic expectation under either explanation is the same, unless under the linked gene hypothesis the percentage of crossing over is greater than the rate of mutation.

In recent years, there have been several reports of the detection of other blood factors in man. Two of these (Sanger and Race, 1947; Walsh and Montgomery, 1947) give evidence for subdividing the
MN groups; the gene for the additional blood factor called S may be closely linked to those for the M and N substances, or may be an allele of the same genes. The others have in general described the detection of new antigenic factors of human blood cells (Andresen, 1947, 1948; Coombs, Mourant and Race, 1946; Callender, Race and Paykoc, 1945; Cutbush, Mollison and Parkin, 1950; Gilbey, 1947; Grubb, 1948; Hubinot, 1949; Ikin, Mourant and Plaut, 1950; van Loghem and van der Hart, 1950; Mourant, 1946). It is hardly necessary to state here that the criteria of both genetics and immunology will need to be met in studying these newly described blood factors before they can be of general use in further studies of human heredity. This in turn rests upon the ability to reproduce the specific antibodies at will in humans or in experimental animals.

There need be no apology offered for the search for new antigenic substances in man, which might appear to smack of “antigen-chasing.” Fisher (1947) has ably presented the case for the use of the substances already known, as quoted below, and these statements apply to others not so well known or as yet undetected.

(i) They constitute stepping stones in human genetics, by providing markers on several chromosomes and facilitating linkage studies and the genetic mapping of the human germ plasm.

(ii) They are of great medical importance, primarily in making blood transfusion possible, but also in guarding against its possible dangers. The Rhesus factor also has elucidated the nature of haemolytic disease, which has, until now, been a serious cause of infantile and foetal mortality.

(iii) They are of forensic importance in the recognition of individuals, in the recognition of parenthood as in the mistaken interchange of children, and in the recognition of disputed paternity.

(iv) All three factors show important differences of frequency among different human races, and cannot in the future be ignored in ethnographic studies.

Their value in other related studies has also been discussed by both Boyd (1949) and Wiener (1943a).

One attribute of these cellular antigens which makes them well adapted for use in studying their distribution in man and in other species is that they are expressed in the cells quite independently of
the genetic complex in which they are found. Nor does there appear to be any effect of the environment upon them. Since the immunological reactions—between antigen and antibody—are primarily surface reactions, it is highly probable that there are many antigenic substances below the surface of the cells which are not detectable by the usual technics. These statements apply equally well to the cellular substances of species other than man.

HUMAN CELLULAR ANTIGENS IN RELATED SPECIES

Following the studies of many investigators on the differential frequency of the four blood groups O, A, B and AB in various races of mankind, it was natural that a search for them in other species of animals should follow. In fact, many workers more or less blindly sought in other species for the same reciprocal pattern of cellular antigens and antibodies as existed in humans. The literature contains many reports of failure to discover iso-antibodies (iso-agglutinins) by which antigenic factors of the cells in a particular species could be detected.

In 1925, Landsteiner and Miller (1925a, 1925b) extended to bloods of chimpanzees, orangs and a gibbon the limited observations of von Dungern and Hirschfeld (1911) on the relationship of chimpanzee blood to that of man. Chimpanzees were found to belong to human blood groups A and O, the orangs to A, B and AB, and the gibbon to group A. Moreover, the A and B factors as they occurred in each of these species of primates were indistinguishable by antibody-absorption tests from those of human cells, and these substances presumably are identical in these four species. The causative genes for those antigenic factors which are present in the different species may also then be considered to be identical to those in man.

It is of course no more surprising to find that the chimpanzee has only A and O substances than that the bloods of the North American Indians are almost entirely made up of groups O and A. However, the bloods of twelve species of New World monkeys (Platyrhina) and six species of the genus Lemur possess a substance similar to, but not identical with, the B factor of human cells; whereas those
tested of eighteen species of Old World monkeys (Cercopithecidae) seemingly had no substance related to either A or B.

For those species which contain substances only similar to the B of humans, a causative gene, but not necessarily a homologous gene in each species, similar to the gene effecting human B may be proposed. Following the above studies it was shown (Wiener, Candela and Goss, 1942) that the blood cells of the gorilla were not reactive with the reagents for the human A and B substances, but that certain organs and secretions gave evidence of B-like reactions, and the serum contained anti-A agglutinins. This finding brings up a question discussed by Boyd (1949) as to which proteins of the living organism should be considered as most indicative of its relationship to other plants or animals. This question may also apply to any substances which are detectable in the blood cells of one species, and only in the tissues or secretions of another, or vice versa.

As stated, the respective genes for the A and O blood factors in chimpanzees and in humans are probably homologous. However, it appears that a single antigenic factor similar to both M and N of humans may be present in all chimpanzee bloods (Wiener, 1938). If further observations substantiate this finding, it would seem that the most reasonable explanation is that a single gene product in chimpanzee cells is antigenically related to the heterozygote MN of humans. That is, it is similar to, but definitely not identical with, the product of the respective genes for M and N of man. If one would assume that in man the allelic genes for M and N affect substances which are more similar than dissimilar—they are recognized by virtue of the differences—in chimpanzees it is probably a single gene that affects an antigenic character which combines similarities of both M and N. Such variations in the products of alleles would not be at all surprising.

THE CHEMICAL NATURE OF BLOOD GROUP SUBSTANCES

In addition to being present in various species of higher apes and monkeys, substances related to the A, B and O of man have been noted in other species of animals, also in plants. For example, an A-
like substance is present in hog gastric mucosa, in the fourth stomach of the cow, in commercial preparations of peptones, and in swine pepsin; a B-like substance has been found in the bovine stomach, while both A- and B-like substances have been noted in the saliva and stomachs of horses. Following chemical fractionation, principally of hog gastric mucosa, various investigators have obtained extracts which have serological activity related to the A-substance of humans. These preparations have been largely polysaccharide in nature, although even the purest extracts contained traces of amino acids. (For references to specific papers on this subject, see Kabat, 1949). Thus, in the present state of our knowledge, the O, A and B substances appear to be nitrogenous polysaccharides.

Some very interesting results from various laboratories have been obtained in studies on preparations from hog stomachs, as reviewed by Kabat (1949). It has been found that purified products from individual hog stomach linings may possess either A activity alone, or O activity alone. Others have shown both A and O activity, and such preparations might be from animals heterozygous for AO. Of the products from ten individuals, there were seven with A, and three with O activity. But there was no difference among these ten in the yield of nitrogen, glucosamine, reducing sugar, acetyl and in electrophoretic mobility. From each of these ten preparations it was possible to isolate derivatives of L-fucose, D-glucosamine and D-galactose. Although the chemical tests on these extracted preparations did not distinguish between those possessing A and O, respectively, it was possible to differentiate them by immunological procedures, and by special procedures (analyzing for glucosamine the specific precipitates between the A preparation and anti-A serum) to establish that the preparation with A activity definitely was related to human blood group A, and that with O activity was related to human O. That is, the amount of glucosamine in the precipitate between the preparation with A activity and anti-A was much greater than that of the precipitate between a preparation with O activity and anti-A. Similarly, analyses of specific precipitates of blood group A preparations from human saliva have shown that practically all the glucosamine was precipitated by anti-hog A.
It is known (Finland and Curnen, 1935) that type XIV anti-pneumococcal horse serum will agglutinate all human bloods. This antiserum will also precipitate purified blood group A and O substances from individual hog stomachs, as well as purified human A, B and O substances. This is not surprising, since the specific polysaccharide of pneumococcus type XIV contains two of the sugar constituents present in the blood group substances, namely, N-acetyl D-glucosamine and D-galactose.

There were variations noted among the individual preparations of A substances from either hogs or humans in precipitating the type XIV antibody. For example, 500 μg of blood group A substance from hog 10 precipitated only 19 μg N from 0.5 ml. of type XIV antibody, while parallel amounts of comparable preparations from three other hogs precipitated 39, 72 and 72 μg N respectively. In searching for an explanation of this phenomenon, it was noted that heating the blood group substance A (hog) at a low pH (1.5 to 1.8) resulted in a complete loss of group A activity, but that there was a striking increase in the capacity of the preparation to precipitate with type XIV antibody. The same phenomenon was obtained with hog O substance, as well as with both human A and B substances.

Further investigations have shown that the principal effect of heating the group A substance (hog) was that the major proportion of the fucose was split off. The increased activity of the product with type XIV anti-pneumococcus serum, following heating, seemingly was by virtue of exposing the N-acetyl-glucosamine and galactose residues. As Kabat (1949) states, there is evidence that the fucose residues seem to be attached as end groups to the main polysaccharide chain of the blood group substances. Their removal might have provided additional cross-reacting sites on the main chain or have allowed a closer approach of the antibody molecule to the reactive groupings on the main chain, thus increasing the extent of the cross-reaction.

These experiments in immunochemistry provide just a fleeting glimpse of the fundamental basis of the specificity of these antigenic substances, and may be thought of as a first step towards the unraveling of the structural differences in related antigens. Furthermore, we
may justifiably assume that one of the actions of the gene for substance A in man and in hogs is to put fucose as a terminal attachment to D-glucosamine and D-galactose, as distinguished from a possible gene effect in type XIV pneumococcus which is only on the D-glucosamine and D-galactose, not on fucose. In this respect the genes for the A substance in man and hogs would be much alike in their products, but the differences are as yet undetermined.

SPECIES DIFFERENCES

As was stated earlier, much of our knowledge of the specificity of proteins depends upon the findings of immunology, and these findings in large part resulted from comparisons of the proteins of various species. One of the earliest examples of such comparisons was the classical work of Nuttall (1904) who compared the taxonomic relationships of many species of animals, based on morphological similarities, with those deduced from similarities of the serum proteins of these species, and found them to be in close agreement. The differences between the proteins of related species were so universally found as to cause Loeb (1917) to raise the question of whether these species specificities would be found to be gene-determined. He held out little hope that an answer to this question could be obtained experimentally, since the proteins of species which would hybridize were difficult if not impossible to distinguish by immunological technics.

Comparisons of differences between related species in the antigens of the blood cells rather than in those of the serum were first made by Landsteiner and van der Scheer (1924a), using cells of the horse, donkey and mule. These authors (1924b) had proposed that “the peculiarities in specificity manifested by precipitinogens and agglutinogens suggest an essential difference in the chemical structures which determine the specificity of the two kinds of antigens.” It is probable that the red blood cells were not previously used in tests of species relationships because of the assumption that the species specificities of the precipitins and hemagglutinins were of the same order, and that species specificity meant protein specificity. In preceding
pages we have seen that this need not be the case, since certain differences between man and some species of lower monkeys are differences in a cellular substance (B) which is largely polysaccharide.

In order to make a genetic analysis of species specificities, the first requirement naturally is to be able to produce fertile species hybrids between the species under comparison. Fertile species hybrids in animals are less frequently found than in plants, so the hybrids and backcross hybrids in pigeons and doves which had been produced by the late Professor L. J. Cole, and which were made readily available by him for such studies, were ideal experimental material.

At the beginning of the studies in 1930, there were available species hybrids from the matings of male Pearlnecks (Streptopelia chinensis) to the domesticated Ring dove, or Ringneck dove (St. risoria), of males of the domesticated form of the common pigeon (Columba livia) to Ring doves, and from reciprocal matings between an African species of pigeon (C. guinea) and the common pigeon (C. livia). The hybrid males from the cross between Pearlneck and Ring dove had been backcrossed with greatest success to Ring dove, although a few backcross offspring had been obtained from mating the hybrid males to Pearlnecks. The species hybrid females of this cross gave a limited amount of fertility, but no viable offspring were obtained from them in matings to either parental species.

Table 1. Antigenic relationships of the blood cells of Pearlneck, Ring doves and their hybrids.

<table>
<thead>
<tr>
<th>Immune serum</th>
<th>First serum dilution of test</th>
<th>Absorptions by cells of</th>
<th>AGGLUTINATION TITERS WITH CELLS OF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pearlneck</td>
<td>Ring dove</td>
</tr>
<tr>
<td>Pearlneck</td>
<td>1:90</td>
<td>F1</td>
<td>23040</td>
</tr>
<tr>
<td>Pearlneck</td>
<td>1:90</td>
<td>Ring dove</td>
<td>11520</td>
</tr>
<tr>
<td>Ring dove</td>
<td>1:90</td>
<td>Pearlneck</td>
<td>15360</td>
</tr>
<tr>
<td>Ring dove</td>
<td>1:90</td>
<td>F1</td>
<td>0</td>
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<tr>
<td>Ring dove</td>
<td>1:90</td>
<td>F1</td>
<td>0</td>
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<tr>
<td>F1</td>
<td></td>
<td>---</td>
<td>15360</td>
</tr>
<tr>
<td>F2</td>
<td>1:90</td>
<td>Pearlneck</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
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<td>Ring dove</td>
<td>7680</td>
</tr>
<tr>
<td>F3</td>
<td>1:90</td>
<td>Pearlneck and Ring dove</td>
<td>0</td>
</tr>
</tbody>
</table>
It was generally not possible to differentiate the erythrocytes of any two species of pigeons and doves of the same genus by the use of untreated immune sera, for the highest dilution (the titer) of the antiserum— which would agglutinate the cells of the two species would usually be the same. A typical example is given in Table 1 of the means required to differentiate the cells of the Pearlneck, Ring dove and their F₁. [For a description of the technique employed, see papers by Irwin and Cole (1936a) and Irwin, Cole and Gordon (1936)]. Only after absorption of an antiserum against the cells of one species (as Pearlneck) with an excess of the cells of the other (as Ring dove) was a “reagent” obtained which would differentiate the cells of one species from those of the other. Actually this reagent would agglutinate the cells of both Pearlneck and the F₁ at a high titer, but not the cells of Ring dove at the first dilution (1:90) of the mixture of cells and reagent. The antigenic relationships of these three kinds of cells deduced from Table 1 are given diagrammatically in Fig. 1. The proportions of specific and common substance in the diagrams are only approximations.

From the data of Table 1 and from Figure 1 it may be seen that each species possesses one or more antigenic substances peculiar to itself (“species-specific”) as well as those common to the other. Furthermore, the species hybrids contain (a) all the substances
common to the parental species, (b) nearly all, but not quite all, the species-specific substances of both parental species, and, in addition, (c) one or more antigenic components not found in either parental species, the so-called “hybrid substance.” The basis for stating that the species hybrids do not contain quite all the cellular substances specific to either parental species is that the cells of the species hybrid do not by absorption remove all the antibodies for Pearlneck cells from the Pearlneck antiserum, nor for Ring dove cells from Ring dove antiserum. It seems reasonably certain that individual differences among the hybrids do not account for this difference between the hybrids and the parental species, for this difference was evident even in individual hybrids with equivalent antigenic components (Irwin and Cole, 1936a). It is probable that, to produce the hybrid substance, there is an interaction in the hybrids of genes which in both parental species produce species-specific effects. Such an explanation could account for the observation that the species hybrids do not contain all the specific substances of the parental species.

Since backcross offspring were obtained from the mating of species hybrid males to Ring doves it would be expected that, if the species-specific substances were heritable, a separation of those peculiar to Pearlneck would be noted in the first backcross generation. If but one antigenic substance peculiar to Pearlneck were involved, with the causative gene or genes being located on a single chromosome originally derived from Pearlneck, there would be two kinds of offspring in the first backcross generation—those with and those without the antigen. If there were two species-specific substances of Pearlneck, there would be four kinds of offspring expected—those with both substances, those with one or the other, and those with neither. The expectation as to the number of kinds of offspring in the first backcross generation would be $2^*$, in which * equals the number of antigenic components specific to Pearlneck.

Actually, following successive matings to Ring dove of selected backcross offspring, at least nine and probably ten different antigenic components specific to Pearlneck have been obtained in unit form, as is shown diagrammatically in Fig. 2. That is, a backcross bird containing any one of these ten substances in unit form produced, in
a backcross to Ring dove, only two kinds of progeny in approximately equal proportions—those with the cellular character and those without. These have been called ("d" standing for dove) d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-4.d-8, d-11, and d-12. Each of these substances has been shown to be immunologically distinct from each of the others (Irwin, 1939), except that no comparison has been made between d-4.d-8 and d-12. The d-8 substance was not obtained apart from the combination with d-4, and the last bird carrying d-4.d-8 died before d-12 was identified. There is therefore a possibility that d-8 and d-12 are the same character.

![Diagram of antigenic substances specific to Pearlneck and Ring dove](image)

**Fig. 2.** The separation in the backcross hybrids of antigenic substances specific to Pearlneck in contrast to Ring dove.

The relative quantitative reactivities of these different substances specific to Pearlneck are displayed in the blocks in Figure 2, based on their agglutinations at different dilutions with Pearlneck antiserum absorbed (usually at 1:60) with Ring dove cells. For example, cells containing the d-1 character were reactive at a dilution of the reagent of 1:2880 or 1:3760, those with d-6 at 1:180 or 1:360, while those with d-11 had the highest reactivity, at 1:11,520 or 1:23,040.

It is not proposed that these nine or ten characters necessarily represent all that are specific to Pearlneck, in relation to Ring dove, for there may be others which cannot be detected at the dilutions of the reagents used in the tests. However, it is probable, but not certain, that these nine or ten substances constitute all or nearly all the so-called "major characters" which differentiate Pearlneck from Ring
dove. With certain exceptions, each of these antigenic characters was passed on to the backcross offspring as expected if it were (a) produced by a single gene or (b) by two or more genes on a chromosome from Pearlneck which exhibited no crossing over with a Ring dove chromosome (Irwin, 1939). One exception involved the d-7 character which was contained seemingly in combination with d-3 in the cells of a single backcross male (D458A2). This male was mated successively to five Ring dove females and produced 46 offspring, 38 of which had either d-3 or d-7, or both, while eight had neither. Most of the offspring died before adequate tests could be made to detect those which carried only d-7 on their cells. The other exception involved the d-4 character, for which definite evidence of a fractionation of some sort was noted. From matings to Ring dove of backcross hybrids with the d-4 substance, 207 offspring have been obtained of which 106 carried d-4 and 101 did not. At least two of the 106 birds possessed only a part of the d-4 substance (Irwin, 1949). That is, the cells of these two birds by absorption did not remove all the antibodies for the d-4 antigen, whereas absorptions by the cells of other birds with d-4 removed antibodies for each other and also for these two birds. It seems reasonable to ascribe these cases of fractionation of the d-4 substance to a separation of two or more genes involved in its production, by virtue of a crossing over of the chromosome carrying them with a homologue in Ring dove. The assumption of a mutation would require the assumption of a very high rate of mutation, and seems much less plausible. If it be accepted that two or more genes act together to effect the d-4 substance, it seems plausible to believe that the majority or perhaps all of the specific substances of Pearlneck are also the result of the joint action of two or more genes.

These nine or ten antigenic substances of Pearlneck thus represent the action of a gene, or the joint action of two or more genes, on nine or ten of the 30-odd pairs of chromosomes of Pearlneck. [Although the number of chromosomes in Pearlneck is not known, it is probable that the number (30-odd pairs) found by Painter and Cole (1943) in the pigeon and Ring dove species obtains also for Pearlneck.] There are two questions which are pertinent at this point. One of these is
concerned with the relation of these species-specific substances, and therefore of their causative genes, to substances and genes of Ring dove. For example, are there in Ring dove components of the blood cells which are antigenically related to these nine or ten specific antigenic characters of Pearlneck? If so, the causative genes would likewise be related, perhaps in a manner for each such gene comparable to the relationship of the gene in man producing the B substance to the presumed gene in certain species of lower monkeys which have a B-like cellular substance. There is no way available to date to determine what proportion, if any at all, of the so-called "common" antigens of Pearlneck and Ring dove are in reality only antigenic similarities of total gene products, a part of which are species specific. Thus, (a) the Pearlneck substance d-1 may be entirely unlike any substance in Ring dove, implying that the causative gene or genes produce dissimilar antigenic substances, or (b) there may be definite similarities between it and a substance of Ring dove but no reagent is available to detect the similarity. In brief, it is not clear at present whether the genes affecting the substances common to the two species are on the same or different chromosomes as those which produce the species-specific effects.

The second question is relative to the observation that only about one-third of the chromosomes of Pearlneck (nine or ten of 30-odd chromosomes) carry the genes which have species-specific effects on the cellular antigens. If to this number is added the probable three or four chromosomes with a gene or genes affecting the specific components of the serum, presumably the serum proteins, of Pearlneck (Cumley and Irwin, 1942), there still are somewhat less than half the number of chromosomes of Pearlneck with genes which differentiate it from Ring dove by virtue of antigenic substances of the blood. In contrast, the generally accepted concept of genetic differences between species, as expressed by Muller (1940) is that such differences generally depend upon multiple genes, each having small effects and presumably scattered over most, if not all, of the chromosomes.

Observations on eight morphological characteristics which conform entirely with this general concept have been made on these two
species, Pearlneck and Ring dove. These characters are over-all length, extent, width of band, length of beak, tail, wing, middle toe, middle toe and claw, and diameter of tarsus. Unpublished analyses of these measurements, which were taken under the supervision of the late Professor L. J. Cole, have shown that the average values for each of the measurements of the species hybrids were intermediate between the respective averages for the two parental species. Likewise, the average values for each of these characteristics in the successive backcross generations to either parental species showed a gradual approach to that of the species to which the backcross was made. This is precisely what would be expected if, in the production of each characteristic, there were multiple genes involved with individually small effects.

THE HYBRID SUBSTANCE

The cellular antigens previously considered have seemingly corresponded directly to their causative genes, thereby giving evidence in favor of the proposal that such antigens might be the direct or primary products of the genes. Such a relationship has been suggested by Irwin and Cole (1936a) and by Haldane (1938), by the latter author in large part because the genes for cellular antigens produce the same effect no matter what other genes are present. The finding that there are antigenic substances (the so-called hybrid substance) in the species hybrid not detectable and therefore presumably not present in either parental species represented an exception to the above relationship. A hybrid substance has likewise been found in the cells of the hybrids between the pigeon and Ring dove (Irwin and Cole, 1936b), and of those between Muscovy (Cairina moschata) and Mallard (Anas platyrhynchos) ducks, as demonstrated by Gordon (1938) and McGibbon (1944). A hybrid substance of the serum of the hybrids between Mallard and Muscovy has also been reported by Sokalowskaja (1936).

A recent article by Fox (1949) reports the results of serological studies of three strains of Drosophila melanogaster— isogenic, ruby (rb) and vermilion (v). Fox proposes that his results require the assumption that there has been an interaction of the rb+ and v+
genes in the isogenic strain to produce an antigenic effect which would be changed if either gene mutated, as in either ruby or vermilion strains.

Not all species hybrids contain a hybrid substance as a cellular constituent, as evidenced by the observation that only about half of the different species hybrids in pigeons and doves possess one or more antigenic substances of the cells different from those of the parents (Irwin, in press). Thomsen (1936) reported that cellular substances not present in either parent had been detected in chickens. However, pertinent observations (by Mrs. Ruth Briles) in unsuccessful attempts in our laboratory to duplicate his findings suggest that peculiarities in the technique may be the explanation of his results (Irwin, in press) rather than the presence of a hybrid substance. Briefly stated, both parents A and B were immunized against the cells of one or more offspring. If the antiserum of parent A was absorbed by the cells of parent B (containing antibodies reactive with the cells of A), the absorbed fluid would then agglutinate the cells of the A parent. In other words, the serum from parent A would then agglutinate its own cells. Seemingly, the blood cells of B had attached to their surface some of the circulating antibodies (anti-A) and these were released into the serum of A from the cells of B during the absorption.

The hybrid substance of the cells of the hybrids resulting from the cross between Pearlneck and Ring dove was fractionated definitely into two and probably into three parts, by virtue of its association with certain specific antigens of Pearlneck (Irwin and Gumley, 1945). One part, called dx-A, was always associated with the d-11 character of Pearlneck. Another, dx-B, was associated, but not completely, with the Pearlneck antigens d-1, d-2, d-3, d-7, d-9, d-10 and d-12. A probable third component was always associated with the d-4 antigen of Pearlneck. The association of the dx-B fraction with several Pearlneck specific characters provides strong evidence for duplicate or repeat genes in Pearlneck—genes on different chromosomes which by interaction with a gene or genes in Ring dove effect a particular part of the hybrid substance. An alternative but rather improbable explanation is that
the association between the dx-B fraction and the various antigens specific to Pearlneck was entirely by chance, and that the dx-B fraction was produced by a gene or genes on only a single chromosome of Pearlneck interacting in the species hybrid and in the backcross hybrids with a gene or genes of Ring doves.

As has been stated (Irwin and Cumley, 1945), there is cross-reactivity between the hybrid substances of the hybrids between the Pearlneck-Ring dove and the pigeon-Ring dove. Further, this cross-reactivity in the Pearlneck-Ring dove hybrid substance appears to be largely if not entirely limited to the dx-A fraction, since this fraction will absorb the antibodies for the hybrid substance of the pigeon-Ring dove hybrid. Also, the reagent for the hybrid substance of the pigeon-Ring dove hybrids (antihybrid serum absorbed by the cells of both pigeon and Ring dove) will agglutinate the cells of backcross birds carrying d-11—presumably by virtue of the presence of the dx-A fraction of the hybrid substance—but not those of Pearlneck which definitely contain the d-11 specific substance. There is no evidence to date that pigeon cells possess any more than a very small fraction, probably none at all, of the d-11 substance of Pearlneck, so the explanation hardly seems valid that the hybrid substance is simply a rearrangement of a species-specific character, which in Pearlneck is d-11. Additional cross-reactivities of the hybrid substance and other substances are discussed elsewhere (Irwin, 1951).

FURTHER RELATIONSHIPS AMONG SPECIES

Following the same general pattern which obtained for the separation in the backcross offspring of antigenic substances particular to Pearlneck and Ring dove, respectively, a comparable separation into unit form was made of cellular antigens peculiar to guinea in comparison with livia (Irwin, Cole and Gordon, 1936). These were called A, B, C, E and F of guinea. Evidence has been presented that the A and F substances, and fractions of C and E—but not necessarily the same fractions—are found in both Pearlneck and Ring dove (Irwin, 1938). The two substances A and F are therefore common to guinea, Pearlneck and Ring dove, in contrast to livia, thus providing
evidence that the antigens common to two species, as Pearlneck and 
Ring dove, are heritable.

The hybrids between pigeon and Ring dove produced less than ten 
backcross hybrids during the past twenty five years in matings to 
Ring dove, none at all in matings to pigeon. Hence no separation in 
unit form has been achieved of the antigens specific to livia in con-
trast to Ring dove. However, a separation of antigens of both cells and 
serum has been noted in the few backcross hybrids available (Irwin 
and Cole, 1936; Irwin and Cumley, 1942). From the evidence avail-
able it appears that the genes which affect the serum proteins are 
different, and probably not linked, with those affecting the cellular 
antigens.

Following the isolation in unit form of the antigen components 
of Pearlneck, in comparison to Ring dove, it was possible to determine 
by immunological tests alone which of these components, if any, 
were present in other species of doves. (The tests were carried out by 
absorbing anti-Pearlneck serum with the cells of both Ring dove and 
another species, and then testing each such reagent with the cells 
representing each of the unit-substances of Pearlneck. If the anti-
bodies were completely removed by the cells of one species for a Pearl-
neck substance, the inference would be that such a species possessed the 
particular substance). An African species of dove, the Senegal (St. 
*senegalensis*), was found to be a very close relative of Pearlneck, in 
that its cells would remove from Pearlneck antiserum all or nearly 
all the antibodies for Ring dove, and also for each of the specific 
Pearlneck substances except d-6 and d-11. The offspring of matings 
to Senegal of the hybrids between Pearlneck and Senegal should 
then show a segregation of the d-6 and d-11 substances, and this 
expected result was actually observed (Irwin and Cole, 1940). Ac-
tually, additional specificities to these have been obtained in other 
Pearlneck antisera, so that it is now known that, in addition to d-6 and 
d-11, there are parts of d-1, d-2 and perhaps of d-7 peculiar to Pearl-
neck in contrast to Senegal.

It has been possible to make a precise analysis of the relationships 
of the cellular antigens between Pearlneck and Senegal, following 
the isolation in backcross hybrids of unit-antigens specific to Senegal.
in contrast to Ring dove. These, as shown diagrammatically in Figure 3, are called (s for Senegal) s-1, s-2, s-3, s-6, s-7, s-8, s-9, s-10, s-11 and s-12 (Irwin and Cumley, 1947). Each of these, with but two exceptions, has behaved in inheritance as expected if produced either by a single gene or by two or more genes on the respective chromosomes of Senegal. These exceptions will be discussed below.

![Diagram of Senegal and Ring dove antigens](image)

Fig. 3. The separation in backcross hybrids of antigenic substances peculiar to Senegal in comparison with Ring dove.

Having given the antigenic characters in unit form of Pearleneck and Senegal, respectively, in contrast to Ring dove, it then becomes possible, following reciprocal absorptions, to compare these for their identity or similarity. The details of these comparisons have been given elsewhere (Irwin, 1949a), and are presented diagrammatically in Figure 4. Since the detailed report of these relationships was published, an anti-Pearleneck serum has been obtained which differs from that used in the previous comparisons in that the antibodies for the d-1 substances are not completely removed by the absorption with either Senegal cells or those carrying the s-2.s-12 of Senegal. Also although s-12 was identified as a separate entity in the first backcross generation, it is now reasonably certain that the genes responsible for s-2 and s-12 are linked. That is, from backcrosses to Ring doves of birds carrying both s-2 and s-12, 62 offspring have been obtained of which 28 had s-2.s-12, two had s-12, and 32 had neither. Similarly, of 16 backcross hybrids obtained from a mating to Ring dove of birds carrying s-6, one bird had only a part of s-6, eight had s-6, and seven
had no part of s-6 (Irwin, 1949). The most plausible explanation of these two cases is that there was a separation of genes for s-2,s-12 and s-6, respectively, by virtue of a crossover.

It has been found (Irwin and Cole, 1940) that the major proportion of the antigenic components of the blood cells common to Pearlneck and Ring dove are shared also with Senegal, and most of those common to Senegal and Ring dove are shared with Pearlneck. Hence the differences in these substances between Pearlneck and Senegal will be largely those specific to either species in contrast to Ring dove, and the majority if not all of these for both Pearlneck and Senegal have been obtained in unit form.

![Diagram](image)

**Fig. 4.** Diagrammatic representation of the relationships of the cellular antigens peculiar to Pearlneck and Senegal, respectively, in contrast to Ring dove.

From the diagrammatic representations in Figure 4, it may be seen that the following substances of each species are indistinguishable or nearly so, and may be considered to be identical: d-3 and s-11, d-4 and s-6, d-5 and s-1, d-12 and s-9. The causative gene or genes for the respective substances may also be considered to be homologous. There was a definite similarity, but not identity, of substance
d-2 and s-7, d-11 and s-8, and possibly of d-6 and s-3. The d-1 antigenic character of Pearlneck has always behaved as a unit in inheritance in the backcross generations, yet its similar substance in Senegal, s-2.s-12, has definitely fractionated, undoubtedly following a crossing over. The d-1 component has certain specificities peculiar to Pearlneck in comparison with Senegal, as stated above, and the s-12 substance of Senegal contains most or all of the antigenic specificities of the s-2.s-12 antigenic complex which are peculiar to Senegal as compared to Pearlneck. If but one gene were involved in the production of the substances of the two species which have antigenic similarity but not identity, the genes presumably would not be homologous but would be similar in part and each would have its specificity. On the other hand, if two or more genes on a single chromosome in each species acted jointly to produce each species-specific substance, one or more genes could effect the common parts of the antigenic substances, and one or more could effect the specific parts. Under this explanation, it would also be possible for any or all the genes on any such chromosome to affect similar but not identical components.

No substance related to the s-10 of Senegal has been noted in Pearlneck. Thus, of the nine chromosomes in Senegal with one or more genes which distinguish Senegal from Ring dove, there is one with a gene or genes whose products differentiate Senegal from Pearlneck, four with genes whose products are similar to Pearlneck and yet serve to distinguish Senegal from Pearlneck, and four with genes which seemingly are homologous in the two species. Likewise, of the nine chromosomes of Pearlneck which carry one or more genes differentiating Pearlneck from Ring dove, there are definitely four and probably five with genes whose antigenic effects are similar but not identical to antigenic substances of Senegal, and four with genes which presumably are homologous to an equal number in Senegal, since their effects are indistinguishable and therefore presumably homologous.

As stated earlier, there are three or four components of the serum, and a gene or genes on a corresponding number of chromosomes, which further differentiate Pearlneck from Ring dove. Similarly, evi-
In dence has been obtained that three and probably four antigens of the serum (or rearrangements of the proteins affected by the causative genes) peculiar to Senegal have segregated freely in the offspring of backcrosses to Ring dove (Cumley, Irwin and Cole, 1943). And, further, definite differences in the serum antigens have been observed in backcrosses to Senegal of hybrids from matings between Pearlneck and Senegal (Cumley, Irwin and Cole, 1941). The genes in these three species—Pearlneck, Ring dove and Senegal—with species-specific effects on the serum appear to segregate quite independently of those with effects on the cellular antigens. And while the cellular antigens which are species-specific may be thought of as being more or less discrete substances, the species-specific differences of the serum antigens may represent changes in the proteins, but not necessarily “determinant groups” in the protein molecule. In order to make a complete analysis of antigenic similarities and differences between related species, it appears highly desirable to make use of both serum proteins and cells. Either system by itself may give only a partial picture of the antigenic relationships of the species.

The different cellular substances which distinguish Pearlneck from Ring dove, Senegal from Ring dove, and C. guinea from C. livia may now be utilized as “tester substances” to detect their presence, in whole or in part, or their absence in other species of birds. From such studies a pattern of phylogenetic relationships should emerge which would be impossible to obtain without the use of these unit-substances.

**Cellular Substances Within Species**

Because of the finding that one or more genes on several chromosomes of pigeons and doves had effects on the *species-specific* antigens of the blood cells, and that other genes had such effects which were *common* to any two species under comparison, it seemed plausible to believe that many genes within a species should have effects on the blood cells. Exploratory tests in both cattle and chickens have indicated that this belief was a reasonable one. In fact, if the gene itself has an individuality, it is to be expected that the number of antigenic substances which may be present in various cells of the body...
will be limited only by the number of genes. If there is interaction between genes as a general rule and not as an exception, the number of possible antigenic substances naturally is changed, and depends upon whether a gene will effect a substance by itself as well as another by interaction. Nevertheless one limitation on the ability to detect many such gene effects may well be that of the position of the antigenic substance on the cell—at the surface or under the surface—since antigen-antibody reactions are primarily surface reactions.

As was stated earlier, the criteria of both genetics and immunology need to be satisfied before an antigenic substance may be called a single genetic character. Thus, in the detection of the substance called A in cattle cells, a cow was transfused with the blood of her calf (Ferguson, 1941). An antibody was obtained which was reactive (produced lysis upon the addition of complement to the serum-cell mixture) with the blood cells of some individuals, but not with the cells of others. From the point of view of immunology, if but one substance were involved, as A, the reactive cells from any individual should, when added in excess to its specific reagent, remove the antibodies for themselves and for the reactive cells of all other individuals. Since it was not possible to make the various matings to satisfy the usual genetic requirements, the analysis of the data by the gene-frequency method was employed, with the example below taken from a report by Ferguson, Stormont and Irwin (1942).

<table>
<thead>
<tr>
<th>Type of Mating</th>
<th>Number of Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With A</td>
</tr>
<tr>
<td>( A \times A )</td>
<td>217</td>
</tr>
<tr>
<td>( A \times - )</td>
<td>76</td>
</tr>
<tr>
<td>( - \times - )</td>
<td>0</td>
</tr>
</tbody>
</table>

Following further isoimmunizations in cattle and immunizations of rabbits, and selective absorptions of the resulting antisera, some forty-odd reagents have become available for typing the blood cells of cattle. The first antigenic substance detected was called A, the next B, the next C . . . Z. Those which followed Z were called \( A' \), \( B' \) . . . \( K' \), with no implication of a relationship between A and \( A' \), B and \( B' \), etc. Each of these has been subjected to the criteria of both genetics and immunology, as given above. That is, the use of the
specifically reacting cells, from usually ten to twenty individuals, in absorbing the respective reagents presumably satisfied the immunological criterion for a single substance. Also, with the possible exception of the substance called J (which was detectable by its reactivity with a normally occurring antibody in the serum of some cattle) each antigenic substance behaved in inheritance as expected if it were controlled by a single gene in contrast to its absence (Ferguson, 1941; Ferguson, Stormont and Irwin, 1942; Stormont, 1950). Of considerable importance is the fact that no one of these substances appeared in the cells of an individual unless either or both parents also possessed it. Furthermore, if each of these substances were produced by a single gene, as at one time appeared to be the case, the number of possible combinations staggered the imagination. (One of the unexpected developments in these studies was the need, and the demand, particularly from the dairy cattle herd associations, for such a test in settling disputed cases of parentage in cattle. A laboratory for the purpose of rendering this service to various breed associations as one of its functions has been established at Ohio State University, under the leadership of Dr. L. C. Ferguson.)

However, instead of these 40-odd substances being genetically independent, each from the others, definite associations have been noted among many of them. For example, Ferguson (1941) reported that the C and E factors were not independent, for of these two only C occurred alone, whereas E was present always with C, and such cells therefore carried CE. Ferguson postulated that there were three alleles involved—one for the absence of both C and E, one for C alone, and one for CE together. Other examples of these so-called subtypes have been given by Stormont (1950).

Shortly thereafter it was noted by Stormont that certain additional factors in the cells of an individual appeared only if one or more other components were also present. Thus, the substance B occurs alone, as does that called G. But a third substance K has never been definitely found to be present unless both B and G were also present. This association of K with both B and G has been observed in nearly two thousand individuals. In other words, the combination of the BGK factors has always appeared as a unit. The following insert,
from previously unpublished data, shows the hereditary behavior of this combination, or “antigenic complex.” Notwithstanding the fact

<table>
<thead>
<tr>
<th>Type of Mating</th>
<th>Number of Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGK x BGK</td>
<td>With BGK</td>
</tr>
<tr>
<td></td>
<td>151</td>
</tr>
<tr>
<td>BCK x –</td>
<td>185</td>
</tr>
<tr>
<td>– x</td>
<td>0</td>
</tr>
</tbody>
</table>

that B, G and K are each recognized by respective reagents, these observations are almost indisputable evidence that the factors B, G and K in the cells are a unit.

Furthermore, the offspring of some individuals possessing BG on their cells have been of two classes, those with B and those with G, as would be expected if the causative genes were alleles. But another individual with BG has produced progeny of two quite different types—those with the complex BG and those with neither, as if a gene controlling B and G together were allelic to one not effecting either B or G. Furthermore, another substance, I, may be present in an antigenic complex with either B or G, but never has been observed in a complex with K. Substances I and K may be noted in the same individual, but separate in the progeny as expected of contrasting characters.

There are recognized in cattle two series of these antigenic complexes, called the “B” and “C” series, respectively. In the B series there are 21 of the 40-odd antigenic factors which are associated in various combinations. Seven of these may occur singly, as was described above for B and G. The other 14 have been found only in various antigenic complexes, each of which may be made up of from two to eight of the 21 substances. In each of these two series of antigenic complexes, the evidence for the existence of the complex is that no separation of a recognized complex has ever been observed in the cells of the progeny of an individual possessing it. The details of this evidence are given elsewhere (Stormont, Owen and Irwin, 1951), but one example from the data will be illustrative. A Holstein bull, H-4, had 35 offspring with the complex B\textsubscript{(0,0,Y,F)}\textsubscript{2} and 31 with B\textsubscript{(0,Y,K)}, exactly as expected if the antigenic complexes were alternatives. Considering all the available evidence, it seems more reasonable to assume
that each antigenic complex is produced by a single gene than by linked genes. Under this explanation, the various antigenic complexes in each of the two systems, or series, would then be produced by a series of multiple alleles. The possibility of pseudo-alleles must be kept in mind, but for the present may be assumed for convenience not to be a complicating factor.

Parallel findings to these in cattle have been made by Briles in chickens (Briles, McGibbon and Irwin, 1951). Different antigenic specificities in chickens corresponding to those of the antigenic complexes in cattle described above have been observed, and in controlled matings these have behaved as expected of multiple alleles. In the light of these findings in cattle and chickens, it seems probable that the further fractionation into several components of what appeared to be a pair of contrasting substances of Muscovy ducks (McGibbon, 1944, 1945) may rationally be explained as the respective products of allelic genes.

Previously in this paper attention was called to the fact that the similarities between the A and B substances of man may be even more pronounced than the differences, but that each is recognized by virtue of the differences between them. Our concept of the causative genes, then, may be that they in turn are more alike than different, as expected of alleles. A pertinent question, for which an answer is lacking, is what constitutes the difference between the two antigenic substances A and B of man, from which inferences might be drawn as to the specificities of the gene materials. Undoubtedly, if in cattle the B series is controlled by genes in an allelic series, something of the same sort of relationship between B, G, BG, BGK and other members of the B series in cattle may be proposed as exists between A and B of man.

Our most precise knowledge of antigenic relationships which may be somewhat analogous to the antigenic complexes in the B series of cattle cells is found in the immunochemical studies of the pneumococcal types. This information has become available in large part because of the ability to link these substances to proteins, thus making them antigenic. As a part of the experimental work in analyzing the specificities of the pneumococcal types, cross reactions have been
observed between the respective antiserum (from horses) to type III and type VIII pneumococci. As was stated previously, the specificities of the pneumococcal types depend largely if not entirely upon the carbohydrates of their capsules. It has been found (Reeves and Goebel, 1941) that the carbohydrate of type III is a polyaldbionic acid. The knowledge of the structure of the polysaccharide of type VIII is not as complete as for type III, but about 60 per cent of the molecule of the carbohydrate of type VIII appears to be aldobionic acid. Cross reactivity may therefore be expected between the soluble specific substances of types III (S III) and VIII (S VIII), by virtue of the presence in each of multiples of the same aldobionic acid as a structural unit. It is probable that the serologically reactive unit in each of these two types is a larger portion of the polysaccharide molecule than a single chemical structural unit. Type S VIII also contains approximately two glucose molecules for every aldobionic acid residue, thereby presumably accounting for at least a part of the specificity of type VIII in contrast to type III. Thus it may be seen that serological cross reactions may be expected when the antigenic substances under comparison are closely related chemically. Also to be expected is the ability to distinguish between such substances, as was actually possible in the case of types III and VIII (see Heidelberger, Kabat and Meyer, 1942, for data and for leading references).

From these and related findings has come the concept that a single antigenic substance may engender a multiplicity of antibodies of varying specificities. Also, antigenic substances of related but not identical chemical constitution may—but sometimes do not—incite the production of cross-reacting antibodies. With these points in mind, what can be inferred concerning the antigenic complexes in cattle cells? To use BGK complex as an example, is the reactivity of cells containing BGK with the respective reagents due to the presence of specific so-called "combining groups," or "determinant groups" in a single substance, or otherwise? Does the BGK complex represent (1) three different and separate antigenic substances? If so, according to the hypothesis of multiple alleles, the causative gene for BGK would accomplish the work of the alleles for B and G, respectively, and for K in addition. Or does the BGK complex represent (2) a single
antigenic substance with (a) a possible common base and three more or less different determinant groups accounting for B, G and K respectively, or (b) a single substance capable of inciting many specificities of antibodies, of which those reactive with B, G and K represent only a part of the reactivities of the spectrum of antibodies which may be produced? These possible explanations are not mutually exclusive, and a combination of two or more may well be more nearly correct than any one alone.

In terms of the action of the causative genes, apart from the possibilities of linkage and pseudo-allelism, the question seems to resolve itself around two main aspects. (1) Do the genes controlling an antigenic complex, as a single gene for BGK, have separate specificities for B, G and K, or (2) does this gene produce a single substance with no such separate specificities, the similarities between such complexes as BGK and BG1Y being due primarily if not entirely to the general similarities in their chemical structure? The writer is inclined to adopt a combination of these two possibilities as a current working hypothesis. No matter what may eventually prove to be the correct interpretation of the chemical structure of the antigenic complexes, and the action of the controlling genes, it appears that these studies have given some insight into the complexities of the gene products and perhaps also of the causative genes.

Twins in cattle

As was stated earlier, each of these 40-odd cellular substances, whether by itself or in a complex, has been found in the blood cells of an individual only if one or both parents possessed it. Furthermore, one would say with assurance that all the red blood cells of an individual have the same antigenic content. In man, identical twins have the same blood types, while non-identical twins are no more alike than brothers or sisters born at different times (see Wiener [1943a] for references to pertinent articles). It was believed that this same condition would obtain in cattle twins.

The opportunity presented itself a few years ago to test twins from a case of superfecundation in cattle, in which a Guernsey cow had given birth to twins with different markings. One twin had the usual
Guernsey color, the other had a white face as expected if a Hereford were the sire, and the herd records showed that the cow had been bred by a Guernsey bull and later by a Hereford bull. The blood tests by Owen (1945) on the five animals involved are as shown below.

<table>
<thead>
<tr>
<th>Guernsey sire:</th>
<th>A</th>
<th>HI</th>
<th>S</th>
<th>X₅Z</th>
<th>E₁H₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereford sire:</td>
<td>ACE</td>
<td>RW</td>
<td>I'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dam:</td>
<td>ACEG IÒQW</td>
<td>ZD'E'H' Z'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guernsey twin 1:</td>
<td>ACEG IQRSWX₅ZD'E'H'Z'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereford twin 1:</td>
<td>ACEG IQRSWX₅ZD'E'H'Z'</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Not only did these twins have identical blood types, but the Guernsey twin possessed factors R and I' which could have been transmitted only by the Hereford sire, and the Hereford twin carried factors S and X₅ which could have been inherited only from the Guernsey sire. Subsequent tests on many other pairs of twins (Owen, 1945) have shown that about 90 per cent of twins in cattle have identical blood types. It is now known that the antigenic factor called J is an exception to this rule.

The most rational explanation of the above phenomenon is that proposed by Owen (1945). In brief, the union of the circulatory systems in about 90 per cent of bovine twins (Lillie, 1916) provides a mechanism for the migration from each twin to the other of the so-called primordial blood cells, and these become established in the hematopoietic tissues of the co-twin. From that time on they produce blood cells and each such twin then will have throughout life a mosaic of blood cells—those from its own blood-forming tissues and those from the tissues of the co-twin. A differentiation of the two kinds of cells in such twins can be made in the laboratory, thus establishing experimentally that there are two kinds of cells in each such twin. Identical blood types were also demonstrated in bovine quintuplets (Owen, Davis and Morgan, 1946), and in other multiple births (unpublished data). One may well ask the question at this time whether the phenomenon of migration and establishment of primordial blood cells is limited to cattle and to the primordial blood cells. It seems reasonable to believe that any primordial cell which may migrate could become established in the co-twin.
One result of the recognition of this phenomenon is that it seems probable that those heifers, born twin to bulls, which will be sterile may be set apart by the blood test from those which are potentially fertile. That is, the female of unlike-sexed twins will not be sterile unless there has been a fusion in utero of the circulatory systems of the twin embryos. Thus those heifers from twins of unlike sex which do not have the two kinds of blood cells should be those in which the fusion of the circulatory systems in utero did not occur. Such heifers should be as fertile as those born singly. Also, although non-identical twins in cattle in which there had been a fusion of circulatory systems in utero will have identical blood types and an admixture of bloods, the majority or all of those twins which have identical blood types but do not have an admixture of blood cells should be identical twins.

It was stated previously that the majority of twins in cattle have identical blood types, except for the substance called J. This substance is recognized by virtue of its reaction with a normal antibody which is present in the serum of some cattle lacking the J substance (Ferguson, 1941). It was noted by Stormont (1949) that the cells of one of a pair of twins had J, those of the other twin did not, although the blood types were otherwise identical and showed a definite admixture of blood. Previous observations had been made that the J substance was poorly expressed, if at all, on the cells of a young calf, although it could be demonstrated in the plasma. Thus it was reasoned that the J substance was not given to the cells by genes in the hematopoietic tissues, but that it was taken up by the cells following their entrance to the blood stream. Stormont (1949) demonstrated that non-J cells, carrying appropriate antigenic markers, would gradually “acquire” the J substance following their transfusion into an animal with J in the plasma and on its cells. Similarly, non-J cells would take up the J substance in vitro from plasma possessing it. At the present writing this substance is unique in that it is actually a constituent of the plasma of those individuals possessing the causative gene, and is detected on the cells seemingly because of its “sticky” qualities. It is probable that the so-called “R” substance of sheep cells (Ycas, 1949) is also primarily a constituent of the serum, and
sticks to the blood cells, since certain of its characteristics parallel those of the J of cattle.

GENERAL CONSIDERATIONS

Very little has been said in the preceding pages about antigenic substances other than those in the blood stream. Other cells and tissues of the animal body do not in general offer the same advantages for experimental material as do the blood cells and serum, particularly in the ease with which they may be obtained. Nevertheless, it may be expected that in the future the antigenic components of tissues and cells other than those of the blood will be subjected to genetic analyses. From such studies precise information will be obtained of the accuracy of the more or less general belief that the basic chemical constitution of all cells of the body is very largely the same, but that there are tissue and organ specificities. It also seems reasonable to believe, as has been forecast by Weiss (1949) and others, that the combined techniques of genetics and immunology may well cast some light upon the biological mystery of cellular differentiation.

Genetics and immunology have other points in common than have been given herein. Some of these have been mentioned in another review (Irwin, 1949b), such as the intriguing findings concerning antigenic types in Paramecium aurelia. These will be described fully in a paper by Sonneborn in this volume. In the field of tumor transplantation, Gorer (1938) has shown that success or failure in transplanting certain tumors depended in part upon antigens demonstrable in the red blood cells, and presumably also in the transplant. Further studies of this kind are indicated for the information that they may give in the general field of tissue transplantation.

In relation to the problems of growth, a very interesting proposal has been made by Tyler (1947) that "the various macromolecular substances that form the basis of cell structures bear the same sort of relationship to one another as do antigen and antibody." It had been noted that a substance on the eggs of sea urchins and other marine animals, called "fertilizin," combines with the so-called "anti-
fertilizin' of the sperm, thus simulating an antigen-antibody reaction (for references to the pertinent articles, see Tyler, 1947). Antifertilizin was found not only on the surface of the sperm, but Tyler obtained it from below the surface of the egg. This antifertilizin from the egg possessed the same properties as that from the sperm, so Tyler concluded that "the finding of antifertilizin within the egg means that below the surface of the cell there is a substance that is essentially an antibody to the surface substance." Various other findings have led Tyler to propose that many mutually complementary substances may be found deeper within the cell. If this concept applies to cells in general, it means, among other things, that serious consideration, with some change in the details, should be given to the main idea in the hypothesis of Lindegren and Bridges (1938), namely, that the specificity of attraction between the chromatides during the synopsis of homologous chromosomes could be explained as an antigen-antibody interaction.

There is another area in which genetics and immunology meet, that of the possibility of inducing gene mutation by means of antibodies. The early work of Guyer (1924) reported that certain genes could be altered by immunological influences. Specifically, it was reported that antibodies to lens protein, when given to, or induced in, a pregnant female would in some manner have an effect upon the lenses of some of the newborn rabbits, and that this condition was heritable. These findings have not been duplicated by other workers, but recently Emerson (1944) reported evidence for a mutation in Neurospora presumably induced by an antibody. Further, the changes, probably somatic, in antigenic types of Paramecium as reported by Sonneborn (1948) can be effected by antibodies, and can also be controlled to some extent by temperature and rate of fission. Since chemical means of inducing gene mutations have recently been added to those of irradiation, the question is pertinent as to how drastic an agent must be to produce a change in a gene.

The areas of interest to biologists in which genetics and immunology may meet with profit are much broader than can be considered in this limited discussion. In general, unless the chemical substances of plants and animals which are as yet undetected and therefore un-
known can be studied directly by the techniques of chemistry, it appears that a first step in their discovery may well be made by the use of techniques of immunology. To some workers, immunology is actually a branch of chemistry. There are powerful tools in both genetics and immunology which can aid in satisfying man’s curiosity about many biological problems. To the writer, the few offspring which have come from the marriage of these two disciplines are taking their first steps after getting out of their cradles. May they and later offspring grow rapidly and prosper!

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CHEMICAL GENETICS

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BECAUSE it is not and should not be an isolated branch of the general field of genetics or of biology as a whole, it is not easy to define chemical genetics in a simple way. We may say that it consists in the study of genes as units of function at the molecular level and contrast it with that branch of the subject which emphasizes the mechanisms by which units of inheritance are transmitted from one generation to the next. Chemical genetics is concerned with the structure of genes, the nature of their primary functions in living systems and the manner in which they direct these functions. We are a long way from knowing the answers to the questions implied in these objectives but we have made some progress and can see at least a few of the ways in which more can be made.

During the many hours he spent in formulating and experimentally testing his hypotheses concerning inheritance in the garden pea, Mendel must have reflected on the nature and physiological manner of action of his postulated factors. But what he thought in this regard, he did not record for us to read. Many of those who followed belatedly in his footsteps, however, did put down their speculations and in so doing began to accumulate the experience, the knowledge, the interpretations and the hypotheses that are now chemical genetics. Bateson, rugged and dynamic champion of the new science of Mendelism or genetics as he named it, and his coworkers Punnett, Saunders and others soon began to talk in terms of presence and absence of active units and of a possible relation between these units
and the "ferments" of the biochemists. Bateson pointed out in 1902 for example, that the human disease alcaptonuria, then being actively studied by the English physician and biochemist Garrod, behaved as a Mendelian recessive trait. Garrod was to a large extent responsible for demonstrating that this metabolic anomaly, characterized by excretion in the urine of alcapton (2,5-dihydroxyphenyl acetic acid), was the result of a discrete block in the sequence of chemical reactions by which the amino acids phenylalanine and tyrosine are metabolized. He also clearly formulated the hypothesis that the progress of the specific reaction by which alcapton is further oxidized in normal individuals is somehow dependent on the normal allele of a specific gene. He furthermore recognized and made effective use as a biochemical tool of the principle that failure of a chemical reaction resulting from a genetic defect may lead to the accumulation of the compound normally transformed by the reaction.

In 1909 Garrod published his now classical Croonian Lectures, delivered before the Royal College of Physicians in 1908 under the title "Inborn Errors of Metabolism." In this he summarized and interpreted the existing knowledge about the four hereditary diseases of man, albinism, alcaptonuria, cystinuria and porphyrinuria. Fourteen years later a second edition of "Inborn Errors" appeared. By this time Garrod was able to add two additional metabolic defects, viz., steatorrhea and pentosuria. Also he was able to extend in a significant way the hypothesis as to the cause of alcaptonuria, for in 1914 the German chemist Gross had found that alcaptonurics are deficient in an enzyme present in the blood serum of normal persons and catalytically concerned with the oxidation of alcapton. Thus, while he did not express it exactly so, Garrod was able to conclude that in a person homozygous for a particular gene defect, a specific enzyme is absent, alcapton is not further oxidized and as a consequence accumulates and is excreted in the urine. The relation gene-enzyme-specific chemical reaction was certainly clearly in his mind. It is proper that he should be recognized as the father of chemical genetics.

It is a fact both of interest and historical importance that for
many years Garrod’s book had little influence on genetics. It was widely known and cited by biochemists, and many geneticists in the first two decades of the century knew of it and the cases so beautifully described in it. Yet in the standard genetic books written in the twenties and thirties, including Goldschmidt’s *Physiological Genetics*, few mention its cases or even give a reference to it. I have often wondered why this was so. I suppose most geneticists were not yet inclined to think of hereditary traits in chemical terms. Certainly, biochemists with a few notable exceptions such as the Onslows, Gortner and Haldane were not keenly aware of the intimate way in which genes direct the reactions of living systems that were the subject of their science.

In spite of rather general neglect of Garrod’s work by geneticists, there was progress in chemical genetics in the early part of the century. Onslow in England became interested in the coat colors of mammals and made important contributions to an understanding of the genetic control, through enzymes, of the formation of the melanin pigments. This line of work was continued and greatly extended by Sewall Wright and his students in this country (1942).

Under the influence of Bateson, Miss Wheldale in England—later Mrs. Onslow—early began a study of the genetics and biochemistry of the anthocyanin pigments of higher plants (1925). The facts established through her work and that of the Robinsons, Scott-Moncrieff, and many others (see review by Lawrence and Price, 1940) have been of the greatest importance in the development of our present concepts as to how genes act in regulating chemical processes.

In addition to Bateson, and Garrod, a number of geneticists and workers in other branches of biology speculated on the possibility of a relation between genes and enzymes. It was often suggested that the genes might themselves act directly as enzymes in at least some instances, a view that may still be entertained in special cases (see Horowitz, 1950). Moore (1910), Hagedoorn (1911), Leeb and Chamberlain (1915), Goldschmidt (1916), Wright (1916) and Troland (1917) were among those who early thought and wrote along this vein.
EYE COLOR IN DROSOPHILA

The writer's own interest in chemical genetics developed through several years' association with Boris Ephrussi beginning in 1933. In the hope that progress could be made through a study of development in the organism best known genetically—Drosophila melanogaster—investigations of the development of eye pigments were initiated in 1935. In this choice of material we were strongly influenced by Sturtevant's previous report of the non-autonomous behavior of vermilion eye color in gynandromorphs (1920). Our studies soon led to the discovery of two sequentially related gene-controlled diffusible substances called eye color hormones. The idea of single gene control of individual steps in a sequence of chemical reaction first took clear form in our minds as a result of this work, although in retrospect I am sure Garrod, Wheldale, Wright, Haldane and others many times previously entertained or at least gave consideration to this view.

The Drosophila eye color work led naturally to a chemical study of the eye color hormones and eye pigments, studies that through the efforts of Ephrussi and Khouvina, Tatum, Butenandt and Becker, and others (see review by Ephrussi, 1942) led to the chemical identification of the first v+ eye color hormone with the tryptophane derivative kynurenine. Although further studies by these workers and by Kikkawa (1941) in Japan made it obvious that kynurenine was oxidized to the second or cn+ hormone, all efforts to isolate and identify this second hormone in insects were unsuccessful.

The great difficulty of making progress in the identification of the cn+ hormone was a blessing in disguise as far as Tatum and the writer were concerned, for in frustration we were led to abandon the eye color work in Drosophila and undertake a series of studies on the gene control of biochemical reactions in the red bread mould Neurospora. With the new organism our approach could be basically different. Through control of the constituents of the culture medium, we could search for mutations in genes concerned with the synthesis of already known chemical substances of biological importance. Success with the new approach was even greater than the first fond hopes
—and they were high. We soon found ourselves with so many mutant strains unable to synthesize vitamins, amino acids and other essential components of protoplasm that we could not decide which ones to work on first.

It is perhaps a matter of considerably more interest to the parties personally concerned than of scientific importance that the chemical nature of the second eye color hormone of Drosophila was ultimately discovered through work on Neurospora. Mitchell and Nyc (1948) suggested that hydroxykynurenine is a product of kynurenine oxidation and a precursor of nicotinic acid in Neurospora. Butenandt, Weidel and Schlossberger (1949) then isolated this compound from Calliphora, made it synthetically and showed that it had cn+ hormone activity in Drosophila.

CHEMICAL GENETICS TODAY

The chemical nature of genes

In summarizing our present position in chemical genetics a natural first question is “what do we know about the structure of genes from a chemical viewpoint?” In spite of the fundamental importance of this question to all of biology and in spite of the great amount of effort that has gone into attempts to answer it, our knowledge is discouragingly little. That this is so should not really be surprising for genes are made up of the most complex compounds known to chemistry—proteins and nucleic acids. No one yet knows how the peptide linkage that binds adjacent amino acid components of a protein is established in a living organism and no one yet knows the sequence of amino acid components of even the simplest protein. In the case of the nucleic acids too our ignorance is great. In spite of these difficulties which chemists are working hard to remove, new ways of increasing our knowledge of the chemical nature of genes and chromosomes are being found.

Aside from pointing out that various lines of evidence, many of them indirect and circumstantial, agree in indicating that genes are largely if not wholly composed of nucleoproteins in which the nucleic acid component is of the deoxyribose variety, I shall leave the subject of chromosome and gene structure to those participants of this
symposium especially charged with its consideration. (See chapters by Mirsky, by Caspersson and Schultz, and by Darlington.)

**Genes as units of function**

In relation to heritable characters capable of being described at the present time in chemical terms, genes are known to control the specificities of many antigens such as the cellular antigens responsible for the many serologically distinguishable blood types in man. In many cases the antigenic specificity is known to reside in polysaccharides combined with amino acids (mucoids), a fact of great interest in connection with specificities of non-genic materials. The one-to-one relation so often observed between genes and antigens has led Haldane (1942) and others to suggest that antigens may well be direct gene products. The direct transfer of specificity between nucleoprotein gene and polysaccharide mucoid seems so improbable (Morgan, 1950) that one is tempted now to look for agents, possibly enzymes, that serve as intermediaries in the transfer. (See also chapter by Irwin for discussion of gene-antigen relations.)

A number of instances are now known in which protein specificities are gene directed. One of these recently described by Pauling et al (1949) and by Neel (1949) concerns the hereditary disease of Negroes known as sickle cell anemia. Here it appears that the electrophoretic mobility of the hemoglobin molecule is gene-controlled. Genetically homozygous affected individuals have homogeneous hemoglobin of an aberrant nature; the molecules being relatively more positively charged than are their counterparts in normal individuals. Heterozygous individuals, said to have the sickle cell trait, have a mixture of normal and aberrant hemoglobin molecules. It appears that the normal allele of the gene concerned directs the synthesis of normal hemoglobin molecules while the mutant gene is concerned with the elaboration of the aberrant molecules of hemoglobin. Red blood cells carrying abnormal hemoglobin in whole or in part are physically distorted under conditions of low oxygen tension.

Cases similar to alcaptonuria in which specific chemical reactions are known to be gene-dependent in relatively simple ways are now so numerous and have been reviewed so many times (Wright, 1941;
Tatum, 1944; Beadle, 1945, 1946; Horowitz, 1950) that there is little need to further discuss them in detail here. In two recently reported cases in Neurospora there is evidence of an enzyme difference between mutant strain and wild type. One of these involves pantothenic acid synthesis (Wagner and Haddox, 1950) and the other the formation of tryptophane from indole and serine (Gordon and Mitchell, 1950).

In the first case the enzyme concerned catalyzes the union of \( \beta \)-alanine and pantoyl lactone to form pantothenic acid. A mutant type is known in which the reaction does not go on at a sufficient rate to permit growth except under special conditions of culture. The enzyme can be demonstrated to be present in the mutant strain, under conditions that do not permit growth, by partial isolation followed by \textit{in vitro} tests for its activity. In the first reports of the tryptophane desmolase case it was found that enzyme could be demonstrated in the mutant strain unable to make tryptophane by dialyzing away certain substances present in the whole mycelium homogenate. In subsequent attempts to repeat these observations Mitchell (1950) was unable to demonstrate the enzyme in the mutant strain under any conditions, including those previously reported to yield active enzyme preparations. It therefore remains uncertain whether the mutant strain lacks tryptophane desmolase completely or possesses a modified enzyme inactive under normal \textit{in vivo} conditions.

\textbf{The one gene-one function hypothesis}

The numerous instances in which a single gene substitution in an organism results in a block in a single metabolic step have led to the hypothesis that many or all genes have single primary functions. The origin of this hypothesis is not easy to determine. It is implied in the writings of Garrod, Scott-Moncrieff, Wright, Haldane, Wheldale, and is explicitly stated by Grünberg (1938). Regardless of how and where it originated, the one gene-one primary function hypothesis has had a strong influence in the development of chemical genetics. It is fairly evident that almost every worker in the field who has set out to describe genetically controlled deviations from normal, whether or not he thought about it in these precise terms, has at least hoped to define the difference in some simple chemical way.
In addition to its value as a working hypothesis the one gene-one function hypothesis is a basically important concept in biology and as such deserves careful consideration. What is it exactly, how can it best be stated, and what evidence is there for it and against it?

Because enzymes so often appear to serve as intermediates between the gene and chemical reaction, the hypothesis has often been called the one gene-one enzyme hypothesis. To take into account the possibility that the specificities of non-enzymatic proteins, nucleic acids, and possibly other compounds are gene controlled, the hypothesis is better expressed in the more general form, one gene-one primary function. With respect to the specificities of enzymes the hypothesis requires that a given gene be concerned in a primary way with only a single enzyme. It is the same with respect to the specificity of other molecules, hemoglobin for example. The hypothesis does not require that the entire specificity of a given enzyme or other molecule be determined solely by a single gene, although this is a possibility and is often erroneously thought of as a necessary consequence.

How can the one gene-one function hypothesis be tested? Fortunately there are a number of ways and no doubt more will be thought of. An obvious first question is, can we be sure that there are at least some genes with single primary functions? While it cannot be proved rigorously, I believe it is generally agreed that the answer is yes. In the many mutant types found in Neurospora, and other forms, in which the metabolic deficiency is repaired through the addition to the medium of a single compound—the substance that would normally have been formed by the blocked reaction—it is difficult to avoid the conclusion that the primary action of the gene concerned is simple. It is true that there are so many possible sources of error that in any particular case a proposed interpretation is likely to be incomplete or incorrect. For example, it has often been pointed out that the repair of the organism through replacement from the outside of the deficiency that is the immediate result of genetic block is not likely to be complete. As in alcaptonuria, one or more intermediates normally preceding the block are likely to accumulate and produce secondary modifications. Such intermediates may accumulate as such or they may be metabolized through pathways not followed by the
normal organism. In either case they are likely to influence other reactions in one way or another. It would in fact be surprising if manifold end effects were not the usual result of mutation in single genes even though all of them had single primary functions. In spite of this type of difficulty, the examples are so numerous that collectively they constitute strong evidence that at least some genes have single primary functions.

A second question then is, what proportion of the genes have single primary functions? The hypothesis, in its simplest form, requires that the answer be, all. The most direct way to determine whether there are genes with multiple functions is to look for them in the same way in which those with single functions are looked for in Neurospora. This has been done and none has been found in which there are two obviously independent primary chemical deficiencies. But this is not a complete answer for if there were genes with multiple functions, the probability of finding them and interpreting them is obviously less than that for single function genes (Horowitz, 1950). There could be at least two reasons for this. In the first place, regardless of how many functions individual genes may have, we know that many functions are irreparable under any given set of environmental conditions. Many mutant types are lethal and in an organism like Neurospora cannot be saved for study. If a high proportion of gene functions are irreparable, say 9 out of 10, then it is evident that the technique of selecting mutants will strongly favor single function genes in which the function is reparable. The chance of a randomly chosen two function gene having two reparable functions will be only 1 in 100 if each function has 1 chance in 10 of being reparable.

On first thought there would seem to be no way to estimate the proportion of gene functions in Neurospora that are irreparable since whenever a gene with such a function mutates the single spore strain carrying it is lost. Horowitz has pointed out, however, that the so-called temperature mutants provide one way of making this estimate. These are mutant types which are normal over one part of the temperature range permitting growth of the original wild type but are synthetically defective over another part of the range. Temperature mutants affect a number of different syntheses and often involve
mutant genes allelic to non-temperature sensitive mutant genes concerned with the same synthesis. It is a possibility that these so-called temperature sensitive genes do not represent a random sample of genes concerned with function, but there is no reason at present to believe that this is so. At the temperatures at which these mutants fail to grow without a supplement, about half of them are reparable and half not so. In other words, this suggests that of the total gene functions represented about half are reparable. If these do represent a true random sample, then this proportion can be used to correct for loss of mutants in which one or more of the gene functions are irreparable. Of the several hundred biochemically mutant strains found in Neurospora, about 84 percent have single requirements. This means that a maximum of 16 percent will be mutants in which the genes concerned have two or more reparable functions. This is a maximum figure because many of those in the 16 percent will require single substances not yet tried, will be two-gene mutants, or will have secondary effects of a single impaired function that make them difficult to interpret. Based on the estimate that half the gene functions are reparable and that 16 percent of the mutant strains of Neurospora carry two-function mutant genes, Horowitz's calculation leads to the conclusion that about 73 percent of all the genes of Neurospora have single functions. The obvious and known sources of error could all work to make this a minimum figure. In other words the evidence is not necessarily inconsistent with the hypothesis that all genes have single functions. On the other hand because of several possible sources of error it does not prove beyond reasonable doubt that even 70 percent of the genes of Neurospora have only one function.

A second reason for failure to detect two-function gene mutants in Neurospora in which both gene functions are reparable is that the growth requirements of such mutants are less likely to be determined in simple tests than are those for single function genes. As a result such mutants will tend to appear among the “unknown” mutants. But if there is an appreciable proportion of genes with two or more functions and the proportion of total functions that are reparable is as high as one half, then the number of two function gene mutants in which both functions are reparable should be high enough so that
an appreciable number would have been discovered by the methods used. The fact is that while a number of mutants with more than a single growth factor requirement have been carefully studied in Neurospora, no clear case of a gene with double primary functions has yet been found.

Both of the above lines of argument indicate that most or all of the genes of Neurospora have single primary functions. It is of course possible that with a special distribution of separable and irreparable functions among genes with varying numbers of functions, many or even all genes might have more than a single function, for then the tests and calculations outlined above would be misleading. While there is no good reason to assume such a situation, it does seem to me that there is a special possibility that may deserve consideration. This involves the assumption that in addition to its specific catalytic function an individual enzyme protein in some other way simultaneously serves the organism in an indispensable respect. In this case, if the elaboration of the enzyme protein were under the direction of a single gene, gene mutation might result in loss of specific catalytic function without complete loss of the protein. Or a more extreme gene change, or one of a different kind, could bring about impairment in the non-catalytic function and result in the loss of an irreparable function. If such dual function proteins do exist, they could of course show a one-to-one relation to genes and at the same time each possess two functions, one separable and one irreparable. Mutations in a gene in control of such a protein might lead to loss of separable catalytic function only or to loss of irreparable non-catalytic function as well or separately. Complete loss of such a gene would invariably be lethal to the organism. If genes of this type do exist, it would to a certain extent be a matter of definition whether they were said to have one function or two.

Consideration of the above possibility is occasioned by the finding that many of the biochemically deficient mutants of Neurospora are capable of back mutations. If a particular gene has a single separable function, that is, is concerned in a primary way with a reaction the product of which can be supplied the organism from the outside, then it would seem that a frequent change induced by a mutagenic
agent, such as x-rays, would be complete loss of the gene. A single gene deficiency should not be lethal if the gene concerned has only a single repairable function but would be incapable of back mutation. While the number of genes studied carefully for back mutation in Neurospora is not large, such a high proportion of them appear to be capable of back mutation that one is tempted to wonder whether this might not indicate some relation such as that just suggested. Stadler has found that several x-ray induced mutant changes at the A locus in maize are lethal in the gametophyte while ultraviolet induced mutant changes at the same locus are not lethal. This has led him to suggest that all x-ray mutants may represent losses or re-arrangements of hereditary material rather than true reversible point mutations. Assuming this to be correct one might still expect non-lethal single gene deficiencies for the A gene if this has a single function not essential for survival of the organism. Failure to obtain these may of course mean only that all the changes analyzed by Stadler involve loss of more than the A locus. On the other hand, if the A gene is concerned with the elaboration of a protein the presence of which is essential to the life of the organism as well as being concerned catalytically in the biosynthesis of anthocyanin, it would not be possible to produce a viable A-gene deficiency.

In connection with hypotheses of gene action, an understanding of the so-called compound loci known to exist in a number of organisms is important. Among these are the A and R loci in corn (Laughnan, 1950); the lozenge and bithorax loci in Drosophila (Green and Green, 1949; Lewis, 1948) and the albino and "Q" loci in Neurospora (Hungate, 1945; Bonner, 1950). In each of the cases of the A, lozenge, bithorax and albino loci there is evidence indicating that two or more related and closely linked genes are concerned. It seems probable that in the bithorax case, at least, the proper functioning of the three genes that appear to be involved is dependent on their close proximity (Lewis, 1945). The R locus in corn can be interpreted as a single gene with at least two mutation sites and at least two closely related functions or as two or more separate genes with closely related functions, located so close together in the chromosome that there is no crossing over between them. Compound loci of these types may
well be of more general occurrence than is now realized. In any event
a fuller understanding of them will certainly contribute in an im-
portant way to an understanding of gene action.

As pointed out earlier it is possible to imagine that the entire
specificity of a given enzyme is determined by one and only one gene.
On first thought, it would seem easy to test this special form of the
one gene-one function hypothesis, for if it were correct, a series of
mutants showing absence of activity of a given enzyme should all
show mutation in the same gene. Such a test has been made by
Markert (1949) using color mutants in the fungus Glomerella. These
are easy to detect and most of them seem to result from altered
tyrosinase activity. The experimental result is clear—if a series of
colorless mutant types are induced with ultraviolet for example, they
are found in most instances to result from single gene changes. Their
tyrosinase activities are found to approach zero. When tested against
one another many of them are found to be genetically distinct. In
other words, it is quite clear that tyrosinase activity is reduced or
done away with completely through mutation in any one of a num-
ber, probably a relatively large number, of genes. Unfortunately the
interpretation is not so simple. One possibility is that the specific
property of tyrosinase is dependent directly on the simultaneous ac-
tivity of a large number of genes, each contributing in a small way
to the final specificity. But this is by no means the only possibility.
Since tyrosinase is determined by its catalytic activity, it is possible
that in the mutant strains under discussion there is a normal amount
of tyrosinase which is inactive for secondary reasons. This is made
improbable by the findings of Markert and Owen (1950) that all
mutant strains tested serologically in which tyrosinase activity is
absent are found to lack detectable amounts of the antigenic activity
characteristic of tyrosinase. A third possibility is that tyrosinase is an
enzyme the formation of which is extraordinarily sensitive in Glomer-
ella and that a mutation in any of a large number of genes will
decrease or abolish its production in indirect ways. That this may
indeed be the case is suggested by the fact that a change in the cul-
ture of the standard strain from agar slants to liquid medium may
abolish tyrosinase production both as determined by activity measurements and as estimated serologically.

The tyrosinase studies of Markert and Owen emphasize a general difficulty in all attempts to identify primary gene functions. There is no sure way of knowing when the gene effect with which one is concerned is a primary one and when it is not. This is a difficulty that has been pointed out before, for example by Emerson (1950) in connection with his studies of a sulfonamide-requiring mutant strain of Neurospora. And, unfortunately, it is not a difficulty that one sees an easy way around. Intuitively, I feel that in a great many cases in Neurospora the reaction under primary gene control has been identified, but I confess I know no way of proving it in even a single instance.

In summary it can be said that while the one gene-one function hypothesis has without doubt served a useful purpose and while there are no compelling reasons for abandoning it at this time, neither should it be accepted without reservation. Even if it should prove correct in principle, like many useful working hypotheses in science it may well be found to err in the direction of oversimplification.

**CHEMICAL GENETICS TOMORROW**

While obviously no one can foretell with any certainty what developments the future will bring in chemical genetics or just how such developments will bear on the many problems we see today, at least some of the general directions along which future progress will be made seem clearly marked.

As is made clear in other chapters of this book (for example, Mirsky, Caspersson and Schultz) there can be no doubt that chemical genetics of the future will be greatly assisted through advances in the general knowledge of the chemistry of proteins, nucleic acids, and other large molecules of key biological importance. As new methods are developed and old ones improved they will find their application in the study of chromosomes and genes. Combined with approaches from the biological side such advances in chemical knowledge are essential for any real understanding of the manner
in which genes reproduce themselves and produce their characteristic effects.

A direction in which progress in understanding gene action seems highly probable is represented by the recent work of McClintock (1950) in which a striking relation is shown between gene function and proximity to heterochromatin as well as between heterochromatin and gene mutation. Relations have been observed in Drosophila that are sufficiently similar to make it clear that the phenomena involved are of general importance. An increase in understanding of them may well go a long way toward clearing up the question of function of heterochromatin, its relation to the ribose nucleic acid of the cytoplasm, and a host of vexing related problems.

In connection with gene action in general and in relation to differentiation in particular, it will certainly be essential to learn more about cytoplasmic constituents. As Wright, Sonneborn, Spiegelman, and others have suggested, it seems probable that there exist in the cytoplasm gene-initiated semi-autonomous self-duplicating agents that are essential in differentiation within higher organisms and in function in all living systems. In full recognition of the outstanding achievements of Sonneborn, Ephrussi, Billingham and Medawar, and others in this direction, it cannot be too strongly urged that new ways of investigating such cytoplasmic agents be searched for. This is an attack in which geneticists, cytologists, enzymologists, and biochemists all have essential parts to play (see chapters by Sonneborn and by Ephrussi.)

In connection with studies in the gene control of metabolism it is evident too that much more than is now known must be learned about working with enzymes and enzyme systems in organisms well suited to genetic study. In Neurospora, as Mitchell has pointed out (1950), relatively little has been done in the direction of developing methods of isolating and otherwise studying enzyme systems. The classical methods of animal enzymology often apparently require drastic modification when applied to microorganisms, possibly because of a lesser tendency toward localized accumulation in specialized cells. Many of the problems of gene-enzyme interrelations could be solved if instances were known in Neurospora, for example, of
gene-controlled easily-crystallizable enzymes for which simple and sensitive activity tests were known. It does not seem unreasonable to expect that if sufficient effort is made to do so, these will be found.

With the recent rapid extension of the approach of chemical genetics to problems of bacteriology (see chapter by Lederberg), it is certain that the unique advantages of bacteria will continue to be made use of in the solution of important problems. The demonstration of recombination phenomena by Lederberg and Tatum has already modified our thinking about evolution in bacteria and has provided a powerful tool for the solution of many important genetic problems in these relatively simple cells. The new knowledge of transforming principles in bacteria has certainly introduced another chapter in genetics, and one that promises to be among the most exciting.

It has given chemists new incentive to learn about the nucleic acids, compounds which everyone recognizes to be extremely important biologically and about which so little is yet known.

In the field of virology to which genetics has now been extended with such spectacular results we can expect achievements that will greatly increase our understanding of the ways of genes. For example, in the simpler viruses, such as tobacco mosaic virus, the direct chemical approach seems admirably suited. Knight and Stanley (Knight, 1947) have already shown that strains assumed to have originated from a common source show definite differences in amino acid composition. There seems to be no reason in principle why such studies cannot be extended to virus strains known to differ through a single mutational step.

In the T-series of bacterial viruses of Escherichia coli, Luria, Delbrück, Hershey and others have shown that virus units are made up of subunits that mutate, recombine, and otherwise behave very much like genes. There are many tricks by which these subunits can be studied (Delbrück, 1950).

The bearing of chemical genetics on theories of evolution has been considered many times. It has already contributed in a significant way to our appreciation that there are remarkable similarities in the basic biochemical properties of diverse groups of organisms and to our
understanding of how biosynthetic reactions may have evolved (Horowitz, 1945). Unquestionably it will continue to play an important role in our thinking about the processes of evolution and their significance.

These brief considerations on the future of chemical genetics are obviously incomplete and for two good reasons—limitation of time and space and limitations in the writer’s foresight.

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REMARKS ON CELL HEREDITY

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IT is appropriate, I feel, to begin today as Professor Muller did with a quotation from the writings of one of the great proponents of modern genetics, E. B. Wilson. The following lines conclude the historical sketch given in the Introduction to the last edition of "The Cell in Development and Heredity," revised by Wilson himself:

1 The determinative action of the nucleus in development was thus finally placed beyond doubt, but probably no investigator would today maintain that the nucleus or the chromosomes are the sole agents of heredity. On the contrary, both cytological and experimental research have clearly demonstrated that the protoplasm (cytoplasm) plays an important part in development. This has been directly proved on the cytological side by experiments on the development of egg-fragments by Boveri, Driesch, Fischel and later investigators, while indirectly the same conclusion is indicated by genetic experiments on the part of Correns, Toyama and others. With reference to this problem much interest has been aroused in recent years by cytological studies on the mitochondria or chondriosomes, cytoplasmic structural elements now widely believed to play an important part in the chemical activities of cells and perhaps also in differentiation; by some authors, accordingly (Benda, Meves) they have been regarded as representing a mechanism of "cytoplasmic heredity" comparable in importance with that represented by the chromosomes. This view, still very far from substantiation, remains a subject of controversy and must be taken with proper scepticism; but in spite of its doubtful status it should be kept clearly in

view in all cytological discussions of these problems. To some extent, perhaps, our conclusions concerning the chromosomes have thus far been more definite and fruitful because we are able to follow their history more readily. (Wilson, 1928)

Twenty-five years after these lines were written, the statement still holds. However, the rapid rise of genetics, resulting in the ever widening recognition of the universal character of Mendelian mechanisms, has indubitably overshadowed both the recurrent claims of evidence for the occurrence of extranuclear heredity, and the theoretical need for postulating its existence. No doubt, to be effective, revolutionary ideas, whether political or scientific, have for a time to disguise themselves in simplified forms and to pretend to the monopoly of virtues. Moreover, since science proceeds by successive approximations, a prevailing theory has of necessity to explain all the facts it can account for, before a new theory can be allowed in. It is only natural therefore that the complete exploration of the Mendelian principles should have delayed the recognition of cytoplasmic heredity.

Wilson was right in assuming that, as he said in the last quoted sentence, “perhaps our conclusions concerning the chromosomes have thus far been more definite and fruitful because we are able to follow their history more readily.” The role of plastids as extranuclear units endowed with genetic continuity is unquestioned precisely because their fate can be directly observed. But this need not be the case of all cytoplasmic self-perpetuating elements. The rigorous demonstration of their role in heredity is therefore primarily dependent on the differentiation of their effects from those of the nuclear genes. Only now that the limits of possibilities offered by Mendelian mechanisms are known to an appreciable extent, can their operation be effectively excluded in some cases. Thus we have the right to say that whatever progress has been made in the study of non-Mendelian inheritance, it is ultimately due to . . . Gregor Mendel

As the title of the present paper suggests, it is not my intention to give an exhaustive review of the present status of the problem of cytoplasmic heredity. A number of cases of cytoplasmic heredity will be discussed in other connections by Sonneborn at this symposium. I
should like rather to limit myself to a few considerations concerning cellular differentiation, a process most compelling with respect to the previously mentioned theoretical necessity of postulating the existence of extranuclear heredity.

**THE PROBLEM OF CELLULAR DIFFERENTIATION**

Since the meristic character of mitosis lends no support to the early theories of sorting out of nuclear units during the orderly process of ontogenetic development, the explanation of the gradual specialization of the different cell lines must be sought either in terms of variation by means of mutation, sorting out or intracellular selection of autonomous cytoplasmic components, or in terms of specific responses of nuclear genes to local differences prevailing in the different regions of the egg cytoplasm, or a combination of both mechanisms.²

The fact that the initial anisotropy of the egg can be set up, in some cases, by very trivial external stimuli, offers no escape from this conclusion, for it seems to be well established today that the differentiation, that is the specialization of potencies in the different definitive tissues, is persistent if not irreversible. This fact, established in particular by experiments of grafting and tissue culture, indubitably shows the existence of cell heredity. On the other hand, the existence of transitory states of labile determination, preceding the irreversible phase, points to the necessity of mechanisms allowing for various degrees of reversibility. This requirement makes the hypothesis of directed gene mutations or activation, although not impossible, less likely than the alternative interpretation mentioned above. This view is further supported by the fact that apparently irreversible cytoplasmic differentiation often is apparent even before the division of the cell nucleus.

Turning then to the other interpretation, I wish first to repeat that whatever mode of cytoplasmic variation is postulated, it must be based on the assumption of self-reproducing units. This is what Delbrück (1948), in a very alluring recent suggestion, has attempted to

² The recent findings of McClintock (1950) may, however, call for a revision of the notion of the equational character of mitosis.
avoid. Instead, he resorts to the notion of flux equilibrium between mutually exclusive reactions, capable of shifting the cell, under the influence of transitory changes of the environment, from one state of stable equilibrium into another, alternative one. Aware, no doubt, of the irreversible character of certain cell differentiations, he emphasizes that systems of this sort would be capable of all possible degrees of stability, and that the shifts from one stable state to another could be either reversible or irreversible. I feel however that, as soon as the occurrence of irreversible change is assumed, there is no real escape from the conclusion that a self-reproducing unit or a part of it is involved. The simplest way of visualizing such a unit consists of course in picturing it as limited in space, that is in attributing to it a particular nature.

Plasmagenes and differentiation

Once the existence of such units or plasmagenes, as they sometimes are called, is taken for granted, two general types of hypotheses are possible and have been proposed as to their behavior in the process of differentiation. At the basis of one of these is the view that differentiation results in the restriction of the manifold potentialities of an originally totipotent egg and can therefore be achieved for example through the segregation or sorting out of an initially mixed population of plasmagenes. The alternative scheme depicts differentiation as the orderly widening of the potentialities of an initially nullipotent protoplasm. Such a result, which is really equivalent to the directed mutation of plasmagenes, could be achieved, as Sewall Wright (1941) has pointed out, for example by the activation, in the course of development, of physiologically inactive plasmagenes by their combination with prosthetic or haptenic groups of independent (either nuclear or extraneous) origin. I will leave open for the moment the problem of the ultimate origin of the plasmagenes themselves, and will only add now that the combination of several of the mentioned mechanisms, including that of Delbrück’s flux equilibria, seems to me to provide the basis of a general theory of differentiation and cell heredity. The detailed elaboration of such a theory must naturally await the verdict of facts as to the validity of its postulates.
Turning to the relevant evidence, we find that the existence of self-perpetuating cytoplasmic units in most types of cells needs no demonstration. Centrioles, blepharoplasts, plastids, kinetoplasts and ketosomes unquestionably belong to this category, which probably includes also the mitochondria. The first open question which requires a solution is rather that of whether the variations of these elements can account for the differentiation of cell lines. Were we not especially concerned with somatic cells, a great deal of the required evidence could be contributed by the classical genetic technique of crossing. Its equivalent, nuclear transplantation and the transfer of portions of cytoplasm from one irreversibly differentiated type of somatic cell to another, similarly could provide answers to the major problems raised by cellular differentiation. But neither of these experiments has been achieved as yet on metazoan cells and consequently the required direct information is not at hand. The closest approximation to it is the indirect evidence provided by crosses between clones of organisms possessing no isolated germ line. Such studies have been performed on several organisms. Some have revealed highly interesting nucleo-cytoplasmic relations, most suggestive with respect to the problem under consideration, and their bearing on it has been fully discussed by Sonneborn (1950). I will therefore limit myself to some evidence which can be adduced from the study of yeasts, a material with which I am more familiar.

EXTRACHROMOSOMAL HEREDITY IN YEASTS

Since in what follows emphasis will be placed chiefly on extrachromosomal phenomena, it may be well to begin by stating that, in spite of the lack of satisfactory cytological data, the occurrence and widespread operation of Mendelian mechanisms in yeasts is established beyond doubt by the work of Winge, of the Lindegrens, and of their collaborators. Dependence on simple Mendelian genes has been demonstrated for a series of morphological and biochemical characters. But almost from the very beginning of breeding work with yeasts, cases have been encountered, the explanation of which in Mendelian terms appears difficult if not impossible. The first facts of this
kind have emerged in Winge and Laustsen’s study of the effects of inbreeding in *Saccharomyces cerevisiae*, var. *ellipsoideus*. Winge and Laustsen (1940) observed that, depending on their mode of origin, genotypically identical and homozygous diploids produce spore progenies widely differing in their germination power. When, in particular, “illegitimate” diploids produced by “cell zygotes,” that is by zygotes formed by the fusion of two haploid sister cells, are compared with diploids formed in the same clone through “direct diploidization,” that is nuclear fusion in a single undivided cell, it is found that the germination frequency of ascospores is much higher in the former (Fig. 1). Furthermore, the loss of germination power is apparently permanent and cannot be restored by the intervention of other methods of “illegitimate” diploidization (Fig. 2). This fact, together with the genotypic identity of the two sorts of diploids, points to the operation of a non-chromosomal hereditary mechanism. Winge and

![Fig. 1. Effect of different modes of diploidization on the germination power of ascospores. (After Winge and Laustsen, 1940.)](image.png)
Laustsen therefore suggested that the difference between these diploids may depend on a difference in their chondriosome content and offered, for the origin of this difference, the hypothetical scheme represented in Figure 3. It is assumed that the chondriosomes divide once per cell cycle, shortly after the nuclear division. Since, in direct diploidization, the two nuclei resulting from mitosis immediately fuse to form a diploid nucleus, chondriokinesis is suspended. Thus the number of chondriosomes is unchanged in the diploids produced by direct diploidization, while it is doubled in cell zygotes. It is worth noting that, according to Winge and Laustsen (1940), direct diploidization in S. validus does not produce similar effects. This is interpreted by assuming that in this species the chondriosomes divide almost simultaneously with the nucleus. The facts described by Winge and Laustsen thus lead to the assumption of the existence in yeast cells of a numerically rather constant quota of autonomous

Fig. 2. Loss of germination power of ascospores following “illegitimate” diploidization.
cytoplasmic elements, possibly chondriosomes, the multiplication of which is closely correlated with nuclear division.

Omitting several non-confirmed cases of non-Mendelian inheritance of enzymatic characteristics in yeast, I now come to a most interesting phenomenon observed by Lindegren and Lindegren (1947) in a red yeast produced by Tatum and Reaume by mustard gas treatment of a haploid white yeast. The red yeast is adenine-dependent. Since in crosses with adenine-independent white yeast,

the adenine-dependence and the red color segregate together in a purely Mendelian fashion, these two characters obviously are the effects of a single gene.

The red yeast in its vegetative reproduction frequently gives rise to white colonies. The cells composing these colonies retain however the adenine-dependence. Similar white clones occasionally originate also directly from adenine-dependent ascospores. The new character of these exceptional clones, that is the absence of color combined with adenine deficiency, is retained indefinitely in the course of vegetative reproduction, but instantly vanishes on crossing to either adenine-dependent or adenine-independent non-exceptional clones.

These results of the Lindegrens have been confirmed by our own data (Ephrussi, Hottinguer and Tavlitzki, 1949) obtained with a red yeast of independent origin. Furthermore, our crosses of the
adenine-dependent white clones with normal, adenine-independent white clones, were followed by four successive backcrosses to the mutant clone. In spite of the repeated backcrosses, the mutant character did not reappear regularly in the ascis. The explanation of these results in Mendelian terms seems impossible, unless the concomitant mutation of at least 20 genes is postulated. Such an assumption however appears most unlikely in view of the very high spontaneous mutation rate. Indeed, it appears more likely that a non-Mendelian mechanism is at work. But since, as will be presently shown, the behavior of red color is but a corollary of a much more fundamental cellular change, the discussion of this mechanism will be deferred until after the presentation of some data unavailable at the time of Lindegrens’ publication.

Mutation in a cytoplasmic element

Any population of baker’s yeast contains a small proportion, roughly one per cent, of cells which give rise to dwarf colonies (Ephrussi, Hottinguer and Chuménès, 1949). The slow growth of these cells is due to their inability to utilize glucose by the more efficient method of respiration, so that, even in the presence of oxygen, this sugar is fermented instead of being oxidized (Tavlitzi, 1949; Słominski, 1949). The respiratory deficiency is due, in turn, to the lack in the mutant cells of two essential respiratory enzymes, cytochrome oxydase and succinic dehydrogenase (Słominski and Ephrussi, 1949), and is a permanent property of the mutant cells; it is retained indefinitely in the course of vegetative reproduction. The mutation appears to be irreversible.

The exceptional white, adenine-dependent yeasts referred to earlier belong to this type of respiratory mutants and the absence of color in this particular case results from the fact that the formation of red pigment is indirectly dependent on the presence of the cytochrome system. Under certain cultural conditions it does not take place in the mutants lacking two essential components of this system.

Chemical induction of cytoplasmic mutations

The respiratory mutants appear during vegetative reproduction of yeasts, whether red or white, with a frequency which corresponds to
a mutation rate of approximately 2 per 1,000 and per cell generation. Moreover, this high mutation rate can be enormously enhanced by growing the yeast in the presence of certain acridines. This fact offers a unique opportunity for the study of the mutation process, as shown by the following experiments:

Single cells from a normal diploid yeast strain are isolated with the help of a micromanipulator in droplets of culture medium containing one part of eucflavine per million. Their budding is observed under the microscope and the successive daughter cells are detached from the mother cell as they form, rinsed and removed to droplets of normal medium. The mother cell, on the other hand, is transferred at intervals to fresh drops of the same medium. Similarly, one second generation bud, formed in normal medium, is isolated from each of the daughter cells. Finally, at the end of the experiment, the mother cell itself is placed in normal medium.

Similar isolations are performed in the absence of eucflavine to provide controls. After the isolated cells have formed sizeable clones, the latter are transferred to test tubes containing the ordinary culture medium. Thus stock cultures are established which can be tested, immediately or after a series of transfers, for the presence of indophenol oxidase.

The results of five such experiments and their controls are given in the form of "pedigrees" in Figure 4. It can be seen that, whereas no spontaneous mutants were found in the controls, more than half of the descendants of the cells placed in eucflavine gave rise to mutant clones. Since the experimental setup offers no opportunity for selection aside from cell mortality and since the latter was nil in the described experiments, we have here an example of induced mutation, about the mechanism of which further inferences can be made by considering the pattern of the cell lineages shown in Figure 4. We see that, in the experiment, all mother cells after producing several mutant buds, remained capable of producing some normal ones and, in any case, remained normal themselves. This makes the hypothesis of a gene mutation, or of a mutation of any cell constituent for that matter, very unlikely, for it is difficult to understand how qualitatively modified replicas are continuously produced by an element which, itself, remains unchanged. The appearance of mutant
cells following eukaline treatment seems to be more easily accounted for by assuming the failure of a self-reproducing cytoplasmic component necessary for the synthesis of the respiratory enzymes in question to be included in some of the buds. Since a cell can alternately produce mutant and normal buds, this cytoplasmic component must

be assumed to be of a particulate nature, and its occasional loss in untreated cells may be taken as the cause of spontaneous mutation.

Such an interpretation is, you will note, in line with the results of the crosses between normal and mutant cells to which I referred earlier, in speaking of the red yeast. If these two cell types are identical with respect to their genes, and if they differ by the presence in one of them, and the absence in the other of a self-reproducing
cytoplasmic agent, it is to be expected that, as is actually found, the mutant character should disappear in such crosses.

I will be excused, I hope, for making at this point a short digression. Figure 5 shows the normal division of a Trypanosome with its nucleus, kinetosome and kinetoplast, and the division of the same organism in acriflavine which, as shown many years ago by Werbitzki (1910), induces the appearance of individuals devoid of kinetoplasts. It can be seen that the dye selectively inhibits the duplication of this autonomous element. Is it not tempting to regard this

![Fig. 5. Effect of acriflavine on a dividing Trypanosome; inhibition of the division of the kinetoplast.](After Lwoff, 1948.)

as a model for the mechanism by which acridines induce in yeast cells the deficiency of the units responsible for the synthesis of certain respiratory enzymes?

ARE THE CYTOPLASMIC UNITS MITOCHONDRIA?

Returning to the yeasts, one may wonder whether microscopic examination of the cells gives support to the hypothesis of cytoplasmic particles. When the so-called Nadi reaction, characteristic of indophenoloxidase, is performed on suspensions of normal and mutant yeasts, the former develop a deep blue color, the latter remain white. Microscopic examination of normal cells reveals that the blue color is concentrated in a rather small number of cytoplasmic granules. There can be seen many granules in each cell, but only a few of them are
colored. The cytoplasm of the mutant yeast also contains many granules, but none of them are colored. Unfortunately, taken by itself, this evidence does not justify the very tempting conclusion that the blue granules actually correspond to the postulated self-reproducing units, for, on one hand, there is no proof that the color is actually produced in the granules; and, on the other, were this proven, we would be entitled to say that the visible granules carry the enzyme, but the identity of the enzyme or of the structure supporting it with the postulated particles would still require demonstration. However, in spite of the lack of such proofs, the presumption that the blue granules actually are the hypothetical particles remains strong, for it is supported by indirect evidence: I am referring to the results of many recent cytochemical studies performed on the so-called "large cytoplasmic granules" separated by centrifugation and identified with the mitochondria.

In many cells, the major part of several enzymes or enzyme systems have been shown to be carried by the mitochondria. Examples are the enzymes responsible for the oxidation of fatty acids and those operating in the integrated reactions of the Krebs' tricarboxylic cycle (Kennedy and Lehninger, 1949; Schneider, 1948; Schneider and Potter, 1949). Particularly interesting is the presence in mitochondria of many organisms, including yeast, of cytochrome oxidase and succinic dehydrogenase (Hogeboom et al., 1946; Chantrenne, 1943). This is true however only for what I have called "normal yeast": the mitochondrial fraction of the respiratory mutants lacks these enzymes (Slonimski and Ephrussi, 1949). This is in line with the interpretation of the "blue granules" as the actual bearers of indophenol oxidase. It appears therefore that, as suspected by some cytologists, the mitochondria of a cell represent a heterogeneous population, and this seems to be further supported by the fact that in the rat liver, according to Schneider and Hogeboom (1950), cytochrome c is also carried by the mitochondria. Cytochrome c is present in both normal and mutant yeasts. Thus, if cytochrome c in yeast were also carried by the mitochondria, one would have to consider the possibility that the mutants contain the cytochrome c carrying mitochondria, but not those carrying cytochrome oxidase and succinic dehydrogenase.
If we remember now that many cytologists, on good grounds, consider that the mitochondria do not arise *de novo*, it appears that the weight of the combined evidence is in favor of the identification of the postulated self-reproducing units with one class of a mixed population of mitochondria.

If this view is accepted, we have then a consistent interpretation of how the two cell lines of identical nuclear constitution are differentiated from each other in a way which satisfies the requirements of the lasting differentiation of metazoan cells. At the cell level, the differentiation of the two lines of yeasts appears as a mutation; in terms of intracellular mechanism however, if the ensemble of the population of intracellular units is taken into account, it is to be regarded rather as the result of a segregation. This segregation, accidental in the case of spontaneous mutation, can be directed by an environmental factor, which causes an irreversible restriction of the potencies of newly formed cells without apparently affecting the totipotency of the generating cells. It may be rash to see in this relationship an exact equivalent of the relations existing in the metazoan organism between the cells of the germ line and those of somatic tissues, but such a parallel does indeed suggest itself.

**THE RELATION OF NUCLEAR AND CYTOPLASMIC ELEMENTS**

These views are not to be taken however as implying a complete duality of the cell with its chromosomal genes controlling numerous functions, on the one hand, and a heterogeneous system of cytoplasmic units entirely escaping genic control, on the other. In this connection, another (slightly abbreviated) quotation from Wilson is interesting:

Whether all hereditary traits are represented in the nuclear organization is an open question. At least a partial exception seems to be offered by plastid-inheritance; and should a similar conclusion apply to the chondriosomes, Golgi bodies and other formed elements of the cytoplasm . . . we should still have to reckon with the possibility that such self-perpetuating cytoplasmic bodies might take their first origin in the nucleus or be influenced by its activity.
The establishment of any of these two types of dependence of a cytoplasmic component on the nucleus naturally depends on the accident of the occurrence and discovery of a gene mutation affecting the component in question. Numerous gene mutations affecting the activity of chloroplasts are known, whereas none have been found, to my knowledge, which prevent their multiplication.

Similar relationships between the genome and the cytoplasmic units responsible for the synthesis of respiratory enzymes recently suggested themselves when a yeast strain was found in which nearly one half of the ascospores give rise to respiration-deficient clones. The respiratory deficiency was shown to be again caused by the lack of the same two enzymes and it was thought that, in this case, it might be due to a gene mutation, the phenotypic manifestation of which is identical with that of the loss of the cytoplasmic units. This interpretation was born out by crosses between mutant and normal haploid cells. The diploids produced by the cross are normal and, as shown in Figure 6, a simple Mendelian segregation is observed in the hybrid asci, which contain two normal and two mutant spores. Thus, the respiratory deficiency is clearly the effect of a recessive gene in the case of these particular mutants, which I shall call "segregational mutants" in order to distinguish them from the "vegetative mutants" discussed earlier and which are also encountered in the yeast strain under consideration.

The rather extraordinary coincidence that the recessive gene causes the simultaneous absence of the same two enzymes which are lost in the vegetative mutants, is certainly a further indication of their close structural association.

Interaction of gene and cytoplasmic particle

While the just quoted results trace the respiratory deficiency of the segregational mutants to the presence of a Mendelian recessive, they do not, in themselves, reveal the relationship between this locus and the cytoplasmic units. This relationship emerges from the cross between segregational mutants and vegetative mutants, derived from the same normal strain. This cross leads to an entirely new result: the fusion of the two deficient cells results in the reconstitution of
FIG. 6. Results of crosses between normal, segregational mutant and vegetative mutant in strain B-II. Small black circle indicates dominant gene; small white circle indicates recessive gene. Black cytoplasm indicates the presence of active cytoplasmic particles, grey cytoplasm that of inactive cytoplasmic particles; white cytoplasm indicates loss of the particles.

a normal diploid cell. Moreover the asci formed by the latter, as shown in Figure 6, again contain two normal and two mutant haploid spores. These results, obviously incompatible with any purely Mendelian scheme, can be brought into agreement if it is recalled that, as shown earlier, there is no genic difference between normal cells and vegetative mutants, and that the phenotype of the latter is due to the loss of self-perpetuating cytoplasmic units; and if it is further assumed that the segregational mutants do contain these units, which are here physiologically inactive, owing to the presence of a recessive gene.

I will refrain from quoting the results, given elsewhere (Chen, Ephrussi and Hottinguer, 1950), of the many crosses undertaken in order to verify these assumptions, and will only say that they all are
in harmony with the view that the synthesis of the two respiratory enzymes is under the joint control of a nuclear gene and of cytoplasmic particles. These particles, independent of the genome in their reproduction, depend on it for their function; they are active only in the presence of the dominant gene.

I have earlier presented evidence which supports the view that differentiation of cell lines can be achieved by the segregation of cytoplasmic components. How near the just quoted new evidence has brought us to supplying a basis to the opposite view of widening cell potencies in the course of ontogenetic development, can be judged by comparing our conclusion with the following suggestion of Sewall Wright (1941):

It may be . . . that the more or less complete early isolation of the germ line of higher organisms has come about in evolution to maintain a line of cells with plasmagens lacking in prosthetic groups and hence in specialized activity but capable in somatic cells of combining with such groups emanating from the nucleus to form molecules that multiply thereafter as plasmagens of a more specialized sort.

You can see that the validity of two postulates of this theory has been established, namely: first, the ability of propagation of plasmagens lacking in specialized activity (in the segregational mutants), and, second, the possibility of their being activated by something emanating from the nucleus (in the heterozygous cell zygotes).

**GENERAL DISCUSSION**

As I said at the outset, the purpose of this discussion was to show that the study of inheritance in microorganisms can supply some elements of a general theory of differentiation and cell heredity. The elements I have discussed concern only one of the two demonstrated types of cytoplasmic variation which may be thought to play an important role in cell differentiation. While the examples I have selected have all been taken from yeast genetics, the same principles could have been illustrated by the Kappa system of Paramecium. The other type of cytoplasmic variation, which is exemplified by the antigenic system of Paramecium, and which I believe also plays a
prominent role in cell differentiation, will be presented by Sonneborn.

I hope we will have shown that the elements of a general theory of differentiation are thus being accumulated; that once more the genetic tool can be effectively used in the exploration of a field previously considered foreign ground; and, above all, that the problem of differentiation now definitely lies within the province of the geneticist.

This view the geneticists have for a long time been reluctant to accept, and one of the reasons for this is clearly expressed by a remark of S. Wright (1941) which really prompted him to write the sentence quoted earlier. He writes:

The usual and most probable view is that cellular differentiation is cytoplasmic and must therefore persist and be transmitted to daughter cells by cytoplasmic heredity. The chief objection is that it ascribes enormous importance in cell lineages to a process which is only rarely responsible for differences between germ cells, at least within a species.

Let us briefly consider this paradox. One possible solution of it has been given, as you have seen, by Wright himself, by his suggestion that the germ line carries "plasmagenes lacking in prosthetic groups." In addition, several other reasons for the rarity of cytoplasmic heredity, or for the difficulties of its demonstration, inherent both in the mechanisms involved and in the methods used for their detection, can be suggested. It must be emphasized that some of the criteria commonly adopted in the search for cytoplasmic inheritance are hardly tenable. It is usually assumed that, were the cytoplasm the carrier of self-perpetuating units, its abundance in the egg and scarcity in the spermatozoon should result in differences in reciprocal crosses. This view neglects however both the microscopic or sub-microscopic size of cytoplasmic elements such as the chondriosomes and the fact that the latter are, aside from apparently rare exceptions, carried in the middle-piece of spermatozoa and thus introduced into the zygote. It is usually assumed also that the random distribution of cytoplasmic units should frequently result in their loss. But one must not forget that many cells are sheltered against such accidents by mechanisms guaranteeing the rather even distribution of the elements under con-
sideration during the process of cell division. It may well be, indeed, that the high frequency of such losses in yeasts, as compared with other cells or organisms, is correlated with their reproduction by budding.

In view of these considerations, and unless the loss of cytoplasmic particles occurs in early gametogenesis (where it is least probable), the emergence in a higher organism of the equivalent of our "vegetative mutant" would be a rather unique event, and the individual in question would be considered as a phenotypic variant, for it would be offered no chance to transmit its character.

Further possible difficulties can be inferred from our study of yeast mutants. Assuming that the presence in a cell of inactive cytoplasmic particles confers on its carrier no selective advantage, it was to be expected that clones of segregational mutants will in the long run almost inevitably lose the inactive particles. Indeed, unless special precautions are taken, this is what most frequently happens. The crosses of such "dual mutants" with vegetative mutants are sterile; diploid cells resulting from the fusion of two cells devoid of cytoplasmic particles never sporulate. Since, on the other hand, the crosses between normal and either segregational or dual mutants give identical, purely Mendelian results, it is evident that we owe the most convincing demonstration of both the existence of cytoplasmic units and of their activation by a Mendelian gene to a most fortunate turn of events.

We may finish this enumeration of the possible reasons for the apparent rarity of cytoplasmic heredity by the question: could it be that cytoplasmic heredity is more specially concerned with fundamental cellular functions? If this were so, its variations would be most of the time incompatible with survival and therefore escape detection. The discovery of the mutants lacking a complete respiratory mechanism in yeast was possible because of the ability of these organisms to derive their energy through the alternative fermentation channel as well. But such an extraordinary adaptation is hardly duplicated by many organisms or functions.

Lastly, I wish to return briefly to a problem previously left open. It will have been noticed that the properties of independent repro-
duction and gene-dependent function ascribed to the cytoplasmic elements of yeasts, are shared by plastids, and it will be recalled that many cytologists consider the latter as derived from mitochondria or mitochondria-like structures. This no doubt increases the likelihood of the identification of our cytoplasmic particles with mitochondria.

Although mitochondria are claimed by some to unite de novo, and although others believe in their ultimately nuclear origin, there is no conclusive evidence in favor of either view; and indeed some cytological pictures suggest a direct division of these elements, which may be self-perpetuating by the majority of workers.

There is no evidence either for the ultimate nuclear origin of the postulated cytoplasmic units of yeasts. Indeed, the very existence of the so-called vegetative mutants is against the initiation of these elements by the nucleus. No valid argument can be presented, however, against an interpretation postulating for example that, just as the functional activity of these units is awakened by something produced by the genes, so the capacity of reproduction is conferred upon them by, say, ribonucleic acid emanating from the nucleus. Such an hypothesis could be supplemented by the further assumption that the purely cytoplasmic component is a protein, for there is no real proof, I believe, of genic control of protein synthesis, and indeed some arguments against it. No doubt, many would prefer to refer to such a protein as a “starter” rather than as a self-reproducing unit.

Nothing can be said at present against such a not altogether unlikely interpretation, which, if confirmed, would really affect only our stand with respect to the degree of autonomy ascribed to the plasmagenes. But then, it is obvious today that the autonomy of any cell constituent can only be relative, and that even “the gene as the basis of life” is only a revolutionary slogan which must not overshadow the concept of “the cell as a whole.”

REFERENCES


Remarks on Cell Heredity


GENETIC STUDIES WITH BACTERIA

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GENETIC RECOMBINATION

GENETIC investigation is by no means confined to breeding experiments, nor even to organisms exhibiting a sexual stage which makes such experiments possible. But recombination provides such a powerful tool for the analysis of the genotype as to be almost a sine qua non for decisive research. Of the three main criteria for the dissection of the genotype—physiological effect, mutational relationships, and recombinational separation—the last is at once the most utilitarian and the most delicate.

Bacteria, for obvious reasons, provide exceptionally favorable material for physiological research. Fortunately, it is no longer necessary to deplore their apparent exclusively asexual reproduction as a limitation to genetic applications. In at least one bacterium, Escherichia coli (the common colon bacterium), genetic recombination can be shown to occur. In addition to providing a sound basis for phenogenetic research in bacteria, the demonstration of genetic reassortment necessitates a search for the presumably sexual morphological processes which can be inferred from it.

We may suspect that if recombination occurred frequently, it would have been detected and confirmed long since. It is, therefore, desirable to design a selective method for the screening of large populations of bacteria for genetic recombinants. Such a method was applied by Tatum and myself at Yale University in 1946 with the help of nutritional mutants of E. coli, strain K-12 (Tatum, 1945).
A nutritional mutant is a culture which has become auxotrophic, that is, dependent upon an external source of some metabolite for its growth. It differs from the nutritional wild type, or prototroph, by its inability to effect the synthesis of some essential protoplasmic constituent which the prototroph can manufacture from simple compounds. Wild type or prototrophic E. coli cells can synthesize all needed metabolites from glucose and inorganic salts, and therefore grow well in such a medium. Nutritional mutants, on the other hand, require that the medium be supplemented with their specific growth factor requirement, which may be a vitamin such as biotin, an amino acid like methionine, or purine or other compound, as the case may be. Nutritional mutants of E. coli are essentially similar to those first produced in Neurospora by Beadle and Tatum (1941, 1945) a decade ago. However, technical advances permit much more efficient and direct recovery of bacterial mutants than is possible for filamentous fungi (Davis, 1950a; Lederberg, 1950c).

As shown in Figure 1, plating mixtures of auxotrophs into the minimal glucose-salts medium permits the selective isolation even of infrequent prototrophic components (Tatum and Lederberg, 1947). Since prototrophs form a set of the recombinants to be expected from genetic exchanges between two different mutants, the plating of such mixtures on minimal agar constitutes a sensitive test for genetical recombination. Precautions are needed, of course, to ensure that these prototrophs do not arise merely by reverse mutation of one of the parents. This complication is most readily disposed of by using multiple, rather than single, mutants as parents.

The prototrophs provide the putatively sexual progeny which can be surveyed for factor recombination. It must be emphasized that the demonstration of recombination does not rest directly upon the mere observation of prototrophs, but upon the occurrence of a series of new combinations of unselected markers introduced with the nutritionally differentiated parents. In Figure 2, B, M, T, L represent nutritional factors (biotin, methionine, threonine, and leucine) which are used for the selective isolation of prototroph recombinants. B₁, Lac, and V represent factors for thiamin requirement, lactose fermentation, and virus resistance. In a glucose-synthetic medium to which thiamin is added, these markers are unselected, and should be
free to assort in any combination, parental or new. Prototrophs occur in such a "cross" with a frequency of about one per million cells inoculated. All of the possible combinations of the unselected markers, as listed, have been found among the prototrophs, although not with equal frequency, suggesting mechanical limitations on recombination (Lederberg, 1947). The same type of result has been obtained with great regularity with many other selected and unselected markers, and in the hands of workers in a dozen different laboratories. About forty mutant characters have been used in these experiments, each conforming to this general behavior.

The prototrophs produced in these experiments are ordinarily pure and stable from the time that the prototroph colony is initiated,
although, of course, different prototrophs may carry different combinations of unselected markers. Segregation thus occurs prior to the growth of the recombinant prototroph. From this it follows that the typical vegetative form is haploid, and that the diploid "zygotic" stage is transient, and undergoes immediate segregation, associated with recombination which sometimes produces a detectable prototroph.

**FACTOR RECOMBINATION IN PROTOTROPHS**

![Diagram](image)

**TYPES**

<table>
<thead>
<tr>
<th>B₁ Lac V</th>
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<td>+ + R</td>
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<tr>
<td>+ - R</td>
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<tr>
<td>+ + S</td>
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<td>+ - S</td>
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**REACTION**

- P

**Fig. 2.** Diagrammatic representation of a cross in which B₁, Lac, and V are unselected markers. All eight of possible recombinations of these factors are found among the prototrophs. The phenotypic appearance of various combinations with respect to Lac and V is shown at the lower right (test carried out by cross-streaking culture with virus on lactose-indicator medium).

Since zygote formation has not yet been directly observed, owing to its relative rarity, the evidence for its occurrence is largely based upon indirect genetic inference. However, the efforts of several workers besides ourselves to accomplish the genetic transfer with nonliving products of parental cells have been entirely fruitless. Davis (1950b) has shown that the interposition of a sintered glass filter between potentially interacting cultures completely prevents recombi-
nation, although the culture medium be forced repeatedly from one chamber to the other. This result suggests that the gametic unit is of the same dimensions as the bacterial cell. Other genetic experiments have shown that the gametic unit must likewise be large enough to encompass most or all of the genotype since so many factors can be exchanged at once. Experiments involving mixtures of three well-marked parental types have shown further that recombination occurs only between single pairs of genotypes, that is, that the gametic units remain intact and do not mix with each other prior to the formation of the putative "zygote."

Before the statistical distribution of markers among prototrophs can be interpreted, it is necessary to verify that this distribution is based upon the mechanical properties of the system, rather than upon the physiological effects of the markers. This can be done in "reverse" crosses, in which a mutant marker is introduced first with one parent, and then the other. With a variety of markers, the segregation of two alleles among prototrophs is quantitatively inverted when the alleles are introduced in reverse combinations. This verifies the concept of the haploid life cycle, and also supports the use of these mutants as indifferent markers or tags for the corresponding parts of the genotype throughout the mechanics of recombination (Lederberg, 1947).

By the nature of these crosses, it is difficult to map these markers, and simultaneously to prove their linear arrangement in linkage without a certain element of logical circularity. However, a number of workers, including Newcombe and Rothfels, L. Cavalli, Gordon Allen and myself, have been able to show with reasonable certainty that many factors can be placed in an unambiguous linear order, although in some instances a sufficient excess of multiple-crossover types has been found that Newcombe has suggested the presence of a certain degree of negative crossover-interference. Unfortunately, however, there is, as we shall see, some suspicion that the segregation behavior of single markers in these crosses may be more complicated than has hitherto been thought, so that detailed linkage interpretations should perhaps be postponed until the behavior of single factors is further clarified.
The analysis of linkage is, of course, greatly impeded by the fact that only a particular recombination subset, the prototrophs, is available with this technique. It would certainly be preferable to have strains or conditions in which zygote formation occurred frequently enough that the selective method could be dispensed with. This has not yet been accomplished, although Cavalli (personal communication) has found unusually "fertile" strains, which recombine perhaps a hundred times more frequently than type. In a related study, T. C. Nelson working at Columbia University, has examined the kinetics of prototroph formation as a function of parent concentration and the time of their contact. His results, which show that the initial reaction between parents can be treated kinetically as a "bi-particulate" reaction, should place on a sound base the study of recombination rates as a function of genetic constitution and environmental conditions.

Prototrophs are not the only recombinant types which can be selected. Gordon Allen has demonstrated the feasibility of searching for complementary genotypes by nutritional selection of recombinants on synthetic medium to which certain supplements are added, followed by the plating of such recombinant colonies into medium with the complementary supplements.

It is also possible to dispense with nutritional selection altogether, by preparing two mutants each resistant to one inhibitor such as streptomycin and sodium azide, respectively, and plating mixed cultures into a medium containing both inhibitors. Dually resistant recombinants can be distinguished from less frequent spontaneous mutants by the recombination of other, unselected markers (Lederberg, 1950a).

NON-DISJUNCTURAL EXCEPTIONS

A more accurate, if not a clearer picture of segregation mechanisms has been obtained from the behavior of certain non-disjunctural exceptions, or diploid hybrids (Fig. 3). Certain stocks have been found in which segregation is delayed, so that a large proportion of isolated prototrophs continues to segregate marker differences introduced with the parents (Lederberg, 1949). This is in distinction to
the typical behavior of prototrophs, mentioned earlier, which are already segregated and genetically pure. The exceptional prototrophs are unstable during culture, and segregation occurs from time to time, with a frequency of the order of once per twenty fissions (Zelle and Lederberg, unpublished). Thus, exceptions heterozygous for lactose fermentation (Lac+/Lac−) form variegated colonies

![Diagram]

Fig. 3. Inferred pathways of segregation of normal and non-disjunctional hybrids. A and B refer to nutritional characters; q, r and s to unselected markers. Q includes those markers (Mal,S) which are frequently eliminated from the diploid cells. The elimination leads to a deficiency symbolized with a delta.

on lactose indicator medium, the colonies consisting of cells still heterozygous in addition to apparently haploid, genetically pure Lac− and Lac+ segregant types (Fig. 4).

If these exceptions segregated solely for the Lac+/− character, one might suspect that the variegation was due simply to mutational instability at one locus. Indeed, a unique example of such an unstable gene, for maltose fermentation, has recently been found. However, segregation of Lac is invariably accompanied by segregation for the remainder of the hybrid genotype, so that the process must be
thought of as the separation of two genomes. That such genomes are indisputably contained within single bacterial cells has been shown by elaborate single-cell pedigree studies by M. R. Zelle.

The segregation usually results in haploids which carry one or the other parental combination of markers, so that each of the genomes of the hybrid tends to preserve its integrity. However, recombinant segregants also occur, with varying frequency depending upon the stock and the markers considered. This excludes the possibility that the exceptions are simple heterokaryons. All of these facts are consistent with the simplest analogy which we can cite, that the exceptions are diploid heterozygotes entirely comparable to those of higher plants and animals. A more detailed analysis has shown, however, that these exceptions cannot be regarded simply as unreduced zygotes, but that a rather complex history of crossing-over, segregation, non-disjunction, and segmental elimination must be postulated for the exceptions if they are to be interpreted in terms of the chromosome mechanics already familiar to us.

The first anomaly consists in the fact that the unreduced exceptions, selected as prototrophs variegated for lactose, xylose, mannitol, or galactose fermentation, are regularly pure for maltose fermentation. About 95 per cent of the exceptions carry only the Mal allele contributed by the T—L—B— parent; the other 5 per cent carry the alternative allele, from the B—M— parent. Thus the majority of the unreduced prototrophs from a particular cross appear to be

\[
\text{LAC +} \quad \text{LAC +/-} \quad \text{LAC -}
\]

Fig. 4. Appearance of heterozygous and haploid colonies on indicator agar. The mosaic appearance of the heterozygote is due to frequent segregations which unmask the recessive Lac—.
(B–M– T+L+B3+/B+M+ T–L–B3–; Lac+ Xyl+ . . . // Lac– Xyl– . . . but Mal–). The question arises whether the observed purity for Mal reflects hemi- or homo-zygosity for this locus, that is, one or two representations of this gene. This has been tested by observing spontaneous reverse mutations from Mal– to Mal+, which occur infrequently (ca. 10–7 per fission) but detectably. Such reversions have invariably resulted in stocks which became pure rather than segregating Mal+, pointing to the hemizygous condition of this locus. If so, one of the parental Mal genes must have been eliminated or deleted. That the deletion covers a finite segment is suggested by the finding that factors such as S'/S* (affecting resistance to streptomycin), linked to Mal have likewise never been recovered in heterozygous form. In Figure 3, this region is symbolized “Q.”

A second type of anomaly has, perhaps, a simpler explanation. In contrast to the regularity with which Mal occurs pure in diploid exceptions, occasional exceptions occur which are heterozygous for some factors, but pure for Lac or for Xyl. Pure Lac– types have been tested for hemi- versus homo-zygosity by the study of Lac reversions from minus to plus. Here, one always finds that the pure Lac– type reverts to a variegated Lac+, pointing to homozygosity. That is, Lac–// Lac– gives rise to Lac–// Lac+. The consistently different results obtained when this test is applied to Mal-pure and Lac-pure exceptions help to support its validity as a test for hemi- and homozygosity. Homozygosity for factors in which the parents differ suggests that the exceptions are not the original zygotes, which should be uniformly heterozygous, but the result of non-disjunction of two products of crossing over. In this respect, they closely resemble cells of mosaic spots of heterozygous, Minute Drosophila melanogaster (Stern, 1936) except that in E. coli we have only the spots, and must infer the normal fly from them. On the same argument applied by Stern to somatic crossing-over in Drosophila, it may be inferred that crossing-over in E. coli involves a four-strand system.

A third anomaly concerns the aberration of monofactorial segregation ratios. Where an equal separation of segregants into two alternative classes is expected, ratios deviating as far as 15:1 are found, the
magnitude of the deviation depending upon the particular factor. This aberration may, however, be regarded as a simple consequence of the elimination of the "Q"-region already discussed. If "Q" is a segment of a chromosome, other factors not eliminated but linked to it will not appear among viable segregants, unless crossing-over supervenes between "Q" and the factor. This presumes that half the segregant genomes are lost, owing to the haplo-lethal deficiency for "Q," represented in Figure 3 with a delta. Some support for this concept may be gleaned from Zelle's single-cell studies, for he finds a rather high frequency of inviable and morphologically peculiar cells in single-cell pedigrees of diploid cultures. It remains to be seen whether inviable cells are unique to diploid cultures which are presumably segregating a haplo-lethal deficiency, or whether they originate from irrelevant causes.

Still another pathway of genetic change has been found in an exception among the exceptions, an isolated instance (among several hundred tests) of a diploid heterozygous for Mal. This diploid most frequently follows the previously established modes of proliferation: simple fission or segregation to haploids. About one one-hundredth as frequently as it forms haploid segregants, however, it gives rise to reconstituted diploids in which some factors previously heterozygous become homozygous. Whether cells are produced which are uniformly homozygous is not known, but from the standard which is (Lac+/— Mal+/— Xyl+/— Xyl+/— . . . .) reconstituted diploids have been found which are (Lac+/— Mal+/— Xyl+/—) or (Lac+/- Mal+/- Xyl+/-) and so forth. The designation of the Lac-pure and Mal-pure derived types as homozygous (not hemizygous) is based upon reverse-mutation tests like those previously discussed. Of particular interest is a study of reversions of a reconstituted Lac+/— Mal—/— Xyl+—. Mal is quite closely linked to Xyl, so that this stock provides an opportunity to distinguish two kinds of reversion from Mal—/Mal— to the heterozygous condition, namely: Mal+ Xyl+/Mal— Xyl— and Mal+Xyl—/Mal— Xyl+. The Mal reversions of this stock have been found to fall into these two classes (coupling and repulsion) in approximately equal numbers. The result has little decision value, but it may help to support the
premise that we are dealing with heterozygous diploids whose genetic structure is basically comparable to that of higher organisms, although deviations such as segmental elimination interfere with an uncomplicated demonstration.

It is not clear whether immediate segregation yielding "normal" prototrophs follows a different and simpler course. That some or all of the same peculiarities may apply is suggested by a number of findings. Unreduced prototrophs occur most frequently in the progeny of special "Het" stocks (Lederberg, 1949a), but can also be detected in standard crosses with the help of two very closely linked Lac- factors in repulsion. Crossing Lac-1 × Lac- results in prototrophs most of which are lactose-negative. About one-tenth per cent, however, are Lac+. Most of these turn out to be unreduced Lac-1/ Lac-1 + Lac-1 + diploids rather than Lac-1 + Lac-1 + crossovers. Such Lac+ exceptions can be detected by carrying out crosses on a synthetic indicator medium. These exceptions from normal crosses have the same behavior in every particular as the more frequent exceptions from "Het" crosses. In addition, it has been found that the Mal factor which behaves so peculiarly in diploids cannot be placed on a linear linkage map with other factors in haploid prototroph segregations. Mr. Gordon Allen (personal communication) has obtained some preliminary evidence for the occurrence of complementary nutritional types from single zygotes, and these also show unorthodox behavior. Finally, I have recently found several occurrences of segregants (from diploid exceptions) which when used as parents in F-2 or backcrosses give entirely unique linkage relationships, although phenotypically identical with the original parent. These segregants must be regarded as structurally different from their phenotypically similar parent, but it cannot yet be said whether the segregant or the parent carries the standard gene arrangement. At any rate, until these questions are cleared up, it will be necessary to deal very cautiously with the problems of linkage in Escherichia coli K-12.

For this reason, I will not attempt to insist upon the evidence for linear organization (Lederberg, 1947), although as far as it goes it appears to be entirely self-consistent.

A cytological comparison of haploid and diploid cells has been
initiated in this laboratory by Miss E. Lively. Distinctive differences in the texture and organization of the nuclear material have been found, which do not yet, however, admit of a clear interpretation, owing especially to the multinucleate condition of most rod-shaped bacterial cells.

**BACTERICIDAL MECHANISMS**

An application of diploid *E. coli* which may be of some interest is to the problem of the genetic mechanisms which are involved in killing by radiations and chemicals. Although it is not possible to offer a clear picture of the mechanisms which are involved, recessive lethal mutations play a negligible role in the killing of bacteria by ultra-violet light or by x-rays. This is shown by the rarity of balanced lethal types among the survivors of various doses of these radiations. Atwood (1950) has reached a similar conclusion from studies on the irradiation of heterokaryotic Neurospora conidia. Rather complex genetic changes are found following irradiation of heterozygous diploid bacteria, in particular haploid and reconstituted diploid cells. These changes can be detected in populations showing 90 to 95 per cent survival, so that there can be no question of selective survival. The widespread genetic reorganization may be correlated with the accumulation of nuclear material observed in "snake" cells found in populations recovering from radiation (Delaporte, 1949).

Similar genetic effects, that is, haploids and reconstituted diploids, have been observed in survivors of populations treated with such diverse reagents as nitrogen mustard, hydrogen peroxide, formaldehyde, acetic anhydride, dimethyl sulfate, and ethylene oxide. Although, in common with radiations, many of these compounds are known to be mutagens, and all may be, the relationship between genetic reorganization, sterilization, nuclear pathology, and mutation is not clear.

In some laboratories, bacterial cell suspensions have been thought of as naked genes with which to do experiments on chemical modification, that is, mutagenesis. This view is not acceptable without better evidence than is now available. Recent discoveries have tended to weaken the supposition that radiations produce their genetic effects
by direct interaction with genetic material. In much the same light, we must be very cautious in interpreting chemical mutagenesis as a direct chemical reaction with the gene. Cells, including bacteria, react in a very complex pattern to treatment with mutagenic agents. The possibility cannot be excluded that some mutations are produced indirectly as a consequence of accidents during recovery or of non-specific and non-localized disturbances of nuclear structure.

In view of the great diversity of reagents which have mutagenic or other nuclear effects, it is reassuring that some have been found which do not. In experiments with diploid E. coli, heat, iodine, and streptomycin were found to kill cells without any detectable genetic concomitants.

GENETIC ANALYSES IN ESCHERICHIA COLI K-12

At this point, I should like to take up a few examples of genetic analysis using recombination methods in order to illustrate the scope and technical facility of these approaches.

Reverse mutation

Mutations leading to the reassumption of the wild phenotype have been noted frequently in bacteriological studies (Ryan, 1946). In fact, the misconception that gene mutations in other forms were irreversible served, at one time, as an argument against the mutational basis of bacterial variations. Such reversions, however, must be studied by crossing techniques before it can be determined whether they represent reverse-mutations of the mutant gene, or changes of distinct loci or other determinants. This type of analysis has demonstrated reverse-mutation in two systems; the S locus (controlling response to streptomycin), and Lac (fermentation of lactose). Newcombe and Nyholm (to be published) and Demerec (1950) have studied streptomycin-sensitive reversions obtained by selection from streptomycin-dependent mutant strains. Most such reversions yield only sensitive prototrophs when crossed with type sensitive, and may thus be regarded as reverse-mutations. Demerec noted a variety of unrelated differences in some apparent reverse-
mutants, but it appears doubtful that these must all be attributed to
changes at a single locus. E. Lederberg (1948) tested reversions of
lactose-negative mutants to the type lactose-positive. All reversals
which showed close phenotypic resemblance to type proved to be
reverse-mutation as tested by crosses to type.

Suppressor and mimic mutations

In contrast to reversions demonstrably based upon reverse-muta-
tion, a number of reversals have been found to depend upon muta-
tion at loci other than that occupied by the original mutant gene.
This can be shown by crossing the reversal to type and recovering
the recombinant mutant phenotype. This result was obtained by
Newcombe and Nyholm with one streptomycin-sensitive reversion
and by Lederberg (1948) and E. Lederberg (1948) with a variety of
reversals of fermentation mutants. As a rule, "suppressor" mutations
are found most readily in stocks carrying alleles with an intrinsically
low reverse-mutability.

In addition to "suppressors," which mimic the wild phenotype,
many instances of mutations which mimic each other are
known, posing the question of the number of loci involved. For example,
Demerec and Fano (1945) described two mutations, (B/1 and B/1,5)
in E. coli B, both of which manifested resistance to the virus T1,
whereas only B/1,5 was resistant to T5. Without recombination
test, it could not be shown whether these represent changes of
similar or distinct genetic elements. Analogous mutants in K-12 can,
however, be crossed with each other, permitting the identification of
two distinct loci: V₁₄ and V₁ respectively.

Multiple alleles

Three series of multiple alleles have been recognized. Mutations
from type (Sr) leading to resistance to (Sr) or to dependence (S₅)
on streptomycin (Fig. 5) have been shown to be uni-local in unpub-
lished work by Demerec and Zinder and by Newcombe and Nyholm.
A series of Lac⁻ (lactose-negative) mutants which can be differenti-
ated by the rates at which they reverse-mutate to Lac⁺ have been
shown to be allelic by E. Lederberg (1948), (Fig. 6). Finally, a third
Fig. 5. Multiple alleles at the S locus, showing appearance of streptomycin-sensitive, -dependent, and -resistant forms when cross-streaked with streptomycin on agar medium.

Fig. 6. Multiple alleles at the Lac locus differentiable by their rates of reverse mutation. The dots represent "papillae" composed of reverted cells.
allele has been found at the $V_1$ locus. This allele $V_{1}^{+}$ results in an intermediate degree of resistance to the viruses $T_1$ and $T_5$, (Fig. 7), but has been studied in detail only from a genetic viewpoint. The compound heterozygote, $V_{1}^{+}/V_{1}^{+}$ shows the $V_{1}^{+}$ phenotype, possibly placing this system in the category of "pseudo-alleles," although no crossovers between $V_{1}^{+}$ and $V_{5}^{+}$ have been found. (Parenthetically,

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PHAGE
T1
---
V1 ALLELE
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S
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P
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R
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PHAGE
T5
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*Fig. 7. Multiple alleles at the $V_1$ locus, showing modalities of response of sensitive, partially resistant and resistant cells when cross-streaked with viruses $T_1$ and $T_5$ on agar medium. The residual growth of $V_{1}^{+}$ cells is due to incomplete lysis, not to the presence of a few resistant cells.*

this "overdominant" phenotype was responsible for the first recognition of non-disjunctive types, Lederberg, 1949a). The structure of such genes cannot now be asserted by the bacterial geneticist any more than by the student of maize or fruit-flies, but at least the question can be put. However, microorganisms possibly provide material in which the physiological differentiation of genic components can be more readily studied.
GENETIC CONTROL OF ENZYME FORMATION

Dr. Sturtevant has suggested that the position effect is the most promising avenue of attack on the problem of gene function, and after having attempted some work on gene-enzyme relationships, I am inclined to agree with him.

As a preliminary approach, attention has been focussed upon a single "adaptive" enzyme, lactase, which can be readily assayed, and for which mutants are readily procured. It is, fortunately, very simple to isolate unlimited numbers of mutants in which this enzyme is affected, simply by plating irradiated populations on indicator medium containing lactose ("EMB-lactose"). Mutants lacking the enzyme can be found by inspection of colonies. Since one can easily score 500 or more colonies on a single plate at a glance, it has required no unusual effort during the last few years to examine perhaps a half-million colonies, from which about 300 mutations have been obtained. These have been tested for allelism by crossing them with each other. At least seven distinct genetic types were found, each giving wild type upon outcrossing with other mutants, but only lactose-negatives when crossed within the same type. It was concluded that mutation at any one of seven loci results in the substantial suppression of lactase formation in cultures exposed to lactose. In order to facilitate enzyme assays and extend their sensitivity, a lactose analogue, o-nitrophenyl-β-D-galactoside was employed. This compound, itself colorless, is split by lactase producing galactose and o-nitrophenol, whose yellow color permits spectrophotometric assay of the enzyme (Lederberg, 1950b). The use of this reagent also allows the investigation to be focussed upon a single enzyme, a simple hydrolytic glycosidase, rather than upon the complex of glycolytic or oxidative systems which are necessarily involved in manometric determinations of enzymatic activity.

More genes appear to be involved with this enzyme than I would have bargained for, but the next step is far more difficult, perhaps impossible: to analyze the mechanism of interference with lactase formation in the mutants, from which the function of the normal
allele might be inferred. Some of the mutants, Lac₂⁻ and Lac₄⁻, show no promise in this direction, because as far as I have been able to determine they are absolutely and irreparably incapable of producing lactase and show no other phenotypic effect. Other mutants which show relative blocks may be more amenable.

For example, Lac₁⁻ cells produce minimal amounts of lactase when grown on lactose, but substantial amounts on analogous substrates such as alkyl galactosides. (This results in the paradox that cells grown on a heterologous substrate are better adapted to lactose than those grown on lactose itself. Since “adaptation” is presumably a physicochemical rather than an entelechist process, such deviations are not surprising but suggest the need for revising “adaptive enzyme formation” in favor of a more general term connoting “enzyme formation under environmental influence.”)

The Lac₅ locus offers even better possibilities, as it presents a clear-cut example of pleiotropic effects at the enzyme level. This mutant is defective in hexokinase (presumably), amylomaltase and lactase, resulting in an inability to split lactose and maltose or to glycolyze glucose (Lederberg, 1948; Doudoroff et al, 1949). In view of the complexity of effects the “single gene” nature of the mutation must be verified—the evidence for this seems to be as conclusive as could be obtained with any genetic analysis. The same phenotypic combination (glucose-, maltose-, lactose-negative) has been observed about twenty times among the three hundred odd lactose-negative mutants isolated. Attempts to find other types of glucose-negative mutants have led repeatedly to Lac₅⁻ mutations. Some Lac₅⁻ stocks throw reversions, selected on media containing any one of the three sugars as carbon sources, but in which the phenotypic effect is reversed en bloc. The reversions have been verified as reverse-mutations by crosses to wild type. Finally, the three effects are inherited as a unit in recombination experiments.

Two temperature sensitive Lac₅ types have been isolated (Lac₅t). Lac₅t shows the wild phenotype at 25°, but the typical Lac₅⁻ at 40°C. The different effects show, however, distinct thresholds so that, for example, the phenotype at 36° is Clu-Mal-Lac+. The possibility of a common underlying mechanism with quantitatively
distinct thresholds for the three effects suggests itself immediately. If so, this might mean either a common "protogen" precursor for the enzymes, or some sort of inhibition. No enzyme-inhibitors have been found in crude preliminary experiments with mixed cultures, but current methods would in no case permit the detection of non-diffusible stimulants or inhibitors of enzyme formation, and there's the rub!

Ignorance is further amplified by a mutation, Cst+, which results in the so-called constitutive production of lactase. E. coli lactase is a classical example (Karström, 1930) of a bacterial adaptive enzyme, because cells grown on glucose show insignificant activity on lactose when tested manometrically, whereas lactose-grown cells glycolyze the disaccharide as rapidly as glucose. However, wild type cells grown on peptone, maltose, or other non-glucose substrates produce lactase in considerable, manometrically measurable amounts, and even glucose-grown cells show definite lactase activity (about one per cent of maximal), readily detected by colorimetric methods. A more adequate description of Cst+ would be, then, that it differs from type Cst− in the production of optimal levels of lactase on glucose and other non-lactose substrates.

The Cst+ mutant was first discovered in a rather interesting way. Neolactose, or altrose-galactoside, appeared to be inert to attack by wild type cells. I attempted, therefore, to select for a mutant with a lactase of altered specificity which could attack this analogue. The experiment was performed in the usual way, by inoculating large populations of cells into a medium with neolactose as carbon source. A "neolactose-positive" mutant was readily obtained in this way, and after purification was subjected to physiological study. Meanwhile, it was found that neolactose was split by type lactase, but that it was unable to elicit the formation of lactase by type cells (resembling the lactose-Lac+,relationship). At first it was thought that the neolactose-positive mutants had an altered specificity of adaptive response so that neolactose would now be recognized as a stimulus. But it was soon found that optimum lactase (= neolactase) activity was developed whether or not a galactoside substrate was included in the growth medium. The necessary inference is that the adaptive
system (or an alternative one) has lost, rather than altered, its specificity of response. It should be noted that the production of maximal lactase activity by growth on glucose, and the absence of galactozymase activity in such cells argue against this mutant's responding to a sort of internal adaptation, viz., by intracellular formation of simple galactosides.

None of these mutations seem to affect the enzyme itself. In Lac+, it is not the enzymes but the conditions of enzyme formation which are temperature dependent. In Lac− and in Cst+ also, it is the specificity of enzyme formative responses, rather than the specificity of the enzyme which is altered. We are left with the rather unsubstantial conclusion that the complex process of substrate-dependent enzyme formation is subject to rather complex genetic control.

The debate of "true" versus "spurious" pleiotropism has been renewed and revised in recent years in the form of the one-to-one theory of gene enzyme relationships. The data presented here, as well as those of Landman (1950) on Neurospora lactase, and of Markert (1950) on Glomerella tryosinase, demand the rejection of this hypothesis in any form which is at the same time simple and general. We may conclude that observed enzymatic changes are often indirect consequences of genic alterations; this is not to imply that they are never direct. However, we lack critical evidence that any biochemically observable enzyme is a direct product of the action of a gene. The one fallacy of which we must be especially wary is the notion that an enzyme is produced by a gene, rather than by an integrated genotype or cell. Nor are we entitled to make a similar sweeping generalization concerning cell antigens, for a variety of secondary products must be expected to show antigenic specificities. Here, however, we have a number of examples where different alleles in heterozygotes behave independently in respect to the specificity of the cell products. This type of evidence of direct gene-to-product relationship is so far lacking for any microbial enzyme, but suggests one of the few workable criteria which can be used.

I am loath, however, to suggest a thorough rejection of the principle of monotropic gene action for the simple reason that there is no adequate alternative. Since any particular case of pleiotropism
may be spurious, the search for a unitary alteration of function is
the only kind of experiment that we can carry out. I suspect, how-
ever, that the worker interested in problems of gene action soon
branches out into an unlimited variety of non-genetic experiments.
It may be wondered whether a geneticist might not function more
effectively as a catalytic or mutagenic agent, attracting the attention
of chemists, embryologists and others to the problems of protein
synthesis and morphogenesis which are now almost insuperable ob-
stacles to the completion of our analyses. Some of the fruits of such
collaborations you have seen in the contributions immediately pre-
ceding.

RECOMBINATION IN THE NATURAL HISTORY
OF BACTERIA

Further study of genetic recombination in bacteria should be both
extensive and intensive. It is particularly pressing for us to learn
some of the details of the morphological processes underlying recom-
binant in E. coli K-12. The genetic analysis has convinced me
that there probably is a fusion of elements, possibly of ordinary cells;
less likely of specialized gametic forms. However, this conviction
cannot be entirely secure without a microscopic observation of fusion
accompanied by a demonstration of its genetic effect. K-12, although
well suited for genetics, does not offer an encouraging prospect for
the cytology of fusion owing to the infrequency of recombination
and the apparent absence of any relevant distinctive forms. Several
workers have, however, described aggregation or fusion processes in
Agrobacterium (Phytononas) tumefaciens (Braun and Elrod, 1946)
which very strongly suggest a sexual process, and are possibly our
best leads toward a cytological demonstration. As these authors point
out, however, "... cytological studies alone will not suffice to clarify
this question. It will be necessary to bring together in a single star
different strains... and determine from this cross whether recombina-
tion of characters results." It is strongly hoped that such a pro-
gram will be executed with this organism, and with others which
show similar morphological hints of genetic exchange.

Tests for genetic recombination have been carried out to too
small an extent to permit any generalizations on the scope of its occurrence. After Tatum and I had made our first observations on K-12, we found that a similar approach had been unsuccessfully attempted previously (see Tatum and Lederberg, 1947, for references). Some years before, a brief note had appeared with the intriguing title "Mendelism among bacteria?" (Brown and Heffron, 1929.) The paper deals with the possible segregation of a single character difference in "Bacillus luteae." However, the details are suggestive rather than convincing, and I have been unable to trace the culture.

The work with K-12 differs from previous investigations primarily in the application of a selective technique for the detection of recombinants. However, the particular choice of this strain appears to have been exceptionally fortunate, for a limited number of attempts to demonstrate recombination in a few other strains have been less successful. K-12 itself is not exceptional in any obvious way. According to Dr. C. E. Clifton of Stanford University (personal communication), it "was isolated by Dr. Blair in the fall of 1922 from the stools of a diphtheria convalescent and was identified at that time as an E. coli by the ordinary laboratory tests. It has been maintained in our stock culture collection since 1925 and is used in our laboratory as the typical coli culture."

Other typical coli cultures used in physiological and genetic studies which have been tested for recombination include the B strain, used in bacterial virus work, Davis' W strain, and the L strain used by Roepke and Ryan and their associates. Unfortunately, these tests have been quite negative, so that various genetic phenomena which have come up in these studies cannot be adequately analyzed. However, Cavalli and Heslot (1949) have discovered an E. coli strain which is moderately cross-fertile with K-12 (one success in about seven tests). In tests of forty E. coli cultures isolated from chickens, I have found two or three which cross with K-12, but at so low a rate as to make analysis difficult. The possibility of intra-fertile systems which do not cross with K-12 has not, however, been tested on a large scale. The extent to which K-12 or other sexual strains recombine genetic characters in their natural habitats is quite obscure. Unless
crossing can occur between rather widely separated, genetically differentiated clones, recombination may make a relatively small contribution to genetic variability since in experimental material it occurs rather infrequently compared to clonal propagation. It must take place between stocks differing in at least two characters to have any effect at all in the production of new genotypes. However, in the laboratory, recombinants dually resistant to streptomycin and sodium azide (Az'S') occur about one hundred times as frequently in mixed single-resistant cultures (Az'S + Az'S') as do mutations of the parental stocks to the same end genotype (Lederberg, 1950a). The potential significance of this finding for chemotherapy needs no elaboration.

Other bacteria have been scarcely studied at all from this viewpoint, and no publications can be cited as yet. However, this kind of investigation is being pursued in several laboratories, and, we may anticipate, not entirely fruitlessly.

"INFECTION" HEREDITARY TRANSMISSION

Two of the preceding papers in this book have considered at length the role of extrachromosomal (so-called "cytoplasmic") factors not only in heredity of microorganisms, but in heredity and development of higher forms. What contribution can a bacteriologist make to this discussion? It will be apparent at the outset that, for the most part, we can ill discuss extrachromosomal inheritance without first building a clear picture of the chromosomal mechanisms, if any. For reasons stated above, this distinction can rarely be made. The bacteria are notable, however, for examples of "infective" heredity, that is to say the transmission of genetic elements despite cellular discontinuity. The most outstanding and the best investigated of these examples is the pneumococcus transformation (McCarty, 1946). Whether or not the pneumococcus also possesses a "chromosomal" or nuclear genotype (and I see no reason to doubt that it does), the analogy which has been drawn between the transforming agents of pneumococcus and cytoplasmic factors in other organisms remains an instructive one (Sonneborn, 1943). I would like to suggest that a rather simple, unitary picture of extranuclear mechanisms can be
developed if we include in our discussion of "infective heredity" agents responsible for "infective pathology" such as intracellular viruses (Altenburg, 1946).

We have already heard many comments upon the difficulty of deciding upon the evolutionary origin or contemporary taxonomy of deleterious parasitic viruses at one extreme, and integrated cytoplasmic genes like plastids, at the other. Within this interval, we find a host of transition forms; kappa, lysogenic bacteriophages, genoids, tumor-viroids, male-sterility factors, Ephrussi's yeast granules, etc. These differ in several parameters, particularly the frequency with which spontaneous "disinfection" occurs, current experimental success in achieving artificial infection, and the pathology of the infection, that is, the phenotype corresponding to it. None of these differences is really fundamental from a genetic point of view, because the cell-virus complex is at least theoretically capable of adaptive evolution as a consequence of natural selection and mutation in either the virus or the cell component, or both. The objection has been voiced that this viewpoint is an attempt to relegate plasmagenes to pathology. I rather think that it may broaden our genetic insights if we consider the likenesses as well as the dissimilarities between pathogenic viruses and plasmagenes. For example, we would not have learned how to "cure" green plants of their chloroplasts (Provasoli, Hutner and Schatz, 1948) if it were not for more mundane chemotherapeutic investigations.

Extra-chromosomal agents which can be transferred outside the cell obviously provide the most suitable material for experimental study of their composition, numbers, morphological structure and so on. From this point of view, the lysogenic viruses carried by many bacteria provide the best material, especially as infection with such a virus is formally indistinguishable from events such as pneumococcus transformations. (For an amplification of this viewpoint, and further literature references see Lederberg, 1949b, especially pp. 17-19.)

Perhaps less profitably, a further generalization may be erected. I wonder whether we cannot split the organism into smaller functional units than the cell, depending upon their integration into
genetically continuous aggregates. For example, the yeast cell would consist of the interaction of its nuclear genotype, its cytoplasmic granule genotype, and so forth. Our examples of cytoplasmic determination would then correspond to a series of interactions of different genotypes with varying degrees of mutual dependence. In a sense this is equivalent to conferring quasi-organismic status upon each of the different components. The advantage (or drawback) of this unifying view is that it comprehends a continuous spectrum of such genotypic interactions ranging from Ephrussi’s granules, lysogenic viruses, and facultative intracellular symbiosis eventually to the least tangible ranges of genotypic (that is, interorganismal) interaction in, for example, human social relations. At each level of interaction pathological deviations can be found, ranging from sick plasids and malignant tumors (on Darlington’s theory) to human serfdom.

ACKNOWLEDGMENTS

The experimental work from this laboratory has been supported in part by grants from the Committee on Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service; the Research Committee of the Graduate School, with funds allotted by the Wisconsin Alumni Research Foundation; The Rockefeller Foundation; and the Wisconsin Agricultural Experiment Station.

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THE ROLE OF THE GENES IN CYTOPLASMIC INHERITANCE

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Following the independent discovery of cytoplasmic inheritance by Correns and by Baur in 1909, the roles of nucleus and cytoplasm in heredity were long studied separately, as if the cell contained two independent and autonomous genetic systems. However, evidence accumulated during the past quarter century points more and more to intimate interrelations between the bases of nuclear and cytoplasmic inheritance. A survey of 50 years of progress of Mendelian genetics therefore properly includes a discussion of what appear to be five different roles of Mendelian genes in cytoplasmic inheritance. By attending to these relations between genes and cytoplasm, some progress may be made towards an integrated conception of the genetic system of the cell as a whole.

INDUCTION OF CYTOPLASMIC MUTATIONS

First of all, genes can induce mutations in cytoplasmic genetic materials. For example, Rhoades (1943) demonstrated that a gene in maize induces plastids to mutate to a form incapable of producing chlorophyll. Similar results have been reported in barley by Armason and Walker (1949) and in other plants by other investigators (see review by Rhoades, 1946). Generalizing this relation, one might expect genes to be capable of inducing mutations in other cytoplasmic genetic materials. Little is known about this, but the occurrence
The Role of the Genes in Cytoplasmic Inheritance

of mutations in mitochondria has recently been claimed by DuBuy, Woods and Lackey (1950).

CONTROL OF THE INTRACELLULAR CONCENTRATION OF CYTOPLASMIC GENETIC MATERIALS

Second, genes can control the intracellular concentration of certain self-duplicating cytoplasmic particles. The best evidence of this is provided by observations on the concentration of kappa particles in killer paramecia. Mr. P. K. Chao kindly permits me to mention his unpublished discovery that the number of kappa particles per cell is approximately twice as great in killer paramecia homozygous for gene K as in killers heterozygous for that gene.

There are also a few reports (Wilson, 1925; Winge and Laustsen, 1940) that mitochondria are reduced when a diploid becomes a haploid; and Gabelman (1949) comes to a similar conclusion with regard to the cytoplasmic factor for male sterility in maize. Further work along this line is much needed.

SELECTIVE ACTION ON CYTOPLASMIC GENETIC MATERIALS

As a third possible role of the genes, their action as selective agents acting upon self-duplicating cytoplasmic entities should be considered, although, so far as I am aware, no completely satisfactory experimental evidence on this is yet available. However, wherever alternative or mutant forms of self-duplicating structures can occur together in the cytoplasm of a cell, it is to be expected a priori that intracellular selection of one or the other might be influenced by gene action. This possibility seems worth examining in paramecia containing more than one type of kappa, a condition reported by Dippell (1950); in drosophilae containing more than one form of sigma, the cytoplasmic basis of sensitivity to CO₂ (see Goldstein, 1949); in plants containing diverse types of plastids (Renner, 1936); and perhaps in organisms containing diverse mitochondrial types (DuBuy et al., 1950). Michaelis (1949b) believes his studies on Epilobium warrant the
conclusion that the genome can act selectively on alternative, self-duplicating cytoplasmic entities; but Lehmann and Duppies (1950) present evidence that weakens this conclusion. Moreover, the postulated alternative self-duplicating entities in Epilobium can hardly be considered as demonstrated. Almost the only available evidence for selection of alternative cytoplasmic genetic particles under gene action is provided by Renner’s (1936) studies on Oenothera. There it is reasonably clear that the evolution of diverse genomes, now characteristic of different species, has been accompanied by the evolution of diverse plastid types.

INTERACTION WITH CYTOPLASMIC GENETIC MATERIALS IN THE CONTROL OF HEREDITARY traits

The fourth role of the genes is to cooperate, in yet unknown ways, with genetic components of the cytoplasm in the control of particular hereditary traits. Determination of these traits cannot be attributed to the action of either genes alone or cytoplasmic genetic factors alone, but only to their interaction. The traits do not appear when the same genes are combined with another type of cytoplasm or when the same cytoplasm is combined with another set of genes; they appear only when particular nuclear and cytoplasmic genetic components are brought together in certain combinations. This kind of gene action in cytoplasmic inheritance seems to me so important that it warrants extended discussion.

The simplest example is provided by the control of chlorophyll production in higher plants. Renner (1936) found that chlorophyll is formed normally in two species of Oenothera, which may be designated A and B; but that two kinds of plastids appear in the cells, one normal and one failing to form chlorophyll properly, if the genes of species A are combined with a mixture of the cytoplasm of species A and B. These two kinds of plastids persisted through 14 generations of backcrossing to species A, the species that provided the genes. Renner believes that the two kinds of plastids were derived from the two parent species and that the plastids of species B fail to form chlorophyll properly when associated with the genes of species A.
Although Rhoades (1946) and others have pointed out that the data do not absolutely exclude the possibility that some other hereditary cytoplasmic material may be the basis of the effect, it is generally agreed that Renner's interpretation of the observations is the most reasonable one.

In any case, the genes of species A are not defective in an absolute sense, for in their presence the plastids of species A form chlorophyll. Nor are the plastids of species B defective in an absolute sense, for they form chlorophyll in the presence of the genes of species B. Renner showed further that they would again turn green, even after 14 generations of functional failure due to association with the genes of species A, when the genes of species A were replaced by the genes of species B. The hereditary failure of certain plastids to form chlorophyll normally is therefore due to an interaction between genes of species A and plastids of species B. It cannot be attributed to either the genes or the plastids alone.

Entirely comparable results, apparently involving some genetic component of the cytoplasm other than the plastids, have been found on a grand scale in extensive investigations on the willow-herb, Epilobium, performed during the past 30 years by Lehmann, Michaelis, their co-workers and others. In species crosses, the differences in morphological traits are clearly controlled by the genes; but when the genes of certain species are combined with the cytoplasm of certain other species, the leaves and flowers may change in shape, and there may appear reduction in the size of the plant as a whole, of the flowers and leaves, of pollen fertility and of color of leaves and flowers. A definite combination of these traits is characteristic of each particular combination of genes and cytoplasm. These syndromes have been followed without loss or diminution by P. and G. Michaelis (1948) in some cases for as many as 24 successive generations of crossing back to the species that provided the genes. They are therefore seemingly due in part to a self-perpetuated condition of the cytoplasm which cannot be changed by the genes, and in part to the genes themselves. They cannot be due wholly either to the genes alone or to the cytoplasm alone, for they do not appear either in the species that provided the genes or in the species that
provided the cytoplasm. As with the previous example of chlorophyll
deficiency, these traits are due to interaction between the nuclear and
cytoplasmic components of the genetic system.

Comparable examples of interaction effects on various traits have
been reported in a number of other organisms. It now appears that
Ephrussi's beautiful analysis of the control of respiratory enzymes
in yeast, as set forth in the preceding paper of this symposium, should
be included among them. Great interest and importance will attach
to the identification of the cytoplasmic component in this case for,
as chloroplasts and the plastids from which they arise do not occur
in yeasts, this would throw light on the nature of other normal genetic
components of the cytoplasm.

Attempts have been made to specify the respective roles of the
nuclear and cytoplasmic components of the genetic system in inter-
actions of the kind now under discussion. These speculations are
based upon two types of observations. First, the genes are known to
be involved in the control of enzyme specificity. Second, certain phys-
ical and chemical properties of the cytoplasm are known to be cyto-
plasmically inherited. For example, in Epilobium permeability and
viscosity (von Dellingshausen, 1935, 1936), quantity of growth hor-
mone (Hinderer, 1936; Ross, 1939) and the level of peroxidase ac-
tivity (Ross, 1941, 1948) are known to be cytoplasmically inherited.
The cytoplasmically inherited physical and chemical properties of the
cytoplasm are held to have a merely modifying effect, probably merely
a quantitative effect, on gene action. The genes whose effects are
thus presumed to be modified are spoken of as "plasmon-sensitive"
genesis.

This is certainly a plausible view when the gene-cytoplasm inter-
actions yield simple quantitative differences; for permeability, vis-
cosity, pH and the like might be expected to modify the quantitative
aspects of enzyme action. Goldschmidt in 1934 reviewed the quan-
titative effects of cytoplasm on gene action. As the extreme case, it
would not be surprising for the action of certain enzymes to be en-
tirely abolished by large changes in the general physical and chemical
properties of the cytoplasm.

On the other hand, this can scarcely be the whole story. Caspari
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(1948), who has tried to make the hypothesis of plasmon-sensitive genes the key to nearly all of cytoplasmic inheritance, made no attempt to apply it to the plastid cases. This is tantamount to admitting that wherever self-duplicating cytoplasmic materials play a part in the gene-cytoplasm interactions, the hypothesis of plasmon-sensitive genes—of mere cytoplasmic modification of gene action—is inadequate.

Although this hypothesis has been traced to Renner and Kupper (1921), they really had a quite different notion, more in harmony with the type of interaction now under consideration. They suggested that the cytoplasmic part of the genetic system provides some of the substrates for gene action. In other words, materials indispensable for normal developmental reactions were believed to be cytoplasmically inherited. There is another possibility worth considering, though at present it can scarcely be explored. Self-perpetuating cytoplasmic materials may have their surfaces so constituted as to absorb enzymes in the particular pattern required for proper functioning—the arrangement of enzyme systems in the necessary assembly-line fashion.

Regardless of whether the cytoplasm contributes hereditary materials, hereditary surface patterns of particulates, or something else, it is now clear that it sometimes does more than merely modify gene action quantitatively. Genes and self-duplicating cytoplasmic materials interact, in ways yet unknown, to produce effects in heredity that are not entirely attributable to either alone.

The general situation is not unlike the one that prevailed some years ago in discussions of heredity and environment. The observations showed that the same difference in phenotype could in one comparison result from a difference in genes and in another comparison from a difference in environment. The newer observations show that the same difference in traits can, in one comparison, result from a difference in genes and, in another comparison, from a difference in cytoplasm. The old paradox proved too difficult for popular understanding and the new paradox is still more difficult because both kinds of differences are hereditary. This new paradox may therefore be expected to meet with even greater lack of comprehension. But geneticists have been through this once and have mastered it;
they should not find insuperable difficulty when the same spectre raises its complex head in another guise.

The situation is simply this. What is transmitted during reproduction is genetic material with a particular reaction norm. The reaction norm denotes different responses under different conditions, without change in the responding genetic materials. This applies both to nuclear and to cytoplasmic genetic materials. The cytoplasmic genetic materials constitute or control part of the conditions to which the genes respond, and the nuclear genetic materials constitute or control part of the conditions to which the cytoplasmic genetic materials respond. The phenotype is the result of interaction between the two components of the genetic system under the conditions in which they are operating. In the present state of knowledge, one can only speculate as to the material, chemical and physical details of the interaction; those are problems for the future.

The evidence for mutually interacting and integrated genetic components in nucleus and cytoplasm has evolutionary significance. Since changes in either the genes or the cytoplasmic materials of heredity can disturb normal development, and since these two components of the abnormally interacting combinations are found in nature in other combinations that yield normal development, it follows that in the course of evolution changes must occur in both the genic and cytoplasmic materials of heredity.

CONTROL OF A SPECTRUM OF ALTERNATIVE CYTOPLASMIC STATES

The fifth and the last role of the genes I propose to discuss has come to light only recently. Different genes of one and the same nucleus may be capable of determining two or more alternative, mutually exclusive traits, only one of which can come to full phenotypic expression in any one cell. The decision as to which of these potentialities will be realized and which suppressed is dictated, at least in part, by conditions outside the nucleus. Once made, the decision is binding during subsequent cell multiplication for a shorter or longer time, and under certain conditions permanently. Thus two
cells with the same genes and under the same conditions may have
different alternative traits and maintain them during cell reproduc-
tion. In other words, once these traits are developed, they are cyto-
plasmically inherited, but the genes seem to determine which ones
can be developed. As this role of the genes in cytoplasmic inheritance
is the newest to be discovered (Sonneborn, 1947a, 1948, 1949, 1950;
Sonneborn and Beale, 1949; Sonneborn and LeSuer, 1948), is least
widely known, and seems to throw light on a number of old but
puzzling phenomena of much importance in genetics, I shall illustrate
it with the example in which it was discovered and devote most of
my remaining time to pointing out its possible wide applicability and
significance.

A single homozygous paramecium (stock 51) gave rise in the
course of time to eight types of descendants (A, B, C, D, E, G, H, and
J), differing in the kind of antigen carried on their cilia. No one
of these hereditary types can maintain simultaneously two of the
eight antigens on their cilia; they are mutually exclusive traits. Al-
though these types are inherited through both vegetative and sexual
reproduction, they were shown by breeding experiments not to differ
in any genes affecting the antigens; the antigenic types are cyto-
plasmically inherited. Under certain conditions (cultivation at 26°
with only enough food to permit one fission per day), the types A, B
and D have been maintained constantly side by side for over four
years, during which they have gone through about 100 successive self-
fertilizations (autogamies). Other types such as H, which are less con-
stant under these conditions, may be highly constant under other
conditions, for example, cultivation at lower temperatures.

All of the types can be transformed to other types of this series
by growing them under different conditions of temperature or nu-
trition. Under such conditions, certain transformations occur after
a few fissions while others do not occur until after many fissions.
Thus, even under conditions which eventually will lead to trans-
formation, certain types may persist for up to 30 or 40 fissions. This
temporary inheritance can be avoided and the transformations can
be brought about within one or two fissions, by combining the
change of temperature with a brief exposure to specific antiserum, to
ultra-violet light or to certain other conditions. Cytoplasmic inheritance of the antigenic types can therefore be perpetuated indefinitely or terminated at will, depending upon the experimental conditions. Neither the spontaneous nor the induced changes of antigenic type involve any irreparable loss; they can be converted back again to the original type. Thus A can be converted to B and B back again to A.

The situation just described is typical of what is found within each stock of this variety, or physiological species, of *P. aurelia*. Different stocks differ, however, in the following ways. First, corresponding antigens in different stocks may differ slightly. For example, in both stocks 51 and 29 there occurs a type which has its cilia agglutinated by anti-A sera, but these A antigens can be distinguished by titrations and cross-absorptions. Second, the conditions of temperature which bring about transformation to a given type may differ from stock to stock. For example, the type B arises at 19° in stock 51, at higher temperatures in stock 29. Third, types found in one stock have not yet been found in other stocks. For example, type F occurs in stock 29, but as yet has not been found in stock 51. This may simply mean that the conditions for inducing its formation in stock 51 have not yet been discovered; so the third kind of stock difference may turn out to be a consequence of the second.

Some of these differences between stocks have been shown to be due to differences in nuclear genes. For example, the difference between the A antigens in stocks 51 and 29 is due to a difference in the alleles present at one locus. Another pair of alleles seems to determine a comparable difference between the B antigens of these two stocks. A third pair of alleles determines whether type F can or cannot arise at low temperature. Thus stock 51 is homozygous for the genes *A*^51^, *f* and *H*^51^, and stock 29 is homozygous for the corresponding genes *A*^29^, *F*^29^, *H*^29^, Presumably other loci are involved in the control of the other antigenic differences between these two stocks.

The experimental analysis leads to the following picture. The animals of any one stock all contain a number of different loci the same series of genes which control the spectrum of specific antigenic types producible in the stock. In animals of different antigenic types, different genes of this series come to phenotypic expression, while the
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others fail to be expressed. Thus, in stock 29, all animals have the genes \( A^{29} \), \( F^{29} \) and \( H^{29} \); but in type A animals gene \( A^{29} \) comes to expression while genes \( F^{29} \) and \( H^{29} \) do not. Conversely, in type F animals, gene \( F^{29} \) comes to expression while genes \( A^{29} \) and \( H^{29} \) do not; and so on. The fact that each type, once developed, perpetuates itself during vegetative and sexual reproduction until external conditions bring about a shift to another one of the alternative types, implies that cytoplasmic mechanisms (as yet unknown) control the persistence of activity of a certain gene (or genes) and the suppression of the activity of the other genes at other loci for alternative antigenic traits.

The antigenic types in another physiologic species of \( P. aurelia \) ("variety 1") are now under investigation by C. H. Beale, of Edinburgh. He kindly permits me to mention here his still unpublished findings which not only confirm the main features of the system set forth above, but bring to light some new features of much interest and importance. In agreement with our results on variety 4, Beale has found in each stock a spectrum of alternative antigenic types with transformations from one type to another brought about by changes in temperature. Likewise, the occurrence of these transformations may be delayed for shorter or longer periods of reproduction following change of temperature. There is thus temporary cytoplasmic inheritance of antigenic type, but no examples of permanent cytoplasmic inheritance have yet been found. The transformations are reversible. Differences between stocks in the spectrum of producible antigenic types have been shown to be due to differences in nuclear genes.

Beale has added two new features to the analysis of the genetics of antigenic types. First, in the corresponding spectra of antigenic types in different stocks, there is a fundamental similarity in the sequence with which diverse types arise as temperature changes. Thus, if the sequence of types called forth in one stock is A at the lowest temperature, and B, C, D and E, respectively, as temperature rises, then in general the same sort of sequence is characteristic of other stocks. However, the absolute temperature and the range of temperature for calling forth a particular type may differ from stock
to stock. To illustrate the point, the types appearing at different temperatures in two fictitious stocks 1 and 2 might be arbitrarily represented thus:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Stock 1</th>
<th>Stock 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>13°</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>17°</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>21°</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>25°</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>29°</td>
<td>D</td>
<td>C</td>
</tr>
<tr>
<td>33°</td>
<td>E</td>
<td>D</td>
</tr>
</tbody>
</table>

This sort of relation may also hold for variety 4; the matter has not yet been sufficiently investigated. If it does, the failure to find a type in a particular stock, for example, type F in stock 51, may be due to our failure to work at sufficiently extreme temperatures, not to the inability of the stock to produce that antigen. Type F arises at lower temperatures than H in stock 29 and type H arises at the lowest temperature (13°) thus far employed with stock 51. It may therefore turn out that F will arise at temperatures lower than 13° or H may still be the type that arises at the lowest temperatures which the stock can endure. It would then be practically impossible to distinguish two alternatives: whether stock 51 contains a gene for type F which can however act only at temperatures below the viable limit, or whether the stock has no gene for F at all.

Only if this same gene could come to phenotypic expression at more moderate temperatures when in a different genic background, would it be possible to distinguish between the two alternatives. In such a case, hybrids or their progeny might manifest antigens that neither parent could show. This sort of mechanism might be involved in some of the well-known cases of hybrid antigens (Irwin, this volume). Beale's discovery thus appears to open up new vistas with regard to latent variation. These cells may carry genes for antigens which they cannot develop because conditions outside the viability range are required for their manifestation. We have indeed some breeding and serologic evidence suggesting that stock 29 contains genes for antigens E and G although neither of these has yet appeared as a ciliary antigen in this stock.

Beale's second new discovery is that antigens which are homologous in occupying similar positions in the temperature series of different stocks may be so distinct serologically as to give little or no cross-reaction to antisera against each other. That the homology is never-
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theless real is further indicated by the demonstration of their control by allelic genes. For example, type Y is homologous to type W in both senses: these two types never occur together in the same stock; they occupy similar positions in the temperature series; and they are determined by alleles. With such distinct antigens controlled by alleles, Beale has been able to show clearly that each stock contains a number of gene loci which come to phenotypic expression at different temperatures. The situation may be illustrated by comparing stocks 60 and 90 at 25° and 29°. At 25°, type Z arises in stock 90, type X in stock 60; and these two types are controlled by a pair of alleles. At 29°, type Y arises in stock 90, type W in stock 60; and these two types are controlled by another pair of alleles at a different locus from those controlling types X and Z. Yet at 25° (with limited food), cultures of type Y and of type Z may be maintained side by side without change for a considerable period, though not permanently. Hence, stock 90 contains two loci controlling the mutually exclusive types Y and Z; one locus eventually comes to expression at 25°, the other at 29°; but at 25° the two types are temporarily inherited.

Beale's results thus confirm and extend the system of gene-cytoplasm interactions found in our variety 4. This is of interest in yet another respect. I had earlier (Sonneborn 1945) pointed out that previous observations on varieties 1 and 4 indicated marked differences in the rules of inheritance. Variety 1 showed principally gene-controlled heredity, variety 4 principally cytoplasmic inheritance. At that time, it was suggested that there could scarcely be any fundamental difference between the genetic systems of two such closely related physiological species. While the attempt then made to reconcile them has proved untenable, our subsequent work on variety 4 and Beale's work on variety 1 have demonstrated the fundamental similarity in the genetic system of these two "varieties," a system which involves in both cases a close interrelation between genic and cytoplasmic inheritance.

The fundamental genic and cytoplasmic mechanisms in this system of determination of antigenic types are of course not yet understood at all, as will be pointed out more fully later. Only the more
superficial aspects of the system are now evident. But even this much knowledge provides a deeper insight into the relations between genes and cytoplasm in the control of hereditary traits. The three main features are: (1) the cell may contain genes for alternative and mutually exclusive traits so that only one of them can come to expression at any one time; (2) the cytoplasm of the cell embodies mechanisms which perpetuate for longer or shorter periods of cell reproduction (sometimes permanently) the particular alternative trait which happens to have been developed; and (3) environmental conditions can bring about shifts from one gene-controlled alternative trait to another. Without further understanding of the mechanisms involved, it is still of much interest to inquire whether this system is limited to the antigenic types of Paramecium or whether it is likely to prove of more general applicability and significance. In this connection, examples of four kinds of phenomena will be discussed, they seem to show enough resemblance to the system of control of antigenic types to warrant the suggestion of fundamental similarity.

The Problem of Dauermodifikationen

The first is known as "Dauermodifikationen," discovered and named by Jollos in 1913 and subsequently confirmed by many other investigators. Like the antigenic types of Paramecium, Dauermodifikationen are traits which are induced by environmental conditions and then manifest temporary cytoplasmic inheritance. After a time under the original conditions, the induced trait disappears and the original trait reappears. The role of genes in Dauermodifikationen was never analysed. Aside from this, the parallel to the inheritance of antigenic types is complete. It is therefore reasonable to suppose that the basic mechanisms may be similar. If so, the interpretation is quite different from the one set forth by Jollos. He assumed that the temporary cytoplasmic inheritance was terminated as a result of the ultimate "triumph" of nuclear genes over cytoplasmic materials of heredity. If the model of the antigen system is applicable, such an antithesis and struggle between cytoplasmic and nuclear materials of heredity need not be assumed. Both the environmentally induced
trait and the original trait could be controlled by nuclear genes. The shift from one to another would be comparable to the shift from one to another antigenic type in the gene-controlled spectrum of possibilities. That this is the correct interpretation is further attested by the fact that Jollos (1921) himself reported observations on antigenic types which as far as they go, are comparable to ours; and he interpreted them as Dauermodifikationen. His other observations were mainly on changes in resistance or sensitivity to injurious agents. In this connection it is remarkable and suggestive that Austin (in press) finds one such change in resistance in Paramecium to be closely correlated with the antigenic type of the animals.

As pointed out earlier (Sonneborn, 1946), however, other known mechanisms may underlie some Dauermodifikationen, particularly changes in the cellular concentration of self-duplicating cytoplasmic factors of the kappa type, including viruses, plastids, kinetosomes and probably mitochondria. In most cases, the analysis of Dauermodifikationen has not been carried out in such a way as to make clear which of these two possible genetic mechanisms may be involved; but present knowledge of these two mechanisms makes it possible to say that the puzzle of Dauermodifikationen—now nearly 40 years old—is at last solved in principle.

Plastid Variegation

The second case I shall discuss has a very special place in the history of cytoplasmic inheritance for it was the first one to be reported. It is the old example of variegation in Mirabilis reported by Correns in 1909. Today this has renewed interest because Correns (1937) finally came to an explanation which anticipated in one respect the system of gene- cytoplasm interactions now known to control the antigens of Paramecium. The Mirabilis case differs from those examples of variegation interpreted as due to gene mutation or to plastid mutation in two important respects: (1) no one cell ever contains both green and white plastids; (2) although the trait shows maternal inheritance, all cells have only green plastids in early development, white areas developing only later. To explain the uniformity of plastids in a cell, Correns assumed that the failure to form chloro-
phyll was due to an effect of the general cytoplasm outside the plastid. To explain the maternal transmission and delayed development of white areas, he assumed that the cytoplasm was in a labile state in which chlorophyll could develop, but that in some cells it transformed later into another state in which chlorophyll could not develop. These assumed transformations of cytoplasmic state are obviously comparable to our demonstrated shifts from one antigenic type to another in Paramecium. Correns, however, implied that this system of alternative cytoplasmic states was independent of the nuclear genes because it was not apparently transmitted through the male. Nevertheless, it is entirely possible that the two cytoplasmic states were gene-controlled, like our antigens, and transmitted through the male, providing one assumes that the plastids in the variegated strain differ genetically from plastids in non-variegated strains; and that the type of plastid in the variegated strain is unable to form chlorophyll when the cytoplasm is in one of its two alternative states, while in non-variegated strains the plastids could form chlorophyll when the cytoplasm is in either of its two states. This interpretation has the advantage of assuming only conditions known to occur: plastid mutations and gene-controlled alternative cytoplasmic states; and it brings Correns' long puzzling case into line with other known examples of variegation due to plastid mutation.

**Determination of Sex**

Inheritance of sex played such a key role in the development of the chromosome and gene theories that it is of special interest to consider it as the third possible example of gene-controlled alternative cytoplasmic states, each of which can be cytoplasmically inherited.

Sex in the alga Chlamydomonas (Moeis, 1936) and in the fungus Hypomyces (Hansen and Snyder, 1946) provides an almost diagrammatic example of this sort of system. In both organisms, sex seems to be controlled by genes at slightly different loci in homologous chromosomes. As the organisms are haploid, they are normally dioecious. Rarely, however, crossing-over apparently occurs between the sex loci. Clones that contain the chromosome with both sex genes are monoecious and individual cells in a clone can differ in
sex, some being male, others female. The reciprocal cross over class, lacking both sex genes, is non-viable in Chlamydomonas, but viable and neater in Hypomyces. Thus, the role of the genes is to control the possibilities for development. When neither sex gene is present, neither sex can develop; when only one sex gene is present, only one sex can develop; when both sex genes are present, then both sexes are developmental possibilities. In this situation, other factors than the genes decide which sex will be realized, for the two possibilities are mutually exclusive. As with the antigens, transformations occur and are reversible.

While there is little or no persistence of sex in these monoecious cultures, persistence is shown clearly in another alga, Protosiphon (Moewus, 1935). Here the existence of two diverse sex genes in the monoecious haploid culture has not been demonstrated; but other factors do determine which sex will be realized. They act at an early sensitive stage of the life history and thereafter sex is fixed during vegetative reproduction, though the observable sex differences are not manifested until later.

Piecing together thus the evidence from various microorganisms, sex seems to be under the control of a system similar to the one that operates in the control of the antigens of Paramecium. Perhaps the hermaphroditic or monoecious sex condition, wherever found, constitutes a pair of mutually exclusive traits the possibilities for which are gene-controlled, but the realization and perpetuation of which in cell lineages is cytoplasmically inherited on the cellular level.

CELLULAR DIFFERENTIATION

Sex in hermaphrodites and monoecious organisms is of course a developmental differentiation. The system of gene-cytoplasm control involved in that specific case may well be applicable to developmental differentiation in general. In the course of development, different cells, presumably having the same genes, acquire alternative mutually exclusive cellular traits of many kinds: different antigens, enzymes, proteins, and so on. Many of these cellular differences can be perpetuated in tissue cultures; they seem to be cytoplasmically in-
herited. In view of what is now known about the determination and perpetuation of antigenic differences within clones of paramecia, it seems likely that all of the cells of any one metazoan body contain genes for many, if not all, of the series of possible alternative traits; that conditions outside the nucleus, perhaps outside the cell, determine which genes of the series of alternatives will come to phenotypic expression; and that cytoplasmic mechanisms—no better understood here than in the case of the antigens of Paramecium—assure the perpetuation of the developed alternative during cell reproduction.

This may not be—indeed, probably is not—the only mechanism of cellular differentiation. Differential distribution of self-duplicating cytoplasmic particles, behaving like kappa or like the cytoplasmic component in Ephrussi’s beautiful studies on the respiratory enzymes of yeast, could also yield developmental differentiations, as has been pointed out previously (Sonneborn, 1947a; Ephrussi, this volume). Still other mechanisms may be required to account for highly complex differentiation in acellular or unicellular organisms.

As Ephrussi has emphasized, the demonstration of a self-duplicating cytoplasmic particle in the system of determination hinges largely on the irreversible loss or mutation of the particle, detected as an irreversible change of character. Yet, inferences about irreversibility are difficult to draw with assurance. For example, under standard conditions, some of the changes of antigenic type in Paramecium are permanent and irreversible; yet under other conditions they are readily reversible. In my opinion, the same may well be true for developmental differentiations which have up until now usually been considered irreversible. The systems of cellular transformation and of interactions between genes, cytoplasm and environment, now being analysed in Paramecium and in yeast, serve as models of the sort of thing that may be occurring in developmental differentiation of higher animals. This is a step forward in the long struggle to understand the relation of genetics to development. Yet the applicability of any of these models to any specific example of developmental differentiation remains purely formal and it must be recognized that
none of the models seems yet to touch the master problem of the control of the pattern of cellular changes in time and space during the course of development.

SUMMARY; DISCUSSION; PROBLEMS FOR THE FUTURE

The preceding account laid very unequal emphasis on the five roles of the genes in cytoplasmic inheritance. The first three, treated briefly, dealt with effects of nuclear genes on self-duplicating cytoplasmic bodies: genes control their mutation and their cellular concentration; they probably also operate as selective agents when alternative forms of a cytoplasmic factor are present together in a cell. The other two roles of the genes were discussed in more detail. One of these involved interaction of nuclear genes with a cytoplasmic component of the genetic system, such that the same change in hereditary traits followed from a change in either the nuclear or the cytoplasmic component of the genetic system. The other involved a different sort of interaction between genes and cytoplasm: genes for two or more mutually exclusive alternative traits coexist in the same cell; extranuclear conditions (in cytoplasm and environment) determine which genes will come to phenotypic expression and the extent to which the developed alternative will be perpetuated during cell reproduction. In all but the last of these five roles of the genes in cytoplasmic inheritance, it is clear that the genes are interacting with hereditary materials or properties of the cytoplasm; and in the fifth role this possibility is not yet excluded. The genetic system of the cell thus includes closely integrated nuclear and cytoplasmic components.

Although this paper has stressed the action of the genes on the cytoplasmic component, there are also evidences of reciprocal action of cytoplasm on the genes. Stubbe (1935) and Michaelis (1949a) report that cytoplasmic genetic materials influence the mutability of genes. Other reciprocal effects of the cytoplasm on the genes may be due rather to gene-conditioned properties of the cytoplasm. These include selection of chromosomes, accepting some and rejecting others; possibly influencing chromosome concentration, that is, poly-
teny or polysomaty; and determining which genes of a series of alternatives will come to phenotypic expression.

Looking ahead now to the future, one of the main tasks seems to be the discovery of the mechanisms of interaction between the genic and cytoplasmic components of the genetic system. There is little need to stimulate interest in the genic part in these interactions, for most geneticists are already preoccupied with studies on the activities of genes. On the other hand, much further work needs to be done on the roles of the cytoplasm in the genetic system of the cell and, it seems to me, our thinking and experimenting should be directed along the following three lines.

First, the full extent and nature of the participation of self-duplicating cytoplasmic materials in the genetic system of the cell needs to be discovered. Of the three needed lines of investigation, this is the one that has already been most explored. In the class of cytoplasmic self-duplicating materials, the plastids are agreed upon by all to be members in good standing. Kinetosomes and centrioles are in nearly as good standing; there is little ground for doubt as to their self-duplication, but their capacity to mutate, their ultimate nuclear derivation, and their occasional de novo origin are still open questions. Mitochondria are more than ever contenders for admission to the class. Evidence that they can exist in submicroscopic sizes weakens the case for their occasional apparently de novo origin. Evidence for their mutability has already been cited. Their association with fundamental enzyme systems brings them to the forefront of importance. If, as is now indicated, they are indeed self-duplicating and mutable, this would go far toward answering the argument adduced by Muller (this volume) for the restriction of normal, self-duplicating, cytoplasmic genetic materials chiefly to plants.

Second, there is need to discover the material basis and mechanism of action of the cytoplasm in controlling which of an alternative series of genes will come into phenotypic expression, and be maintained so, in the course of cell multiplication. That self-duplicating cytoplasmic materials may be involved here also, at least in part, has by no means been excluded; but neither has it yet been demonstrated. A number of other possibilities have been suggested. Wright (1945),
Waddington (1948) and Delbrück (see Sonneborn and Beale, 1949) have all proposed schemes based on the concept of open systems or steady states, which had been developed earlier by Bertalanffy (1950 and earlier). Kimball (1947) suggested that the presence in the cytoplasm of some direct or indirect product of a gene might increase the rate at which that gene functions. Another variant of the gene-activation hypothesis was put forth by Alexander (1948). There are many ideas on this question. What is now urgently needed is decisive experimental analysis of the problem in a model case such as the antigen system in Paramecium.

Third, there is another possible kind of cytoplasmic role in heredity that has been considered very little, but may turn out to be of the first importance. The cytoplasm may provide a particular self-perpetuating molecular pattern. This could be true both for the surface of self-duplicating particulates, such as plastids and mitochondria, and for the fibrous ground substance of the cytoplasm. There are, it seems to me, reasons for suspecting that the molecular organization of the cytoplasm may be a hereditary property of the cytoplasm, comparable to the hereditary arrangement of the genes in the chromosomes. The rapid and efficient operation of enzyme systems with many enzymes participating in a regular sequence, seems to require a precision of localization on enzyme-bearing particles such as mitochondria; and this arrangement is most easily conceived as a consequence of the surface pattern on which the enzymes are absorbed. As the mitochondria are probably self-duplicating, the pattern too may be perpetuated.

To put the matter as vividly as possible, let us try to make concrete some of the consequences that would follow in an extreme case, even if we have to imagine situations not yet realized in the laboratory. If the nucleus were in complete and exclusive control of heredity, then it would have to be concluded that nuclei, isolated under conditions that permit their multiplication, would be capable of reconstituting cells of the kind from which they were taken. If this did not happen, then it would have to be concluded that the cell, including the cytoplasm, somehow serves as a necessary model for the formation of new cellular material in essentially the same sense as the
genes are necessary models for the formation of new genes. The unicellular colorless flagellate Chilomonas can be cultivated on a few inorganic salts and acetic acid. Would any biologist go so far as to believe that a successful culture of Chilomonas nuclei, provided with such a diet—or for that matter with as complex a diet as one wishes—could reconstitute a Chilomonas cell, or any cell at all? Yet if the cytoplasm is entirely the result of gene activity, if it can all be made de novo, when food is provided, no model of pre-existing cytoplasm should be needed for its new function.

Perhaps it will be objected that there are some self-duplicating cytoplasmic elements which the nucleus cannot make. Then suppose these too can be cultivated in vitro. Is anyone willing to believe that, if all such self-duplicating components of the cell were thrown together in a test tube in the proper proportions with adequate food for their multiplication, a Chilomonas cell or any cell at all would result? Although the whole picture is admittedly imaginary, it makes the nature of the problem sharp and clear. If cells cannot be reconstituted in the way suggested, then it seems to me we are forced to admit that the molecular and particulate arrangement of the cellular materials, their organization into a working system, is itself a part of the genetic system of the cell.

But these are problems for the future. When the mode of interaction between the two or three components of the genetic system of the cell is understood, we shall not only have a deeper genetics, but shall have achieved the long-sought fusion between genetics and embryology. In the course of reaching these goals, the two major divisions of the cell—nucleus and cytoplasm—which have necessarily been torn asunder in the preliminary analysis of the last fifty years, will again be reunited in an integrated, interactional conception of the genetic and developmental system of the cell. Perhaps these great achievements of the future will be celebrated 50 years hence at the 100th Jubilee Celebration of the birth of genetics.
ACKNOWLEDGMENTS

This paper is contribution No. 457 from the Department of Zoology, Indiana University. The work of the author and his associates has been greatly aided by grants for research from Indiana University, the Rockefeller Foundation, the U. S. Public Health Service, and the Jane Coffin Childs Memorial Fund.

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MENDEL AND THE DETERMINANTS

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MENDEL'S DISCOVERY

AFTER 50 years we do well to look back and consider what Mendel's work means for us, and what it is likely to mean, for work of this importance is not necessarily rediscovered in one step or at one moment. If we take the trouble to read Mendel's paper today we find in fact some things very different from what they seemed to be to the rediscoverers.

It was Bateson who probably did most to put the Mendelian theory into circulation and by inventing such terms as "homozygote" and "allelomorph" gave it the verbal resources it needed for exact discussion and legitimate expansion. To him, and to those who have followed him even down to the present day, the essence of Mendelism was segregation. And to some who asked, segregation of what? he would give no answer. To Bateson the Mendelian laws became more and more abstract and less and less related to any concrete substance. So great indeed was the confusion between a "character" and what determined it that we even find an exponent of the chromosome theory like Boveri referring in 1904 to the possibility of the crossing-over of "characters" in chromosomes.¹

¹ He uses Mendel's term "Merkmal." Incidentally it is interesting to note that Punnett (1950) attributes Bateson's rejection of crossing-over as a basis of linkage to a faith in what he believed to be Boveri's idea of the individuality of the chromosome. Evidently, however, Bateson had not read Boveri's paper.
This confusion was not due to Mendel himself. It is true that Mendel cautiously refrains in his experimental description from representing homozygotes (which cannot show segregation) as AA or aa, reserving the double letters for the heterozygotes Aa (which can show segregation). This is a superb example of the attempt to separate fact from hypothesis. But when, at the end of his paper, Mendel comes to draw his conclusions he draws them with complete confidence and logical consistency. Elements in the cells, he says, must determine the characters. These elements must be double after fertilization, and only at germ cell formation will the maternal and paternal elements separate.

This grand speculation is not Mendelism as we ordinarily picture it. It is what has been called Morganism. But, in fact, it is a part of Mendelism, a part which the early Mendelians forgot to notice or refused to accept. They, perhaps inevitably, concentrated on the technical details of segregation—and later of crossing-over. Their concentration gave them a narrow picture of the world of heredity, a picture which aroused an equally narrow opposition. It is all the more remarkable that they had no narrow view of the scope of the science they were creating. The name of genetics which Bateson gave to this science announced to the world their belief in the tremendous implications of what seemed to the impartial bystander to be mere statistical trivialities. That belief we now know has been justified by 50 years of inquiry—but not in the way they expected. For in this time the elements have superseded the ratios. I want to consider how this has come about.

THE THREE-LEVEL SYSTEM

The early Mendelians clung desperately to the idea of a unity of the determinant and the character, the idea they covered with the expression unit character. They seemed to abhor the vacuum in understanding that any separation would create. The discovery of gene interaction by Bateson in 1907, of plastid determination by Baur in 1909, and of the chromosome basis of linkage by Morgan in 1911 forced the determinant and the character wide apart. In these circ-
circumstances Johannsen's term gene slipped in just when and where it was needed, as it were, to register and confirm the separation. Its use distinguished those who believed from those who did not—Johannsen himself falling in the latter group. Ever since that time we have been concerned in filling up the vacuum created in this way. We have been occupied in giving the gene a concrete reality in place of the merely Euclidean character of the original concept.

The results of our labors have been to show the pre-eminence of the nucleus and the chromosomes in determining heredity. Our opinions in this matter have been decisively influenced by the permanence of chromosome organisation and the exactitude of chromosome division, already established in the nineteenth century. The influence has been twofold. In the first place, however important cytoplasmic determinants might be, the precision of the chromosome mechanism makes it very much easier to analyse and describe in precise terms. Nuclear action had therefore to be described first as a frame of reference for any non-nuclear action there might be. In the second place, the process of evolution itself has depended on having a precise mechanism for registering the effects of natural selection. Nature happens to need for evolution the same frame of reference that the experi-
menter needs for analysis.

There is however something more than mere precision in the chromosome mechanism which distinguishes it from any cytoplasmic system and accounts for its pre-eminence in long-term government. It is that the inheritance, the distribution, and the survival of nuclear genes is independent of their individual function. The whole nucleus is transmitted as a unit and survives as a unit. The coordination in multiplication and division of all the genes in the nucleus means that they all sink or swim together.

It is a paradox, perhaps the outstanding paradox, of genetics (albeit one which we have now liquidated), that the genes, which are the centers of the highest physiological activity in the cell, are subjected to a mechanical discipline in distribution which entirely overrides their individual physiology. The units which are most powerful in physiological action as parts of the nucleus are most obedient in mechanical action as parts of the chromosomes. They are, as I have
Mendel and the Determinants

said elsewhere, like the members of a legislative assembly, subject as individuals to the laws they enact as a body.

The coordinated reproduction of the genes of a nucleus in mitosis means that the selective value of each gene in evolution is related to a fixed nucleus for a period of time.

This enormous increase in the complexity of the unit of heredity would have been of no value in evolution without the means of regulated breakdown in that unit which is provided by crossing-over and recombination at meiosis. And indeed the life of a nucleus is limited by the occurrence of fertilization at one end and of meiosis and crossing-over at the other end of the sexual cycle. Meiosis and crossing-over are both necessary for the recombination of parts of chromosomes. But we now recognise that they are themselves bound up together in an elaborate and elastic relationship. Crossing-over, which is the means of recombining parts of chromosomes, is also the basis of chiasma formation. And chiasma formation in turn is the basis of the pairing of chromosomes and hence of their segregation to alternative haploid nuclei. In other words crossing-over, in determining the means of recombination, also determines the unit which will be recombined.

A remarkable situation arises from these interlocking causal sequences. Over one sexual cycle the unit of selection and evolution is the whole nucleus. But over a period of many sexual cycles the unit of selection and evolution is reduced, step by step, to the unit of crossing-over and recombination, the mechanical gene.

The unit of selection is elastic in time. But it is also elastic in another way. It has to be considered in relation to the variety of nuclei into which recombination can introduce it. Structural hybridity interferes with crossing-over and thus expands the size of the mechanical gene. But structural hybridity is related in turn to the frequency of crossing and the size of the mating group. The size of the recombining unit, and the types of nuclei into which it can enter, thus depend on the breeding system of the group and the variation occurring within it.

The organization of the chromosomes, the system of pairing by chiasmata, and the alternation of mitosis and meiosis therefore en-
able selection to operate at three levels—gene, nucleus and species—all of which vary in relation to one another. This three-level system (hinged, as Weismann realized, on recombination) constitutes the central fact of the life of sexually reproducing organisms.

Thus we see that crossing-over whose importance for us in the last 50 years has lain in its use as the mechanism of discovering genetics is likely in the next 50 years to be more important as the mechanism of adaptation and evolution of the three-level system.

In itself the three-level system is simple enough. But its implications are without number. To discover them is our continuing task. The first implication is that to the three-level system the nucleus owes its long-term genetic pre-eminence both in nature and in research.

GENETIC PARTICLES

As we have learned more about what nuclear genes can do, and cannot do, we have also learned how to assign particular types of heredity to the cytoplasm. Perhaps the clearest example is in Bateson's rogue peas. The rogue character as Bateson saw it was non-Mendelian, which put it outside the nucleus. But it was also biparental; indeed the pollen might be more effective than the eggs in transmitting the character, which put it outside the cytoplasm. Hence, for Bateson, refusing to adopt the chromosome theory, the problem was insoluble. To us, certain as we are of the limits of nuclear uncertainty, it is merely a question of finding out how a small amount of cytoplasm may contain more determinants or plasmagenses than a large amount, a problem which the kappa particles of Paramecium with their different rates of multiplication have found no difficulty in solving for us.

Cytoplasmic particles must depend for their propagation, since they are not attached to the nucleus or to one another, on their particular chemical character which must in turn be related to their particular physiological activity. It might seem that this activity must vary in the course of differentiation in proportion to its significance. In this case no organisms with a high degree of differentiation could have genetic particles of high significance in the cytoplasm. But is it
possible that there are highly significant genetic particles in the cytoplasm which do not vary in physiological activity in the normal course of differentiation? This is true, I am inclined to think, of particles which by mutation become responsible for cancer both in plants and animals: latent plasmagenses not brought to light in the normal course of development (Darlington, 1948).

We are gradually being driven to conclude that there is a wider range of cytoplasmic determinants of greater power than our predecessors had dared to suppose. And just as, in laying the foundations of Mendelian or nuclear genetics, new terms were needed to establish new concepts and avoid confusion with older ideas, so we have to adopt new terms for cytoplasmic determinants. When we try to do so we find that the same precision is not at once possible. We may begin by referring to particles important in heredity as plasmagenses, and to those important in infection as viruses. But what is heredity and variation for a protozoan is development and differentiation for a higher organism. And what is heredity for a particle of Rickettsia transmitted through the egg of a bug is infection when the same particle is transmitted by the bug to man. Again, how are we to speak of transmission by cell-free filtrates, of plant diseases like the King Edward Potato leaf curl, of bird tumors like the Rous sarcoma, and of l'Héritier's genoid in Drosophila? These we have no reason to suppose are transmitted by infection in nature. And again there are, as Billingham and Medawar have shown, nuclear products which are self-propagating in the cells of piebald mammals and are capable of infecting cells which do not produce them. Finally, we may recall that new plant viruses or virus-like entities are continually arising under controlled conditions, and even, as Gautheeret has shown, under the action of specific agents like Phytomonas tumefaciens or heterauxin (Darlington, 1949). How then can we doubt that particles propagating or transmitting themselves in one way may not, in the course of evolution, turn over to another way of life as opportunity offers or as mutation determines?

In these circumstances would it not be wrong to insist upon absolute distinctions in every case? And would it not be dangerous to argue on the basis of such distinctions? There is, it seems, one dis-
tinction we must make before taking over the data of pathologists and others who are unfamiliar with the importance we attach in genetics to natural selection and its consequences. There is one kind of particle which is propagated by natural infection and is therefore adapted to natural infection and owes its character in part to this adaptation; and there is another kind of particle which is capable of artificial cell-free injection in the laboratory but cannot infect in nature. The one kind are true viruses: the other, the virus-like entities, we may describe as proviruses since, though their ancestors were not viruses, their descendants might be; they have the making of viruses in them if the opportunity arises—as in fact it sometimes does.

If a plasmagene can become a virus, can the opposite change from a virus to a plasmagene perhaps occur? The kappa particles of Paramecium would offer themselves as candidates for admission to such a class in transition. The disease in this case is unique in being transmitted to all offspring, in being carried by all and carried presumably with advantage, and in being scarcely infectious at all in nature. The kappa particle is therefore much more of a plasmagene than a virus. The fact that some difficulty arises in the distinction is what makes the situation so significant, more so than the difficulty arising in the distinction between plants and animals. It means that the pathologist cannot dismiss a particle as "only a plasmagene" and the geneticist cannot dismiss a particle as "only a virus." Both have to take both kinds of particle as their own serious concern.

Viruses are of interest in relation to the history of genetics in two other ways. In the first place our knowledge of viruses has grown up in the same half century as genetics. But the concepts used have been quite independent until recently. No one has been bothered to distinguish in viruses between germ plasm and soma or between genotype and phenotype. It is only most recently that they have appeared as anything more than naked genes. Now we can see them, perhaps beginning in this way, but ending up in organization of great complexity, adopting chromosome nucleic acid as the vehicle of their propagation and clothing themselves (in the nuclear viruses of insects) with a protective capsule, an adaptive covering, a body distinct from their germ plasm. So the notions of genetics at last
become applicable to viruses long after the difficulties from which they arose have been solved.

In the second place we often hear the question whether viruses are living or not. The relationship with plasmagene shows us the answer although it is not an answer that will satisfy everybody. Life has two aspects: the physiological or momentary, and the genetic or continuing. Physiologically a virus is living only as a part of a cell in which it momentarily maintains itself as part of a particular dynamic equilibrium. Genetically it can be said to be living by itself. For a virus, unlike a plasmagene, is an independent unit of propagation and, genetically, life is the capacity for continuing propagation. The process of infection which separates the plasmagene from the virus also separates the physiological from the genetic conditions of life in genetic particles.

**PHYSIOLOGICAL UNITS**

The discovery of gene interaction by Bateson was the beginning of the task of filling up the vacuum between determinant and character. This task has now developed into a great industry ramifying over the whole world of biology. The pioneer experiments of Ephrussi and Beadle with Drosophila pigments, and the instructive analyses of Lawrence and Price with Dahlia pigments have been followed by the great achievements of Beadle and others with Neurospora. All these have arisen from our applying the classical Mendelian method. As usual in genetics, however, the problem can be tackled from the determinant beginning, as well as from the character end.

Observations of the interactions of whole nuclei and their effects on the life of the cell are necessarily crude but their interpretation is indispensable to an understanding of gene action. The two main types of nuclear interaction, competition and cooperation, take effect, it seems, subject to conditions which we may define. In the first place, proteins must be being produced. In rapidly growing root tips (as Vaarama has found) or in animal tumors (as Koller has found) where protein may well have been over-produced, sub-haploid cells can survive and even multiply. In the second place, cooperation is limited to nuclei between whose cells a ready exchange
of nuclear products is permitted, that is, where a membrane or wall is not too well developed. Further the nuclei must together make up something like a balanced set, that is, the normal haploid set of the species. The observations which lead to these conclusions (Darlington, 1931) thus confirm the general view that the production of proteins by cells depends on their possession of a set of genes which may equally well be in one nucleus or in many, since the genes can exchange products equally well within one nucleus or through the cytoplasm. All growth depends on the cooperation in protein production of this set of nuclear genes which must be selected, adjusted or balanced for their efficiency in this task.

Observations of the chromosomes and nuclei themselves can however give us much more precise information. We can look at the cell and see what the genes are doing and how they are doing it. We owe a great deal here to Caspersson for showing us that the genes produce proteins of different types which pass in to the cytoplasm and for telling us some of the important distinctions that can be made in the cell between euchromatin and heterochromatin and their products. It is now worth while seeing how far this method will carry us when applied to the study of nuclei in all conditions of life.

We are liable at first to be confused in looking at the chromosomes by the double life their responsible positions compel them to lead. On the one hand they have the business of protein production, their public work. On the other hand they have their private work. A chromosome has to reproduce. It also has to undergo a complex cycle of movements in coordination with its brethren. These movements are internal, such as spiralization and crossing-over, and also external, such as the terminalization of chiasmata and the anaphase separation.

But the public and equally the private work of the chromosomes depends on the chemical activities of their constituent genes. Chromosome mechanics, which we can study as a science in itself, and one with specially important bearings on Mendelian inheritance must rest on a basis of gene chemistry. Will it therefore tell us something about gene chemistry? It will, I believe, tell us certain things that we can learn in no other way.
The genes that we can distinguish from their neighbors in the chromosome owe their identification to a particular property; the products of their activity remain attached to them. They are of three kinds—first, there is the heterochromatin which is rendered visible by the accumulation around it of substances it produces. At high temperatures this accumulation dissolves at prophase. At low temperatures however it probably dissolves too slowly since it can be used to prevent the proper attachment of nucleic acid to the heterochromatic segments. In the metaphase chromosome these starved segments consequently appear constricted.

Secondly, there is the nucleolar organizer which McClintock divided and thus showed to be a multiple gene. It secretes material which accumulates in the resting nucleus, dissolving only during prophase and leaving its mark on the chromosome (as heterochromatin can do) in the form of a constriction.

And thirdly there is the centromere which is the multiple gene responsible for the organization of the spindle. This also by its activity, no doubt, prevents the attachment of nucleic acid and appears as a constriction in the metaphase chromosome.

Now a remarkable fact has come to light in the last ten years: all these three types of gene, characterized by having non-detachable or non-diffusible products, under special conditions, can be persuaded to release them.

The association of nucleolar organizers with constrictions in many plants by Heitz revealed the existence of exceptions for example in Eremurus, Tradescantia, Tulipa, Trillium, and Paris polyphylla (cf. Darlington 1947,) and in Allium ampeloprasum (Levan, 1940). In all these plants the nucleoli arise at the ends of chromosomes and there is consequently no evidence of specific organizers. A corresponding situation arises in Pisum where nucleoli in certain nuclei are formed, not at the ends, but near the centromeres, of all the chromosomes. Then a number of plants were discovered by Kattermann, Müntzing, Rhoades and others in which, at meiosis, the function of the centromeres was shifted to the ends of the chromosomes. In this case the specific and original site of the centromere is still revealed by the non-division of the chromosome at that point at the second meta-
phase. The condition of the abnormality can be specified. It depends on an abnormal nucleic acid metabolism and a particular genotype. It seems in maize (Rhoades and Kerr, 1949) that this action is due to something secreted at the centromere which flows along the chromosome to the ends to generate the spindle there and to set up the anaphase repulsion. Normally it seems the secretion is localized at the centromere and, by unfolding proteins in the cytoplasm, turning them into spindle fibres on the spot. Diffused centromere action in a number of plants and animals may arise by just such a diffusion of the centromere secretion.

We might suppose that the centromere or the nucleolar organizer was, like an end, and no more than an end, in being an obstacle to the further diffusion of enzymes produced elsewhere in the chromosome. This would work for the nucleolar organizer. But it would not work for the centromere which must be held to do the real work, for its absence is fatal to the movement of fragments of chromosomes arising from x-ray breakage.

In both cases, the displacement in the product of nucleolar and of spindle organizers, it is more likely that the particular substance, presumably an enzyme, secreted by their particular organising genes, diffuses along the chromosomes to an end (or in Pisum to the centromere) and there takes effect.

In the third case, that of the heterochromatin, we find related species, for instance in Fritillaria, or opposite sexes, as in the Orthoptera, in which the corresponding chromosomes or segments behave, or do not behave, as heterochromatin. On the present view the products of the heterochromatic gene are either attached to, or detached from, the genes according to conditions. The same genes, or their products, must be behaving differently in different circumstances, just as the centromere behaves differently in different parts of the nuclear cycle.

However we represent these relationships we get a picture of gene action and interaction in the nucleus which, in some respects, confirms our knowledge from macroscopic events and, in other respects, extends our knowledge. It shows genes doing a particular job under special genotypic conditions. This job consists in secreting enzymes
which act on the products of other genes or on the cytoplasm. The consequences of their activity depends on their diffusibility. The chromosome itself is very often the vehicle of diffusion. And the genes are usually reduplicated in linear sequence, in a block of similar or identical parts producing units of effective action.

So much for confirmation. Extending our knowledge, we see that that action is controlled by variations in the genotype, that is, in the nucleus as a whole and in the nuclear cycle. One type of gene, the centromere, is evidently doing different things at different times. At one moment it is influencing the nucleic acid attachment of its chromosome and hence its reproduction and its pairing at meiosis, its breakage and reunion under x-ray treatment. At another it is influencing the spindle. The enzyme it secretes may be the same at these different times; but it may very well be different.

These observations bring us into contact with the super-cellular analysis of gene action.

D. F. Jones (1947) has shown that these types of relation are revealed by the diffusion of gene products occurring in cell mosaics of the maize aleurone layer. First, there are those in which adjoining cells differ in regard to a gene whose product does not diffuse through cell walls and consequently gives a clear boundary. Secondly, there are those where an ultimate gene product diffuses from a cell that produces it to an adjoining one that does not, and so gives a blurred border. Thirdly (as I would suppose), there are those where an intermediate gene product, A, diffuses from a cell that produces it to an adjoining one that lacks the gene, and reacts to give the visible pigment with the likewise diffusible intermediate product of a second uniformly distributed gene, B, which in homogeneous tissue is not produced in sufficient quantity to use up all the supply of A. There is thus an accentuated border where the unused B has invaded the AB cells:

<table>
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<tr>
<th>gene product</th>
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In the light of these relationships it would appear that the characteristic differentiation of cells is conditioned by the fact that all
diffusion of gene products between cells is somewhat limited even where diffusion occurs. And where many genes are concerned in any particular process some will be entirely cell-limited and thus limit the process itself. It is the existence of this non-diffusible system which gives individuality to the cell and irreversibility to differentiation.

For cytoplasmic determinants the same distinction with regard to attachment and detachment of products also arises. Centrosomes, plastogenes, flagellar bands and other organella determinants are attached to their products. The melanophore determinants of mammals and other infectious plasmagenes such as the genoid of Drosophila and also the kappa particles of Paramecium yield detachable or diffusible products (Table 1).

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<th>TISSUE</th>
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<td>NUCLEAR</td>
<td>Heterochromatin</td>
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<tr>
<td></td>
<td>Nuclear Organiser</td>
<td>Terminal Nucleoli</td>
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<tr>
<td></td>
<td>Centromeres localised</td>
<td>Neo-centrics and diffuse types</td>
</tr>
<tr>
<td>CYTOPLASMIC</td>
<td>Centrosomes</td>
<td>Melanophore determinants</td>
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<td></td>
<td>Plastogenes</td>
<td>Kappa particles</td>
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<td></td>
<td>Organella-genes</td>
<td>Genoid</td>
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We are now considering genetic particles from their second aspect—that of physiological units and here the biochemist has something valuable to tell us. The nucleus is a closed world which operates under different conditions, physical and chemical, from the cytoplasmic world. The nuclear membrane presents a very tangible barrier whose disappearance, for example, at once changes the action of the centromeres by giving them a substrate for spindle formation. But are there not more than two worlds in the cell?

The inconsistency of the many simultaneous reactions of the cell has been explained by Peters (1949) as due to its organization in independent sacs close to the limit of visibility to which statistical aver-
ages such as the disassociation constant no longer apply. These hypothetical fields of enzyme action are comparable with the microsomes of Claude and the plastid precursors commonly assumed in plant cells. Where their size is not concerned, in their special properties of surface action, semi-permeability, and individuality of content, these granules or sacs, organellae or fields of action—little worlds containing only a few thousand million atoms—will be not unlike the master-organ, the nucleus itself.

The nucleus is undoubtedly a world to itself. But it is a large and standardized world enjoying no spurious indeterminacy from its marginal size. The smaller organellae provide that intermediate twilight world between the invisible chemical molecule and the visible cell which is perhaps necessary to explain organization and development. By considering the cell as a hierarchy of vesicles or organellae we may ultimately understand its spatial differentiation, underlying as it must the spatial differentiation of the whole organism.

THEORIES OF HEREDITY

Philosophers and biologists have equally been inclined to attribute general rules to heredity and to its variation, adaptation and evolution. Such rules are implied by the classifications of Ray and Linnaeus as well as by the theories of Lamarck, Darwin, Weismann and Morgan. But we now know that these rules depend on the chemical nature and physical state of the substance of heredity which in both these respects is differentiated and in both respects undergoes cyclical changes at mitosis and manifests many different types of behavior at meiosis.

Thus the substance of heredity is composed in part of plasmagenes, particles which lie in the cytoplasm protected, some of them only by their numbers and the laws of chemical reaction and equilibrium and others perhaps by ultra microscopic vesicles. As to its larger part it is composed of materials, the chromosomes, which are cyclically protected by a nuclear membrane and exposed in the cytoplasm. The greater part of the chromosomes is coated with nucleic acid when exposed during mitosis and is thus protected from many external sources of danger.
including direct chemical action; but the centromeres are not and are therefore (as Vaarama found) vulnerable like plasmagenes. They can be permanently modified by colchicine. Again one part of the chromosomes, the heterochromatin, is heavily coated with nucleic acid and with its own products in the resting stage. This makes it more vulnerable to nitrogen mustard (Loveless and Revell, 1949) less vulnerable to radiation (Darlington, 1950). The degree of polyploidy of the nucleus, the subdivision of chromosomes into genes and the differentiation of nucleus and cytoplasm follow somewhat different rules in different groups of organisms—especially in the three groups viruses, bacteria and cellular organisms. And finally in higher organisms the cytoplasm is protected from the outside world but not in Protista, Algae and Fungi. Beneath all this variety, however, the self-reproducing properties of genetic particles, and the principles of heredity, variation and natural selection which arise from these properties, remain as the enduring foundations of biology.

Genetics began as a study of the relations of parents and offspring in sexual reproduction. It continued by examining a deeper layer of events, the movements and activities of the determinants responsible for these relations. It then passed to consider unicellular and unimolecular organisms in which the distinction between determinant and phenotype almost lapsed and in which sexual reproduction entirely lapsed only perhaps to reappear again at a lower level. Genetic notions then transferred to the study of cell-lineages within organisms in which development took the place of heredity and differentiation the place of variation. By these stages it will be found that genetics has gradually broken down the barriers between the departments of biology. It has done so by introducing those rigorous notions of constant particles constantly determining verifiable effects which Mendel so clearly set forth nearly a hundred years ago. Now indeed in 1950 we may claim to have rediscovered Mendel. We not merely understand segregation and recombination; we have discovered the elements which segregate, and recombine and, above all, determine.

Apart from these positive assertions there is a negative method of assessing our debt to genetics. In many countries today we have biological societies, experimental and otherwise, where genetic ideas and
techniques, although not excluded from discussion, are not understood. In consequence the activities of these societies are confined to non-genetic experiments, interpretations, arguments and conclusions. It is interesting to observe from time to time how such societies go to work. Their proceedings, like our own, involve the pursuit of knowledge; but the pursuit is like a game of blindman’s buff conducted in an unlimited space. The physical exertion is evidently refreshing but nothing is ever captured.

For us, on the other hand, genetics removes the bandage from our eyes when we set out in pursuit of knowledge about living things. Accustoming our eyes to the uncovered light is difficult, its consequences are likely to be violent. Men working in the dark seclusion of the older disciplines fear its revealing effects on their established habits. The systematic botanist and zoologist are shocked at the possibility that the microscope will disclose faults in their elaborate bibliographic constructions and reveal the sacred mystery of the species to the vulgar view. The assumption of inescapable determinants is terrifying to the anthropologist and the psychologist, the immunologist and the practicing physician. Apparently, in some countries, it is even embarrassing to the agriculturist. At least as terrifying and embarrassing as it was 50 years ago to the founders of genetics when they feared to face the question of Mendel’s elements. We must therefore expect in the future that the fears of our fellow men will provide at least as great an obstacle to the development of our subject as do the inherent difficulties of our research.

If we look at the work of Mendel as part of the larger pattern of the history of science we see, what Mendel himself understood, that it was the necessary consequence of the cell theory propounded only twenty years earlier. Those who have from time to time rejected Mendelism and genetics have always been found to be ignorant of the function and meaning of cells. By their rejection they have won the applause of all those who happened to be similarly situated. An interesting implication is then demonstrated to us. Instead of particles, matter and causes we find ourselves back with humors, forces and supernatural purposes; back, in fact, in the meaningless and fruitless arguments and speculations of the Middle Ages, back with the al-
chemists, the astrologers and the quack doctors. By our close and necessary attention to detail we may easily lose sight of the greatness of the intellectual change which began 100 years ago in the time of Mendel and which has borne fruit in the achievements of our present genetics. It is not merely that everything of value in biology will gradually take its place in genetics. It is that by the achievements of genetics the commonplace words that we use—such as heredity, variation and evolution—have lost the simple but vague meanings that they had and acquired new meanings, meanings which we ourselves still realize only in part. Nothing less than a reshaping of human thought is now taking place.

SUMMARY

Genetic principles are derived from Mendel's work and from the cell theory both of which are closely related to, and continue to sustain the development of, scientific determinism. They have enabled us to replace in biology forces, humors, and purposes, by matter, particles, and causes. We must not suppose that this result was achieved suddenly and that Mendel's work was entirely rediscovered in 1900. In fact the original rediscoverers only rediscovered his segregation. It has taken 50 years to rediscover the determinants which he called elements and which we call genes. The process of doing so has depended on studying the exceptions to his rules. Crossing-over, the first of these, has been the main weapon of experimental analysis in genetics in the past. Its importance in future however will depend on something else, on its control both of recombination itself and of the units of recombination, the mechanical genes. The second exception has been cytoplasmic inheritance which will in future enable us to see the relations of heredity, development and infection and thus be the means of establishing genetic principles as the central framework of biology.
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EVOLUTION OF CYTOGENETIC MECHANISMS IN ANIMALS

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THE development of the very extensive field of animal cytogenetics during the past half-century has been so complex that, within the limits of the present paper, it will be possible to deal with only one aspect, namely the growth of our ideas on the evolution of cytogenetic mechanisms. Especially during the past twenty years we have learned to realize the importance, both from the theoretical standpoint and from that of animal and plant breeding, of understanding, not merely the properties and mode of action of single genes, but also the functioning of integrated gene-systems. The whole development of what we may call the neo-Darwinian trend in biology in recent years, typified by the work of men such as Sewall Wright, Dobzhansky, Ernst Mayr, Stebbins and Simpson, would indeed be incomplete if it did not rest on a firm understanding of the broad lines of the evolutionary history of the cytogenetic mechanism itself.

Such an understanding rests, of course, on a basis of knowledge concerning the structure of the chromosomes at various stages in mitosis and meiosis, but we have no intention of discussing problems of chromosome structure here, except in so far as they are directly related to our main theme. It is important, however, to note that our knowledge of the anatomy and physiology of the chromosome body has grown immeasurably during the past fifty years. In order to have a clear idea of the condition of cytology at the beginning of the century
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we cannot do better than to turn to the second edition of E. B. Wilson's great work *The Cell in Development and Heredity* (1900).

**EARLY SEPARATION OF CYTOLOGY AND GENETICS**

At the time that book was written cytology was generally regarded as a branch of histology or embryology, rather than as an independent field, and its significance for genetics and evolutionary theory was, at most, only dimly sensed. This tendency persisted for a considerable time, since the early cytologists, both here and in Europe, had all been trained in the morphological sciences and were mostly histologists or anatomists who had simply specialized in what they regarded as a particular aspect of their main field of study. The first generation of geneticists, on the other hand, were, for the most part, far too busy carrying out actual breeding experiments to devote themselves to microscopical studies at the same time. Thus, for a time, we had cytologists who knew very little of genetics and geneticists whose knowledge of cytology was second-hand and frequently limited.

This is not to say that each school did not do excellent work in its own particular field. It was, after all, during this period that the geneticists worked out the fundamental principles of heredity in Drosophila and a few other selected organisms, while the cytologists explored the physical basis of genetics in these and a great variety of other animals and plants which were not easily amenable to genetic analysis. During this "exploratory" period of cytology a vast amount of information concerning chromosome structure and behavior was accumulated. A great deal of this knowledge, however, could not at that time be interpreted in terms of genetics (or, for that matter, interpreted in any way whatsoever). Thus for a while cytology tended to become a mere accumulation of largely unrelated facts about cells and chromosomes. It is true that some of this information did provide striking confirmation of genetic principles. Such was Janssen's work (1909) on the chiasmata of amphibians, which provided the essential clue to the mechanism of crossing-over, and Carothers' work (1913, 1917, 1921) on the independent segregation
of structurally heterozygous bivalents in grasshoppers. Many other cytological investigations carried out in the first two decades of the century seemed, however, to be irrelevant to genetical theory, being concerned with mere cytological curiosities of no general significance.

The cleavage which existed between cytology and genetics, in the early days of both fields, was unfortunate in several respects. Many opportunities were undoubtedly lost which might have been grasped if the two disciplines had been more closely integrated from the beginning. For example, the salivary gland chromosomes of the Diptera were discovered and studied by Balbiani (1881) and Carnoy (1884) and were reinvestigated from an embryological standpoint by Alverdes (1912); but their significance for the cytogenetics of Drosophila was not appreciated until the work of Painter (1933). We can only guess how different the history of genetics would have been if Morgan and Bridges had developed the salivary chromosome technique twenty years earlier.

**SYNTHESIS OF CYTOLOGY AND GENETICS**

The first real change in the relationship of cytology and genetics came through the work of Bridges in 1916-1920 on non-disjunction, deficiencies and duplications in Drosophila, followed by his work on triploid interspecies, the genic balance theory of sex-determination and on inversions and translocations, during the 1920's. This was followed by the work of Belling on the cytogenetics of the liliaceous plants and Datura, which was important, not merely because of its results, but because it demonstrated the usefulness of the acetocarmine technique, the forerunner of all our modern "squash-methods," essential for the salivary gland investigations and labor-saving in so many types of cytological study.

With the work of these men and others too numerous to mention here the era of synthesis between cytology and genetics may be said to have begun toward the end of the 1920's. The new field of cytogenetics received a tremendous impetus from the discovery of Muller (1927) that X-rays would produce structural changes in the chromosome as well as mutations of individual genes. From this time
on it became possible to produce inversions, translocations and other structural rearrangements at will, instead of waiting for them to occur spontaneously. Karl Belar's book Die Cytologischen Grundlagen der Vererbung (1928) represented the first general synthesis of cytogenetics but contained relatively few new ideas apart from the author's interesting theories on the mechanism of anaphase movement of the chromosomes.

The Beginnings of Cytogenetics

By 1930 the time was ripe for the formulation of the main laws and principles of cytogenetics and the construction of a coherent system which could be used as a firm frame of reference for future work. This was the task which was attempted by Darlington in the first edition of his book Recent Advances in Cytology (1932) and which he has brought up to date in subsequent works. From that time onward we have undoubtedly had a frame of reference, but there has been considerable doubt as to its firmness. If, in retrospect, we are particularly conscious of those parts of the framework which have had to be discarded as insecure, this should not be allowed to obscure the great value of having a framework of some sort. This was definitely not the case so long as the protagonists of telosynapsis and parasyntapsis were arguing (as they were as late as 1928-29) about the elementary question of whether the chromosomes underwent pairing side by side or end to end. Moreover, the most important aspect of Darlington's original system, from the standpoint of genetics, namely the equivalence of the cytological chiasma and the genetic crossover, still survives virtually intact, in spite of some recent attempts to overthrow it.

Geneticists have always been impressed by the similarities between the main principles of heredity in organisms as widely separated phylogenetically as maize and mice, molds and man. They have hence been predisposed toward acceptance of a system of universal laws and principles of cytogenetics. Nevertheless, it has always been known that the cytogenetic mechanism differed from organism to organism. Already in the last century some of the peculiarities of the chromosome cycle of Ascaris had been worked out, and it was soon
discovered that such insects as Aphids and Hymenoptera possessed anomalous genetic systems. Even Drosophila was found to be anomalous in having no crossing-over in the male. At a slightly later date the work of Metz began to reveal the astonishing complexities of the cytogenetic system of Sciara, and the Schraders did the same for the Coccids, while the Whitings investigated the genetics of the haplo-diploid wasp Habrobracon. The work of these investigators, as well as that of Renner, Cleland and others on the plant Oenothera, during the 1920's and 1930's was of the utmost importance, because it kept alive a spirit of scepticism as to the universality of the laws and generalizations which were being put forward by the cytogenetic school, since Sciara, the Coccids, Habrobracon and Oenothera seemed to constitute flagrant exceptions to all such laws and generalizations. Today, with more knowledge and experience, we can take a broader and more balanced view: these organisms are perhaps not quite so aberrant as we once thought them, nor are the "normal" cytogenetic mechanisms all poured from the same mold.

VARIETIES OF CYTOGENETIC SYSTEM

Broadly speaking, we may distinguish two types of evolutionary modifications of the cytogenetic system. On the one hand we have those groups of organisms in which the fundamental mechanisms of meiosis, fertilization or cleavage have been radically altered. On the other hand, we have those species of animals in which the formal mechanism of heredity is of the normal type, but in which some degree of chromosomal polymorphism is present in the natural populations, the individuals being distinguished by the possession of different inversions or other structural rearrangements, or by the presence or absence of supernumerary chromosomes. We shall endeavor to describe some examples of both types of systems, selecting particularly those which have only been investigated rather recently, and which are presumably less familiar than the classical cases previously mentioned.
**Anomalous Cytogenetic Mechanisms**

Most of the more highly anomalous cytogenetic mechanisms which have been worked out are found in the class of insects. There are, however, a number of other invertebrate phyla, such as the nematodes and the rotifers, in some of the members of which highly anomalous types of chromosomal cycles occur. In the vertebrates, on the other hand, the chromosome cycle seems to be of a “normal” type in all forms hitherto investigated.

The more primitive insects, such as the orthopterous orders, the mayflies, dragonflies, etc., all seem to possess normal chromosomal cycles. These are the modern survivors of the paleozoic types of insects. In many of the more modern orders of insects, however, we find highly anomalous mechanisms of meiosis, either throughout the whole order, or in particular families or genera. Some of these are associated with some form of parthenogenesis (such as the cyclical parthenogenesis of the aphids or the arrhenotokous parthenogenesis of the Hymenoptera); but many of the anomalous types of chromosome cycle occur in groups in which reproduction is strictly bisexual.

**CYTOGENETIC SYSTEMS IN THE DIPTERA**

The two-winged flies (Diptera) are an order of insects which constitute exceptionally interesting material from the standpoint of understanding the principles underlying the evolution of genetic systems, since within this one group we find at least four different types of such mechanisms.

There can be little doubt that the earliest Diptera had a fairly normal meiosis in both sexes, with chiasmata in the males as well as in the females; this is the condition still met with in the primitive crane-flies, the mosquitoes, the true midges (Chironomidae) and some other families. At a very early stage in the phylogeny of the order, however, in one phylogenetic line the chiasmata were lost from the meiosis of the males. This line includes the fungus gnats (Mycetophilidae) and some other morphologically primitive forms, as
well as all the families of “higher” Diptera, including, of course, Drosophila.

The evolutionary significance of this loss of chiasmata from spermatogenesis is somewhat obscure. Obviously, it led to a reduction in the total amount of recombination in the species in which it first occurred. The amount of recombination varies so much, however, as between members of the same group of animals, that we can hardly state on a priori grounds whether a large or a small amount of recombination is a good thing for any particular type of species. Of course, in most groups, changes in the amount of recombination have been brought about by changes in chromosome number and in the total chiasma-frequency, without the abolition of chiasmata in the male.

Another consequence of the loss of crossing-over from the males of most Diptera is that it has apparently permitted certain species to become heterozygous for inverted chromosomal segments without paying the penalty, in the form of acentric and dicentric chromatids, which would otherwise result from crossing-over in such mutually inverted segments. It is possible that this fact resulted in the type of spermatogenesis without chiasmata having a positive evolutionary value in those Diptera in which it first arose.

Meiotic Mechanisms in Sciarids and Cecidomyids

Apart from Diptera with chiasmata in the male and those in which chiasmata are lacking from spermatogenesis, there are two families in which the whole chromosome cycle has undergone the most bizarre modifications. These two families, which probably arose from the phylogenetic line in which the chiasmata had already been lost from spermatogenesis, are the Sciaridae, studied by Metz and his associates (see Metz, 1938) and the gall midges (Cecidomyiidae) which we have investigated in the past few years (White, 1946a and b, 1947a and b, 1948, 1950). The former consist of a single large genus, Sciara, together with a few much smaller genera which have not been studied cytologically. The latter are a much larger and more diversified group, containing several genera and at least 3000 described species, with the most varied types of life cycle. On morphological
grounds, these two groups are probably rather closely related to one another and to the Mycetophilidae. Nevertheless, the three families possess very different types of cytogenetic mechanisms. In the Mycetophilidae there are no chiasmata in the males (Frolowa, 1929; Le Calvez, 1947; Fahmy, 1949) but the chromosome cycle is otherwise normal. In the Sciariidae and Cecidomyidae, on the other hand, the first meiotic division in the male is of an asymmetrical type, which has usually been described as unipolar, and there are certain chromosomes which are confined to the germ line, being eliminated from the somatic nuclei during some of the early cleavage divisions. In the Sciariidae the meiotic divisions in the female are “normal” in type, while in the Cecidomyidae they are also anomalous, although in quite a different manner from those of the males (White, 1950).

We do not have time to describe all the complications of the chromosome cycles of these Sciariids and Cecidomyids, and will only discuss certain of the theoretical implications of these bizarre cytogenetic mechanisms.

In the Sciariids the chromosomes which are confined to the germ line (called ‘limited’ chromosomes by Metz) are relatively few in number and are even absent altogether (perhaps secondarily) in some species. They are described as heterochromatic, but we know nothing as to their genetic properties.

In the Cecidomyids the corresponding elements (which in this case we call E-chromosomes) are far more numerous and are present in all species so far examined (about 20 altogether). Thus, in the Cecidomyids there are always far more chromosomes in the oogonia and the spermatogonia than in the somatic nuclei. The matter is, however, still further complicated by the fact that the somatic chromosome number is not the same in the two sexes. Thus, in Miastor metraloas there are 6 chromosomes in male somatic nuclei, 12 in female somatic nuclei and 48 in the gonidia of both sexes. The chromosomes which are present in the soma may be called S-chromosomes and we may say that the male Miastor has a haploid set of S-chromosomes in the soma and a diploid set in the germ line together with 36 E-chromosomes, which are not represented in the soma at all. In the female there is a diploid set of S-chromosomes in the somatic
nuclei, the cytological composition of the germ line nuclei being the same as in the male. In another Cecidomyid, Taxomyia taxii, the situation is a little different: here there are 6 chromosomes in the male soma, 8 in the female soma and 40 in the germ line of both sexes. Oligotrophus pattersoni also has 6 and 8 chromosomes in the male and female soma respectively, but has only 34 chromosomes in the germ line (that is, there are only 26 E-chromosomes instead of 32 as in Taxomyia). As far as we have been able to determine, the fertilized egg always contains the full or gonial number of chromosomes initially, the somatic cells losing all the E-chromosomes (and in the case of the males some S-chromosomes as well) during the early cleavage divisions. The actual course of the meiotic divisions varies to some extent from one genus to another in the Cecidomyids, but in the most typical cases the sperm only receives a haploid set of S-chromosomes and no E-chromosomes. The egg-nucleus, on the other hand, receives a haploid set of S-chromosomes and a complete set of E-chromosomes as well. Thus in Trishormomyia helianthi, where the germ line number is 24, the sperm transmits 4 chromosomes and the egg 20. The S-chromosomes form chiasmata in the female, but the E-chromosomes do not do so in either sex.

The genetic significance of these vegetatively inherited E-chromosomes presents a fascinating problem, quite unsolved at the present time. They exhibit a type of heteropycnosis which might suggest that they are genetically inert, but the fact that they are apparently quite constant in number within each species renders this doubtful. One might suppose that they contain genes whose only functions are related to the growth, division and differentiation of the germ line cells themselves (as suggested by Haldane (1932) in the case of Ascaris). Alternatively it is possible to imagine that the E-chromosomes in the germ line give rise to diffusible substances which pass out into the soma throughout the lifetime of the individual. In our opinion, however, the most probable explanation is that the E-chromosomes which are eliminated from the somatic nuclei and become broken down in the egg-cytoplasm continue to influence the development of the individual for at least the earlier part of the life cycle,
either as "free genes" in the cytoplasm, or as genic products of some kind.

Whatever the precise genetical interpretation of this extraordinary type of chromosome cycle, there can be no doubt that it has proved extremely successful from the evolutionary standpoint, since the Cecidomyidae are one of the largest families of Diptera, exhibiting considerable morphological diversity and having invaded a large number of different ecological niches.

One consequence of the peculiar chromosome cycle found in *Seiara* is that each individual, although having two parents, has only three genetic grandparents, all the chromosomes of the paternal grandfather having been discarded during the spermatogenesis of the father. Whether the same situation occurs in the Cecidomyids is not known, but appears probable. A similar condition probably obtains in most of the "higher" scale insects, according to the work of the Schraders. Such a situation would seem to place the evolution of the male at a disadvantage, as compared with that of the female, since in each generation half of the chromosome sets which have been "tested" by natural selection in the male and found satisfactory are lost from the species forever. This might conceivably be advantageous in a group like the coccids, where sexual dimorphism is extreme, and where genes or gene-combinations which have a high adaptive value in one sex might actually lower viability in the other sex (we are assuming here that the female is more "valuable" to the species than the male!). On the other hand, it is less easy to imagine that such a mechanism could be advantageous in the Sciarids, where sexual dimorphism is much less pronounced.

ANOMALOUS MECHANISMS IN HOMOPTERA

By way of contrast with the anomalous cytogenetic mechanisms which have been developed in the Diptera, we may consider some of those which have been found in certain of the Homoptera, and particularly in some of the scale insects.

In none of the Homoptera have localized centromeres ever been demonstrated and it seems probable that in this order of insects, as
well as in the closely allied order Heteroptera, the chromosomes are attached to the spindle along their whole length, at all divisions, whether somatic or meiotic. It is still uncertain whether such chromosomes have numerous invisible but discrete centromeres distributed along their length or whether they have a "diffused centromere-activity"; but, in view of the observations of Hughes-Schrader and Ris (1941) on the mitotic autonomy of chromosome fragments produced by x-rays in Steatococcus, it is no longer possible to deny that Homopteran chromosomes have a structure which is fundamentally different from that of chromosomes with single, localized centromeres. Just how many other groups of animals have chromosomes with "diffused centromere-activity" is somewhat doubtful: the Heteroptera almost certainly do, and we believe that the same is probably true of the scorpions of the genus Tityus (Piza, 1939, 1941, 1943) in which Rhoades and Kerr (1949) have observed the mitotic autonomy of fragments produced by irradiation, although some other scorpions such as Opisthacanthus clearly have a single, localized centromere-region in each chromosome (Wilson, 1931). The situation in the Lepidoptera and some other groups is still obscure.

M. J. D. White

Meiosis in Coccids

Although the coccids are famous for their anomalous meiotic mechanisms, the phylogenetic relationships of the different cytogenetic systems met with in this group are very obscure (Hughes-Schrader, 1948). Puto sp. appears to have a relatively "normal" (and hence presumably primitive) type of meiosis, but it belongs taxonomically to one of the more specialized families of coccids (Eriococcidae). The males are XO and the autosomes form six bivalents during spermatogenesis; chiasmata are apparently present (Hughes-Schrader, 1944).

The members of the tribe Llaveini and related forms investigated by Schrader (1931) and Hughes-Schrader (1931, 1940, 1942) exhibit various cytological specializations over the primitive conditions met with in Puto, but these are principally concerned with the spindle apparatus during the male meiotic divisions, and there seems no reason to believe that such forms as Protortonia, Llaveia, Llaveiella
and Nautococcus have genetic systems which differ greatly from that of Puto. Asynapsis occurs regularly in the case of some of the autosomal pairs, but since it apparently never leads to meiotic nondisjunction, the only genetic effect is presumably a reduction in the amount of crossing-over.

The coccids of the tribe Iceryini have an entirely different type of cytogenetic system, since the males are haploid and arise from unfertilized eggs, there being no special sex chromosomes (Hughes-Schrader 1930, Hughes-Schrader and Ris, 1941). In Icerya purchasi and two other species the "female" has become converted into a self-fertilizing hermaphrodite which is a haplo-diploid chromosomal mosaic, in which the cells of the female part of the gonad have four chromosomes, while those of the male part have only two chromosomes (Schrader and Hughes-Schrader, 1926; Hughes-Schrader, 1927, 1948). Apparently a reduction division occurs in the early embryology of the testicular portion of the gonad.

In the members of the family Eriococidae (except for Puto, already mentioned) both males and females are diploid but one set of chromosomes is heteropycnotic in the males, while the other is not. Neither set shows heteropycnosis in the females. No pairing of the chromosomes takes place during the spermatogenesis of such forms as Phenacoccus (Hughes-Schrader, 1935) and Pseudococcus (Schrader, 1923). The second meiotic division is a 'unipolar one,' at which the heteropycnotic and non-heteropycnotic sets are separated from one another. The nuclei which receive the heteropycnotic chromosomes degenerate, so that only two sperms, instead of the usual four, are formed from each primary spermatocyte. The mechanism of sex-determination in these forms is still a mystery.

Any attempt to interpret these peculiar types of meiosis in terms of their effects on the population-dynamics and speciation mechanisms of the forms in which they occur would be premature at the present time. We believe, however, that they are in all probability functionally interrelated with the extreme development of sexual dimorphism in the coccids—a dimorphism which is manifested in an extremely early stage of the life cycle in some species.
MALE HAPLOIDY IN HYMENOPTERA

In the coccids male haploidy is confined, as far as we know, to the few species in the tribe Iceryini, and in the Coleoptera it is known to occur only in a single species, *Micromalthus debilis* (Scott, 1936). Haplo-diploidy is also known to exist in some Aleurodidae and in some mites and it may also exist in the Thysanoptera, but we do not really know how widespread it is in any of these groups. In the Hymenoptera, however, male haploidy seems to be universal; and since this is one of the largest and most successful orders of insects, with about 150,000 described species, it seems worth while to consider what the genetic and evolutionary implications of haplo-diploidy are. In the first place, it is clear that in a species where the males are strictly haploid there can be no "reservoir of hidden variability" (in the form of numerous recessive mutations in the wild populations) such as exists in *Drosophila* species and presumably in other groups where both sexes are diploid. As far as male Hymenoptera are concerned it does not matter whether a mutation is dominant or recessive; it will in either case have to face the test of natural selection from the very beginning of its history in the species. Deleterious mutations will hence be eliminated from the population very rapidly while advantageous ones will become incorporated in the genotype of the species somewhat more rapidly than in organisms where both sexes are diploid. The theoretical consequences of this are that wild populations of Hymenoptera must be expected to lack a "reserve capital" of hidden genetic polymorphism upon which the population can draw in the event of gradual changes in the physical or biotic environment. We might therefore expect that the Hymenoptera would show little evidence of adaptability. Obviously this is not so. The group has been extremely successful in evolution and has invaded a wide variety of different types of ecological niches. What is the explanation of this apparent paradox? It seems to us that there are several possible explanations but these are only put forward very tentatively at the present time.

On the one hand, it may be that the chromosomes of the Hy-
menoptera contain many "repeats," so that the males are, from the genetic standpoint, diploid for these regions, the females being tetraploid for the same chromosome segments. It must be admitted that there does not seem to be any direct evidence for this hypothesis from the genetics of Habrobracon, but our knowledge is not extensive enough to eliminate it from the field of possibilities.

A second hypothesis is concerned with mutations whose effects are limited to the female sex. Obviously, the above considerations concerning the absence of a reservoir of hidden variability will not apply in such cases, since there can be a reservoir of recessive mutations which affect females but not males. We are inclined to consider this as the most probable explanation of the paradox. If it is true that most species of Hymenoptera possess a "reservoir" of mutations whose effects are limited to the female, we should expect that sex to show a somewhat greater evolutionary plasticity than the males. Certain taxonomic data seem to be in accordance with this: in some groups of Hymenoptera the males of related species seem to be much more similar than the females.

We have discussed the cytogenetic systems of Sciara, the Cecidomyiids and the coccids at some length because they represent clear-cut deviations from the normal scheme of things, and because their evolutionary consequences, although still largely unknown, are certainly not unknowable. Certain biologists appear to believe that, because such groups as the Cecidomyiids and the Hymenoptera are composed of species which seem to be of the same general nature as the species of other organisms, the details of the cytogenetic mechanism must be of little importance as far as speciation and evolution are concerned. To a certain extent this is no doubt true; we must not expect the mechanisms of speciation and evolutionary change to be entirely different from the normal in these groups with anomalous chromosomal mechanisms. What we may expect are differences in the relative importance of different factors and in the ways whereby the end-results of adaptation and speciation are brought about.

As far as the cytologist is concerned, the most difficult part of his work lies in deciding which of his observations are truly significant.
and which are relatively unimportant. By neglecting the distinction between profound deviations from the norm of chromosomal behavior and trivial peculiarities of appearance at particular stages of mitosis and meiosis certain cytologists seem to have reached a point where they consider every species of animal to have a distinct type of cytogenetic system. Now we have tried to show that the variety of such systems in the animal kingdom is considerable; but this does not alter the fact that, in general, the cytogenetic mechanism is extremely uniform throughout most of the major groups of animals, always provided that we neglect those petty morphological details which obviously do not entail any genetic consequences. Such cytological non-conformists as Sciara, the gall-midges, the coecids and the Hymenoptera are definitely exceptions to the general scheme of things, and it is for that very reason that they are significant and deserving of much further study.

POLYPLOIDY IN ANIMALS

Whereas in plants genetic systems involving polyploidy are extremely common (at least in most families of Angiosperms) only very few polyploid species of animals are known. A pioneer attempt to explain this difference between animals and plants was made by Muller (1925) who suggested that polyploidy could hardly be expected to establish itself in bisexual organisms, since it would immediately abolish the mechanism of heterogamety upon which sex-determination depends. Muller's argument still possesses considerable cogency. If this were the only barrier to polyploidy we should expect to find polyploid species among hermaphrodite and parthenogenetic animals while, conversely, we should not expect to find any polyploid species of dioecious plants, unless dioecism had established itself in an already polyploid species (which may be what has in fact occurred in the hexaploid dioecious Rumex acetosella).

As far as animals are concerned, there are many instances of polyploidy in parthenogenetic forms, for example the tetraploid and octoploid races of the brine shrimp Artemia (Barigozzi, 1935, 1944), the triploid Isopod Trichoniscus provisorius (Vandel, 1940), the tetra-
poloid grasshopper *Saga pedo* (Matthey, 1946) the tetraploid races of the solenobia moths (Seiler, 1923, 1943) and the triploid, tetraploid and pentaploid Otiorhynchus weevils (Suomalainen, 1940, 1947).

Hermaphroditism, as a normal reproductive mechanism, is rather sporadically distributed throughout the animal kingdom. We may take the flatworms, the earthworms, the leeches and the pulmonate mollusca as the outstanding examples of groups in which the overwhelming majority of the species are normally hermaphroditic, although we have far too little information as to the extent to which self- and cross-fertilization normally occur in these groups.

Future work will no doubt determine more exactly the extent to which polyploidy has occurred in these hermaphroditic groups of animals. At the present time it seems probable that there are a few polyploid species of Turbellaria (White, 1940; Benazzi, 1949), while the situation in the other groups of flatworms is not clear. In the earthworms, according to the work of Muldal (1948, 1949), there are probably no polyploid species among the sexual (that is, hermaphroditic) forms, but several apparently tetraploid forms and one octoploid or decaploid species are known among the facultatively or exclusively parthenogenetic species. Finally, in the pulmonate mollusca, which are virtually all hermaphroditic, no cases of polyploidy (apart from one which has now been disproven) have been recorded. We may conclude, therefore, that, in general, the hermaphroditic groups of animals resemble such plant groups as the conifers and the oaks, rather than the grasses or the compositae, in showing little or no polyploidy. This may result from the rarity of self-fertilization among such forms, or the fact that hybridization between different taxonomic entities (leading to allopolyploidy) only occurs very rarely or not at all—or it may depend on factors which are still unknown. At any rate, it seems clear, from the cases we have already cited, that there are no strong barriers to the establishment of polyploidy in parthenogenetic animals, while it is equally obvious that barriers do operate to some extent in the case of the hermaphroditic groups.
Polyploidy in Bisexual Animals

In the bisexual groups of animals, the barrier to polyploidy seems to be complete. There seems to be no valid evidence for the establishment of polyploidy in any group of bisexual animals, although claims to have demonstrated its existence in various Heteroptera, fishes, Lepidoptera and even in man have been put forward from time to time, often on the flimsiest kind of evidence and will no doubt continue to appear in the future. Such extensive investigations as those of Pfäler-Colander (1941) on the Lygaeids, Hacker (1948) on the spiders and S. G. Smith (1950) on beetles provide no support for the occurrence of polyploidy in any of these groups (except in the parthenogenetic species of beetles mentioned above) and Matthey (1949) finds no satisfactory evidence that it has occurred in the phylogeny of the vertebrates.

"NORMAL" CYTOGENETIC MECHANISMS

Having discussed the problem of the evolution of anomalous cytogenetic mechanisms, we may now turn to consider those cases where species with relatively "normal" genetic mechanisms are cytologically polymorphic in nature.

The work of Dobzhansky and his associates on the distribution of paracentric inversions in populations of Drosophila pseudoobscura and D. persimilis, culminating in the striking demonstration that these inversions furnish the physical basis for an elaborate mechanism of heterosis or hybrid vigor, whereby these species adapt themselves to the many different habitats they occupy, has provided the first real evidence of the role of structural rearrangements in wild populations of animals. We may now ask ourselves whether such a mechanism is peculiar to Drosophila, or whether it exists elsewhere in the animal kingdom. Most cytologists, I believe, are convinced that inversions are rather frequent in many wild species of animals. The evidence on this point is, however, mostly negative. The question is whether they are really absent from many species or whether we are simply unable to detect them. Apart from genetic studies on
linkage relationships, there are, of course, three methods of proving the existence of an inversion, namely an examination of the salivary gland chromosomes—restricted in applicability to the Diptera flies—the study of pachytene bivalents, and the finding of “bridge” and “fragment” chromatids (the result of crossing-over in mutually inverted chromosomal segments) at meiosis. As far as the first method is concerned, we have evidence that paracentric inversions are present in the wild populations of many and probably most species of Drosophila. The champion species in this respect is undoubtedly D. willistoni. In some other species of Drosophila, however, such as D. meridiana and D. bifurca, inversion heterozygosity is either very rare or completely absent from the natural populations.

Since crossing-over in paracentric inversions leads to the production of bridge and fragment chromatids, heterozygosity for such inversions would lead to a considerable degree of sterility were it not for the fact that these chromatids are excreted in the polar body nuclei during the oogenesis of Drosophila and that they do not arise at all in spermatogenesis, owing to the absence of crossing-over in the male. Drosophila thus avoids paying the penalty for the possession of inversions. The same is probably true of other “higher” Diptera, which lack chiasmata in the male. In the Agriomyzid Liriomyza, at any rate, Mainx (1949) has demonstrated the presence of numerous inversions.

Turning now to the “lower” Diptera, we find that in a few species of Sciarids and Cecidomyiids (in which, as we have already seen, the male meiosis is highly anomalous and no chiasmata are formed in spermatogenesis) inversions are fairly common, while in most species they seem to be rare or absent, as in Drosophila meridiana. In the maculipennis species-group of the mosquito genus Anopheles inversions do not seem to occur in the natural populations, although several of the sibling species are distinguishable by inverted sequences, according to Frizzi (1947). In the midge genus Chironomus, however, we find the somewhat surprising situation that, although chiasmata are formed in the male, certain species contain rather numerous inversions in their wild populations, although, as in Drosophila, there
seem to be some species which show few or no inversions. Whether those species of Chironomus with inversions actually do produce a certain proportion of lethal sperms (that is, sperms which kill the eggs which they fertilize) is not known. Possibly the distribution of the chiasmata along the chromosome is localized in such a way that they seldom or never occur in the regions where the inversions lie. But in this case the inversions could hardly provide the basis for a mechanism of adaptive heterosis of the Drosophila pseudoobscura type unless chiasma formation were suppressed in the inversion regions of the heterozygotes but not in the corresponding regions of the homozygous individuals of the population.

Detection of Inversions in Other Animals

Outside the Diptera, we have no salivary gland chromosomes to help in the detection of inversions, and we consequently have to rely on the much less satisfactory procedure of looking for bridge and fragment chromatids at meiosis. This method is only applicable in organisms with fairly large chromosomes and in which the details of meiosis are clear. The urodele amphibia are one group that fulfills these conditions. Here, in spite of the large amount of work that has been carried out, I know of no evidence for the existence of inversions in heterozygous form in wild populations, although Spurway and Callan (1950) have shown that several subspecies of the European Triturus cristatus differ in respect of inverted sequences in much the same way as the sibling species of the Anopheles maculipennis group.

Turning to the grasshoppers, on which a vast amount of cytological work has been carried out, I know of only two workers who have claimed to have found inversions. Darlington (1936) obtained evidence of several inversions in the European species Chorthippus parallelus and Stauroderus bicolor, while Coleman (1947) has stated that inversions are common in grasshopper material, without giving any details as to what species he has in mind, or what the nature of the evidence is. I, myself, have carried out cytological studies on thousands of individual grasshoppers, belonging to many species and all the main subfamilies, without finding a single instance of an undoubted inversion, although “false bridges” (due to the sticking
together of chromatid ends) and "pseudo-fragments" (due to lagging of some of the smaller chromosomes on the spindle) may occur from time to time and sometimes look deceptively like true bridge and fragment chromatids.

We may draw two alternative conclusions from these rather negative data on anopheline mosquitoes, urodeles and grasshoppers. Either paracentric inversions do not occur at all frequently in the natural populations of animals with chiasmata in the males, or else if they do occur they must usually be restricted to chromosomal regions in which chiasmata are seldom or never found (in the latter case they would, of course, be undetectable by the "bridge and fragment" method). Such a conclusion is not unexpected, since in a population in which many "lethal" sperm were formed there should be a very strong selection against newly arisen inversions. It thus seems improbable that there can be many species of animals (other than in those groups where there is no crossing-over in the males) which have been able to develop the particular structural mechanism which underlies the adaptive heterosis of Drosophila pseudoobscura.

**STRUCTURAL REARRANGEMENTS IN GRASSHOPPERS**

Although inversions seem to be generally absent from grasshopper populations, as far as we can determine from the evidence available, there is one group of grasshoppers in which it has been known ever since the pioneer work of the McClung school (Carothers 1917, 1921; King, 1924; Helwig, 1929) that structural rearrangements of a different type were responsible for the existence of a bewilderingly complex degree of natural cytological polymorphism. This group, which we shall refer to as the tribe Trimerotropi, includes the three North American genera Trimerotropis (ca. 44 species), Circotettix (7 species) and Aerochoreutes (1 species), the first of which also has a couple of species in South America. During the past two years we have begun an investigation of the conditions in these cytologically polymorphic species of grasshoppers, guided by the idea that polymorphism on such an elaborate scale must surely be of some adaptive value to the species in which it occurs. The remarks that follow
should be regarded merely as a progress report on an investigation which is still in its initial stages.

We may begin with a description of the situation in the genus Trimerotropis itself. There are two sections within the genus, a primitive one (A) in which all the chromosomes are invariably acrocentric and a "derived" one (B) in which some of the chromosomes have become metacentric. With certain exceptions to be noted later, there are always 11 pairs of autosomes in the members of this genus, so that the metacentric elements found in the members of section B have not arisen as a result of fusions between acrocentric elements, but in some other manner.

We have thus far studied 19 species belonging to Section A. Apart from a few species in which supernumerary chromosomes or chromosomal regions are found in certain individuals, they are cytologically monomorphic, that is, we have not been able to detect any visible cytological differences between one individual and another. Their only interest thus lies in the fact that they represent the primitive condition, from which the type of genetic system found in Section B arose.

At present we know of the existence of twelve species in Section B. Five of these seem to be cytologically monomorphic and are probably closely related to one another. T. pallidipennis and three other species are characterized by a metacentric X-chromosome, three pairs of metacentric autosomes and eight pairs of acrocentrics. T. schaefferi from the gulf coast of Texas has four pairs of metacentric autosomes and only seven pairs of acrocentrics.

The remaining species in Section B, seven in number, all show some degree of cytological polymorphism, the numbers of acrocentric and metacentric chromosomes varying from individual to individual. Since several of the individual chromosomes can be of either type, structural heterozygosis is extremely common, and in fact in many populations nearly all the individuals are structural heterozygotes for one or more chromosome pairs, sometimes for as many as seven.

Four of the seven species of Circoctettix exhibit the same general type of population structure (the other three have not been studied) and so does the single species of Aerochoreutes. These smaller genera may
hence be plausibly regarded as evolutionary offshoots of Section B of Trimerotropis.

It is still not entirely clear what kind of structural rearrangements have converted the originally acrocentric chromosomes of these grasshoppers into metacentrics. Apparently pericentric inversions are not involved, since inversion loops are not seen at pachytene (Coleman, 1948). The most probable explanation is that we are dealing with centromere shifts of some kind. In the cytologically monomorphic species of Section B the situation seems, so to speak, to have stabilized itself, so that in these species structural heterozygotes do not occur. In the cytologically polymorphic species, on the other hand, both the "old" acrocentric and the "new" metacentric types of chromosomes have persisted alongside one another in the case of some of the elements of the genome, thus giving rise to a condition of balanced polymorphism. The complexity of the situation in the natural populations varies from species to species and also, to a considerable extent, between different microgeographic races of the same species. At one end of the scale we have such forms as Circotettix crotalum, which is apparently confined to two mountains in southern Nevada. Here only one of the ten pairs of autosomes is heteromorphic. At the other extreme, we have certain populations of Trimerotropis sparsa, in which as many as seven of the eleven bivalents may be structurally heterozygous in some individuals.

Apparently, in such heterozygous bivalents no chiasmata are ever formed in the region between the "old" and the "new" centromere positions. Structural heterozygosity thus seems to suppress crossing-over in certain rather extensive chromosomal regions, as happens in the case of paracentric inversions in Drosophila, although the detailed mechanism is almost certainly different. There are thus strong grounds for suspecting that, as in the case of the Drosophila inversions studied by Dobzhansky, the structural heterozygosity of the Trimerotropine grasshoppers may constitute the physical basis for a self-perpetuating mechanism of heterosis of some kind. This suspicion is strengthened by the fact that in species which exhibit this type of cytological polymorphism, we have never found a colony which is
M. J. D. White

cytologically monomorphic, although some of the colonies are in all probability sufficiently small and isolated to have become monomorphic as a result of “drift” if there were not some rather powerful agency promoting heterozygosity.

Structural Polymorphism and Heterosis

There are a number of ways in which, theoretically, such a system could give rise to heterosis. We might be dealing with differential viability of homozygotes and heterozygotes, in either or both sexes and at any stage in the life cycle, up to the end of the reproductive period. Alternatively, we might be dealing with some type of differential fecundity. In the first case, it should be possible to test for differential viability, by comparing the numbers of homo- and heterozygotes actually found in the natural populations with the numbers to be expected on the basis of the Hardy-Weinberg equilibrium, as was done by Dobzhansky and Levene (1948) in the case of Drosophila pseudoobscura. In practice, it is not possible in grasshoppers to determine the constitution of large numbers of females, cytologically. It is also difficult to study very old males, in which the testis contains very little but ripe sperms. We are therefore restricted to a study of adult, but not senile, males. A very serious difficulty is the fact that, in a population in which as many as six or seven chromosomes may be either acrocentric or metacentric, it becomes extremely difficult to be certain of identifying the different elements correctly in every individual. So far we have only been able to analyze two populations from the standpoint of comparing the numbers of homo- and heterozygous bivalents with the numbers to be expected if all types were equally viable. In both these cases the numbers of the different classes of bivalents actually found agree almost perfectly with the statistical expectation and therefore provide no support for the hypothesis of a mechanism of heterosis based on differential viability, at least in these particular populations, in the year 1949. We believe, however, that it is necessary to study much more extensive material before the hypothesis of differential viability can be definitely abandoned as an explanation for the cytological polymorphism of these grasshopper populations. In particular, we need to study species and
populations where only one or two chromosome pairs are heteromorphic. Most of the samples of such species as Trimerotropis sparsa and T. suifusa which we have studied were too highly polymorphic for a complete analysis to be possible. We also need to know if fluctuations in the frequency of particular chromosomal types occur within individual populations from year to year.

RECI PROCAL TRANSLOCATIONS

Reciprocal translocations seem, in general, to be very rarely present in the heterozygous condition in animal populations. This is no doubt due to the intense selection pressure against their establishment (see mathematical discussion of Wright, 1941). There are nevertheless a few instances in which undoubted reciprocal translocations have been detected through the observation of rings or chains of four chromosomes at meiosis (Carothers, 1931; Helwig, 1942; Dobzhansky and Dreyfus, 1943). Unfortunately, in none of these cases do we have adequate data on the frequency of the different classes of homozygous and heterozygous individuals, so that we have no means of determining the role that these translocations were playing in the populations in which they were found. It is, of course, clear on general grounds that most of the changes in chromosome number which have occurred in the evolution of animals were due to translocations of one kind or another. On the other hand, we must accept Wright’s conclusion that reciprocal translocations involving genetically active segments of chromosomes can hardly be expected to establish themselves in sexually reproducing species except under very special demographic conditions, for example in species represented by many small isolated populations.

Until recently no case of multiple translocations leading to an Oenothera-like condition was known in any animal species. However, Piza (1947) has described such a situation in the scorpion Isometrus maculatus, where he found a ring of eight chromosomes and two bivalents at meiosis: since only a single individual was studied it is not clear whether this condition is characteristic of the species as a whole. The chromosomes of Isometrus are probably polycentric, like
those of Tityus, but this is not absolutely certain from the published figures.

**SUPERNUMERARY CHROMOSOMES**

Apart from those types of chromosomal polymorphism which result from the presence of structural rearrangements such as inversions, translocations or centromere shifts in the natural populations of a species, there are relatively large numbers of animal species in which so-called supernumerary chromosomes are present. Such chromosomes may be defined as elements which are present in some individuals of the population but not in others. They are hence not necessary for life, and their genetic effects must be relatively slight, since it has never been possible to distinguish individuals with supernumerary chromosomes from those lacking supernumeraries on the basis of external appearance.

Some supernumerary chromosomes have been described as euchromatic, but the majority are undoubtedly heterochromatic, at least throughout most of their length. In certain cases they are minute relative to the other chromosomes, but in other species they may be quite large.

Supernumerary chromosomes have been studied in about 40 species of animals, but most of the early observations were very fragmentary, and do not help to elucidate the role of the supernumeraries in the population dynamics of the forms in which they occur. The earliest studies do suggest, however, that there are a number of different types of supernumeraries, some of which have been derived from autosomal material, while in other cases they have probably arisen through duplication of parts of the X- or Y-chromosomes. Thus the supernumeraries of the bugs of the genus Metapodius (Wilson, 1907, 1909, 1910) resemble the Y's very closely, while those of *Cimex lectularius* (Slack, 1939, Darlington, 1940) behave like X's at meiosis, and were hence probably derived from true X's. The problem of determining with complete certainty the exact mode of origin of the supernumerary chromosomes in any particular case seems, however, to be almost insoluble.

In the Trimerotropine grasshoppers heterochromatic supernumerary
elements have been found in nine different species. In some of these they are acrocentric chromosomes, while in others they are metacentrics whose meiotic behavior suggests that they are isochromosomes. At least in *Trimerotropis suffusa*, *T. sparsa* and *Cicotettix undulatus* both types coexist in the same species. The behavior of the supernumeraries is very similar in all the Trimerotropine grasshoppers in which they occur, suggesting that they may have had a common origin at a relatively early stage in the evolutionary history of the group, having been subsequently lost in those members of section B and the genus *Cicotettix* in which they do not now occur.

In *T. gracilis* and *T. sparsa* supernumerary chromosomes are definitely absent from some natural populations, although present in others. In neither case, however, do we as yet have sufficient data to define the geographical range of the supernumeraries in relation to the total distribution area of the species. In *T. suffusa* and *C. undulatus* present data suggest that the supernumeraries may be co-extensive with the species, that is, they may occur in all populations. In some populations of *T. suffusa* supernumeraries are present in only about 10 percent of the individuals, while in a population of *C. undulatus* from Ashton, Idaho they were present in 28 percent of the males.

The Trimerotropine supernumeraries seem to be constant in number in all the cells of an individual, at least in the germ-line. There is thus no indication that they undergo mitotic non-disjunction in the spermatogonia. Most individuals with supernumeraries have only one such element, but in *T. sparsa*, *T. suffusa* and *C. undulatus* individuals with two and three supernumeraries have been found; so far no individual with four such elements has been encountered.

In individuals with a single supernumerary this element behaves as a univalent in spermatogenesis, dividing in either the first or the second meiotic division (usually the latter), but not in both divisions. Thus such individuals produce equal numbers of sperms with and without the supernumerary. In individuals with two or three isochromosome supernumeraries these form bivalents or trivalents in only a small proportion of the spermatocytes the majority of the first meiotic divisions having all the supernumeraries present in the univalent condition. Thus an individual with two supernumeraries
produces sperms with 0, 1 and 2 supernumeraries and one with three gives sperms with 0, 1, 2 and 3 extra elements, the exact proportions of the different kinds of sperms depending on the percentage of cells in which pairing of supernumeraries has occurred, a figure which seems to vary considerably from cyst to cyst and from individual to individual.

Two views have been advanced concerning the role of the supernumerary chromosomes in natural populations. By some of the earlier workers such chromosomes were held to be entirely inert, that is, completely neutral from the standpoint of natural selection. This viewpoint was put forward at a time when heterochromatin was generally regarded as completely inert, a view which modern work on the Y-chromosomes of Drosophila species and the B-chromosomes of maize has rendered untenable.

An entirely different conception of the role of supernumeraries in the case of certain plant species has been put forward by Östergren (1945) and is supported by Müntzing (1946) and Melander (1950). These authors have been principally concerned with explaining the case of the supernumerary chromosomes in certain varieties of rye (Secale cereale) where a peculiar mechanism of directed non-disjunction at the gametophytic mitoses would lead to a considerable increase in the number of supernumeraries in the population were it not for the fact that there is a compensatory loss of supernumeraries at the meiotic divisions (in individuals with single supernumeraries). The Swedish workers also believe that the supernumeraries exert a deleterious effect on the viability of the plants so that there is a three-fold equilibrium between accumulation at the gametophytic mitoses and loss at meiosis and, through natural selection, the supernumeraries living a "parasitic" existence in the strains in which they occur. Östergren and Müntzing's ideas on "parasitic" supernumeraries have been extended to the case of the flatworm *Polycelis tenuis* by Melander (1950).

The concept that chromosomes which are not only unnecessary for life but actually deleterious to the organisms bearing them persist in natural populations on account of an inherent accumulation mechanism is an extremely interesting one. We do not believe, how-
ever, that it can be regarded as finally proven, even in the case of rye, since there are as yet insufficient data on the rates of accumulation and loss in individuals with different numbers of supernumeraries under varied environmental conditions. In an English strain of rye the transmission of supernumeraries is apparently lower than in the Swedish varieties, so that Darlington (1950) concludes that since they are maintained in the population they must be favored by selection, rather than the reverse. On a priori grounds we would expect that, if a supernumerary were deleterious, its rate of accumulation would fall, as a result of selection operating over a number of generations, to a level at which the supernumerary would be lost entirely from the population. The analogy with a parasite seems to us a fallacious one, since a supernumerary chromosome is not an independent organism with an adaptable genetic mechanism of its own.

In the case of the supernumeraries of the Trimerotropine grasshoppers there is no evidence of an accumulation mechanism and we feel sure that no accumulation takes place during spermatogenesis (no observations have as yet been carried out on the behavior of grasshopper supernumeraries in oogenesis). We believe, however, that the known facts with regard to the Trimerotropine supernumeraries can be explained if we assume that in most cases a single supernumerary raises the viability of the individual slightly, at least under certain conditions, while higher numbers decrease viability to some extent. In some species and populations, where individuals with two supernumeraries are relatively common, it may be that the deleterious effect does not begin until there are at least three such chromosomes present. If this explanation is, in general, correct, we should expect the frequency of supernumeraries in the population to be kept down by selection to a level at which individuals with a deleterious dosage are relatively rare.

We have already stated that in the Trimerotropine grasshoppers the number of supernumeraries present seems to be constant for all the primary spermatocytes of the same gonad. In the case of some other animal supernumeraries, however, such as those of the grass-
hopper *Camulida pellucida* studied by Carroll (1920) and those of the flatworm *Polycelis tensa*, investigated by Melander (1950), mitotic non-disjunction occurs in the germine, so that the number of supernumerary elements varies from one primary spermatocyte to another, even in the same individual. In the latter case there is the additional complication that the supernumeraries are lost from most of the somatic cells during development, so that they are in some respects analogous to the E-chromosomes of the Cecidomyidae. A similar state of affairs seems to occur in the case of the supernumeraries of some plant species.

**PRESENCE OF EXTRA CHROMATIN**

Apart from supernumerary chromosomes in the strict sense, there are a number of species of animals where supernumerary chromosome regions may be present in some individuals but not in others, attached to or inserted into members of the regular chromosome set. Such cases differ from those in which extra chromatin is present in the form of independent chromosomes in that the mitotic and meiotic behavior of the chromosomes bearing the extra segment is entirely regular. An example of this state of affairs we may take: a certain population of *Trimerotropis bilobata* (a member of Section A) from Las Vegas, Nevada, in which 6 out of 56 individuals were heterozygous for the presence of such an extra chromosomal region in one of the smaller autosomes. The other 50 individuals were homozygous for the absence of this extra segment and no individual homozygous for the supernumerary region was found in the sample. In a case like this it is obvious that no individual can exist with more than two supernumerary segments; whereas in cases where the extra chromatin is in the form of a separate chromosomal element there is no limit (other than that imposed by lowered viability) to the number of supernumerary chromosomes which may be present in one individual. We believe that the case of *T. bilobata*, and others of the same general type which have been described in the literature, can best be accounted for on the supposition that one extra region raises viability while two depress it slightly.
We do not propose to discuss here the future development of the whole field of animal cytogenetics. As far as the study of cytogenetic mechanisms as such is concerned, we believe that the exploratory phase is largely past. It is, of course, probable that more types of meiotic mechanisms remain to be discovered, either in groups of the animal kingdom hitherto unstudied by cytologists or in cytologically aberrant members of groups which have already been investigated to some extent. But the main types of cytogenetic systems are, in all probability, already known, and such mechanisms as remain undiscovered at present are likely to be minor variants of already known types.

Two great gaps in our knowledge remain, however. In the first place, as rightly pointed out by Schrader (1944), we still do not properly understand the physicochemical basis for any of the maneuvers of the chromosomes in mitosis and meiosis. Any satisfactory explanation of the phenomenon of meiotic pairing must explain why the chromosomes do not pair in the spermatogenesis of such forms as Sciara and the Cecidomyids and, similarly, any causal explanation of crossing-over must also make clear why it is that crossing-over does not take place in males of the "higher" Diptera. It may well be that the anomalous varieties of mitosis and meiosis will furnish essential clues to the understanding of the more frequently occurring types.

In the second place most of our existing knowledge on the genetical aspects of population dynamics comes from the work which has been carried out in the past twenty years on Drosophila, and we have as yet very little information on the genetic structure of natural populations in other groups with different types of cytogenetic systems. In this field, which is not strictly within the domain of cytology, but requires the use of cytological methods and a full understanding of cytological principles, we are still in the pioneering stage. At first sight it may appear as if immense labors will be required before any considerable advances can be made in this direction. We believe, however, that if the cytogenetical investigations of the future are
planned from the standpoint of testing hypotheses and of obtaining answers to specific unsolved problems, rather than from the laudable but less rewarding viewpoint of simply adding to the sum total of knowledge, they will greatly enlarge our understanding of the mechanisms of biological evolution in a much shorter time than one might think at present.

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OLD AND NEW PATHWAYS IN HUMAN GENETICS

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THE year 1950 rounds out the first half-century of the modern science of genetics. During these fifty years this branch of biological science has grown and developed until it, too, has its own branches and subdivisions. Meanwhile, lines of demarcation originally existing between genetics and other sciences have gradually disintegrated until now genetics merges imperceptibly into many other fields. Agricultural genetics, physiological genetics, developmental genetics, biochemical genetics, radiation genetics, cytogenetics, population genetics, and human and medical genetics have all emerged as co-ordinated fields of research, and each has made notable contributions to knowledge and to human welfare.

Although speculations on matters of heredity may be found far back in human history, and although the pioneer researches of Mendel are now nearly one hundred years old, modern genetics dates only from the turn of the century. When Mendel’s principles had been rediscovered and had been tested on various experimental organisms, when the cytological basis for the phenomena had been recognized, and when the earlier basic principles had been expanded, extended, and woven into an understandable pattern, it was inevitable that an attempt should have been made to determine the applicability of these principles to man.

1 Presidential Address to the American Society of Human Genetics, delivered as part of the Golden Jubilee Celebration of Genetics at Columbus, Ohio, September 12, 1950.
During the first decade of the century the literature on human genetics began to appear under such names as Ballowitz, Bulloch, Davenport, Drinkwater, Farabee, Fischer, Folkar, Galton, Garrod, Gossage, Guthrie, Guyer, Harmon, Holmes, Hunt, Nettleship, Pearson, Vogt, Weinberg and Wilson. During this decade there was begun the publication of the valuable *Treasury of Human Inheritance* under the editorship of Karl Pearson. While some of the studies had a strong eugenics slant, others dealt with human genetics *per se*.

It quickly became apparent that Mendel’s principles were indeed applicable to man, and the discovery of new and more complicated principles through the years has served only to strengthen and further document the conclusion that the genetics of man is essentially like that of other organisms. Virtually every basic law and principle of modern genetics can be illustrated with human material, and some of these principles have in fact been derived from data on man.

In spite of the flurry of interest in human heredity during the first decade or two of the century, this interest, particularly in America, was short-lived. The rise of the experimental method in genetics with its prospect of definitive results within reasonable time limits caused many geneticists starting out on their careers to feel a hesitancy toward forsaking experimental work and to fear that they might be embarking on non-scientific procedures in taking up the study of human heredity. As a result, the programs of the Genetics Society of America for the ten years following its inception carried a scant dozen papers on the genetics of man.

On the last day of the year 1941 there was held at the Dallas meetings of the American Association for the Advancement of Science a symposium on human genetics, jointly sponsored by the American Society of Naturalists, the Genetics Society of America, the American Society of Zoologists, and the Botanical Society of America. Following this symposium, interest in the study of human heredity in America gradually increased. The old Eugenics Record Office at Cold Spring Harbor, which had faded from the picture in the face of a growing scepticism regarding the eugenics movement on the part of many geneticists, was replaced in this country by such centers of research in human genetics as the Ohio State University, the University
of Michigan, the Bowman Gray School of Medicine, the University of Minnesota, the University of Chicago, the New York City Examiner's Office, and the New York State Psychiatric Institute. Other centers are developing rapidly. The culmination of these activities in America was the formation of the American Society of Human Genetics in 1948, an event which holds promise of a true flowering of the subject on this side of the Atlantic.

Meanwhile various European laboratories had been far-sighted enough to perceive the importance of the study of human heredity, and centers of activity there had grown apace. Those in Germany and Russia were unfortunately detoured or terminated due to considerations of war or of conflicting ideologies, but those in England, Sweden and Denmark, and to some extent in Switzerland, Holland and other parts of Europe, are continuing to function at a high degree of efficiency. The recent formation by the International Union of Biological Sciences of a subsection on human genetics offers great hope that there may soon be a complete revival of international cooperation in the study of human heredity.

Within the last few decades remarkable progress has been made in the analysis of the genetics of man. The earlier studies dealt almost exclusively with individual families, and were concerned largely with conspicuous phenotypic discontinuities. If the trait were sufficiently striking and readily identified, data were often obtainable for several generations back. The families were of necessity selected, only those families containing at least one affected member being included in the studies. This, of course, created a bias in the data, but in spite of it many anomalies and diatheses were shown to have genetic bases, and for some of them presumptive evidence was obtained for specific types of hereditary behavior.

The discovery of the human blood groups by Landsteiner and the remarkable extensions of the original discovery by Levine, Wiener, Hirsfeld, Bernstein, Race and others opened the way to an entirely new approach to human genetics: the population approach. The blood groups have been to human genetics what Drosophila has been to classical genetics. The first of the many important contributions which the study of blood groups made to the field of human heredity
was the providing of "test characters" for analysis. Test characters are normal traits which can be ascertained easily and accurately, which are dependent upon a single pair or set of alleles, which are relatively uninfluenced by nongenetic impacts, and in which both or all alleles of the set are present in the population with reasonable frequencies.

In the study of test characters, the data are collected wholly at random, that is, without regard to the phenotypic composition of the individuals. The data may represent only individuals in the population, without regard to genetic relationship, or they may be in the form of families or kinships. Both kinds of data are valuable, each in its own way. When families are investigated in this manner, it is with the assurance that the majority of them will exhibit intrafamilial variation. The many blood agglutinogens are of this nature, as is the taste deficiency to phenyl-thio-carbamide. (Cotterman and Snyder, 1939; Snyder, 1931)

Test characters present the student of human heredity with problems not encountered by the laboratory geneticist. In the laboratory or field plot, where selection and inbreeding may be freely practiced, relatively isogenic, truebreeding lines may be established. From such lines we may, by controlled matings, obtain classical genetic ratios, and thus determine the mode of inheritance involved in any specific trait, and the genotypes of particular individuals, with relative ease.

In man, however, we must work with highly heterozygous material which is frequently restricted to two generations, and sometimes to but one, and in which the genotypes are in many cases capable only of incomplete specification. Under these circumstances even well-classified data will often contain mixtures of genotypically different types of matings.

The approach to the solution of such problems constitutes one of the most important milestones in the history of human genetics. In 1925 Bernstein, by means of cleverly designed extensions of the Hardy-Weinberg law of equilibrium, showed that data on test characters are subject to rigorous mathematical analysis on the basis of the frequencies of the postulated genes in the population. The immediate outcome of Bernstein's studies was his proof that the A, B,
and O blood groups are inherited on the basis of a set of triple alleles, and not, as had previously been thought, on the basis of two pairs of genes. The long range outcome of these studies, however, has been the development of population genetics as an important area of research.

Gene-frequency analyses for many types of transmission in populations were rapidly developed by Cotterman, Haldane, Lenz, Hogben, Snyder, Wellish, Wiener and others. To such an extent have these methods developed that, as Cotterman has pointed out, unit factor inheritance may be detected in data comprising but a single generation, that is, in sibships of completely unspecified parentage. Thus in the course of the development of methods for analyzing the genetics of man, the required number of generations has been reduced first to two, and finally to one, while the requisite knowledge of parental genotypes has been gradually reduced and finally eliminated altogether.

It must not be thought that methods which lessen the required number of generations or which minimize knowledge of parental genotypes are as desirable or efficient as classical methods. Such methods merely serve, as efficiently as possible, in an area in which test matings with precisely known genotypes are not available.

It is obvious that the methods of population genetics are not applicable to single families, and will not automatically correct the bias resulting from the selection of families on the basis of the inclusion of at least one affected member. Haldane, Hogben and others, however, have provided correction factors for cases of this sort, and such instances may now be analyzed efficiently.

Meanwhile a determined effort was being made to study linkage in man, and to map the human chromosomes. Here further new problems presented themselves. It was soon apparent that in dealing with human material, just as a single phenotype will often include two indistinguishable genotypes, so a single heterozygous genotype will include two indistinguishable phases, coupling and repulsion. This seemingly insurmountable difficulty was solved with increasing degrees of efficiency by Bernstein, Wiener, Haldane, Fisher, Penrose, and finally by Finney. The mathematical methods used are monu-
ments to the ingenuity and ability of biometricians, and have helped to repay the debt which human genetics owes to other sciences. By the use of such methods a start has been made on the mapping of human chromosomes. (Snyder, 1949.)

The original methods devised for studying the dynamics of gene frequencies were based on the assumption of infinitely large populations in equilibrium. But man is a gregarious animal and tends to cluster in groups of limited size. Within restricted populations sampling fluctuations, which constitute a characteristic of the genetic mechanism as inherent as any of its other properties, may have rather surprising effects.

Natural populations which at first sight may appear to be very extensive are in fact often composed of numerous local and more or less self-contained breeding units (isolates). In a completely self-contained unit, the gene frequency dynamics will be determined by the size and mating pattern of the unit. Where some intermigration occurs, the evolution of the over-all population will depend in important ways on the numbers in the individual sub-groups and on the extent of the intermigration. Among the hundreds of millions of human beings whose distribution is practically continuous over the earth’s surface there are countless isolated rural groups which contribute only occasional migrants to other populations. Even in urban areas isolates occur on the basis of social class, religious affiliations, culture patterns and other isolating mechanisms. The mating patterns of the world of mankind are in fact those imposed by the occurrence of many partial isolates. Dahlberg has roughly estimated from the frequencies of various types of consanguineous marriages that the average effective mating number of such quasi-isolates in Europe lies somewhere between 400 and 3000, and this figure may be taken as applicable to America also. In earlier centuries the numbers must have been even smaller.

Considerations such as these lead to important conclusions regarding human genetics. While sampling fluctuations would be likely to result only in negligible shifts in gene frequencies from generation to generation in large populations, they may in small populations of
a few hundred or less bring about radical alterations in gene frequencies quite aside from mutation or selection.

It happens, moreover, that the sampling fluctuations tend to be cumulative in their effects. Since the sample of genes drawn from the supply of any generation must in turn generate the supply from which a new sample will be drawn when the progeny reproduce, the allele which is less frequent in the parental generation tends to have its frequency further reduced in progeny resulting from samples drawn from the supply. Such cumulative changes result in what is known as genetic drift. It can lead to the spread of a new mutant gene through a small population, or to the loss of a new allele before it has had a chance to spread appreciably or at all.

The human geneticist today must therefore describe variability in two categories: that of the individual and his kinship, and that of the population. The individual and the members of his family are to be described in terms of Mendelian genetics; that is, in terms of the presence or absence of specific alleles, plus, of course, the results of environmental circumstances. The population, on the other hand, is to be characterized in terms of population genetics; that is, in terms of the relative proportions of various alleles, and of the overall environmental impacts.

Among the basic principles of population genetics which are essential to the working materials of the modern student of human heredity are the following (Snyder, 1947):

1. Classical Mendelian ratios are not to be expected in random samples drawn from a population, nor even necessarily from classified data including the pooled offspring from phenotypically similar matings. Classical ratios are to be found only among the offspring of an individual family if there are sufficient children, or among the pooled offspring of a series of families where the parental mating type of each family is in fact genotypically identical with that of every other family. Since the genotypes of human beings are seldom capable of specification, even the best classified data will usually contain mixtures of genotypes and must not be expected to yield Mendelian ratios.

2. In spite of the absence of classical ratios, predictable ratios of
another sort do occur under the conditions stated above. These ratios
are population ratios and are expressed in terms of the proportions
of the alleles. They are dynamic ratios in contrast to static Mendelian
ratios. Among random matings of individuals exhibiting a trait due
to a dominant gene, for example, there will be some in which one
or both parents are homozygous, resulting in the Mendelian ratio
of 1:0, and others in which both parents are heterozygous, resulting
in the Mendelian ratio of 3/4: 1/4. In a random-mating population
these static ratios are jointly expressed as one dynamic population
ratio, 
\[
\frac{1 + 2q}{(1 + q)^2} : \frac{q^2}{(1 + q)^2},
\]
where q is the proportion of the recessive allele in the population (Snyder, 1934). Many similar population
ratios have been derived and are of the utmost value in the analysis
of human genetics.

3. The comparison of predicted and observed population ratios
can be used to estimate the number and kind of genes responsible
for a hereditary variation in a population just as the comparison of
predicted and observed Mendelian ratios can be used to estimate
the number and kind of genes involved in a laboratory experiment.
Goodness-of-fit tests have been formulated for many such estimations.

4. The inherent characteristics of the Mendelian mechanism are
such that in a large population in which the effects of mutation,
selection, and in-and-out migration are either negligible or balancing
each other, the proportions of the various genes will remain constant
from generation to generation, regardless of the dominance or re-
cessivity of each gene. If there is in addition random mating in a
constant environment, the proportions of the genotypes, and thus
of the traits produced by them, will likewise remain constant.

5. Changes in the proportionate occurrence of genes or traits can
be brought about, however, by various phenomena including muta-
tion, selection, inbreeding, assortative mating, migration and, par-
ticularly in isolates, genetic drift. The rates and extents of such
changes are capable of mathematical estimation. The methods have
been summarized in recent books by Dahlberg, Hogben, Li and
others.

6. The occurrence of genetic linkage between the genes for two
traits does not change the association between those traits in the population from what it would be if they were not linked. Stated conversely, a correlation between two traits in a free-breeding population does not indicate genetic linkage between the genes responsible for the traits.

7. The inherent characteristics of the linkage mechanism are such, however, that the occurrence of linkage may be detected without recourse to the classical distinctions between coupling and repulsion phases, by means of specially constructed methods applicable to human material.

Unfortunately theoretical considerations of population genetics are far in advance of empirical investigations. There is as yet insufficient realization of the necessity of carefully collected field data on the genetics of human populations. The realization is growing, however, and is even beginning to invade the related fields of anthropology and medicine. The recent Cold Spring Harbor symposium on the origin and evolution of man brought together anthropologists, physicians, and geneticists, and did much to point the way to cooperative field efforts.

Even with the limited population data now available; however, one clear conclusion seems to be emerging (Snyder, 1947, 1948). Human populations differ one from the other almost entirely in the varying proportions of the allelic genes of the various sets of hereditary factors, and not in the kinds of genes they contain. The extreme positions held by those who on the one hand maintain that there are no significant genetic differences between human races, and those who on the other hand hold that certain races are “superior” and others “inferior,” require drastic modification in the light of the accumulated data on the gene frequency dynamics of human populations.

Throughout the growth and development of human genetics there have been other mistaken beliefs. One of the earliest and farthest-reaching fallacies in the philosophical approach to human problems was the belief that if a genetic basis were demonstrated for a certain trait, that trait could not be subject to environmental modification; and conversely, if a trait were shown to be influenced by the
environment, it could not at the same time be genetically determined. Although many of us have for years called attention to this fallacy, it still crops up in the literature, especially that of medicine, sociology, psychology, and education.

There is usually an element of fear in the case of the heredity-environment fallacy. For the physician, there may be a certain reluctance to accept the genetic basis for a disease or anomaly on the grounds that it would thereby be useless to attempt therapy. For the sociologist or psychologist, the reluctance involves the fear that new or changed attitudes could not be brought about if there were any genetic basis for the original development of individual differences in behavior. The fallacy appears in subtle ways and is not always easy to detect. In a recent book on intergroup relation centers (Clinchy, 1949), the author states that hostilities and methods of expressing animosities are not born in children, and therefore they can be controlled (italics mine). It may indeed be true that such things are not born in children, but even if they were, this would not determine that they could not be controlled. The same author states that science proves that differences in knowledge, customs, and personality are not transmitted biologically. Science proves no such thing. There is little significant scientific evidence either way on the possible genetic basis for personality, but there is a patent fear, on the part of the author of the statement, that if there were such a basis, intergroup relation centers might lose their usefulness.

The psychologists who recently maintained that massive doses of vitamin A would cure colorblindness wrongly concluded from their studies that it is obvious that colorblindness is not the simple Mendelian trait that popular theories assume it to be (Dunlap and Loken, 1942). When the results were attacked by another psychologist (Murray, 1942), this worker upheld the postulate that certain anomalies of color vision are both hereditary and incurable (italics mine), thus using the same misconceived argument, but the other way around.

We must keep constantly in mind the fact that each person with all his characteristics is the cooperative result of genetic and environmental agencies. The genes and their accompanying cytoplasm do
not alone make a man or woman. There is always an environment in which the individual develops, although the relative effects of differences in gene substitutions and differences in environmental forces will vary from trait to trait.

There are human genes, such as those responsible for the blood antigens, which express themselves rather uniformly within any known range of environment. There are other genes, such as those for resistance and susceptibility, the expression of which may vary considerably in different environments. It is probable that, in general, the fewer biochemical steps that intervene between a gene and its resulting trait, the less significantly will it be environmentally influenced, and the closer will be the correspondence between the presence of the gene and the presence of the trait.

It is important to make the distinction between what the gene actually does, which is apparently of a biochemical nature such as antigenic or enzymatic activity, and what the end result may be, under extragenic influences. This point of view is especially important when it is realized that the environmental events intervening between gene actions and finished characters in man may range from such overt occurrences as trauma and infection to the most subtle embryological, immunological, and psychological phenomena. Furthermore, there is no reason to doubt the feasibility, and in many cases the desirability, of attempting the control of such environmental agents. The recognition of a genetic potential in many traits should serve only to broaden rather than to narrow the scope of activity of the physician, the psychologist, the sociologist, and the educator.

Moreover, the recognition of the dual action of genetic and environmental agencies in the production of finished characters makes it possible to frame the question as to how much of the variability in a given trait in a specified population in a specified environment is due to differences in the genotypes of the individuals concerned, and how much is due to variations in the environment under discussion. The mere collection and analysis of families will offer little information on these points. Similarity or variability within families may conceivably be due as well to non-genetic agencies as to genetic
factors. In addition to pedigree studies we must use methods designed to discriminate between the potential causes of the variation. Such methods involve the observation of a series of genetically diverse individuals in the same environment, and of genetically similar individuals in diverse environments.

The twin studies by such investigators as von Verschuer, Newman, Rife, and Kallmann are examples of this type of procedure. So also are the institutional and foster home studies of students such as Burks, Freeman, and Leahy. Investigations of this nature mark the beginnings of an attempt to analyze traits other than those dependent on single-gene substitutions.

It was logical that the earlier studies and even the first of the new methods devised for analyzing human genetics should concern themselves with single-gene effects. Such effects are often striking, easily recognizable, and are usually sharply discontinuous. They are found to occur in practically every structure, organ and tissue of the body, and in nearly all physiological processes. We now have presumptive evidence that more than 100 variations (mostly pathologic) in the skin and its derivatives, more than 100 eye abnormalities, and a comparable number of skeletal anomalies are attributable to single-gene substitutions. There is reasonable evidence of single gene determination for a score of blood dyscrasias and for comparable numbers of aberrations of the muscular system, of nervous disorders, and of metabolic and endocrine disturbances.

On the other hand, there are remarkably few non-pathological variations in man which have been demonstrated to be the results of single-gene substitutions. The blood group antigens, the taste ability and deficiency for phenyl-thio-carbamide, the direction of the fine hair of the forehead and one or two others represent a major portion of normal human characters affected by known single genes. Recent studies by Spuhler, Reed, Rife, and others offer some hope that this list may be augmented.

At any rate our precise knowledge of human genetics is largely confined to the effects of single gene substitutions. Among other known facts about such characters is the discovery that they can be simulated by the effects of appropriate environmental agencies in the
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absence of the gene. The resulting "phenocopies" are often indistinguishable from the effects of the genes themselves. In human material, certain conditions apparently occur as single-gene effects in some families and as phenocopies in others; for example, the skeletal and neurological anomalies following irradiation (Murphy, 1947), the eye defects as sequellae to infection such as rubella (Gregg, 1941), and the simulated heart troubles brought on by environmentally conditioned anxiety reactions (Snyder, 1948).

Finally it has been shown that the effect of a gene substitution at any locus may be influenced by the genes at many other loci, and that conversely most single genes have multiple effects, often on viability, in addition to some major action.

It is tempting, in the face of the striking and variable effects of single-gene substitutions, to suppose that collectively they play a major role in human genetics. It is becoming more and more apparent, however, that single-gene differences resulting in marked phenotypic discontinuities are not in fact the most common type of genetic variability. Among the effects of radiation on gene mutation, for example, are some conspicuous phenotypic effects apparently identical with spontaneous mutations; a larger number of lethal mutations; and the largest number, by far, of mutations, the only discernible effects of which are slight changes in viability, vigor, or other physiological activities. Natural mutations appear to be distributed over a similar spectrum.

Probably both morphological and physiological characters affect viability, and there seems to be a general parallelism between the conspicuousness of the visible effect of a gene and the degree of viability impairment which results. Broadly speaking, the more striking the effect, the greater is the reduction in viability. Thus the largest class of mutant genes is composed of those genes which individually do not have readily discernible phenotypic effects, but for which the cumulative effects may be quite demonstrable.

Although most of the characteristics in man which have been adequately analyzed from the genetic standpoint are pathological discontinuities, the significant differences between individuals and between populations are to be found in such things as intelligence, special
abilities, social behavior, size and features. These are traits of a non-pathological nature; moreover, they follow a continuous distribution rather than a discontinuous one. Insofar as they have a genetic basis, therefore, they must depend upon multifactorial heredity involving cumulative effects of genes.

The importance of the problems of dealing with such cumulatively acting genes has so impressed Mather and his colleagues that they have designated genes of this sort polygenes. It is assumed that the effect of an individual gene of a polygenic series approaches the limit at which it cannot be distinguished from an environmental increment. In contrast, the single genes responsible for conspicuous phenotypic discontinuities are called major genes. It is possible to identify the latter individually, and to assign them to precise loci and specific linkage groups.

Polygenes, on the other hand, cannot be individually identified and localized. Their effects appear to be quantitatively equivalent and cumulative. Their existence must be accepted, however, if for no other reason than the effectiveness of selective breeding for almost any continuously variable quantitative character in a genetically heterogeneous population. There is, moreover, evidence that polygenes, like classical major genes, undergo segregation and crossing-over.

If then, as seems clear, genes with individually minute but cumulatively appreciable effects constitute the largest class of available mutant genes, much of the genetic variability of man is contingent upon multifactorial inheritance. Not only most of the genetic variability, but most of the resulting phenotypic variability as well, must depend upon such genes, since natural selection tends to keep at low incidences the large discontinuities produced by major genes, because of the viability impairment connected with them.

Almost the only discernible single-gene differences which are found at appreciably high frequencies in human populations are those which appear to be approximately neutral in their viability effects. The genes concerned are those for the blood group antigens, taste deficiency, color vision, hair whorl, and similar neutral factors. The genes for thalassemia and for sickle cell anemia which have recently
been so carefully studied by Neel, present exceptions for which we do not at present have a clear explanation. In these instances a relatively high frequency of the traits has been maintained in the face of the elimination or decreased fertility of the affected homozygotes. Whether an inordinately high mutation rate, a selective advantage of the heterozygote, or some other explanation, will be found to apply, only time will tell. Recent evidence from Reed’s laboratory (Reed, 1949) that fibrosis of the pancreas presents a similar situation raises the possibility that mutation rates in man may prove to be higher than has been realized. Nevertheless, of the hundreds of severe abnormalities in man which may be attributable to single-gene substitutions, only a few have population incidences above one in ten thousand, and the vast majority have very much lower occurrences than this.

In a recent discussion of these problems, David and I (David and Snyder, in press) suggested that if two unrelated people were picked at random from a population, or even one from each of two populations, they might be found to differ in respect to one or another of the blood antigens, and one might be a taster of phenyl-thio-carbamide and the other a non-taster. Beyond this it is highly probable that few if any of the observable phenotypic differences between them would be referable to known major genes. And yet we know at least the physical differences to be largely genetic because of the almost complete physical identity of the members of any pair of monozygotic twins. It follows that the major part of such genetic differences as are involved in the non-pathological range of human variability is most probably multifactorial in nature. Recognition of this fact may set limits to the amount of information which we may expect to gain through continued analysis of single-gene differences.

I would be the last one to suggest that we should cease searching for and describing individual gene effects. It is essential that we continue to develop and refine the methods for detecting and analyzing the activities of major genes in human populations. Such analyses provide, among other things, as I shall shortly describe, valuable practical applications in medicine. But, as David and I have recently pointed out, if human genetics is to progress along fresh pathways,
the traditional atomistic approach must be supplemented by new methods which will provide information on multifactorial inheritance. We must be able to analyze genetic variability without recourse to classical single-gene analyses. The newer types of twin and twin-family studies appear to be a fruitful approach for this kind of investigation. Although the techniques need elaboration and refinement, much progress is being made through quantitative comparisons of intra-pair differences in monozygotic and dizygotic twins separated and together, twin-family analyses, and the use of co-twin controls.

If the anthropologist, the psychologist, the sociologist, and the geneticist are to join forces in the genetic analysis of racial traits which are significant on the level of intelligence, personality, and social behavior, the implications of multifactorial inheritance must be carefully studied. Such transmission would seem, for example, less likely to bring about phenotypic differentiation through genetic drift than would the transmission of major genes, since the effects of individual multifactorial genes are apparently in large part mutually interchangeable. Even though genetic drift should result in the accumulation of different constellations of such genes in various populations, the over-all phenotypic manifestations would tend to remain constant, since the relative proportions of plus and minus genes should be about the same from one population to the next. Even in occasional instances where genetic drift does lead to phenotypic differentiation between small populations, this would have little chance of persisting after the populations expanded, unless it has a high adaptive value. As Dobzhansky and Montagu have pointed out, flexibility and plasticity of behavioral adjustments are likely to have had selective advantages over fixed or stereotyped responses in human intelligence and social behavior.

I cannot close this discussion without some reference to the important advantages to the health and welfare of mankind which have accrued from our increasing knowledge of human genetics. In past years those persons who have been concerned with the progress of medicine have given their chief attention to the problems of the alleviation and control of unfavorable and deleterious agencies of the environment. Throughout the decades increasing degrees of mastery
have been achieved over the harmful and debilitating effects of infectious agents, malnutrition, trauma, emotional stress, and occupational hazards. These achievements properly stand as significant monuments to the abilities, energies, and enthusiasms of those who have accomplished so much for medicine and human welfare. The decisive roles played by the bacteriologist in facilitating the control of infectious disease, by the biochemist in outlining the regulation of nutritional disorders, by the psychologist and psychiatrist in helping to overcome the harmful effects of emotional stresses, by the physician in combating the ravages of physiological aberrancies, and by the surgeon in repairing damage due to trauma and irritation, are now being paralleled by the medical geneticist, who is making possible the understanding and control of genetic disease.

The pioneering studies of such investigators as Macklin, Lenz, Roberts, and Allan have led to a widespread attack on the problems of medical genetics and have resulted in a series of practical applications. Those applications include the clinical detection of genetic carriers of disease (Neel, 1947), the earlier and more accurate identification of morbid genetic entities (Macklin, 1941), the instituting of therapeutic and preventive measures based on such detection and identification (Snyder, 1946), the developing of genetic prognoses (Snyder, 1946), and the solution of immunological problems such as hemolytic disease of the newborn (Levine et al, 1941), and of medicolegal problems such as the determination of disputed paternity (Wiener, 1943). Rapid progress is being made along all these lines, and it would be impossible to present the details here. The increase in sound genetic information in the newer medical text books and the growing number of courses in medical genetics in medical schools, however, testify to the appreciation of the genetic viewpoint by the physician. I would remind the medical members of the society of a consideration which Macklin has clearly stated. When it is realized that the very early signs of a disease, so often at present unrecognized, are to be found more frequently in relatives of a patient with an overt condition, then these early signs will be more actively searched for. Slight but significant deviations from normality, which have in the past been ignored by physician and patient alike, will
take on new and important meanings in the light of genetics, giving rise to new criteria for diagnosis, earlier identification, and consequent new opportunities for prevention and therapy. Furthermore, the detection of genetic carriers will make possible a sharpened approach to the physiology and biochemistry of disease, by providing for study a relatively large number of people in the very early stages of the conditions.

The physician is in a strategic position to obtain critical genetic data for prognosis. It is especially important that physicians should record consecutive series of family histories on various anomalies as the patients appear in the office, regardless of whether there is a patent familial occurrence in every case. Too often only those families are recorded and reported in which several members exhibit the disease, while the sporadic cases are not considered worthy of publication. Yet these are the very data which make accurate genetic prognosis possible.

To both the medical and anthropological members of the society I would point out that empirical data have not nearly caught up with theoretical considerations of human genetics. We need accurate data on the frequencies of all sorts of characteristics in populations, on their distributions within families, on the physiological and biochemical activities of the genes responsible for them, on the formation of isolates within larger populations, and on the extent of inbreeding and assortative mating within such isolates and of intermigration between them. The recent reports of Böök, Boyd, Kemp, Mourant and Pearson are excellent examples of what can be done along these lines.

The human genetic studies of the future must be cooperative efforts. Only by teamwork involving scientists from many areas can the understanding of the genetics of man be expected to advance appreciably. To those of you in related fields who are willing to lend your aid and advice to such teams, it may be confidently promised that in direct proportion to the data and information thus provided there will emerge a deeper and more significant understanding of human biology and new practical ways in which to use the information for the improvement of the health and welfare of all mankind.
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IN the first enthusiastic application of Mendelism to man, Bateson (1906) and Garrod (1902) were able to recognize the significance of the patterns, in pedigrees of human diseases, which had been unintelligible to previous observers. The new interpretations were convincing and for a time it seemed as though the chief object of studies in human genetics would be merely to discover as many single gene traits as possible. It was soon realized that metrical characters like stature and intelligence would not fit into this plan. Divergences of opinion arose and Pearson at first opposed the use of Mendelian interpretations. Many difficulties were resolved when effects of the principle of random mating were understood and the properties of additive genes investigated. From these controversies a new outlook emerged emphasizing the fundamental importance of the concept of gene frequency. The idea was soon exploited mathematically by Haldane (1932), Wright (1931) and Fisher (1919). Thenceforward the gene could no longer be regarded as a static element in the population. Its qualities had to be understood in relation to evolutionary trends. If a gene increased the fitness of its possessors, its prevalence should increase; a gene which diminished fitness would tend to be eliminated. These principles have great importance in human genetics both for the understanding of the existing genic composition of the population and in the prediction of future developments.
Many people have been tempted to assume that civilization, by means of medical and social care, has suspended or at least reduced the force of natural selection, so that genes causing unfitness in the natural state are becoming artificially established. It is also widely believed that the infertility of certain groups with distinguished social and intellectual attainments together with the high birth rates of those less successful economically will lead to deterioration in civilized populations. Such arguments have been used to justify programmes of eugenic sterilization. However, when Galton (1889) first proposed the idea of national eugenics, he had in mind the positive selection of favorable characters, and he envisaged the possibility of breeding a nation of exceptionally gifted men and women.

Eugenical thought has tended to accept uncritically certain assumptions. The first is that we know what phenotypes are desirable, and alternatively, that some social or national groups are essentially more desirable than others. Secondly, it is believed that extremely desirable and undesirable characters breed true; that is to say, the influences of environment and of dominance, overdominance and genetical modification are neglected. Furthermore, fertility is supposed to be a quality depending upon social environment rather than upon genetical constitution. Our knowledge of human genetics at the present time, however, is sufficient to raise serious doubts as to the truth of these assumptions. The relationship between gene and phenotype is often so complex that, even if we are satisfied about the undesirability of a given phenotype, the genes contributing to it may not necessarily be bad for the species. It is difficult to decide whether any gene is good or bad.

Take, for example, the case of a lethal recessive phenylketonuria. The effect of natural selection cannot be much improved by artificial means because the fertility of the homozygote is nearly zero. Yet the disease is prevalent in some European populations with a gene frequency of about 1 in 200. No less remarkably, it is absent from other populations. It seems reasonable to suppose that genes as frequent as this are nearly in equilibrium. The explanation that there is a very high mutation rate of the order of 1 in 20,000 per gene per generation is improbable. Equilibrium would be produced if the
heterozygous carriers were very slightly favored in some circumstances so as to have one half of one per cent more children than the average person. In general, the unfavorable effects of a gene in one context may be balanced by favorable effects in another.

If we turn to metrical or graded characters, in which the genetical contribution is multiple, the same problem presents itself in a different form. Physique and intelligence are among the qualities which are generally recognized to be eugenically significant, but great alarm has been caused by finding that in Western countries the intellectual and skilled groups do not have a high enough birth rate to maintain their numbers. Galton drew attention to the relative infertility of men of exceptional intellectual stature. The same is probably true of those with extremely tall physical stature. It is, however, possible to take the view that these phenomena are genetical and that they represent a biological tendency for phenotypes with extreme measurements to be infertile as compared with those in the center, with more normal measurements. Imbeciles and dwarfs are much less fertile than people of average mental and physical stature. The group with medium measurements and the greatest fertility might be more heterozygous than either extreme and, if so, equilibrium would be maintained in any given population with fairly random mating. Differential fertility with respect to intelligence is not necessarily an indication of degeneracy (Penrose, 1950).

These important problems can be investigated in endogamous communities and evidence may be found, which points towards or away from balanced genetical systems, in national or cultural groups. The problems, however, extend beyond local populations. With the growing facilities of transport and communication, no groups of the human family are likely to remain biologically isolated indefinitely. Thus, investigations concerning genic equilibrium must eventually be considered in the human race as a whole. The natural geographical groups form a set of relatively but not absolutely isolated populations. Furthermore, language, color, stature, occupation, culture and aptitude produce types of assortative mating within as well as between these groups. At the same time, inbreeding, if one can judge by trends in Western countries, is gradually diminishing. It may be
that the human race is gradually replacing local inbreeding by assortative mating and the genetical consequences of such a change require most careful study.

The investigation of the dynamic genetical processes in the human race presupposes accurate knowledge of world phenotype frequencies coupled with data on fertility. Moreover, by making an inventory of the genic composition of the human race, a useful base line for comparison with any local population is obtained. In considering the conditions of equilibrium of a given gene, the ultimate unit is the whole human race, not a local population. Indeed, in one area a gene may be advancing while in another it may be receding. Differential birth rates between people in diverse geographical areas may gradually alter the world frequencies of genes, but the decline of one population and the advance of another could be regarded objectively as an adaptive process due to selection. The value of a given gene in one part of the world may be quite different from its value in another. Particularly interesting in this connection is the study of antigenic genes. The selective force against such antigens as D in the Rh system depends in a peculiar manner upon its local frequency (Haldane, 1941). The selective effects depend also upon size of family and probably upon the presence or absence of other genes.

The success of any attempt to establish world gene frequencies is limited by the data which have already been collected. Anthropologists have been preoccupied with the differences between various extreme types and little effort has been made by them to find out what the average man is like. Martin (1928) estimated that mean male stature is 165 cm, that is about five feet five inches. The frequency distribution of all adult stature, however, has not been estimated, though Martin gave 121 cm and 199 cm as physiologically normal limits. The average hair color and skin color also could probably be determined. In these cases, however, phenotype is influenced by environment and is not precisely related to genotype. Something is known about the total frequency of taste deficiency and of color blindness though, when we come to rare hereditary diseases, it is almost entirely a matter of guesswork. There is, however, much interesting data about the frequency of rare traits in different popula-
tions. For example, Africans have more genes for drepanocytosis and fewer for phenylketonuria than Europeans. One of the few cases, in which there is sufficient data to make a good estimate of total gene frequency, is given by the O, A, B, and AB blood groups.

From existing data on phenotype distribution, it can be calculated that, in the whole human population, the alleles O, A and B have frequencies p, q and r, 0.623, 0.215 and 0.162 respectively (Penrose and McArthur, 1950). Thus, antigens A and B are not far from being equally represented (see Fig. 1.). Any population can be rated according to its divergences from these standard frequencies by summing the squares of the differences between standard values and those in the specific population. This index of distance from the mean is equivalent to an estimate of divergence from mean stature or any other mean measurement. The peculiarities of any local population can thus be accurately appreciated. Everyone is accustomed, quite erroneously, to regard the group from which he has originated as being the normal. Judged by world standards, his group is likely to be abnormal and he may have to fall back on the assumption that, though unusual, it may represent a specially desirable set of gene frequencies. He is perhaps justified only in inferring that his group has a genetical structure well suited in the past to its environment, else it would not have maintained itself.

Fig. 1. World blood groups. Perpendicular distances p, q, and r represent gene frequencies; areas show phenotype frequencies.

The peculiarity of a population in the statistical sense also depends upon its numbers; that is to say, the significance of a deviation from the world average has a different meaning in a large from that which it has in a small group. If the population of Great Britain consisted only of two or three hundred people, their blood group distribution
could reasonably be supposed to be a chance variation. Among the possible explanations of the divergent frequencies in different parts of the world are: colonization by very small groups in prehistoric times, varying mutation rates or geographical differences in selective values of alleles. In the case of blood antigens, there can be instability of a complicated kind favoring any gene which is possessed locally by the great majority of people. The fact that some populations are known which are entirely of group O, but none which are entirely A or B, suggests that A and B are capable of being eliminated from the human populations. Stability could be obtained if A and B heterozygotes had biological advantages of some kind; for instance, increased vitality or fertility. Little evidence is available on this point but some recent data suggest that the loss of A and B genes due to antigenic incompatibility may be compensated by increased fertility of group AB females (Bryce, et al, 1950).

The number of genes of any given sort in the world population is usually so large that any changes induced locally by natural or artificial selection will usually have no appreciable effect on the total frequency. The exception to this would occur, if a gene were found only in one locality. Until such a condition is discovered, the idea of national or local eugenics is seen to be futile. The wider problem of genetical improvement of the human race must be viewed against the background of gene frequencies in the world population and the relative fitnesses of different phenotypes in different environments. To this purpose the present paper is intended to make a modest initial contribution.

REFERENCES


Unlike most of the other topics on this program, the influence of inheritance on disease has been under observation for at least 5000 years. The records are chiefly medical. The Smith (Breasted, 1930), and Ebers (Ebbell, 1937) Papyri record the surgical and therapeutic knowledge of the Egyptians of 3400 to 2800 B.C. Their views are modern in that they emphasize the importance of gathering data and then interpreting them rationally; materia medica prescribe the use of medicinal plant, animal, and chemical products in terms of today; ricinus seed, liver, and antimony; their statues portray chondrodystrophic types in their god, Pan; and their tombs record interest in cattle, sheep, and donkey breeding with specific mention of horn-poled characters in cattle. In the Euphrates valley, Hammurabi developed the oldest code of laws 1000 years before Moses. The physicians' seals show forceps and other surgical instruments. Biblical literature describes some 20 diseases. Marriage of a man into an epileptic family is proscribed, and priests are disqualified for polydactyly.

The Greeks built on the accumulated data of their neighboring civilizations. Leucippus (Gomperz, 1913-1929) developed the idea that the multiplicity of things, both living and dead, in this world was built up from simpler distinct units, atoms. Sex determination for Democritus (Gomperz, 1913-29) was due to a preponderance of male or of female generative materials. Aristotle systematized and laid the foundation of comparative anatomy, systematic zoology,
embryology, teratology, botany, and physiology. These stupendous contributions made him the authority by whose works all arguments, including those for spontaneous generation, entelechy, and for the responsibility of nutrition for sex determination, found their settlement for nearly 2000 years.

The Hippocratic Corpus gave us an insight into the advances of medicine and the Greek dependence on earlier Egyptian work. Disease syndromes became recognized. The idea of a constitution for or against a disease came into being. The concept of contagion was present in the minds of Aristotle and Varro, in attributing malaria to invisible miasma, and of Galen and the Church Fathers in the spread of tuberculosis. But nothing came of these ideas for another millennium. The revival came with a great plague, an anatomist, and the microscope. Fracastoro, in studying the syphilit of 1500 came to the first clear understanding of seeds of contagion passing from one person to another. Solid foundations of anatomy, embryology, physiology, and many other branches of science contributory to genetics and pathology were being formed. The tubercular constitution of the Hippocratic Corpus took on the connotation of the inheritance of disease. Doctors and others in the succeeding period became much interested in this phase of disease causation, collecting more or less valid pedigree material. Maupertuis' study (Glass, 1947) of the handicapping condition, polydactyly, was outstanding. He investigated the normals as well as the polydactylous, applied probability theory to his results, formulated a particulate theory of inheritance with attraction of particles derived from the parents, and implied segregation, dominance, independent inheritance, and mutation as causes of variation. But the thought of the times was unable to visualize the significances of the results. The hereditary influence came to mean that the ancestral heredity was itself responsible for disease. With this concept came a certain hopelessness that because the disease was hereditary, the probability of finding a satisfactory treatment was negligible.

The principle that a specific contagion could cause a specific disease had to enter medical thought through a side door. The farmers of France in 1797 recognized that rust on wheat was such a specific
contagion, but it took the persistent substantiation of this principle by Pasteur, a chemist, for the idea to become established, first within the younger students and later with the older medical group. The breakdown of disease into separate entities, commenced by the ancient surgeons in classifying wounds, and expanded in the Hippocratic Corpus to include specific clinical syndromes, was further aided by the isolation of distinguishable entities specific for particular diseases. Through the efforts of Pasteur and his students and Koch and his students, the importance of the microorganisms for disease was up over the horizon where all could see. Yeasts, bacteria, protozoa, and viruses followed the mites and round worms into their proper niches as “causers” of specific diseases. It is little wonder that the dominant element in the medical thought of 1900 held that when the means of infection and vehicles of infection were identified the problems of disease outbreak would be solved. The concurrent brilliant successes of vaccination built up this position. Enthusiasm ran so high as to obscure other factors in the problem save in the minds of a few leaders, as when Koch pointed out that some facts still remain difficult or impossible to interpret, compelling us for the present to accept the view of a varying liability.

The revitalizing of thought on inheritance began with the reading of Mendel’s papers at the beginning of this century. Genetics as a designated science had its birth six years later, 1906. As genetics is an integral part of all life, there is no wonder that its first findings immediately impinged on man, his medicine, and his diseases. But it entered medicine when medicine was marking up one advance after another through the developments in bacteriology and acquired immunity. It entered as a somewhat discredited approach, as the older concepts of inheritance of a disease were considered refuted by finding disease vectors as specific bacteria, protozoa, etc. Advances in serology had further undermined inheritance in showing that disease resistance could come about through vaccination, or introduction into the host, of protein specific for the disease organism. Passive and active immunization, it was reasoned, would account for the passing of resistance from parent to offspring or ancestor to ancestor, and for the observed variations in resistance within different populations.
In the days when techniques were such as to make contamination easy, Koch had quite properly emphasized that purity of the organism is an essential step in the proof that a disease organism is truly the agent in that disease. This emphasis did have the effect of discrediting mutation, temporarily removing from the field of discussion this most significant element in genetics of disease.

The fusion of Mendelian concepts with the wealth of accumulated observations on cellular behavior in reproduction, begun just before 1900 and becoming a rapidly accomplished fact by 1906, was a contributing factor to the ultimately favorable acceptance of genetics in medicine. Prior to 1900 nutritional influences, ovulation order, phases of the moon at conception, etc., had been seriously proposed by various physicians as causative mechanisms behind the differentiation of the sexes. The observed sex chromosome differences in male and female cells, and the behavior of the cells in gamete formation and subsequent fertilization gave observed facts and the rational explanation for sex determination which medicine required. Genetic research continued this development with the demonstration that the balance of the sex chromosomes and autosomes in some animals or the X- and Y-chromosomes in dioecious plants are important elements in sex determination. Specific genes were isolated for particular sex or hermaphroditic types. Research on the problem was going in worthwhile directions.

The second influence favoring the genetic attack came as the result of work on plants. Medical treatment of the sick organism is only worth while when the individual has large value, and when a valid control method is known. Most economic plants are individually of low value and for many diseases the control methods are of questionable effectiveness. These facts forced the plant workers in their search for disease protection into adopting techniques whereby genetic resistance to one or more diseases is bred into the different varieties.

THE PROBLEMS OF DISEASE RESISTANCE

The status of genetics in relation to medicine and man when the term "genetics" was coined may be summarized as follows: the older
concept of the direct inheritance of the disease was largely discarded, and a feverish search was on for the external agents, which, by invading the host, could cause the numerous diseases to which the species was susceptible. The earlier concept of disease heredity carried with it the fatalistic view that such diseases could not be successfully treated, one had to live with them. The Hippocratic idea of a constitution for and against a disease had been transmuted by some into the concept that individuals had over-all constitutions—some resistant, some susceptible—for all diseases and vicissitudes of life.

Spontaneous generation of life as a common occurrence on this earth had been effectively disproved by 1900 but left in its wake a grave question. The written records show that man has been scourged by sporadically appearing pandemics beginning from Thucydides’ description (Crawley, 1934) of the plague of Athens in 430 B.C. Lesser epidemics appear from time to time. The events which led to these far-reaching disasters were not clear. Resistance had been induced in certain forms through vaccination. The facts of this acquired resistance were carried over to explain natural resistance, thus pointing up the question whether natural resistance was like acquired resistance in showing the same serological pattern. Schools of opposing thought represented by Metchnikoff ideas of cellular resistance, and Ehrlich emphasis of humoral elements of the blood, were in a turmoil of activity, each pressing its case.

The following paper will deal with the contributions of rising genetic knowledge to the rational understanding of these problems.

DEPENDENCE OF THE DISEASE SYNDROME ON THE INHERITANCE

As so often happens, the collection of data on this problem shows that the facts are apportioned to each side of the earlier controversy. Genetic research since 1906 has shown that inheritance affects disease reactions according to the processes necessary to bring them about. Inheritance of disease was made specific by relating each case to specific entities, the genes making up the inheritance system. In any one case the morbid condition may be due to a gene or a group
of genes working on a substrate of tissue formed by the remaining normal genes. The substituted gene in truth parasitizes the normal development of its host and leads to death or morbidity. It comes as no surprise to those who have studied the extensive pedigrees before 1900 that the more critical work fully confirms that inheritance alone can lead to definite diseases without outside intervention. Bateson’s 1906 paper on brachydactyly, congenital cataract, albinism, alcaptonuria, haemophilia, and color blindness in man received increasing support with the passing of time. Table 1 presents some of these cases. The introduction of the Mendelian idea of specific genes for particular jobs clarified the part inheritance played in disease causation.

<table>
<thead>
<tr>
<th>CONDITION</th>
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<th>ANIMAL</th>
<th>INVESTIGATOR</th>
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<td>Cataract</td>
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<td>r</td>
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<td>r</td>
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<td>D</td>
<td>Cattle</td>
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<td>Porphyrinuria</td>
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<td>Cattle</td>
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<td>r</td>
<td>Fowl</td>
<td>Warren 1940</td>
</tr>
<tr>
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<td>r</td>
<td>Fowl</td>
<td>Lamoreux 1942</td>
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<td>Short mandible</td>
<td>r</td>
<td>Dog</td>
<td>Grüneberg and Lea 1940</td>
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<tr>
<td>Hemophilia</td>
<td>r</td>
<td>Dog</td>
<td>Hutt, Richard, and Field 1948</td>
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<td>High uric acid</td>
<td>r</td>
<td>Dog</td>
<td>Trimble and Keeler 1938</td>
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<td>D</td>
<td>Rabbit</td>
<td>Wheeler, Sawin, and Stuart 1939</td>
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<tr>
<td>Amputated legs</td>
<td>r</td>
<td>Swine</td>
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The morbid conditions of Table I are representative of many hundred different, simply inherited disease types attributable to single
genes working within the rest of the animal's inheritance complex. The disease condition resulting from the same gene has a characteristic pathological syndrome. It is well to remember that in each species where the morbid gene occurs, there is a mate producing the healthy type which, in its inheritance, is allelic to the morbid gene. In man, the effects of some 200 such genes have been examined. In Drosophila, the species which has been scrutinized most, pathological changes attributable to morbid genes or small deficiencies number several thousand. Most normal genes in the fly seem capable of change to an allele or a loss which will cause morbid types identifiable as morphologic types or through embryologic development. These genes remain endemic in the population over long periods of time and may spread to other regions of the earth. An important consideration is attached to these cases which is not true of the other disease effects. For them a true immune type may be said to exist. The individual with proper genetic constitution will not show the disease defect, the immunity being conferred by the normal alleles of the pathological genes.

The isolation of these genes in particular chromosomes demonstrated anew that clinically similar disease syndromes may be due to quite different causes acting through different paths. Haldane (1948) points up the significance of this problem in discussing retinitis pigmentosa. The first pedigrees of this disease introduced the puzzling fact that some showed the disease dependent on a dominant, others on a recessive, gene. Localization of the inheritance showed the clinically similar diseases were in truth distinct. One locus for the disease has ordinary sex linkage, another is partially sex linked, and the third type occupies loci in the other chromosomes. Evidence at hand indicates that the genes in these loci control different steps in the disease development. The etiology of the disease is not worked out, but it can hardly be done until the genetic types are studied separately and each gene control step understood. Work in Drosophila and Neurospora points to the likelihood that the steps are different. Scarlet, cinnabar, vermilion and cardinal are indistinguishable eye colors controlled by four different genes. The vermilion allele cannot oxidize tryptophane, the cinnabar gene cannot conduct a
further stage in the pigment formation, and scarlet and cardinal are unable to work upon the pigment precursor. As Haldane (1948) says, "just as the methods for the cure of bacillary and amoebic dysentery are very different, so it is unlikely that the same therapeutic measures will succeed against diseases, however similar in their symptoms, which are due to different genes."

TREATMENT OF HEREDITARY DISEASES

Surgical repair has been a long-standing method of treating hereditary conditions such as hernia, hair lip, etc. Where no such obvious method was available, as for instance in mental conditions, the prognosis of hopeless was common. A growing recognition that diseases of this type may be amenable to treatment is a favorable sign. The proper treatment is often discovered through devious paths. A case which is illustrative of what may be accomplished to alleviate inherited disease is found in the dwarf mouse. Dwarf mice are weak, sterile, only one third the adult size of normal mice, they show basal metabolism reduced to one half normal, with repression of thyroid, adrenal, and reproductive organs. The pituitaries lack most of the eosinophil cells. The pleomorphic disease syndrome suggests the eosinophil cells of the pituitary as the focus of the dwarf gene's action. Transplantation of pituitaries from normal mice causes a resumption of growth and a return of thyroids, adrenals, and reproductive tract (save in the females) to normal. Anterior lobe extracts from cattle, or prolactin, and an ethanol precipitate when recombined, are equally effective. The gene works through its action on hormonal secreting cells of this gland. Replacement therapy with products of the normal dominant allele make it possible for the dwarf mice to attain normal size and function. Replacement of defective gene actions through proper therapy has been accomplished for the frizzle fowl, for lethal anemia in mice, and for diabetes in man, as a few examples. Many physiological and mental diseases unstudied as yet from this viewpoint, as Parkinson's disease, Huntington's chorea, arthritis, etc., suggest like possibilities of control when enough is known of gene action. Phenylketonuria is particularly suggestive, for a lack of a single
enzyme seems a likely cause of the change in the metabolic cycle at phenylpyruvic acid and the causation of the idiot complex. Further study of these possibilities offers new vistas in preventive medicine. The return of the defective type to normal, however, does not alter the bad inheritance that is transmitted to the next generation.

**CONSTITUTION IN INFECTIOUS DISEASE**

To be effective in infectious disease morbidity and mortality, inheritance must cause variation in resistance of the different individuals throughout the population. This variation must be apart from such things, known to affect disease resistance, as pathogen dosage, etc. With this in mind, the Genetics Department of Iowa State College started experiments in 1925 to measure mortality variation and to form lines of mice and fowl of characteristic resistances to mouse typhoid, *Salmonella typhimurium*, and fowl typhoid, *Salmonella gallinarum*, respectively. The pathogens were introduced intraperitoneally and the dose was held constant for the particular experiments. Parents of the succeeding generation were the best survivors of their test. Intense inbreeding was practiced to purify the genetic types.

Figure 1 shows that both the mouse and fowl populations were highly variable in their constitutions for disease resistance (Schott, 1931; Hetzer, 1935; and Lambert, 1936). In the first generation the population of mice had 18 per cent survivors, of fowl, 11 per cent. By the second generation, the population had 35 per cent survivors for the mice and 60 per cent for the fowl. Both graphs show that the host populations increased rapidly in resistance with the successive generations of selection for resistance.

Resistance increased rapidly at first, then somewhat more slowly for six or seven generations, the ultimate survival value of each group being 80 to 90 per cent. In the eighth generation for the mice the dosage of organisms was increased to $2 \times 10^8$. This increase was accompanied by a 10 per cent reduction in survival. From that point on the resistance increased again, 93-96 per cent resistant animals being reached in the fourteenth generation. The results show that
Fig. 1. Survival value of mice (Schott, 1931, 1932 and Hetzer, 1935, 1937) and domestic fowl (Lambert, 1931, 1936) for successive generations of selection toward the resistant types. Solid line for fowl, dot-and-dash line for mice. Eleventh generation of fowl selection was untested. Eighth generation of mice had dosage changed from \(5 \times 10^4\) to \(2 \times 10^5\).

despite continuous selection and inbreeding for eight generations there was further residual variation within the strain. Chicks of the eleventh generation were not tested. Subsequent tests showed high resistance within the chickens. Testing of the parents is not necessary for the resistance in the progeny. The chickens and mice have kept their high genetic resistance despite the fact that the inbreeding has led to the accumulation of genes for smaller size, lower fertility, and some apparent lack of vigor.

Two possible explanations might account for this change in resistance: (1) selecting of small variations due to genes for resistance already in the parental lines or (2) selecting of mutations toward higher resistance, each mutation being partly dependent on the total
genetic constitution of the host for disease resistance. Either explanation would lead to the disease resistance observed in these lines. Actually both factors appear important. There is some experimental evidence to indicate that if a sufficiently large population of mice is chosen it is possible to pick out from this population individuals which carry very high genetic resistance to mouse typhoid and actually make the change from a relatively susceptible population to a highly resistant population in a very few generations. This sudden change could favor the view that disease resistance may be accomplished in one step or may be due to a single gene pair. The results of Hetzer (1937) show that this is not the case. The circumstances leading to the choice of resistant mice are fortuitous, a result of a combination of several genes for resistance in one animal brought about by chance segregation.

A second question of interest is why a completely resistant strain is not attained. The strains which have been formed would be considered completely resistant if bacteria of low virulence were used to initiate the disease. With highly virulent organisms, some deaths do occur. It seems that no species of animals having a native disease has yet established a completely immune race through genetic means or any other means for that matter. Highly resistant animals have been produced but with highly virulent organisms of the pathogen it is possible to produce some deaths in all cases.

Since the fourteenth generation the selected strains have been maintained without testing. The resistance of the present generation is as high as it was under testing. Genetic resistance when made homozygous for the strain becomes a permanent attribute.

These results, while not the simplest from an inheritance point of view, are typical in showing the importance of genetic constitution in many diseases. Like results have been attained in the liver disease of mice due to *B. piliformis*, foulbrood of bees due to *B. larvae*; *P. suis*, and *S. cholerae suis* infections in guinea pigs; *S. enteritidis* typhoid of rats and mice, *S. pullorum* of the fowl. Comparable patterns are evident in diphtheria, scarlet fever, appendicitis, and are indicated in tuberculosis, leprosy, and other human diseases of bacterial origin. Similar results have been attained for large and small
infectious pathogens, the tapeworms of rats, the roundworms of chickens, protozoans for cattle tick fever, malaria of the mosquito, virus mosaic of tobacco, tumors of the fowl, and poliomyelitis of man, etc. That the disease host's inheritance plays an important part in the morbidity and mortality from different infectious diseases has now become a well nigh universal conclusion. Genetic research since 1906 has led to a redefining of its part in disease. The host genotypes furnish the nutrient patterns on which the pathogens may work readily, with difficulty, or not at all. The inheritance specifies the grade of susceptibility which then must await the invasive organism for its expression.

The question of an overall constitution for resistance to all disease has been investigated for several combinations of animal diseases initiated by various pathogens, for instance, S. typhimurium, pseudorabies virus and ricin poisoning, P. avicida, B. Friedlaenderi, pneumococcus, and loping ill. The results point to a correlation between the resistance of one disease and that of another when the organisms initiating the diseases are closely enough related to have some genetic similarity. When they are far apart taxonomically the resistances for the two diseases are nearly or quite independent. Recent unpublished work of our laboratory with Dr. Plough shows that the independence can extend to strains of S. typhimurium which differ by single mutations.

The independence of genetic constitutions for different diseases has long been settled for many plant diseases. In many instances specific resistance is traced to single-gene differences. Disease resistance or susceptibility is specific for each disease.

**ORIGIN OF PANDEMICS**

The sudden appearance and the fearful toll of life in a pandemic have always led to supernatural explanations of its cause. It seems highly probable, and was certainly true in the past, that, by the time the seriousness of the disease was recognized, the opportunity for determining its origin was past. In a small way, an attack on this problem has begun recently. Three elements could contribute to dis-
case variation: the general environment, the resistance or susceptibility of the host population, and the peculiar character of the pathogen. Epidemic history records the outbreaks under so seemingly ordinary conditions that environment does not appear likely as the general cause. Similarly the subsequent spread of the disease to all regions of the earth indicates that the disease is satisfied with widely varying conditions. Genotypic variations in host susceptibility undoubtedly contribute to variation in morbidity and mortality during the course of the epidemic. Susceptibles die first, thus creating a peak in the mortality curve. Removal of the susceptible contacts reduces the chance of infection in the genetically more resistant population and lowers the mortality curve. As will be shown later, acquired active and passive immunity phenomena play some part in this decline, but as the power to become immunized is closely related to the animal's own natural resistance genotype, it too is a function of the genotype. Freely breeding populations in which there has been little or no selection for resistance to a disease generally have a high proportion of genetically susceptible hosts, which are ready to accept an infection easily and react violently to it. The studies of epidemic measles on the Faroe Islands (Panum, 1940) in 1846 bear this out. All told, the effect of the host genotype on the epidemics is pronounced, but generation time in man appears too long for host genotype to be the main factor in the disease incidence. This narrows the problem down to the pathogen. A pathogen having high virulence for its host must enter the population. The organism may be introduced from without through contact with people and products from foreign lands. This method, in a sense, only postpones the question of where the organism comes from. Koch early focussed attention on the invariant nature of pathogens. But by 1904, Barber, by his famous pipette method, demonstrated that pure strains of coli do produce variants in proportions similar to those of mutations found in other forms. Over the succeeding years this fact has received confirmation from many quarters.

Virulence and its dependence upon the inherited bacterial constitution may be examined by searching out phenotypical variants in an original pure stock as in Zelle, 1942, Lincoln and Gowen's (1942) work. Two different lines of the corn-wilt organism, Phytomonas
were searched for naturally occurring colony mutations as well as for those following x-ray irradiation. A quantitative measure of virulence was obtained by comparing the green weights of inoculated and uninoculated plants. Twelve colony mutants from the dark yellow rough parent ranged from white to light yellow, rough to extreme smooth through several grades, mucoid to dry, and small to large. The parental virulence was 31. Of the 12 mutants, 3 were below the parent, 9 were of greater virulence, the highest being 70 with a mean of 45. The other type had 9 mutants, seven of the 9 had less virulence than the parent index 75. Two were above the parent index, 81 and 78. The average was 62. The mutants were stable. These results show that changes in virulence of a pathogen may arise through mutation. The mutants tend to remain fairly close to the parental type, but the range from the highest to the lowest may be wide. The variation which is observed is directional. When the original parent is of low virulence, the mutants tend to be of higher virulence. When the virulence of the mutant is high, the mutations tend to be of lower virulence. Similar facts have been observed for mutants in viruses and other forms.

Changes in virulence similar to those which appear above occurred under field conditions. In November, 1940, our culture of S. gallinarum was completely avirulent, whereas it had been highly virulent the previous May. This result demonstrated that a large population of virulent bacteria may be replaced by avirulent bacteria in about seven months, a fact which became of greater interest since this line had previously retained its pathogenicity for several years.

Serological and cultural tests showed the culture, S. gallinarum. When this culture was inoculated in a highly susceptible chicken, no disease was observed, but five isolations were recovered. Two lines were soon lost. The other three lines were passed separately and blindly through 6 successive ten-week-old birds of the susceptible strain. The inoculating dose was two billion organisms, and the bacteria remained in the birds one week. At the seventh passage virulence tests were made on 11 ten-day-old chicks; line D7 killed 11 in less than ten days; line C7 killed 6 out of 11, but took twenty-one days; line E7 killed 8 of 11, but also took twenty-one days. On ex-
tensive tests over a period of some years, line D has proved to be a highly virulent culture. Planned experiments on larger numbers of chickens gave similar results.

From the avirulent stock culture described above 20 colony isolations were made. As this organism does not clump appreciably, each of these colony isolations probably represents the descendants of a single bacterium. Ten of these avirulent lines were exposed to the environment of our inbred, highly resistant chickens described above. These inbred lines are capable of surviving nearly 1000 times the number of bacteria which will cause death in most flocks. The other ten lines of avirulent bacteria were grown in a strain of chickens marked by susceptibility to fowl typhoid. Two chicks were used at each passage for the resistant host line and one chick for the susceptible host line. The avirulent strains of S. gallinarum were thus exposed, on the one hand, to the intensely unfavorable environment of the resistant strain of host, and on the other hand, to the more favorable environment of the highly susceptible host.

Each culture was passed successively through 16 different ten-day-old chicks. Despite the fact that twice as many chicks were available for recovering the organism at each resistance passage, 24 passages were lost in the transfers through the resistant host compared to 10 for the susceptible series. The avirulent line has great difficulty in establishing itself even to making a mild disease in the resistant host strain. This fact suggests that the resistant host would be a potent selecting force tending to pick out the progeny of any variants characterized by increased virulence. This did not turn out to be the case.

Small tests were made throughout the passage experiments. When changes did occur they came suddenly during a single passage and resulted in a substantial gain in virulence, the total amount of change differing for different lines. The subsequent tests showed a retention of the new change in virulence. These results favor mutation and subsequent replacing of the avirulent type by the virulent mutant.

Tests of the surviving lines at the end of the sixteenth passage give further support to this conclusion. Two of the lines, I and R, had not changed in virulence as the result of growing in their natural host for half a year (Fig. 2). One line was carried in the resistant host. The
Fig. 2. Changes in virulence of different lines of S. gallinarum originating from the same avirulent line, after successive passages through resistant chicks A to J and susceptible chicks K to T. Ordinate at left shows virulence when passage culture is tested on susceptible strain chicks.
other line was passed through the susceptible host. If virulence is
due to chance mutation, the expectation would be essentially equal
numbers of mutations in each group. Two lines of medium virulence
have been established from the resistant host against three lines for
the susceptible host. Seven highly virulent lines came from resistant
host passage and six from susceptible host passage.

Ten lines passed through the resistant hosts showed virulence as
follows: a dose of 100,000 organisms inoculated into 74 resistant
chickens led to 20 per cent death; inoculated into 203 susceptible
chickens led to 70 per cent death. With 100,000,000 organisms as the
dose, 70 resistant chickens had 22 per cent death, 124 susceptible
chickens had 88 per cent death. For the lines derived by passage
through the susceptible host the 29 resistant chickens with 100,000
dosage had 7 per cent death and the 186 susceptible chickens had 84
per cent death. For the 100,000,000 dosage, 54 resistant chickens had
22 per cent death, and 98 susceptibles had 86 per cent death. These
data show that passage through either host is equally favorable to
establishing of virulence. The degree of increase in virulence may be
judged by the fact that the original avirulent culture inoculated in
a dose of 100,000,000 organisms showed no death on the resistant
host and only 34 per cent on the susceptible host.

Chicks of either strain are highly efficient selective agents favoring
any variants toward virulence and encouraging them to multiply at
the expense of the avirulent type. The population within the host
becomes rapidly purified toward the virulent type. The genetic con-
stitution of the domestic fowl, the natural host to this disease, is
sufficient to create the necessary conditions for this selection process.
The culture media, on the other hand, appear to favor those organ-
isms whose genetic constitution is for a saprophytic type of growth.

Like results were observed for S. typhimurium in mice. In essence,
the different forms show changes in virulence to be analogous to
mutations in higher forms. The rate of mutation for a given type
is small. The changes are sporadic in their appearance and when they
do occur, they are permanent and true-breeding. The variation can
go in either direction—toward higher virulence or toward avirulence.
The environment of the host or culture medium acts as a selective agent for the genetic type which fits the environment.

These data point to the spontaneous origin of virulence as mutation of a pathogen's genotype, which is then selected for by the host to become the dominant virulent type capable of invading the whole host population.

The development of plant genetic research in this country offers a grand demonstration of the significance of these genetic factors to pandemics. Oats or wheat have passed through similar phases, the general observations on one species being confirmed by those on the other. For our purpose the development of oat types in Iowa will be considered (Hughes, 1945; Murphy, 1948-49). The breeding program commenced in 1906. The varieties planted were farmer and seedsmen selections and introductions. In 1910 acreage planted to Early Champion, Green Russian, Kherson, White Russian, Silvermine constituted 26, 28, 8, 6 and 4 per cent of the total. Oats are largely self-fertilized, so each variety approaches to a pure line genetically. The fact that no variety occupied more than 28 per cent of the state showed that specific inheritance for disease types was pretty well scattered. Pure-line oat selections were introduced in 1913 and by 1920 made up most of the acreage of the state. As they were largely selections from Kherson—Albion, Richland, Iowar, Logold—the state became blanketed with but one genotype. In 1930 crossing of Victoria—a variety from Argentina but probably originating in Uruguay—and Richland, was accomplished. Victoria was a poor variety agronomically, but it was outstanding in having high resistance to 76 of the then known 82 races of crown rust, the six to which it was not resistant being rare in the United States at that time. It was also resistant to 29 of 31 known races of loose smut, and the 14 known races of covered smut. From this cross came the selections Boone, Control, Tama. Again these varieties were closely similar in genotype, but different from that of the earlier 1910 to 1920 varieties. Bond is a variety from Australia which Murphy (1948-49) has shown is resistant to 75 of 82 races of crown rust, most races of loose and covered smut. The seven races of crown rust to which it was susceptible were rare in the United States at the time of its use in crossing with
other varieties. Crosses of Bond to Richland x Green Russian hybrids gave four varieties of similar genotype: Clinton, Benton, Shelby and Cherokee, but with, in turn, a different genotype from that of their predecessors. Each of these varietal groups came to cover nearly 100 per cent of Iowa’s oat acreage. The Richland group to 1940, the Victoria group 1943 to 1947, the Bond group 1948—.

Yield is a good measure of mortality and morbidity from disease. The oat yield shows four major cycles marked by the low yields of the drought and depression years. Aside from these depression points, yields from 1866 to 1910 showed a downward trend, the decrease being from 34.5 in 1866 to 31.0 in 1910. The first varieties, as the products of selections, were introduced in 1912. Since 1915, the yields have been above the 1866 mark. The Kherson selections, Richland, etc., yielded well till 1940, although they were susceptible to common rusts and smuts. In 1941, 1942 and 1943, crown rust races to which these varieties were susceptible increased in numbers, causing a reduction in acreage of the older varieties from nearly 100 per cent to practically zero. The Victoria derivatives, Boone, Tama, etc., which were common rust and smut resistant, were available in 1942. They rapidly gained ascendency in the state representing 97 per cent of the plantings by 1945. In 1946 Helminthosporium victoriae took on an entirely new significance. The Victoria varieties were susceptible, disease incidence became high, and yields were greatly reduced. Bond derivations, Clinton, Shelby, etc., were available. They were resistant to Helminthosporium blight and the common rusts and smuts. These varieties again took over the oat-growing regions of the state making the area a nearly solid block of the same genotype. The immediate result was that a race of crown rust which had previously been rare increased rapidly. It now constitutes nearly 50 per cent of the crown rust races attacking the Bond derivatives and has begun to take a severe toll.

What an epidemiological experiment! It involves all of the factors one would like to examine. Huge numbers of individuals, individuals stratified for their genotypes, diseases of different etiologies, avenues of infection and lesion types, each disease species stratified into different infective races, environmental conditions generally favorable
to each disease, are all here. The diseases involved have different modes of spreading. Rust is airborne over huge areas, starting up from the south and being augmented by seasonal additions as it progresses north toward Canada. Alternate hosts are important. Genetic segregations and recombinations of the genes take place. Helminthosporium blight persists in the soil but moves less rapidly over an area. The contact rate factor, so frequently postulated in human epidemiology, is varying over the period covered, and measure can be made of its effects.

The data show that the diseases run in cycles. The cycles are highly dependent upon the genetic constitution of the particular host and of the pathogen. Fairly close contact between the susceptible hosts is a necessary factor for the establishment of the disease. Amount of inoculum to which the host population is exposed plays an important part in the severity of the epidemics. When the host contacts are close, the host genotypes susceptible, and the inoculum received by each host virulent and large in amount, epidemics of severe proportions follow.

These facts may be stated in terms of the pathogens' genotypes. In order to survive, pathogens must have some susceptible hosts. If these hosts are few or widely scattered as viewed from the infective range of the pathogen, then the disease will pass nearly unnoticed or appear in mild endemic form. But the pathogen also undergoes mutations both toward and away from virulence. The mutation rate is low, but the large numbers of progeny make its effects appreciable. The mutation having virulence for the previously resistant types has a large population, the individuals of which are in close proximity to work upon. Conditions are then right for an epidemic burst of the new disease. In nature the host population is subject to many diseases, each of which has the above possibilities. The result is a constant succession of genotypes in both host and pathogens.

In these oat data, the successive parts played in the epidemics by the genetic factors become fairly clear. Virgin lands which have not been cropped with cultivated plants are relatively free of the diseases of cultivated crops. They are also high in fertility for such crops. Similar genotypes are so widely separated as to restrict disease spread.
High yields result. Disease builds up and fertility decreases. This build-up of the unfavorable elements was expressed in the slow drop in oat yields from 1866 to 1910. The varieties planted in the period were late varieties. The 1913 to 1926 releases were early varieties. Their genetic constitutions for earliness enabled them to escape most of the older diseases, and consequently the yield was larger. Rusks and smuts to which they were susceptible began to catch up and to become divided into many races through mutations. By 1940, the pathogen population capable of attacking the older host genotypes had the upper hand. Genetic research introduced host resistance to the then common rusks and smuts. This resistance would seemingly have come from fairly recent mutation since it was dependent on a single gene, or at least very few genes. The host resistance removed the immediate danger from rust, but allowed the full development of another disease, *Helminthosporium victoriae*. Again the disease course portrays an epidemic rise to a crisis. The introduction of two resistant allelic systems into the oat varieties, one for the common rusks and smuts, and the other for Helminthosporium, again gave a brief respite marked by more satisfactory yields. But again what had been an obscure disease, crown rust 45 which seemingly depends for its pathogenicity on a simple mutation from some one of the earlier rusks, was able to build up rapidly in the homozygous population represented by the state’s and nation’s oat acreage. Fortunately, sources of resistance to this disease have been searched out and the process of incorporating them into the present oat genotypes is well on its way.

The facts reviewed above are illustrated equally well by the wheats of the same period. In infectious disease the genotypes of host and pathogen are in a constant state of flux. The successive events are like those of laboratory-controlled experiments. The close union of genetics and pathology in research has clarified many previously obscure hypotheses and added new theories of significance to epidemiology.

**MECHANISMS IN NATURAL RESISTANCE**

Associated with acquired immunity is an increase in the blood serum agglutinins, precipitins, bactericidins, and opsonins. These
humoral bodies are generated by contact with the specific proteins of the pathogen. In natural resistance this contact is lacking. The question arises whether the reacting system generated by introducing foreign protein into the organism in vaccination is the same as that formed by the genes in the naturally resistant mice before any previous contact with this protein. Cases where the mechanism of natural resistance has been examined show a wide variety of characters significant to the resistance. In a strain of guinea pigs Rich (1923) showed lack of blood complement lead to susceptibility. In fowl duodenal mucus, Ackert (1942) found an antihelminthic substance. Foulbrood resistance appears due to inheritance of instinct for cleanliness of the hive (Park, 1935-48). The height of the normal body temperature may be significant to resistance in the fowl (Bell, 1949). All experiments are against passive or active transfer of natural resistance through circulating substance.

Metchnikoff (1892) favored the reticulo-endothelial system as the active element in resistance. This has been supported by Severens et al. (1944) for pullorum of the chicken, by Reich and Dunning (1941) for general resistance in the rat, and by Gowen and Calhoun (1943) for typhoid resistance in the mouse. The macrophage system of the mouse was studied by Oakberg (1946). Susceptible mice show a rapid disfunction of the liver, after infection. Resistant mice may develop large lesions, but these lesions are rapidly walled off from the rest of the liver. The remaining liver is normal in metabolizing fat and glycogen. The normal cells are protected in some manner from the endotoxin released by the dead bacteria. The macrophages react very differently. In susceptible mice, the macrophages take up large numbers of the bacteria, some cells containing 40 or more. These bacteria stain sharply and characteristically. They give the appearance of being in reproduction. Macrophages containing bacteria are hard to find in resistant mice. When they are found, the bacteria are apparently undergoing rapid digestion by an intracellular enzyme. They stain very faintly and are ragged in appearance. This observation has particular interest for the enzyme which the observations suggest could be generated as a specific gene product in the resistant mice, not in the susceptible animals. The general agreement of this
view with specificity of gene action in biochemical reactions makes
the hypothesis of further interest.

ACQUIRED IMMUNITY

The phenomenon of acquired immunity, brought about by the
introduction of a disease organism, toxin, or antigen into a com-
patible host, was an established principle when Mendel's paper was
rediscovered in 1900. The commonly recognized changes are the
appearance of agglutinins, precipitins, bactericidins, and opsonins
in the blood serum and increased phagocytosis by the blood cells.
Most significant to us as hosts is the increased specific resistance to
the disease immunized against. The facts of the acquired immunity
concept, it was reasoned, would explain the more significant pheno-
mena of natural resistance to a disease. This was pure conjecture.
Not all diseases gave the acquired immunity reactions. There were
unaccountable irregularities in the effects. Many gaps in understand-
ing the mechanism of the process came to be recognized. Possibly
one of the greatest of these is the relation which acquired immunity
bore on natural resistance found in a host prior to any previous ex-
posure to the disease.

Three elements contribute to the initiation and severity of a dis-
ce: the host, the pathogen, and a collective group of influences
causing variation in disease expression, which may be called the en-
vironment. As any one experiment can mark out only a small be-
ginning on this problem, we chose to work with the typhoid
producing bacteria, Salmonella typhimurium, as the disease agent,
and our laboratory mice, Mus musculus, as the host. The opportu-
nity to contribute to the problem came from having separated both the
host and pathogen into several relatively pure-breeding genotypes,
ranging in the mouse from a strain nearly completely susceptible, to
a strain nearly completely resistant, and in the typhoid organism
from a line which was almost a saprophyte having little disease poten-
tialities to a line having high virulence.

Six long-inbred strains, 25 to 60 generations of brother x sister,
of mice were studied. The natural resistance of these strains when
first exposed to 200,000 typhoid organisms of our virulent race was S86, R75, Z58, E53, L13, and B8 per cent survival. The bacterial races have been derived from our virulent line by isolation of mutants for virulence; one mutant, DSCI showed initially about 16 per cent more deaths with our different strains of mice than our standard virulent line 11C. The other mutant, 9D, showed about 21 per cent less deaths than 11C for the different strains. All our cultures of 11C and 9D have shown constant virulence over the three years of these experiments. Unfortunately, DSCI, in the line used, has been replaced by a mutant of less virulence, although in another culture subline DSCI has retained its full virulence.

To analyze the effects of these different genotypes on acquired immunity a factorial experiment including 4212 mice was performed. The variables were the six mouse genotypes; the three bacterial genotypes; three grades of vaccine administered, 1,250,000, 12,500,000 and 125,000,000 heat killed organisms; three types of immunization, one dose, two doses at one week apart, and three doses at one-week intervals, each. There were 6 x 3 x 3 x 3 = 162 different groups of mice. The mice in one group all had brothers or sisters in the other groups, so that any within-litter variation within strains was minimized. Tests for resistance were all made three weeks from last immunization with 50,000,000 living 11C organisms. This dose was 250 times that used for our natural resistance tests. As the experiments were completely balanced, direct comparisons were legitimate.

The results show that the greater the quantity of vaccine used, the more resistance is developed. This is an old fact. A new fact came out of these immunizations, however. In making the immunization it was found that mice may die as a result of inoculating the dead vaccine. These deaths all occurred in the mice which had low natural resistance to typhoid, the L and B strains; the higher the dosages, the greater the likelihood of death. Vaccination with dead organisms was dangerous to mice whose genotypes were highly susceptible to the living organisms. The data repeat a second known fact; immunization in three weekly periods is better than two, and two is better than one. The third result presents clear evidence for the significance of the bacterial genotype in immunization. It shows that lines of the
typhoid organisms which have low virulence are poor immunizers. As these bacterial lines come from each other by mutation, the importance of the bacterial genotype in vaccination is demonstrated. It might be argued that the severity of the vaccine reaction accounted for this result. This conclusion is doubtful in the light of these results which show that the strains most naturally susceptible were most severely affected by the vaccinations, and in the light of the data to follow, which show that these strains have the poorest acquired immunity.

Figure 3 presents the fourth result, the effects of heredity on the reactions to vaccination. The most susceptible strain after vaccination is also the most susceptible genotype when exposed to the living disease prior to vaccination. Similarly, the next most susceptible is
next most susceptible, and so on. The two most resistant genotypes prior to vaccination show the highest resistance after vaccination. In acquired immunity the mouse genotypes react to the bacterial genotypes in a manner like that observed for natural resistance. The level of resistance of all strains is simply raised in a comparable manner. Measured in terms of bacteria, an immunized mouse can resist 100 to 200 times more live bacteria than the unvaccinated mouse.

The effects of vaccination are like those of natural resistance in that they depend on the genotypes of host and pathogen. In our mice, characteristic immune elements of the serum such as agglutinins, bactericidins, etc., are not found in any of our hereditarily differentiated mice prior to their contact with the disease. Cellular elements show high correlations with the natural resistance. This being the case, the results on acquired immunity to typhoid point to cellular elements as being the differentiators in the resistance acquired through immunization. The parallels in the results for natural and for acquired resistance suggest that the same cell elements are ultimately responsible for both immune phenomena.

In this brief review, the writer has tried to portray a few of the manifold ways in which, over 50 years, the nascent science of genetics has clarified and particularized the part played by inheritance in the causation of and resistance to disease. Large omissions have consciously been made in the review. These the reader can, and no doubt will, fill in if he is interested. The space devoted to the significance of the genetic methods as tools in future research is regretfully small. Chemical genetics offers means by which disease problems can be reduced to relatively simple chemical terms. Mutation allows for direct, relatively simple, controlled variation of the reacting system thus facilitating an analysis of the mechanisms by which the system acts. Advances along these lines have already been made by causing the genes of bacteria and viruses to mutate and then studying, in the light of the diseases they normally produce, the altered chemistry of the mutants and the products which they form. Tools for future research are before us and the possibilities for future contributions of genetics to the alleviation of man's diseases appear bright.
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*This paper has an extensive list of references to closely allied subject matter to which the reader is referred for additional material.


John W. Gowing

ANY discussion of genetics in relation to cancer needs a general definition of principles involved.

Cancer itself is a form of unusually rapid growth ordinarily occurring in an organism in an unbroken series of descendant cell generations. In medicine it is accepted as a disease because it occurs in a situation where control and organization, balance and orderly function are essentials. Actually, its biologic value is high in that it has the capacity for unlimited growth if sufficient food is provided. Biologically also it has a close relationship to a number of types of uncontrolled growth, such as wens, moles, warts and other so-called "benign" tumors. Many of the latter differ chiefly from cancer in that they do not invade surrounding tissues and so do not give off cells which spread through the body fluids to secondary sites where they become centers of independent growth.

The invasive and spreading quality commonly considered as characteristic of clinical cancer has so far been evaluated by the geneticist as being secondary in importance to its vigorous and uncontrollable growth either in vivo or in vitro. The change from normal cells to those showing this capacity for more rapid and continuous growth has been observed in tissue cultures by Gey (1941) and by Earle (1943). This shows that it is not necessary to have the internal environmental conditions of the body in order to obtain the carcinogenic change.

The mechanical factor in metastasis was demonstrated by Tyzzer some years ago (1913). In mice, tumors are often malignant in that
they show progressive nonencapsulated growth resulting in the death of the animals. It is relatively unfrequently that such tumors spread by metastasis. Massage, however, was followed by a significant increase in the incidence of metastatic nodules. It would seem that the relative looseness of structure of the tumor, its accessibility to blood and lymph vessels and the size of those vessels in relation to the surface of the tumor from which the cells were liberated are factors in making the metastatic phase of malignancy possible.

In most mammals cancer occurs at periods when central control of an organ or tissue has not yet been fully established or in which loss of control of the function of an organ or tissue has begun. It is very probable that the same factors are operative in the origin of benign tumors, some of which are normally checked and controlled in the organism assumes its full balance. The genetics of cancer is thus a study of the factors transmitted from one generation to another which at any stage in the development of the organism predispose any cell or any group of cells or tissue to a change or changes that lead to independence from organized control of the process of cell division.

This being the situation, it is clear that theoretically genes may be involved in producing some of the internal environmental situations which increase the probability of maintaining controlled growth or of allowing breaks from it. Similarly, one may logically expect that any other elements of the individual cell may be more or less regular or controllable either in quantity, quality or function, thus providing intracellular environmental factors.

Since cell division is a process which involves relationship between the components of the cell, it is evident that variation in one or more of them may create different types or degrees of intracellular unbalance. These in turn may favor, be ineffectual in or decrease the likelihood of the process of cell division. The degree or type of unbalance may also be correctable, permanent without notable consequences or cumulative as a chain reaction. We are thus dealing with a highly complicated field in which a process so fundamental as to be an essential to perpetuation of life is involved.

The striking nature of tumors and cancer as a departure from
the orderly laws of development has given it a power to invade man's calm and studied selection of problems for research and to demand his attention in spite of the difficulties of which his common sense and experience give him fair warning. Genetics, however, does not confine itself to studies of genes alone, and it is encouraging and stimulating to realize how much its methods and techniques have done to help us to begin to unravel the intricate combination of inherited environmental elements of which the constitution of the organism is composed.

Before considering in more detail the mammalian experimentation in which most of our genetic investigation on cancer has been carried out, it may be proper to mention very briefly certain other organisms touched by this field of research.

PLANTS

Although plants present a number of conditions in which tumors and disorganized growth can be studied, there has been surprisingly little experimental work with this material from a genetic point of view. Microorganisms have been identified as one of the most important groups of etiological agents. It has been observed that there are certain typical specific and genetic responses to such agents, but beyond the description of the characteristic reactions for the various types of plants no genetic data have been obtained. Fasciation also, which represents extremely interesting disorganization and disorientation of growth patterns as well as hyperplasia of certain parts of plant structures, has been little studied. There is evidence, however, that genetic influences are present in the production of some types of fasciation and that extrinsic or environmental agents may be the chief factor in other types.

Brieger and Forster (1942) record two interesting species crosses within the genus Nicotiana. The first, between N. glauca and N. langsdorfi, produces tumors of the stem, stem base or roots late in the development of the plant. Another cross, that of N. glauca x N. sanderas, produces early, large tumors of the stem base or root. The leaves on shoots arising from these tumors may be deformed.
The flowers often have abnormal color patches. All of these phenomena suggest unbalanced growth as a result of conflicting growth tendencies. Reference will later be made to hybridization in other forms.

INSECTS

The first genetic work on cancer of invertebrates utilized Drosophila. It was the natural by-product of the extensive and intensive use of that genus in developing and analyzing the chromosomal basis of Mendelian heredity.

Bridges (1916) and Stark (1918) described a melanotic tumor in the larvae which because it was uniformly lethal they termed "malignant." This tumor depended upon the action of a recessive gene at locus 0.1 on the X chromosome. Later E. S. Russell (1940) found that death of the tumor-bearing larvae was caused by a malformation of the gut occurring 65 ± 1 hours after hatching and growing until it completely obstructed the lumen. The tumor itself was found to be benign on transplantation.

Stark and Bridges (1919) described a second tumor which was benign and stopped its growth at pupation. The incidence of this tumor was affected by one or more genes on each of the four chromosomes, although chromosomes II and III combined appeared to be responsible for about 80 per cent of the tumor production and X and IV for 20 per cent. In 1924 Wilson recorded and studied two other benign tumors with multiple factor background. Russell in 1942 added five others. In one of these (36a) a homozygous condition of a gene on chromosome II was essential for production of any tumor while chromosome X had at least one gene increasing tumor production and chromosome III one or more genes decreasing it. In these cases, therefore, we find that many types of genetic action may influence the origin of tumors. A single gene may be involved. So may a combination of relatively indefinite and confused gene effects. Finally, a number of genes each with its characteristic effect may influence the final result.

It will be surprising if very much the same range of genetic relation to cancer is not found in mammals after sufficient studies. In fact
as we shall see there is considerable evidence already available that such is the case.

**TUMORS IN LOWER VERTEBRATES**

Research on both spontaneous and transplanted tumors reported in fishes, amphibia and birds has used the genetic approach, if at all, in broad and general applications.

Avian tumors, many of which are associated with filterable agents, have shown species or at times “breed” specificity. The reaction of the host has not necessarily been confined to an “all or none” level but may involve modifications in the type or degree of growth following the introduction of an agent. There is little doubt that genetic similarities and differences are factors in the origin and growth of avian tumors, but the absence of inbred lines of domestic birds has prevented the identification and analysis of genetic influences with any high degree of accuracy and experimental control.

In fishes the series of observations by Gordon (1932) and others has established the importance of unbalanced growth tendencies in a number of hybrid forms. The malignant neoplasms which may result represent a failure to adjust, in balance, the conflicting capacities for growth introduced by the two different parent species. Histological phenomena related to the neoplastic process in this material are open to investigation under unusually favorable conditions. The numbers and distribution of the pigmented micro- or macromelano-phores can be directly and easily observed and recorded. Since these cells are themselves the elements of which the tumors are composed the opportunity for investigating the process of carcinogenesis is excellent.

In amphibia, where virus tumors in frogs have been the most common and accessible types of neoplasm, the situation is still a very general one. Here, as in birds, the absence of controlled genetic strains provides a limiting factor in the detailed use of analytic genetic methods.

We may next consider the development of research in genetics related to cancer among the mammals.
GENETIC METHODS INVOLVED IN MAMMALS

Studies of heredity in relation to cancer were of interest even before the rediscovery of Mendelism. They were confined to the recording of familial incidence of the disease in humans. It was evident that in certain families the appearance of cancer was so frequent as to suggest a common factor either of relationship or environment in its etiology. Since, however, records of several successive generations of humans lacked either completeness or accuracy or both, such studies could not be considered as being more than stimulative to further investigation under more controlled and uniform conditions. The family or group presentation of data is thus the most primitive level of genetic investigation.

As experimental genetics developed, the idea of the "pure line" or inbred strain as a means of reducing variables and of separating inherited and environmental influences was introduced. Obviously it had and still has little application to human beings, but it was not only a possible but a very promising tool in studies of laboratory plants and animals. By its use has come the definite knowledge, still all too imperfect and inadequate, which we have of those elements of the constitution which are of parental origin as contrasted with those which result from as yet unexplained developmental factors or from both identifiable and unidentified extrinsic environmental causes.

LEVELS OF RESEARCH ON GENETICS OF CANCER

These, in order of their development and degree of analysis, may be described as: (1) transplanted tumors, (2) spontaneous tumors and (3) induced tumors.

Transplanted tumors

The beginning of investigation of transplanted tumors in rodents dates back to the work of Jensen (1903) and Ehrlich (1906), who found that the process of transplanting a bit of tumor which originated in the animal into another might in some cases be followed
by progressive growth of the implant. This obviously was a measure of the tolerance which the host animals possessed for tissues of the donor. It has little to do with the origin of cancer or with the factors which might operate in its production. On the other hand, it was a measure of the biological similarity or difference between donor and host.

Two Americans were quick to recognize the possibilities which the European discoveries contained. Loeb (1908) began a series of experiments which led to the development of his theory of “individuality differentials” to which reference will be made later. The other, Tyzzer (1907a) (1915), was fortunate in having at his disposal a strain of Japanese waltzing mice which at that time was in all probability the nearest approach to a genetically homogeneous strain of laboratory mammals. As a result, Tyzzer found that tumors originating spontaneously in Japanese waltzing mice of this (Lambert) strain would grow on implantation in approximately 100 per cent of the hosts of that strain which received them. When, however, bits of the same tumor were implanted in various strains of non-waltzing domesticated mice they failed to grow. This gave Tyzzer an absolute or almost absolute difference between two unrelated strains of mice in respect to their tolerance of tissue implants derived from a mass of tissue that originated in one of the strains. Tyzzer then crossed the two strains and found that the first generation hybrids between them grew the tumor just as well or better than did the animals of the parent strain in which it originally arose. He then produced, by in-breeding the first generation hybrids, a second generation into which he implanted bits of tumor from the grandparental Japanese waltzing ancestor. The behavior of the first generation hybrids suggested that susceptibility to implants of the tumor was dominant in the newly rediscovered Mendelian sense. It was, therefore, surprising and disturbing that of the second generation hybrids inoculated not a single animal grew the tumor.

Meanwhile, Loeb (1945) and his associates, using both mice and rats, had developed a theory, based on carefully recorded pedigrees, that closeness of relationship was the main factor in determining whether the tissues of one animal would grow in another.
From this work he came to accept the terms of *heterotransplant* (interspecific), *homoiotransplant* within a species but distantly or remotely related, *syngenesiotransplant*, an exchange of tissue between closely related animals of the same species, and *autotransplant*, a transference of tissue from one part of an animal to some other location in the same individual. Loeb's general conclusions were that autotransplants were always or almost always successful; syngenesiotransplants usually so, as between sibs or parent and offspring; homoiotransplants rarely so; and heterotransplants were not successful.

In England, Bashford (1911), Haaland (1911) and others focussed their attention on the tumor used for implantation. They found, in successive groups of genetically unknown mice used as hosts, fluctuations in the percentage of animals that grew the implanted tumor. They carefully charted successive groups and very naturally, due to the mixed ancestry of their mice and the size of their samples, obtained a curve resembling a mountain ridge with peaks (the groups with a preponderance of susceptible animals) and valleys (the groups with a preponderance of non-susceptible animals). On these observations they erected the theory of fluctuating virulence of the tumor. This was based on the mistaken belief that all hosts were alike and that the tumor was the only variable with which they were dealing. This was the situation in 1912. In the meantime, however, genetics had made two great advances. Mendel's law had shown that a given character (reaction) might depend on the simultaneous presence of two or more genes and that when any one of the required combinations was absent, the character was not formed or, in other words, the reaction did not take place. The cross of two white sweet peas (Bateson and Punnett 1908), producing purple in the first hybrid generation and a 9:7 ratio of purple to white in the second hybrid generation, was one case in point.

If one supposed that a given character, "X," depended upon the simultaneous presence of *three* genes, the ratio would be 27:37 actually producing a minority of individuals with the character that all of the first generation hybrids possessed.

As one increased the number of genes, the simultaneous presence of which is necessary, the relative number of second generation indi-
viduals possessing all of them decreased rapidly until with ten genes involved only approximately 1.3 per cent of the second generation will have the combination necessary to produce the character in question.

Keeping this principle in mind for future reference, we may turn to a second great advance of genetics. This was the development of the idea of "pure lines." As far as any one pair of hereditary units (genes) was concerned, Mendel himself showed how to obtain and maintain uniformity. It required, however, the work of Johannsen (1913) to show that a self-fertilized type of plant like the common bean had, by the process of inbreeding, produced genetic uniformity.

This was rapidly taken up by various Americans, East and Jones (1919), Pearl (1914), Jennings (1916), and Wright (1921), who showed that even brother-to-sister matings or any other form of close inbreeding, if persisted in, would eventually reduce the inherited variables to a negligible minimum. This principle, combined with the behavior of characters which are dependent upon the simultaneous presence of many genes, resulted in the transition of investigations on the genetics of transplanted tissue from the level of group or family analysis to that of strains (Little, 1914). The first step in this transition was a careful repetition of Tyzzer's work on a tumor of the Japanese wasting mouse. This confirmed his findings in both parent strains and in the first generation hybrids, but by the production of a much larger second hybrid generation showed that a small percentage of this generation (3 out of 183 inoculated) grew the tumor (Little and Tyzzer, 1916). The reappearance of susceptibility in this generation made the observed data explicable on the simultaneous multiple gene hypothesis. It also suggested that with less specific tumors or more closely related strains, the number of genes involved should be less and the proportion of susceptible F₂ animals should be higher.

Both of these results were realized—the first with a sarcoma of the Japanese wasting mouse (Little, 1920) and the second in crosses of two strains of the domestic house mouse (Strong and Little, 1920; Strong, 1922; Little and Strong, 1924). The number of genes needed to produce susceptibility to tumor transplants was gradually reduced
from seven to three and two. Finally Strong in 1926 obtained conclusive evidence of a “one-factor” or simple Mendelian inheritance in the case of a carcinoma.

Meanwhile, the behavior of tumors originating in F1 hybrids suggested that Loeb’s theory of closeness of relationship required fundamental revision. It was found that while tumors originating in either of the two parent strains would grow in first generation hybrids, the reverse was not true. Since the progeny have the same degree of pedigree relationship to the parents as the parents have to the progeny, the interchangeability of tissue between them should, by Loeb’s theory, be similar. Using normal spleen implants as a test, Little and Johnson (1921) showed that the tissue of first generation hybrids was actually a combination of the two parents. Each parent recognized as foreign to it the elements of the hybrid tissue derived from the other parental strain and, therefore, eliminated the transplant. On the other hand, the first generation hybrid could utilize the elements in its tissue derived from parent strain A to recognize and grow tissue from that strain and simultaneously, if required to do so, could utilize elements in its tissue derived from parent strain B to grow implants from that parental strain. Once these principles were established, other interesting results followed rapidly.

Linkage of some of the genes that determine susceptibility with certain of those that determine coat color was demonstrated by Little and Strong (1924) and by Bittner (1933a, b, c, 1934a). Simple sex linkage of a susceptibility gene was shown by Strong (1929) and a more complicated case involving possibly not only the X but the Y chromosome was reported by Bittner (1932b).

The fact that multiple tumors arising spontaneously in the mammary glands of a single animal differed from one another in the genes which determined their identity was demonstrated by Cloudman (1932).

Reference has earlier been made to the theory of “virulence” of tumors advanced by British investigators on the basis of fluctuation in percentage of susceptible animals in succeeding groups of inoculated mice. A lively discussion of virulence and adaptation was carried
on between 1908-1919 (Calkins, 1908; Bashford et al, 1908; Bashford, 1911; Haaland, 1911; Woglom, 1913, 1919).

The inadequacy of the control of material on which the conclusions leading to this discussion were based was very strikingly shown by Bittner (1932a). Choosing in advance, by knowledge of their genetic constitution, groups of mice which he knew would be susceptible or non-susceptible in proper ratios he repeated almost exactly the irregular curve obtained by the British investigators by chance many years before. His stocks of mice were different and the tumor was different, yet by use of the genetic theory of transplantation, he controlled completely the proportion of susceptible mice obtained. With this experiment the old theory of fluctuating "virulence" and "adaptation" of the tumor met its end.

Evidence that tumors could change in the course of transplantation over a period of time was, however, recorded by Strong (1926b). He used an adenocarcinoma of the mammary gland (dBrD) originating in the dilute brown strain of mice. Three sublines of this tumor were isolated. Two of them arose as a sudden, extensive, and self-perpetuating change fulfilling the accepted broad definition of the process of mutation. Strong (1926a) reported another case of a different tumor (dBrC) in which the original tumor gave a six-factor ratio and "mutated" into three sublines, one of which gave a two-factor ratio, one a one-factor ratio, and one which apparently had lost its strain specificity and grew in all animals of a foreign strain as well as in those of the strain of its origin. This was, of course, clear evidence that not all the factors that determine susceptibility or non-susceptibility were genes. It indicated that the role of the genetic theory of transplantation was like all other Mendelian phenomena. It provided the best and most accurate analysis of the predictable and repeatable type of parental influence but did not provide all the answers to all of the problems in studies in that field.

OTHER PARENTAL INFLUENCES IN TRANSPLANTATION

In all of the genetic experiments with transplantation of either carcinomas or sarcomas, all the changes in the neoplasms had been in
the direction of less specificity or fewer genes involved. In transplantable leukemia in mice, however, MacDowell and Richter (1930, 1931) showed that changes in the leukemia could occur in the direction of greater specificity for the strain of origin. This in turn suggests the interaction of some sort of agent with the implant and the host so that the genetic response to the combination of the two variables may be influenced by changes in either the tissue itself or in the agent accompanying it.

Reference will be made later, in some detail, to an agent known as the mammary tumor inciter (MTI) transmitted in the milk of nursing mothers and affecting profoundly the incidence of mammary tumors in mice. It is paralleled to some extent by milk-borne influences described by Cloudman (1941) which increased the likelihood of successful growth of two mouse tumors, a fibrosarcoma originating in strain C57 black and a malignant melanoma S91 originating in dilute browns. The data are as follows:

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Growth in Parent Strain</th>
<th>Growth in Foreign Strain</th>
<th>Growth in Foreign Strain Nursed by Parent Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrosarcoma</td>
<td>100%</td>
<td>25%</td>
<td>100%</td>
</tr>
<tr>
<td>S91</td>
<td>100%</td>
<td>11.5%</td>
<td>36.6%</td>
</tr>
</tbody>
</table>

Law (1942b) found similar effects of foster nursing in two transplantable lymphoid leukemias of the mouse but failed to find any such influence in one myeloid leukemia tested.

Certain experiments reported by Cloudman (1943) and by Harris (1943) indicate that some influence towards susceptibility can pass from one mouse of a parabiotic pair to the other. The tumor used by Cloudman (1939) was a reticulo-endothelioa of the liver originating in the leaden strain (Ld). On implantation for over 65 transplant generations this tumor grew in all of 1179 leaden stock mice. One hundred and eighteen mice of the C57 black stock all failed to grow it. In 30 of 34 pairs of parabionts a C57 black mouse united with a leaden stock animal grew the tumor. This principle seems to apply to other tumors as well. We must therefore conclude that other influences besides genes may play a part in determining the nature of mammalian tissues and that such agents may also modify or affect
the ability of a host organism to recognize, tolerate or eliminate an implant of foreign tissue. In the present state of our knowledge we must admit that this effect may be on the host, on the implant or on both.

**NATURE OF THE GENETIC FACTOR IN TRANSPLANTATION**

Gorer (1937) (1938) provided the first clear evidence on this point. In a tumor giving a two or three factor ratio in crosses between two strains, he described an antigen which is apparently the result of a gene and called it Antigen II. This gene apparently was identified in determining the fate of the transplanted tumor. His own description follows:

Normal and neoplastic tissues contain iso-antigenic factors which are genetically determined. Iso-antigenic factors present in the grafted tissue and absent in the host are capable of eliciting a response which results in the destruction of the graft. Under special circumstances the response may not be elicited or the grafted tissue may not be destroyed thereby. Antigenic differences between normal and neoplastic tissues are not normally capable of stimulating a defensive reaction.

Recently, Snell (unpublished) has discovered four different alleles of the Antigen II gene with some evidence of a possible fifth allele. These studies of histocompatibility are of great value in providing evidence bearing on the chemical similarities and differences which identify individuals and strains. They also have an important bearing on the cancer problem and represent the first major effort to unravel the complexity of invisible physiological characteristics which are the genetic contributions to the constitution of the individual mammal.

Certain experiments have contributed to our understanding of some of the special circumstances which may upset the orderly behavior of the genetic factor in transplantation. Lewis (1937) has found that repeated inoculation of tumors (induced by dibenzanthracene) into alien strains might lead to changes in both the host and the tumor by which successful transplantation into the alien strain was more frequent than in unmodified tumors. Warner and Reinhard (1939) and Reinhard, Warner and Goltz (1941) used exposure to x-rays
to increase the percentage of successful transplants of an adenocarcinoma (dBtB) of the mouse in the alien strain C57 black. Similar results were obtained with the tumor “New Buffalo” and the Simpson mouse tumor. Higher doses of x-rays decreased the number of takes as compared with lower doses. E. E. Jones (1926) showed that a mechanical factor introduced by a bit of the sterile flannel as an absorbent of the emulsion of transplanted tumor increased the probability of growth of the implant.

Leukemias on transplantation show clear evidence that a foundation of Mendelian genes operate in determining susceptibility. The results, however, indicate that the rule of decreasing specificity which characterizes the behavior of other types of neoplasm, after repeated transplantation, does not necessarily hold for leukemias. It is an almost invariable principle that tumors arising in F1 hybrids as well as normal tissue of animals fail to grow in either parent strain. Furth, Bock and Kalis (1944) showed, however, that successful growth of leukemias of F1 hybrids may be obtained in their parent strains. It would seem, therefore, that leukemia has certain characteristics which differ from those of other neoplasms. This indicates the need of further study.

To sum up, we may say that Mendelizing genes are the chief factor which is predictable in estimating the results of transplanting tissue. These are, however, other influences which may modify the expected results.

Spontaneous Tumors

The term “spontaneous tumor” is used to describe those neoplasms which originate under circumstances of which we are still ignorant. It may safely be assumed that as our knowledge increases, we may expect to find that more and more tumors now classified as “spontaneous” will be attributed to causes which are identifiable and reproducible experimentally. It may, however, be considered proper to include for the present in this group all those tumors which occur naturally without the conscious interference of the experimenter. In this category there will fall all human tumors, although the so-called “industrial” or “occupational” tumors are close to the borderline of
the induced tumor group to which we shall later refer. The influence
of heredity in the incidence of tumors in man is difficult to prove
unless it is very obvious and unavoidable. Although secondary meth-
ods such as statistical analysis of groups of individuals and comparat-
ive studies of twins may provide suggestive evidence of genetic influ-
ence, the order of accuracy in studies of human material does not
equal that of experimental investigation in the laboratory.

STUDIES OF IDENTICAL TWINs

These are based upon observation of parallel series of develop-
mental events extending throughout the life of the two individuals
and culminating in the origin in each of the same type of tumor
at approximately the same chronological age. There has been a suffi-
cient number of such cases reported by McFarland and Meade
(1932) and Macklin (1940) to indicate that the incidence of similar
tumors in both members of a pair of identical twins is greater by far
than would be expected by chance distribution alone.

It must, however, be pointed out that such data really prove little
if anything concerning the genetics of such tumors. There are few
if any facts showing that the twins developed a type of tumor which
had appeared in one or both parents or grandparents. Without such
comparative data on succeeding generations, the evidence provided
by similar histories of twins is limited in application. All that can be
said is that origin of monovular twins from the cells descended from
a single fertilized ovum which develop with a single placental contact
with the maternal parent may produce similar ontogenic histories.
Theoretically, if cancer was due to an agent or agents present in a
fertilized ovum or in maternal blood in a form transferable through
the placenta, the same result would be expected. Such an agent
might be a gene, a part of or a whole chromosome, or any component
of a reproducible type in the egg cytoplasm or a viroid or serological
substance of maternal origin. Similarly, if cancer required as one of
its etiological factors any agent or substance transmitted through the
milk or by direct contact with the mother, twins of any sort would be
exposed under essentially the same physiological and mechanical con-
dations in the mother. The common origin of the monovular twins might then be a discernible factor in producing a similar response to such stimuli.

PEDIGREE STUDIES IN HUMANS

Certain types of tumors show a high incidence in even incomplete or imperfect pedigree data derived from records of several generations of humans. It is clear that some sort of parental influence is operative in these cases. Until, however, linkage between the genetic basis of such neoplasms and some other known gene in man is established, our explanation of their method of transmission must be tentative. In this group one may list: (1) the benign but often precancerous lesions of the rectal polyps (polyposis intestini) which behave much like a simple Mendelian dominant; (2) retino blastoma or glioma retinae, usually found in young children or congenitally (its behavior is that of a dominant frequently with low penetrance); (3) neurofibromatosis, Von Recklinghausen's disease, generally admitted as a relatively clear case of a Mendelian dominant.

As possibilities for further observation and analysis Gates' lists (1946) of a number of other types of neoplasms may be mentioned. Fibroma molluscum, bilateral acoustic neurofibromata, hemangioma, lipomata, cylindromata are among the neoplasms the incidence of which occurs in such distribution as to suggest strongly a basis of parental influence.

None of these tumors is, however, among the commoner types in man. It will, therefore, be of interest to mention briefly certain studies of relatively recent origin on some of the more widespread varieties of cancer. One of the most stimulating of these investigations was that of Wassink (1935) who studied the incidence of cancer among the relatives of cancer patients. Tabulating his data according to the site of the neoplasm, Wassink found considerable variation in the frequency of incidence among the recorded relatives. In his material, cancer of the breast, the stomach or the rectum showed the highest frequency among relatives. Among the lowest incidence groups were the familial populations related to men with cancer of the liver and
women with cancer of the uterus. He interpreted his results as indicating that among the different members of certain families there is a predisposition for cancer to form predominantly in certain organs.

Two excellent studies have been made in Denmark by Videbeck (1947) and by Jacobsen (1947). The former was particularly concerned with leukemia. His experimental group of 4041 relatives of 209 leukemic probands and his control group of 3641 relatives of sound probands were closely parallel as to age distribution and were entirely adequate. From his data he concludes that "leukemia is a matter of chromosomal inheritance," a statement which he amplifies by stating that he believes that a predisposition to the disease is inherited and that one may exclude the hypothesis of either a simple dominant or recessive.

Jacobsen, studying the relatives of 200 breast cancer probands, believes that the tendency to develop that condition is primarily inherited. Both he and Videbeck feel that a general tendency to cancer formation, independent of particular sites, is also inherited. The latter estimates that approximately 20 per cent of the population may have this general tendency.

Kemp (1948) has reviewed the situation in genetic studies on human cancer. He reaches essentially the same conclusions, namely, that breast cancer depends on hereditary factors and that the predisposition is linked with that for endogenous cancer in general. The general predisposition to endogenous cancer seems to be frequent in relatives of probands with cancer of the corpus uteri but that in cases of cancer of the cervix, the only striking incidence of cancer among probands is cancer of the esophagus.

Summarizing the data from humans, one may conclude that predisposition to cancer both as a general and as a localized risk may be due to parental influence as one of the main etiological factors of the disease. In certain types (Von Recklinghausen's disease, polyposis intestini) the genetic factor is relatively direct and clear. In others such as cancer of the breast and leukemia, the complete etiology is a complex of genetic (specific and general) predisposition and other factors of the internal environment either originating there or being stimulated by external environmental causes.
Very little work has been done with controlled genetic studies of spontaneous cancer in guinea pigs, hamsters or rabbits. In rats our knowledge is chiefly limited to the fact that certain types of tumors are more frequently found in some strains than in others. Absence of more detailed information is due either to the fact that too few inbred, genetically homogeneous strains exist or to the absence of data on morbidity and mortality in those that are available.

In mice, however, quite the opposite is true. There has long been active and widespread interest in genetics in relation to spontaneous cancer incidence. As long ago as 1907-1915 several investigators in England and in the United States (Tyzzer, 1907, 1909; J. A. Murray, 1911; Bashford and Murray 1909; Haaland, 1911; Slye, 1913; Lathrop and Loeb, 1915) were publishing results which showed that genetics influenced the incidence of cancer in different strains. This work was carried out with populations far from homogeneous from a genetic point of view. Because the genetic nature of the material varied within itself and between investigators as well, it followed that interpretations were tinged by these facts and that little similarity of points of view obtained. Moreover the fact that mice form a great variety of tumors both as to histological characteristics and as to site was a factor in confusing matters still further. There were different sorts of epithelial and connective tissue neoplasms of the skin, kidney, pancreas, liver, lung, ovary, uterus, mammary gland, testis, thyroid, spleen, thymus and blood forming organs (Slye, Holmes and Wells, 1914, 1915, 1917a, b, 1919, 1920, 1921a, b, 1924, 1926, 1931, 1935; Simonds, 1925). Attempts to treat all of these in various combinations as though the term "cancer" described a single genetic category obscured the more complete analysis by introducing biological and pathological variables.

With the development of the first genetically homogeneous inbred strains, certain facts became evident. First, one could separate different types of cancer and isolate them in certain strains. This showed that various constitutional types had different potentialities for the
production of neoplastic growth. The localization of the center or centers of this growth could also be fixed with a high degree of certainty. Thus, although later investigation may prove that general genetic influences may affect the development of all cancer, the availability of strains which form specific types is proving to be a most useful adjunct to analysis of the internal environment of the animals in which cancer is or is not formed as the case may be.

Proceeding from the more definite genetic types to the less understood we may first consider lung tumors.

LUNG TUMORS

Lynch (1924) was the first to employ for genetic crosses two strains of mice which differed markedly in their natural incidence of epithelial lung tumors. Her work showed that the incidence which characterized the “high” tumor strain tended to persist in the F₁ generation. She recognized, however, that in all probability more than one genetic influence was involved. In a later series of papers (1925, 1926, 1927, 1931, 1937) she supported this general conclusion and showed that the sexes did not differ significantly in the incidence of these tumors or in their power to affect that incidence. Using entirely different stocks, Bittner (1940a) obtained in a cross with F₁ and F₂ generations results which seemed to indicate that in this case a single Mendelian dominant with incomplete penetrance might be involved. The most complete study of these tumors has, however, been made by Heston (1940, 1941, 1942a, b, c). In one of his crosses four or more pairs of genes seemed to be active. There was also evidence of genetic linkage between certain of the “lung tumor” genes and those already identified for “waved 2” and “shaker 2.” Still later (1945) he demonstrated that the gene A² (yellow) had at least a physiological effect in increasing the incidence of lung tumors. Thus the existence of Mendelian genes which affect incidence in this site seems certain.
MAMMARY TUMORS

In the incidence of this type of neoplasm which is the commonest among experimental mammals, four types of influence seem to be involved. Indications that a strong maternal influence exists were obtained by the staff of the Jackson Laboratory (1933). This was later broken down into an important agent, the mammary tumor inciter, transmitted through the mother's milk (Bittner, 1936b, c) and a minor uterine influence exerted during development of the embryo (Fekete, 1947).

Chromosomal factors were also apparently involved (Murray and Little, 1935b, 1939) and were conclusively demonstated by Andervont (1940, 1941) when the maternal influence was controlled and equalized. It has not as yet been possible to identify any individual gene or group of genes involved. It has, however (Little, 1934), been noted that in an inbred stock of yellow (A<sup>y</sup>) mice, the incidence of mammary tumors is lower among the mice with yellow coat color than among their non-yellow sibs. This is probably a question of the physiological effect of the A<sup>y</sup> gene rather than a direct genetic linkage.

Finally there is clear evidence that hormonal factors are involved. These may influence the degree and type of development of the mammary gland itself. The operation of hormonal factors is also shown by the rate of incidence of mammary tumors in three different inbred strains when virgin and breeding females are compared as follows:

<table>
<thead>
<tr>
<th>Stock</th>
<th>Virgins</th>
<th>Breeders</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>dba</td>
<td>30%</td>
<td>60%</td>
</tr>
<tr>
<td>A (Bittner)</td>
<td>4%</td>
<td>78%</td>
</tr>
</tbody>
</table>

We thus can list the following four groups of factors involved in the etiology of mammary cancer in mice as: (1) milk, (2) intra-uterine, (3) genes, (4) hormonal.
ADRENAL TUMORS

These tumors have been very rarely recorded in the many strains of mice observed by many investigators over a long period of time. Slye (1921b) listed only four adrenal neoplasms in 33,000 autopsies and her later data did not appreciably increase that ratio. On the other hand, experimental upset of the hormonal balance between the adrenal, the pituitary and the gonads has proven to be an important etiological factor for adrenal tumors in certain strains of mice and their hybrids. Early gonadectomy of dba mice is followed by hypertrophy and hyperplasia of the adrenal cortex after some months. This phenomenon was the regular and definite result of the operative procedure. Benign tumors which formed as outgrowths of the adrenal cortex pushing down into the medulla gave evidence of hormonal (estrogenic) activity by the incidence of a considerable number of mammary tumors and by a cellular arrangement of areas of the adrenal cortex resembling the zona granulosa of the ovary (Woolley, Fekete and Little, 1939, 1940, 1945a, b, c, d, e).

The genetic difference between strains was shown by the behavior of C57 black mice and ce extreme dilution animals when early gonadectomy was carried out. The C57 black mice showed no profound or critical adrenal change. The ce mice in almost 100 per cent of the operated animals (males and females) showed marked adrenal cortical activity and involvement. An active period of hyperplasia culminated in the formation of carcinoma of the cortex in both sexes. These animals gave evidence of both male and female hormonal activity. The carcinomas themselves were strongly androgenic and bits of them when transplanted grew into masses which could apparently secrete sufficient androgenic material to "masculinize" castrated male mice. In the case of adrenal tumors, therefore, we have a situation where different genetic constitutional types, as exemplified by various inbred strains, react very differently to hormonal unbalance. Studies by Dickie and Woolley now well under way show that apparently these genetic differences are primarily chromosomal and probably multigenic in nature.
LEUKEMIA

By far the most careful and extensive work on the genetics of leukemia in mice has been that of MacDowell and his associates (1940, 1941, 1943). Using crosses between a high leukemic (90 per cent) strain (C58) and various low leukemic strains, several important facts were recorded. There was, for example, a marked difference in the incidence of leukemia in reciprocal F1 hybrid populations. This indicated a maternal influence which was extra-chromosomal. It was further evident that the influence of genes was not a simple Mendelian relationship. Further investigation and analysis of the various influences contributing to the origin of leukemia is needed and should prove most interesting and important.

NON-EPITHELIAL TUMORS

As yet few studies have been made on significant numbers of animals derived from strains in which one type of non-epithelial tumor was predominantly or exclusively present. In one cross between two inbred strains (dba and C57 black) (Little, Murray and Cloudman, 1939) lymphoblastomas (60.3 per cent) endotheliomas of the liver (21.5 per cent) and a small number of fibrosarcomas, lymphangiomas, osteogenic sarcomas, hemangiomas, and one each melanoma, reticulum cell sarcoma, and "undifferentiated" sarcoma were observed. Although small numbers of the F1 generations prevented a direct gauge of maternal influence, the two F2 generations derived from the reciprocal crosses provided evidence bearing on this point as follows:

<table>
<thead>
<tr>
<th>Generation</th>
<th>Total F2 Mice</th>
<th>Tumors</th>
<th>% Tumorous</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2 C57 Blk x dba</td>
<td>468</td>
<td>61</td>
<td>13.1</td>
</tr>
<tr>
<td>F2 dba x C57 Blk</td>
<td>649</td>
<td>90</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Clearly, there is no evidence for maternal influences. It is also clear from the available data that more than one gene must be involved.
HYBRIDS AND TUMOR FORMATION

Reference has been made to the production by Gordon of melanotic tumors in hybrids between two genera of fishes, Xipophorus and Platypoecilus. This process seems to be one of the common results of certain definite types of interspecific matings. It represents a metabolic upset resulting in failure to regulate the production and distribution of pigment forming cells. Reference has also been made to the work of Brieger and Forster (1942) in which tumors are observed in the F1 hybrids of two species of Nicotiana.

Evidence of an increased tendency to tumor formation in mice has also been reported (Little, 1939) in the F1 generation of a cross between *Mus musculus* and *Mus bactrianus*. The two species differ in size, rate of maturity, and in fecundity as measured by litter size. The incidence and distribution of neoplasms are also different in the two parent strains used.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>% with Tumors</th>
<th>No % Tumors</th>
<th>% with Epithelial Tumors</th>
<th>% with Epithelial Tumors</th>
<th>% with Multiple Tumors</th>
</tr>
</thead>
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<tr>
<td>Mice</td>
<td>139</td>
<td>3.8</td>
<td>6</td>
<td>3.8</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Bactrianus Mus</td>
<td>877</td>
<td>14.4</td>
<td>137</td>
<td>13.2</td>
<td>39.66</td>
<td>23.6</td>
</tr>
</tbody>
</table>

Not only did the hybrid mice show a marked increase in tumor incidence but the percentage of multiple tumors was approximately three times as high as that in the "higher" of the two parent strains. The occurrence of increased incidence of tumors following hybridization in plants, fishes and mammals indicates that the influence of unbalanced growth tendencies introduced from different parental types may be a potent and basic etiologic factor in neoplasia.

THE NATURE OF GENE d (BLUE DILUTION):

It is interesting to note the recurring evidence of a relationship between this long recognized and well known gene and certain phenomena of growth. Evidence of linkage between a gene for susceptibility to transplanted tumors and the gene d has already been
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mentioned (Little and Strong, 1924; Bitter, 1933a, b, c, 1934a). Its relationship to increased incidence of non-epithelial tumors has also been reported (Little, 1934; Little et al. 1937). MacDowell et al have (1945) also identified it as a plus modifier for incidence of leukemia. MacArthur (1949) has demonstrated that gene d also tends to increase body size in normal non-cancerous mice. All of this evidence may well combine to suggest the first case of an interesting and significant influence either through linkage or by direct physiological effect exerted by a color gene on fundamental growth processes and their control.

Thus the conclusion to be drawn from investigators of all these types of tumors (lung, mammary, adrenal, leukemia and various non-epithelial neoplasms) is that the genetic constitution of the individual is an important factor in determining the tendencies to uncontrolled growth of the different organs, tissues and cells of that individual. Furthermore, as might be expected, such tendency to uncontrolled growth is influenced by many genes very probably with different degrees of pleiotropism and with different degrees of independence and interaction.

It is fortunate that the number of identified genes in mice is large and is steadily increasing. Their analysis and location should result in more rapid identification of the invisible and subtle genetic complex which affects internal environmental balance and control or its disruption.

INDUCED TUMORS

Up to the present time, with few exceptions, the literature on the genetics of tumors of this type has been relatively confused. Factors which have contributed to this result have been natural and understandable. In the first place, the number, type, dosage and method of application of carcinogens have all introduced variables which have influenced the accuracy of experimental work. No sooner had 1,2,5,6-dibenzanthracene been discovered and utilized as an improvement over tar than 3:4 benzpyrene was found to be more useful in certain experiments. This in turn gave way to the still more active 20-methylcholanthrene. Meanwhile scores of other carcinogens of different
potency and different degrees of specificity were being described. So diverse and active were these substances that most investigators forgot genetics as a means of reducing or controlling the biological variables and enthusiastically painted or injected or implanted pellets in mice and rats in whatever quantity and from whatever strains available. It has also been true that the number of geneticists engaged in studying cancer is decidedly limited and that during the period since the availability of prepared carcinogens the great majority of these experimenters have been chiefly occupied in studies of either transplanted tumors or of spontaneous neoplasms.

At intervals, however, certain investigators in the field of induced tumors have used material in which the genetic background was a recognized factor.

**Chemicals**

Lynch (1931) was among the pioneers in this work. She had previously shown that the incidence of lung tumors could be similarly increased in a “high” and in a “low” tumor strain of albino mice by painting it with tar. She then demonstrated that each of four genetic strains had its own characteristic incidence of skin tumor formation following tar painting. The rate of incidence of skin tumors was independently determined from that of lung tumors. Kreyberg (1934) also established the fact that there was a difference in incidence of tar warts or papillomas between two strains of albino mice. The latent period between painting and tumor genesis was also definitely shorter in the high incidence strain. He later (1935) showed that within a single strain the males showed a distinctly delayed reaction to tar compared with the females. Incidence of tar tumors in these strains was independent from incidence of mammary tumors. Brues and Marble (1939) showed that C57 black was resistant to tar painting but that strain C (Bagg Albino) reacted by an increase in the incidence of characteristic lymphomatosis from a normal 2 per cent to 50 per cent of the experimental mice. The course of the disease was also greatly accelerated. Strain differences in response to dibenzanthracene have been demonstrated by Andervont (1934) in
the case of five inbred Jackson Laboratory strains. Experiments with 3,4-benzpyrene showed strain differences of an unanalyzed nature in the formation of both carcinomas and sarcomas. No definite or critical advance was, however, recorded in a manner that led to further important developments.

With 20-methylcholanthrene, however, much more extensive and important work has been reported. It is an active carcinogen which affects numerous organs and tissues sometimes with and sometimes without extensive local reaction. Strong (1941), using the inbred strain NH, showed that subcutaneous inoculation of a standard amount of methylcholanthrene in sesame oil developed by selection of parents of the proper type resulted in three substrains which were characterized respectively by either skin carcinoma, spindle cell sarcoma, or no tumor. A year later (1941) Strong concluded that genetic factors in each subline determined their response and that the origin of skin carcinoma and of sarcoma depended upon distinct and independent factors. This coincided with the independent behavior of these two types of tumor in work with transplanted tissue some fifteen or twenty years before. Extending this work, Strong (1943, 1944) and Bardette and Strong (1943) produced from advanced hybrid generations resulting from a cross between three inbred strains, many sublines with characteristic and different types of tumor. The dosage and method of applying the carcinogen was constant and similar to that used with the NH mice. The explanation of these results will be considered when the question of mutation and cancer formation is discussed.

Mider and Morton (1940) discovered an interesting difference in response to application of 0.5 per cent methylcholanthrene in benzine between the related strains C57 black and C57 brown. Both strains formed numerous papillomas, but while those on the C57 blacks always regressed, those on the C57 brown in some cases progressed and became malignant. Later work by Strong has indicated a greater sensitivity to carcinogenesis on the part of brown (b) mice compared with those having the (B) black gene.

Engelbreth-Holm (1941) has suggested the use of methylcholan-threne to bring into view the potentiality of the genetic and the
hormonal influences to the exclusion of the mammary tumor inciter (milk factor) in any given strain. The proportion of multiple mammary tumors and the speed of their appearance were significantly increased in female dba mice while males of the same strain lacking the hormonal stimulus remained negative. Experiments by Zimmerman and Arnold (1944) and by Burdette (1943) have failed to show any difference in reaciton of reciprocal crosses between strains. The response to methylcholanthrene like the spontaneous incidence of non-epithelial tumors thus appears to be free of maternal influences as far as present experimental data show.

Results obtained from other chemical carcinogens are too numerous and too widely scattered to allow complete discussion. Certain experiments are, however, of particular genetic interest. Gardner (1943a) tested the reaction of six inbred strains to subcutaneous injection of triphenylethylene. Bonser (1940, 1944) also working with the same carcinogen showed that strain differences existed and that

<table>
<thead>
<tr>
<th>Stock</th>
<th>Gardner Percentage</th>
<th>Bonser Percentage</th>
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<tbody>
<tr>
<td></td>
<td>Tumors of Testis</td>
<td>Tumors of Testis</td>
</tr>
<tr>
<td>JK</td>
<td>53.0</td>
<td>—</td>
</tr>
<tr>
<td>A</td>
<td>41.0</td>
<td>80.0</td>
</tr>
<tr>
<td>C3H</td>
<td>7.1</td>
<td>—</td>
</tr>
<tr>
<td>C121</td>
<td>0.0</td>
<td>—</td>
</tr>
<tr>
<td>N</td>
<td>0.0</td>
<td>—</td>
</tr>
<tr>
<td>CBA</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>RIII</td>
<td>—</td>
<td>39.0</td>
</tr>
<tr>
<td>RIII fostered on CBA</td>
<td>—</td>
<td>0.0 atrophy of testis</td>
</tr>
<tr>
<td>CBA fostered on RIII</td>
<td>—</td>
<td>0.0 hyperplasia of testis</td>
</tr>
<tr>
<td>RIII fostered on A</td>
<td>—</td>
<td>88.9</td>
</tr>
</tbody>
</table>

there was no necessary correlation between the presence of the mammary tumor inciter and the development of testicular tumors. There is, however, sufficient difference in some of the histological conditions in reciprocal hybrids to suggest the possible value of more careful and detailed efforts to detect structural differences possibly indicative of different hormonal or other levels under maternal influence.
In all of this type of work it is at present difficult to distinguish in the effect of the carcinogens what tumors represent new centers of uncontrolled growth created by the action of the carcinogen as such and what represents an increase in incidence of tumors at an already existing site of neoplastic potentialities. As the volume of data contributing to our knowledge of strain incidence of spontaneous tumors increases we should, however, be able to evaluate more clearly the nature of the role which the carcinogen is playing.

**Hormones**

The exact part played by hormones in the origin of certain types of tumor is a matter of opinion and interpretation of some very obvious and widely distributed facts. The almost complete sex linkage of mammary tumors in both men and mice is one such fact. The beneficial results of female sex hormone therapy in cancer of the prostate and of male sex hormone treatment in some cases of mammary cancer are likewise suggestive and significant. The adrenal tumors of mice appearing in the absence of gonadal hormones and preventable when these are restored is another example. Whole ovaries implanted in ordinarily non-tumorous castrated males of a high mammary tumor strain caused mammary tumors to develop (Murray, 1927). Lacassagne (1932, 1934) obtained the same result by injections of diethylstilbestrol. Shimkin and Grady (1940) confirmed this work in another strain of mice. Later Lacassagne and Nyka (1937) demonstrated a selective strain difference in the hypophysis following the injection of estrogens. Miller and Pybus (1942) obtained a large number of uterine tumors in strain CBA following injections of ketoxyestrin. An Edinburgh strain was almost completely resistant. Males of the A strain developed testicular tumors when estrogen was injected (Gardner, 1943b; Bonser and Robson, 1940) and later produced malignant lymphomas in another strain (Gardner, Dougherty and Williams, 1944). How much of this effect is a direct carcinogenic response and how much represents the preparation of a suitable chemical and histological substrate for the
action of carcinogens already present in the body is not known. It is, however, evident that the latter explanation more accurately describes the situation in the case of mammary tumors in mice.

**Physical Agents**

The application of solid carbon dioxide and carbon dioxide snow has produced papillomas and some cancer in some strains of mice (Berenblum, 1929; Mansens, 1931). Heavy exposure to ultraviolet radiation may also result in tumor formation in some strains chiefly albinos (Rusch and Baumann, 1939). X-rays also have an interesting influence on the formation of tumors. They may act as a supplementary and accelerating agent (Mottram, 1938) in combination with benzpyrene when neither agent alone will produce even skin warts. They may also apparently divert or modify the action of the mammary tumor inciter in certain cases. In one strain of mice (Marsh albino) where the mammary tumor incidence is normally 78 per cent (Renhard and Thibadeau, 1934) and the presence of the mammary tumor inciter has been later demonstrated, 970 r of x-rays was applied dorsally. After 12 months the animals had multiple elevated ventral growths some of which developed into epitheliomas. There was only one possible case of the usual mammary carcinoma. An experiment of this type unrepeated to the present day is extremely stimulating and suggestive of the use of irradiation to change the susceptibility pattern of internal structures to the specificity of carcinogenic agents under controllable and analyzable conditions.

The future should see a great expansion of experiments in the induction of tumors in animals whose age, sex and genetic constitution are held constant. Such work would materially advance our knowledge of physiological genetics as its various processes develop the balanced and unbalanced phases of the internal environment.

**RELATION OF MUTATION TO CARCINOGENESIS**

The possible identity of the process of mutation with that of carcinogenesis has long been a most intriguing idea. Actually a description of the two processes coincides. Both are suddenly appear-
ing intracellular changes which are self perpetuating. Tyzzer (1916) was one of the first to recognize this fact.

A broad definition of mutation has included the possibility of such a change being cytoplasmic (including extranuclear bodies and/or structures) or nuclear in origin. Within the nucleus there are a number of possibilities. The change may be in number of chromosomes, in exchange, redistribution, loss or inactivation of regions of one or more chromosomes, or in rearrangement or alteration of a gene or genes. Any and all of these processes might well result in metabolic or physical alteration in intracellular function. Any of them might be the cause or the result of any other alone or in combination.

Boveri (1914) who recognized the increase in neoplastic cells with supernumerary chromosomes as compared with normal tissue cells of that type evolved the theory that the pressure of supernumerary chromosomes was the cause of cancer. Later investigations, however, have led to the feeling that abnormal mitosis including increase in chromosome number may be a result or manifestation of a phase of uncontrolled growth rather than its cause.

Cells with extra chromosomes other than those with orderly polyploidy usually leave fewer descendants than those with the normal number when both types are in competition either in vivo or in vitro. The numerous publications of Blakeslee and his associates (1928) have also shown conclusively that irregular or asymmetrical distribution of extra chromosomes was not followed by tumor formation. Loss of whole chromosomes either individually or to the balanced haploid condition has not been followed by tumor production.

It seems reasonably certain, therefore, that if cancer is the result of mutation it must be a mutational change of some part of the cell other than a whole chromosome or a number of chromosomes.

Jones (1935, 1936, 1937) in a most interesting series of papers has shown the relationship in maize endosperm of intrachromosomal changes to abnormal growth of certain regions. A small, cytologically invisible deletion in chromosome 4 recorded by Jones also showed that areas of abnormal growth involved changes in cell size, depressions, outgrowths, and depressions paired with outgrowths. By using genetic markers, he was able to demonstrate that these conditions
were the result of shifting of genes from one cell to another due to various kinds of intrachromosomal aberrations in the developing somatic tissue. He points out that similar changes occur in Drosophila as the result of somatic segregation.

This type of evidence is supplemented by proof that the rate of cell division resulting in organisms of different size depends upon Mendelizing genes in part at least in both plants and animals. Normal growth may thus be affected by mutational changes in such genes occurring in either germ or somatic cells.

Obviously if there was an inherited predisposition to somatic mutation in a particular type of cell or tissue, the degree to which internal environmental factors could influence such a mutational change would vary with the nature of the cell or tissue and with their degree of independence from or dependence on growth controlling and balancing influences operating in the body. It is not surprising, therefore, that no simple and obvious answer to the relationship between mutation and carcinogenesis has been found.

One of the strongest links in the chain of experimental evidence for the similarity of the two processes is the steadily growing list of agents which are both mutagenic and carcinogenic. In mammals, Little and Bagg (1923) showed that x-ray exposure of adult mice was followed by a genetic change either genic in scope or else confined to a cytologically invisible area of the chromosome (Painter, 1928). Snell and Picken (1935) showed that both x-rays and neutrons could cause visible growth-disturbing translocations. Radiation of this type has long been known to be carcinogenic under certain conditions. Strong (1945) records a number of genetic changes affecting not only color but tumor incidence as well which he obtained in mice treated with methylcholanthrene. Later Demerec (1948) showed that methylcholanthrene was an effective mutagenic agent in Drosophila. Another example of an agent proven to be both mutagenic and carcinogenic is nitrogen mustard (Demerec, 1947; Haddow, 1949).

As the cytological action of agents of this type is more completely analyzed, we may well make real progress towards an understanding
of the common elements present in the mutagenic and carcinogenic processes. It should, however, be remembered that the cancer cell which most successfully leaves its descendants in the resulting tissue mass is not a diseased cell but rather one that performs its life cycle more rapidly and with an effectiveness at least equal to that of the "normal" cell. If then it is produced by the process of mutation, that process has not basically rearranged the elements of cell metabolism or of mitosis in the site in which the neoplasm occurs or to which it can metastasize. If the descendant cells originated from a malignant cell are themselves somewhat variable and in some cases involve departures from orderly mitosis that produce chromosomal aberrations other than orderly polyploidy these variable and atypical cell descendants will eventually lose out in competition with those in which the chromosomal behavior is more normal.

CONCLUSION

The impact of genetics upon the problem of origin and growth of tumors is thus a varied one. It includes genetic control of the constitution of animals by inbreeding and by planned crosses between inbred strains. It reduces the biological variables as far as possible so that chemical and physical modifications can be more exactly analyzed and evaluated. It provides by the use of transplants of normal and tumor tissues a means of comparing the two and of measuring differences in the composition and reactivity of the individual and of its component parts. It isolates strains with localized or generalized tendencies to form tumors of one or more particular types either as the result of intrinsic factors or of application of extrinsic agents. It provides biological hosts of predictable types by which exchange of ova and transplantation of ovaries may become useful aids in determining extrachromosomal influences. It provides a steadily growing body of concrete knowledge on the existence, location and foundation of "marker" genes which may by direct physiological effects, by modifying effects or by linkage help to increase our information concerning parental influence in the etiology of cancer. It uses the rapidly developing field of cancer research to establish principles of
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genetic control of experimental material in a manner directly applicable to the whole field of medical research.

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AS is well known the relations between genetics and plant breeding are so intimate and comprehensive that it is impossible to cover this field in a single lecture. I shall try, however, to summarize some of the essential points, and I shall also mention some examples of present-day attempts to utilize advances in theoretical cytogenetics for plant breeding purposes. Several of these examples represent data acquired by Swedish workers. I must apologize for this one-sidedness, but naturally it is easiest for me to speak of material with which I am more or less familiar.

Very soon after the rediscovery of Mendel’s laws it was realized that the new principles of heredity and variation had far-reaching consequences for practical plant breeding work. Already in 1901 and 1902 de Vries and Tschermak visited the Svalöf plant breeding station in Sweden and told the staff members about the new discoveries. In the first place the principle of genetic recombination must have appealed to the breeders, as it clearly demonstrated the possibility of combining various valuable characters from different parent strains. Genetic recombination is still one of the two cornerstones of modern plant breeding. The other one, which is even more fundamental, is the demonstration by Mendel of stable hereditary units, later on called genes.

On this basis of stable genes which may be recombined, some other conceptions of primary importance were soon established.
Thus, already in 1902 Bateson coined the terms homo- and heterozygote, and soon afterward Johannsen made the distinction between genotype and phenotype. He also clarified the difference between genetically heterogeneous populations and homozygous pure lines and demonstrated that selection was only effective as long as the material selected comprised different biotypes. Selection within a really pure line gave only negative results, even if it was carried on for many generations, and this result was later on verified by other workers using various kinds of material.

The constancy of the genes also explained the constancy of clones, in which the same individual and the same genotype is reduplicated by various types of vegetative propagation.

The possibility of selecting valuable new types by raising offspring from single individuals had already been empirically realized before Mendel and was especially practiced by Louis de Vilmorin in the middle of the nineteenth century. Important results with this method in wheat, oats and barley were later on also obtained by Hays in Minnesota and by Hjalmar Nilsson and his collaborators at Svalöf. Otherwise the Darwinian views predominated among plant breeders in the nineteenth century, and mass selection was considered to be the best plant breeding method. This is very well illustrated by the following statement by the German plant breeder Rümker:

All our cultivated plants constitute a plastic, malleable material which requires only the formative hand of the artist to become modelled into a thousand new racial forms, and just as not everyone can be an artist unless he has the necessary gifts and inspiration, so it is not everyone who is born to be a breeder.

Especially after the work of Johannsen it was realized that the isolation of new varieties by raising progenies from single individuals could be carried out with success only in populations of self-fertilizing species and even in such cases the method had its obvious limitations. It was found that by such selection nothing really new was produced and that only an isolation of biotypes which were already present in the population took place. And further, in many cases the constellation of characters shown by the extracted lines was far
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from ideal. If a new line represented an improvement in one respect it very often had taken a step backwards in another respect.

In a now classical paper Nilsson-Ehle (1906) points out the great difficulties of obtaining really good products by line selection in spontaneous populations and recommends artificial crosses as the best way of combining various desirable characters. He especially emphasizes that the crosses should be undertaken with the definite view of combining one or the other valuable property of one variety with certain other properties of the other parent. Crosses should not be undertaken just in order to increase variation. Nilsson-Ehle gives a number of examples and says that in the offspring of artificial hybrids he had been able to get some derivatives combining valuable characters of the parents but also segregation products combining the poor properties of the parents. Thus, from the cross between the winter wheat varieties Extra Squarehead and Grenadier some forms combined the short stiff straw of Grenadier with the resistance to yellow rust characteristic of Extra Squarehead, but other derivatives combined the tall and rather lax straw of Extra Squarehead with the susceptibility to yellow rust of Grenadier. Already from such examples it became clear that the economically important characters in wheat could be recombined.

THE INHERITANCE OF QUANTITATIVE CHARACTERS

In three papers published in the years 1908-1911 Nilsson-Ehle clearly demonstrated that quantitative characters in general are inherited in a Mendelian way, but that as a rule many so-called polymeric genes or multiple factors are involved in such cases. As this theory is of fundamental importance to plant breeding, it deserves to be considered in some detail.

Nilsson-Ehle's material was wheat and oats, and in both of these species he analyzed numerous crosses very carefully, especially by raising a large number of families in F3 and the following generations. As far as possible the material was used for factorial analyses and in several cases it was possible to establish the action of distinct factors. Several cases of monohybrid segregation were found but also
the new ratios 15:1 and 63:1. In some cases he also believed that he had segregation in the ratio 255:1 but this was not verified by later investigations.

Nilsson-Ehle emphasized that in his crossing material there were all transitions from cases of distinct segregation in sharply separated categories to cases of purely quantitative continuous variation. The inheritance of ear density in wheat represented a typical borderline case, which, however, could be factorially analyzed. The rather continuous variation observed was shown to be controlled by one inhibiting factor and two length factors. After obtaining evidence also in a number of other cases that an apparently single character was conditioned by several genes, Nilsson-Ehle concluded that also in cases of purely quantitative variation, which cannot be genetically analyzed, several or many different genes are involved. These genes are inherited in the same way and are just as constant as the genes conditioning qualitative characters.

This rested not only on analogy, but the theory was supported by comprehensive experimental data. Especially significant is the demonstration that after crossing and recombination it is possible to obtain numerous constant gradations with regard to different quantitative characters. This quantitative differentiation is evident already in F3, though complete constancy is not obtained until later generations.

If the parents were widely different with regard to a certain quantitative character the constant gradations were predominantly intermediate between the parents. If, on the contrary, the parents were alike or almost alike the gradations generally represented transgressions. The frequent occurrence of positive as well as negative transgressions represents a very strong support of the theory of polymerism. The only possible interpretation is that quantitative characters in such cases are conditioned by several or even numerous constant genes which are recombined and that the parents carry different sets of such genes.

The frequent occurrence of transgressions proved to be very valuable for breeding purposes, and Nilsson-Ehle considered the utilization of transgressions as a special breeding method. A very good
example is the transgression in yield represented by "Eagle oats." This variety was produced by Åkerman from a cross between v. Lockows Gelbhafer and Victory Oats. If the yields of the parents are represented by the values 98 and 100, Eagle oats will be equal to 106, thus surpassing the parents by about 7 per cent.

An important consequence of the theory of polymerism, observed by Nilsson-Ehle, is that allogamous organisms are always heterozygous and represent an inexhaustible multitude of different genetic combinations. From the polymerism it became clear that it is not only the relatively few genes, having obvious visible effects, which are segregating, but rather a large number of genes having small visible effects or only physiological consequences. Such segregation is still more masked by the modifying effects of environmental influences. Under such circumstances quite special methods, clonal propagation or inbreeding, are needed in order to reveal the enormous hereditary polymorphism occurring within such an apparently homogeneous material as for instance the plants in a field of rye.

Nowadays, these facts are quite self-evident and it is easily forgotten that 40 years ago the situation was quite different. In this field Nilsson-Ehle's results completed and deepened Johannsen's classical analysis of populations and pure lines which was published already in 1903.

When Nilsson-Ehle had seen that the polymeric genes generally conditioned such quantitative and physiological properties as must be of the greatest importance to the viability of the organisms and to their ability to survive under variable environmental conditions, he concluded (in 1909) that the main purpose of sexual reproduction must be the creation of genetic recombination. This is a thesis which is now rather axiomatic, but not until the theory of polymerism had been developed was such a conclusion fully justified.

There is an immediate connection between the importance of genetic recombination to the viability of organisms and their adaptability to different types of environment. In one of his papers Nilsson-Ehle points out that not in a single case had he been able to find transitions from modifications to heritable variations. Thus, a Lamarckian explanation of the acclimatization of wild as well as
cultivated plants was quite excluded. The mechanism at work was rather crossing, recombination and selection, this process also being promoted by spontaneous mutation. This represents the fundamental ideas of the special branch of science later on called genecology by Turesson and others. Nilsson-Ehle also gives numerous examples of the genotypical adaptation of cultivated plants to special habitats and shows that this is of great importance in plant breeding work. On account of this principle the central breeding station at Svalöf was later on supplemented by a network of branch stations in various parts of Sweden. As far as possible local selection and breeding is carried out at these stations. Only in this way is it possible to get a satisfactory genotypical adaptation to the ecological conditions characteristic of each local region.

The theory of polymeric genes was advanced before 1910 at a time when genetics was a purely experimental science without contact with cytology. Not until 1918 was the chromosome number of ordinary wheat correctly determined by Sakamura, and later on especially, through the work of Kihara, it turned out that wheat as well as oats are allopolyploids, the three genomes of which are differentiated from each other. This differentiation, however, does not exclude essential similarities between the genomes, and there is now reason to assume that the typical polymeric segregation ratios 15:1 and 63:1 are due to this partial homology between the three genomes. In several other cases typical polymeric segregation ratios and about equal effect of the various dominant genes have been met with when the species are polyploid but very seldom when they are diploid.

Thus, Nilsson-Ehle’s theory of polymerism seems to be based on a special phenomenon and, indeed, it is not probable that he would have been able to advance his theory so soon, if his material had been represented by diploid species. This, however, in no way reduces the validity and fertility of the theory. As may be recalled, the attention of the investigator passed from the distinct polymeric segregation ratios to less clear cases of segregation, in which it is probable that the polymerism was caused only partly or not at all by polyploidy. Finally, the main part of the segregations studied by Nilsson-Ehle was impossible to analyze factorially, but proof that segregation really
occurred was obtained, in part by the appearance of transgressions, in part by the establishment in later generations of constant gradations. Even if the starting point may have been of a special kind Nilsson-Ehle clearly realized the essential thing, that the quantitative and practically important characters are "construction characters" shaped by cooperation between a large number of genes. As a rule these genes do not have identical effects and some of them are only modifiers, but they all cooperate in the modelling of a certain character.

In this connection it should be pointed out that during his later experience as a barley breeder Nilsson-Ehle observed and described transgressions in earliness and in other respects of quite the same type as those previously observed in wheat and oats. As barley is diploid it is clear that the polymeric inheritance observed here as well as the corresponding phenomena in the polyploid species represent the essential point in Nilsson-Ehle's discovery. This corresponds to inheritance controlled by multiple genes, to use the term generally used in the United States. Therefore I cannot agree with Darlington and Mather (1949) when they define polymeric genes as non-allelicomorphic genes of apparently identical and cumulative action which are characteristic of allopolyploids. The polymeric genes of Nilsson-Ehle include Mather's so-called polygenes as well as the special cases of identical or homologous polymeric genes depending on polyploidy. It should also be mentioned that originally Nilsson-Ehle used the term "Gleichsinnige Faktoren." Later on he took up Plate's term "Homomerie" for the same thing. The term polymerism or "Polymerie" which was later on generally accepted in Europe was not introduced by Nilsson-Ehle but by Lang, who also spoke of "Dimerie," "Trimerie" and so on.

EFFECTS OF PLANT BREEDING ON CROP YIELD

It is well known that the classical methods of plant breeding—selection in spontaneous populations as well as selection in populations produced by hybridization—have led to numerous improved varieties of great economical importance. This is especially true of
the well-planned breeding by recombination, in which various desirable characters of the parents have been combined more or less successfully. As an example I only need to mention the combination of the high specific yield of the English Squarehead wheat with the winter hardiness and good kernel quality of certain Swedish land varieties. The economic importance of such work may be illustrated by Table 1.

**Table 1 - The increase in value, due to plant breeding, of the cereal harvests in Sweden.**

<table>
<thead>
<tr>
<th>CEREAL</th>
<th>PRESENT VALUE OF THE TOTAL HARVEST (IN KRONOR)</th>
<th>PER CENT INCREASE DUE TO PLANT BREEDING</th>
<th>VALUE OF THE INCREASE (IN KRONOR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter wheat</td>
<td>98,661,750</td>
<td>25</td>
<td>24,965,937</td>
</tr>
<tr>
<td>Summer</td>
<td>45,140,500</td>
<td>12</td>
<td>5,416,860</td>
</tr>
<tr>
<td>Winter rye</td>
<td>67,650,000</td>
<td>15</td>
<td>10,147,500</td>
</tr>
<tr>
<td>Oats</td>
<td>146,544,450</td>
<td>14</td>
<td>20,881,616</td>
</tr>
<tr>
<td>Barley</td>
<td>53,541,690</td>
<td>12</td>
<td>4,300,992</td>
</tr>
<tr>
<td>Mangcorn</td>
<td>(oats + barley) 98,062,200</td>
<td>13</td>
<td>12,748,086</td>
</tr>
</tbody>
</table>

The table shows the value of the total yearly cereal harvest in Sweden, these values representing average values of the latest five-year period. The next column shows the increase in production which may be ascribed to plant breeding. These percentage values have been obtained by comparisons during several years between the old unimproved land varieties and the modern products of plant breeding grown side by side. The increases range from 12 per cent in oats and summer wheat to 25 per cent in winter wheat. The value of this increase may be seen in the last column. The total for all the cereals amounts to about 78 million Kronor. This is the yearly increase in value which the Swedish harvest of cereals has attained thanks to plant breeding. It should also be kept in mind that the breeding has also resulted in other favorable changes which are not reflected by these values. This is, for example, true of the improvements in straw stiffness allowing a stronger nitrogen manuring. Improved kernel quality and an increased earliness are other advantages.

In Sweden much breeding work has also been done with other
agricultural plants. It is not possible to make exact statements about these other crops, but it seems safe to conclude that plant breeding has been able to increase the annual value of the total Swedish crop by about 100 million Kronor which corresponds to about 20 million dollars. This is a large amount for a small country like Sweden, especially if it is compared to the total amounts so far spent on plant breeding. For the period 1886 to 1948 these costs amount to about 15 million Kronor and thus, the yearly profit is several times larger than the costs for the whole period of more than 60 years. The figures mentioned are of course far from exact, but at any rate, it is perfectly clear that the state of Sweden has made a brilliant bargain by its investments in plant breeding. Similar experiences have also been made in other countries and I do not think it is possible to find another business giving as good dividends as this one.

EFFECTS OF INBREEDING

In cross-pollinating organisms it has been more difficult than in the self-fertilizing ones to attain definite knowledge of the genotypical constitution, and the results obtained in inbreeding experiments have been interpreted in various ways. However, most or all investigators agree that the differentiation occurring in inbred material is caused by homozygosity for genes present in the original, heterozygous population, and further, that hybrid vigor is due to genotypical differences between the parent types. It is very doubtful if the cytoplasm has anything direct to do with inbreeding degeneration as claimed by some workers. A rather strong argument against this possibility is represented by results recently obtained by one of my students, Mr. A. Lundqvist. He compares the effects of inbreeding in diploid and autotetraploid rye and finds significant differences, the tetraploids being much more resistant to inbreeding than the diploids. This may, indeed, be expected as quadrivalents are frequent at meiosis in the tetraploid and the segregation of homozygous recessives will be slow.

Time does not permit me to go into details concerning the different theories of inbreeding degeneration and I hope the reports of
the recent conference on heterosis in Iowa will give us good answers to all our questions in this field. I just want to call your attention to the extremely important population studies in Drosophila carried out by Dobzhansky and others, which demonstrate a very frequent occurrence of factors which in a homozygous condition have more or less bad effects. They cause all degrees of reduced vigor from complete lethality to almost normal viability, and there is also preliminary evidence of factors decreasing fertility. Evidently this is all that is needed to explain a typical inbreeding degeneration. The occurrence of such factors in allogamous populations has long been assumed, but now their existence has been definitely demonstrated. The evidence is less definite in other organisms, but there is reason to believe that the situation found in Drosophila is also characteristic of most other cross-fertilizing species. As regards the allogamous plants an indication in this direction is the frequent occurrence of partial pollen sterility in population plants. Such sterility does not occur in autogamous species and these species do not suffer from inbreeding either.

Finally I would like to call your attention to the fact that the inbreeding phenomena cannot be solved by an exclusively genetic analysis. It has been found in rye as well as in other allogamous plants that inbreeding often has a marked effect on the meiotic mechanism and that reduced pairing and other meiotic irregularities may lead to the extinction of some lines and lack of constancy in other lines, even if they have been inbred for a long time.

**HYBRID CORN.**

The practical utilization of all the knowledge gained from experiments on inbreeding and outbreeding is illustrated in the most beautiful way by the magnificent work on corn breeding carried out in the United States. As you all know, the theoretical foundations in this field were created by such pioneers as Shull and East, and by a brilliant cooperation between many workers it has been possible to build up, step by step, a line material which on outcrossing has led to a very marked increase in yield. Concerning the details of this work
I may refer you to the paper by Dr. Mangelsdorf in this volume. I would just like to mention that according to Sprague, 83 per cent of the maize grown in the corn belt in 1944 was hybrid maize, and that the use of this material led to an increase in yield of 600 million bushels.

Even if the details of the inbreeding phenomena are not yet quite clear I think we are fully justified in ascribing the practical results obtained to an intimate and unusually successful cooperation between theoretical genetics and practical plant breeding.

**INDUCED MUTATIONS IN CROP PLANTS**

The development of genetics and cytology during the last decades has given rise to new plant breeding methods. This is especially true of the work on induced mutations and on polyploidy. After the discovery by Muller that mutations may be induced by irradiation, and many other workers had concentrated on this new field of genetic research, attempts were also made to utilize induced mutations for plant breeding purposes. A special difficulty for these attempts is the fact that the induced mutants generally have a decreased viability, this reduction in vigor in the first place being caused by deleterious changes of the chromosome structure. But also many of the finer changes, which are considered to be true gene mutations, have a negative effect and are useless from a practical point of view. There remains, however, a quite small but exceedingly important group of induced mutations which are not destructive but in which viability is normal or even improved. It may also happen that mutations, having bad effects in their original genetical environment, may have a more favorable action after recombination. It is now perfectly clear that the new genes or alleles induced by irradiation are of essentially the same kind as the spontaneous ones, and this really means that the spontaneous rate of genotypical variation may be enormously increased by irradiation or other similar agencies.

Especially through the mutation experiments carried out by Gustafsson at Svalöf it is now perfectly clear that this method is of positive value for plant breeding work. In barley, the main ma-
Genetics and Plant Breeding

Material for this work, it has been possible to induce very marked and favorable changes in straw stiffness and earliness. Some of the mutants also give a higher yield than the original material. This is shown in Table 2 taken from a recent publication by Gustafsson and Mac Key (1948).

Table 2 · Agronomic characters of thirteen induced mutants in barley.

(Gustafsson and Mac Key, 1948.)

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>NO. OF YEARS TESTED</th>
<th>REL. YIELD</th>
<th>STRENGTH OF STRAW (1-10)</th>
<th>1000-GRAIN WEIGHT, GRAMS</th>
<th>RIPENING TIME; DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden barley</td>
<td>8</td>
<td>100</td>
<td>6.5</td>
<td>38.1</td>
<td>103</td>
</tr>
<tr>
<td>Erectoides 1; 44/1</td>
<td>8</td>
<td>102.2</td>
<td>7.3</td>
<td>37.0</td>
<td>104</td>
</tr>
<tr>
<td>Late, tall; 44/2</td>
<td>8</td>
<td>103.3</td>
<td>8.0</td>
<td>38.4</td>
<td>107</td>
</tr>
<tr>
<td>Broad-leaved, late; 44/9</td>
<td>5</td>
<td>100.5</td>
<td>7.6</td>
<td>36.2</td>
<td>109</td>
</tr>
<tr>
<td>Large-seeded; 44/7</td>
<td>5</td>
<td>109.1</td>
<td>6.8</td>
<td>42.2</td>
<td>103</td>
</tr>
<tr>
<td>Broad-leaved; 44/23</td>
<td>6</td>
<td>102.0</td>
<td>6.6</td>
<td>41.5</td>
<td>105</td>
</tr>
<tr>
<td>Maja barley</td>
<td>4</td>
<td>100</td>
<td>8.3</td>
<td>39.0</td>
<td>105</td>
</tr>
<tr>
<td>Erectoides 12; 44/17</td>
<td>4</td>
<td>101.9</td>
<td>9.1</td>
<td>39.0</td>
<td>106</td>
</tr>
<tr>
<td>Erectoides 13; 44/19</td>
<td>4</td>
<td>96.1</td>
<td>10.0</td>
<td>35.6</td>
<td>105</td>
</tr>
<tr>
<td>Erectoides 16; 44/20</td>
<td>3</td>
<td>98.1</td>
<td>9.3</td>
<td>40.1</td>
<td>99</td>
</tr>
<tr>
<td>Bright-green 2; 44/35</td>
<td>4</td>
<td>99.8</td>
<td>9.0</td>
<td>38.8</td>
<td>106</td>
</tr>
<tr>
<td>Stiff-strawed, early; 44/18</td>
<td>4</td>
<td>102.0</td>
<td>8.8</td>
<td>40.1</td>
<td>103</td>
</tr>
<tr>
<td>Seeds differently colored; 44/18</td>
<td>4</td>
<td>103.4</td>
<td>9.0</td>
<td>39.1</td>
<td>106</td>
</tr>
<tr>
<td>Broad-leaved, late; 44/31</td>
<td>4</td>
<td>101.4</td>
<td>8.7</td>
<td>36.8</td>
<td>106</td>
</tr>
<tr>
<td>01513 bl. (“Ymer”)</td>
<td>3</td>
<td>100</td>
<td>9.8</td>
<td>42.1</td>
<td>103</td>
</tr>
<tr>
<td>Bright-green 3; 46/65</td>
<td>3</td>
<td>102.0</td>
<td>9.3</td>
<td>41.0</td>
<td>101</td>
</tr>
</tbody>
</table>

Mac Key (1948). The values in this table have been obtained from field trials, carried out in the same way as in the testing of ordinary breeding material. Three commercial varieties are represented in the table together with thirteen selected induced mutations. The five mutations of Golden barley have been compared with the mother line for 5-8 years and all have higher values for yield as well as for straw stiffness. Some of them have an increased 1000-grain weight and this is especially true of the mutant “large-seeded; 44/7.” On an average this mutant had 9.1 per cent higher yield than the mother strain and yet it has the same earliness. The values of the other mutants in the table are somewhat less reliable, being based on data from only 3-4 years. On an average, however, these mutants have
good values and surpass the mother types either in yield, straw stiffness or earliness.

In practice Golden barley has now been replaced by the higher yielding varieties Maja and Ymer, and therefore the improved mutants of Golden barley are not able to compete with the new commercial varieties Maja and Ymer. Hence, the mutation work must now be concentrated on the varieties at present giving the highest yields. As is evident from the lower part of the table some promising mutants of Maja and Ymer have already been obtained. On the whole I think the greatest value of the new mutants will be as material for further breeding by recombination. Even if an extremely early mutant will have a lower yield than the mother line it is probable that the new mutant gene will be of value after recombination.

In several other plant species besides barley positive results of the same kind have been obtained by Gustafsson and his collaborators. This is true of wheat and oats as well as of yellow lupins and some other species. Efforts are now also being made to induce mutations in fruit trees.

Very promising results with induced mutations in barley have also been obtained by Freisleben, Lein and Hoffmann, working in Halle. From their very large material the following data are especially interesting (Table 3). Though the period of testing is too short to allow safe conclusions these values strongly support Gustafsson’s conclusion that it is possible in barley as well as in other cultivated plants to get induced mutants of agricultural importance.

Table 3 - Relative yields of the best barley mutants in Halle-Hohenthurm 1947 and 1948. (Data given by Kuckuck-Mudra, 1950, p. 164.)

<table>
<thead>
<tr>
<th>NO.</th>
<th>MUTANT</th>
<th>1947</th>
<th>1948</th>
<th>1947-1948 AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>früher, kurzhalzig</td>
<td>123.30</td>
<td>114.4</td>
<td>118.95</td>
</tr>
<tr>
<td>96</td>
<td>früher, breite Bl., kürzer</td>
<td>127.80</td>
<td>101.4</td>
<td>114.60</td>
</tr>
<tr>
<td>120</td>
<td>früher</td>
<td>113.63</td>
<td>104.1</td>
<td>108.87</td>
</tr>
<tr>
<td>92</td>
<td>früher, breite Bl.</td>
<td>110.06</td>
<td>105.6</td>
<td>107.83</td>
</tr>
<tr>
<td>115</td>
<td>standfester, kürzer, dunkler</td>
<td>104.48</td>
<td>104.0</td>
<td>104.24</td>
</tr>
<tr>
<td>3</td>
<td>erectum dunkel</td>
<td>104.20</td>
<td>101.7</td>
<td>102.95</td>
</tr>
<tr>
<td>245</td>
<td>dichte Ahre, gr. Korn, dunkel</td>
<td>101.47</td>
<td>102.5</td>
<td>101.99</td>
</tr>
</tbody>
</table>
INDUCED POLYPLOIDY

For many years and especially after the introduction of the colchicine method attempts have been made all over the world to utilize induced polyploids in agriculture. At the Svalöf institute a special department for such work was started in 1931. During its twenty years of activity plenty of material has been studied by my colleague Dr. Levan and by myself, and our experience may be summarized as follows:

1. Most induced polyploids have no practical value, but there is a small group of material in which the reaction to chromosome doubling is favorable. In such cases new material has been obtained which is already of great importance to agriculture or which most probably will be in the near future.

2. Only in rare cases are the primary polyploids, the so-called raw-polyploids, practically useful. In most cases it is necessary to improve this material by recombination and selection, and by such work vigor as well as fertility may be improved. The situation in this respect is about the same as with the induced mutations. Induction of polyploidy as well as of mutations implies a more or less violent disturbance of the balance, which may be partially overcome by recombination without losing the essential and practically important effect of the primary change.

3. Among the polyploids auto- as well as allopoloids may be of practical value.

In support of these statements I wish to mention the following examples:

Diploid allogamous species in which the vegetative parts represent the agricultural product are an especially favorable starting material for the induction of autopolyploidy. Table 4 represents a summary of field trials with diploid and tetraploid red clover which were carried out by Turesson, Levan and Frandsen. The values in the table are relative values concerning the green matter production, the diploids in each trial having the value 100. Thus values in the table higher than 100 imply that the tetraploid has been superior to the corre-
Table 4 • Relative yield values in different strains of tetraploid red clover. (Data given by Levan, 1948, p. 488.)

<table>
<thead>
<tr>
<th>STRAIN</th>
<th>YEAR</th>
<th>PLACE OF TRIAL</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Øtofte</td>
<td>1945</td>
<td>Øtoftegaard</td>
<td>101</td>
<td>116</td>
<td>131</td>
<td>112</td>
</tr>
<tr>
<td>tidlig</td>
<td>1946</td>
<td>Øtoftegaard</td>
<td>109</td>
<td>147</td>
<td>—</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Frandsen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1944</td>
<td>Ultuna (Turesson)</td>
<td>—</td>
<td>125</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Svalöf</td>
<td>112</td>
<td>127</td>
<td>—</td>
<td>114</td>
</tr>
<tr>
<td>Merkur</td>
<td>1945</td>
<td>Ultuna (Turesson)</td>
<td>105</td>
<td>118</td>
<td>—</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>1946</td>
<td>Svalöf</td>
<td>77</td>
<td>103</td>
<td>—</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Östergötland</td>
<td>115</td>
<td>132</td>
<td>—</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Västergötland</td>
<td>114</td>
<td>135</td>
<td>—</td>
<td>120</td>
</tr>
<tr>
<td>Hersnap</td>
<td>1944</td>
<td>Ultuna (Turesson)</td>
<td>149</td>
<td>148</td>
<td>—</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>1945</td>
<td>„ („„)“</td>
<td>128</td>
<td>120</td>
<td>—</td>
<td>125</td>
</tr>
<tr>
<td>Ultuna</td>
<td>1943</td>
<td>Svalöf</td>
<td>82</td>
<td>98</td>
<td>—</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>1946</td>
<td>„ („„)“</td>
<td>77</td>
<td>119</td>
<td>—</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ultuna</td>
<td>106</td>
<td>57</td>
<td>—</td>
<td>63</td>
</tr>
<tr>
<td>Offer</td>
<td>1943</td>
<td>Svalöf</td>
<td>104</td>
<td>113</td>
<td>—</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>1946</td>
<td>„ („ „)“</td>
<td>102</td>
<td>118</td>
<td>—</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Västermorland</td>
<td>109</td>
<td>—</td>
<td>—</td>
<td>109</td>
</tr>
</tbody>
</table>

Responding diploid. Considering the total harvest we find that in 11 cases out of 15 the tetraploids were superior to the diploids. The superiority is more marked at the second than at the first harvest per year. This means that the tetraploids are somewhat more slow-growing, but in red clover this is no disadvantage. A greater difficulty is the seed production which is lower in the tetraploids than in the diploids. However, according to Levan, selection for higher seed production seems to be successful. In the related clover species, *Trifolium hybridum*, the seed setting in the tetraploids is already sufficient. As also in this species the green matter production in the tetraploids is higher than in the diploids, two different tetraploid strains are already in the market. One of them was produced by Turesson, the other one by Levan.

Relatively favorable experiences with induced polyploids have also been obtained in beets. In sugar beets the triploids evidently represent the yield optimum, whereas the tetraploids are somewhat less
good than the diploids. In turnips Levan has obtained quite good
yield values for the tetraploids.

From my own experience I want to give some information on
tetraploid winter rye. Although this is an autotetraploid with 28
chromosomes ± instead of 14, it is quite vigorous and has remarkably
good properties. The amount of seed setting is somewhat re-
duced, it is true, and also the degree of tillering is less good in the
tetraploids than in the diploids. However, as the kernel weight is
about 50 per cent larger than in the diploids, the total yield is rather
satisfactory. Further it should be mentioned that the big kernels of
the tetraploid give very vigorous seedlings and that the flour from
these kernels has been found to have a better baking quality than the
flour of ordinary diploid rye. Concerning frost resistance, drought
resistance, earliness and straw-stiffness the tetraploids seem to be just
as good as the corresponding diploids.

A difficulty for the evaluation of the tetraploids is represented by
the fact that diploid and tetraploid strains cannot be directly com-
pared in the same field trial. If tetraploid rye is exposed to the pollen
of ordinary rye the degree of seed setting may be severely reduced
owing to the formation of aborting triploid embryos.

It is therefore necessary to keep the diploid and tetraploid rye
strains isolated from each other and to make indirect comparisons by
the use of common standards. I have used different varieties of win-
ter wheat as standards, and trials of this kind have now been under-
taken in five years at seven different places in southern Sweden. The
values of one such trial are shown in Table 5. In comparison to the

\begin{table}
\centering
\caption{Yield trial with diploid and tetraploid rye. Skara, 1948.}
\begin{tabular}{|l|l|l|l|}
\hline
\textbf{VARIETY} & \textbf{YIELD (WATER CONTENT 15\%)} & \textbf{RELATIVE VALUES} & \textbf{1000-GRAIN WEIGHT} \\
\hline
Steel rye, diploid & 4260 & 130.3 & 30.3 \\
Wasa II, " & 4190 & 128.1 & 29.3 \\
Ergo-wheat & 3270 & 100.0 & 41.9 \\
\hline
Steel rye, tetraploid & 5820 & 147.3 & 46.2 \\
Wasa II, " & 5720 & 144.8 & 46.4 \\
Crossing group " & 5610 & 142.0 & 46.3 \\
Ergo-wheat & 3950 & 100.0 & 44.7 \\
\hline
\end{tabular}
\end{table}
wheat standard, Ergo, two diploid rye varieties, Steel and Wasa II, had the relative values 128 and 130 (Ergo = 100). In the tetraploid field trial the corresponding relative values of tetraploid Steel and Wasa II were 145 and 147 and thus clearly higher. The other values in the table demonstrate that in this particular case the 1000-grain weight was 55 per cent higher in the tetraploids than in the diploids. In other cases the values of the tetraploids were not quite as good and more data are needed before we can be quite sure about the relative yield of the diploid and tetraploid rye strains. However, the data already obtained justify the conclusion that tetraploid Steel rye has about the same yield as diploid Steel rye. But since the baking quality of the tetraploid is decidedly superior it has now been decided to release tetraploid Steel rye to the Swedish farmers next autumn. Large propagations of this variety are just now being sown.

My personal experience of induced allopolyploids in agricultural plants is limited to ryewheat, Triticale, having 56 chromosomes, 42 wheat + 14 rye chromosomes. In this connection I just want to mention that the best types available 15 years ago had only about 50 per cent of the yield of the wheat standards. I have now Triticale-types reaching about 90 per cent of the yield of the wheat standard, and the possibilities of raising new and improved Triticale-strains are almost unlimited. I therefore believe that it will be possible to get Triticale-types of agronomic importance later on. A special difficulty is the meiotic lability frequently met with in this material. This is probably a consequence of the fact that Triticale represents a combination of one typically cross-fertilizing and one typically self-fertilizing species. However, it seems to be possible to overcome this difficulty by using selected inbred and self-fertile strains of rye for the crosses instead of ordinary population plants. I can also mention that we have recently found it possible to combine wheat and tetraploid rye, though we do not know anything yet about the properties of this new allopolyploid.

Finally, I would like to say a word about the natural and synthetic allopolyploids in the genus Brassica. One of my students, Mr. Gösta Olsson, is working with various auto- and allopolyploids in this genus and especially with synthetic forms of winter rape (Brassica napus,
var. oleifera). This species has the somatic chromosome number 38 and represents a synthesis of winter turnip rape (Brassica rapa, var. oleifera) with 20 chromosomes, and cabbage (Brassica oleracea) having 18 chromosomes. Synthetic rape has been produced by various workers, and Rudorf in Germany has already reported that hybrid derivatives between natural and synthetic rape forms have given quite promising results. In some cases the yield of these hybrids was 25-30 per cent higher than in the standard, a well-known commercial variety.

All this work on the practical utilization of various induced polyploids would have been impossible without the corresponding theoretical background. It is only thanks to theoretical cytogenetic work that the phenomenon of polyploidy has been discovered and that its great importance to the evolution of plant species has been demonstrated. During this work the difference between auto- and alloplody has been clarified, and it has been shown that both these categories of variation are involved in the evolution of a great number of our cultivated plants. Under such circumstances attempts to produce new types of polyploids in cultivated plants are well justified, and the results already obtained demonstrate, indeed, that this method of plant breeding is possible and may lead to quite valuable products.

From what I have said I think it is evident that the classical ways of plant breeding as well as the more recent attempts to utilize induced mutations and polyploidy have been built up through a close cooperation between theoretical cytogenetics and the experiences of practical breeders and farmers. The theoretical basis is the result of international cooperation and is in the first place founded on Mendel's discovery of stable units of heredity. Especially the Drosophila-workers demonstrated the hereditary function of the chromosomes, and from the same group we have also received the fundamental results on induced mutations and valuable data on the causes of inbreeding degeneration.

Plant breeding based on such premises is not just a theoretical play but a science of the greatest importance to the economy and feeding of the whole world. Besides rational manuring and other agricul-
tural measures, consistent breeding work on this basis is the best way so far known to protect humanity from starvation.

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THE art of animal breeding was already well advanced before 1900. By countless centuries of trial and error, certain practices had come to be recognized as generally a bit more successful than others in producing animals more like the breeder's desire. Yet only a little was known about why things happened as they did; the art of animal breeding was far in advance of the science.

The Roman writings on farming were moderately abundant and included many items about the selection, care and breeding of animals. In his *Roman Farm Management* Fairfax Harrison (1917) has given us a fairly broad view of this in his translations of what was written by Varro, Cato, Pliny and other Roman farmers with a bit of leisure. We even get a few glimpses of the earlier Carthaginian and Greek practices and views. Little else written before the early 1700's has been preserved for us. At that time descriptions of the local races of livestock in Britain began to appear in print. These contained comments on animal breeding practices. Engeler (1936) has given us a brief but clear story of animal breeding practices and thought in the German-speaking lands and in France from around 1700 up to the end of the 1800's.

Biological thought and animal breeding experience were much intertwined during the nineteenth century. Which influenced the other the most is not clear. Darwin's extensive compilation of facts about domesticated plants and animals certainly influenced his biological views. In turn, many of the animal breeders may have modified their
views and even their practices after they had read or heard Darwin. That Galton and his helpers studied data concerning dogs, horses and cattle, as well as data on man, is also well known. Yet animal breeders were using widely the fractions 1/2, 1/4, 1/8, etc., several decades before Galton formulated his "law." Any who think that controversy over crossbreeding is a modern thing might do well to read Buffon's panegyrics on crossbreeding as a means of improving all animal forms toward perfection. The extreme claims of Linnaeus concerning the fixity of species had their still more extreme counterpart in the doctrine of "racial constancy" which was prominent in German writings on animal breeding near the middle of the nineteenth century. It may well be doubted that the breeders themselves ever emphasized race so extremely and neglected individual merit so completely as those who wrote about this doctrine recommended. Rather it is to be supposed that the writers and philosophers went more readily to extremes in such matters than did the breeders who had to be at all times in close contact with many of the facts and whose livelihood depended to some extent on their actions not being further out of balance with the facts than were the actions of their competitors.

**ANIMAL BREEDING IN 1900**

The nineteenth century had seen pure breeding become established as the standard method for producing seedstock. Along with this arose the herdbooks and the breed associations as mechanisms for safeguarding breed purity and for taking other cooperative actions which the breeders thought were in their interests. Very few of these breed associations were formed before 1850. The process reached its peak in the 1870's and 1880's. Only a few new associations have been established since 1900, although the rate seems to be increasing again for light horses and for some kinds of cattle with zebu blood. The expansion in numbers of pure-bred animals continued to be rapid in most countries until the agricultural depression of the 1920's. While it was actively under way, the breeds competed intensely to enlist new breeders and to expand in numbers. Most of the association activity concerned the measures used to keep the
breeds pure, measures for improving the breed, and efforts to expand it in numbers.

Crossbreeding and grading were used widely for producing commercial stock. Indeed in the initial stages of almost every pure breed its advertising literature contained prominent claims about the good results to be obtained from crossing their sires on females of other or ordinary stock.

The main method of improvement was individual selection within pure breeds. Outcrosses had often been made to other stock in the initial stages of forming the breed but this usually ceased when the stock was thought meritorious enough to be recognized and treated as a breed. This gave rise to voluminous discussions of the meaning and usefulness of breed purity. Purity of breeding was often more highly esteemed in the importing lands, where numbers were rapidly expanding, than in the region where the breed was first formed and where many high grades were usually present for comparison.

Selection was based mainly on individual merit, although always there was much talk of pedigree and the selections must have been influenced at least a little by the merits of ancestors and collateral relatives. In many cases the progeny test helped determine whether a mature animal continued to be used or was discarded.

The attempts to use pedigrees in estimating an animal's breeding worth led the users almost automatically to try to make pedigree mean more than it possibly could. It was easy to infer, without realizing that they were doing so, that there simply must be some correct way to interpret pedigrees which, if one could only find it, would be the key to remarkably rapid animal improvement. The dilemma was that every man who bred animals saw that there certainly was something to pedigree and yet there were conspicuous individual exceptions to any rule he could devise for predicting from its pedigree what any animal would actually be. These efforts to force unjustified meaning into pedigrees loom large in the animal breeding literature at least until the 1920's. There was much about families, tribes, male lines, female lines, transmission of prepotency, uniformity of get, topcrosses, outcrosses, sires whose daughters were good but sons were mediocre (or the reverse), whether improvement in the various char-
acters were better sought through the top line or the bottom line of
the pedigree, etc.

Out of this dilemma, that pedigree was obviously important and
yet even full brothers often differed widely, came much of the con-
fusion in the writings about animal breeding and most of the re-
marks about the laws of heredity being so mysterious. For example,
when I entered agricultural college, more than a dozen years after
the rediscovery of Mendelism, I remember being told that the first
principle of animal breeding was: like produces like; while the second
principle was: like does not always produce like! Sometimes the con-
flict was reconciled and also a bow to pedigree was made by stating
it as: Like produces like or the likeness of some near ancestor. Some
writers of that period contrasted “the law of heredity” with “the law
of variation” with the inference that they were opposite or mutually
exclusive forces. Atavism or reversion added to the mystery and
helped provide a fertile soil in which some of the superstitions could
continue to live.

THE FIRST TWENTY YEARS OF MENDELISM

Mendelism and immediately subsequent discoveries had three
major effects in clarifying theory. First, it became clear that identical
pedigrees need not mean identical heredity. Second, it became clear
that genetically caused and environmentally caused variations were
both present and often indistinguishable in the individual but had
quite different consequences for its descendants. Third, it was no
longer necessary to suppose that mutations were so abundant or that
their nature or causes were so important for the results of practical
animal breeding as had previously seemed obvious and unavoidable.

In pre-Mendelian theories of heredity it seemed that full brothers
ought to be identical in their heredity, since they had the very same
parents. Yet every breeder with his eyes open knew that often they
were not. Mendel’s laws of segregation and recombination destroyed
this mystery, as a rising sun dispels a fog, although it took much more
than a decade for all the implications of those laws to become clear.
Likewise this clarified the mysteries of atavism or reversion and dis-
pelled most of the supposed reasons for believing in such fictions as telegony, maternal impressions, schemes for sex control, the mysterious influence of remote ancestors, and the like.

The second major contribution of genetics toward clarifying animal breeding theory was the distinction between genetically caused and environmentally caused variations. To be sure, some of this had been foreshadowed in Weissman's speculations and philosophical discussions concerning the germ plasm, but these had little if any effect on animal breeding theory. Moreover, Weissman appears not to have appreciated the amount of genetic diversity within each race. At least he did not emphasize it. Also Darwin had referred continually to the variations being only partly hereditary. A fairly complete understanding of the situation is probably best dated from Johannsen's work on pure lines in plants. It became widespread when popularizers and breeders began to use "genotype" and "phenotype" to distinguish between the animal's transmitting ability and what it is itself. The implications of this distinction were even slower to be fully appreciated than were those of Mendel's law of segregation. Even today and even among geneticists there are still some who do not appreciate instantly and automatically that the presence of environmentally caused variations makes it almost axiomatic that the very best individuals will not usually have as good progeny as they are themselves and, conversely, that the very worst individuals will usually transmit a higher level of merit than they themselves show. That the former was a fact has long been known by animal breeders, although they were rarely aware of the latter, since they did not often see what happened when the poorest individuals were kept for breeding purposes. That selected parents generally had offspring not as good as themselves was usually regarded as one of the mysteries of heredity or, more discouragingly still, as proof of some kind of automatic and powerful tendency to degenerate and that the breeder trying to improve his stock was in some way fighting against an important law of nature in addition, of course, to having to take the consequences of any mistakes in judgment which he individually might make.

These two major changes in outlook cleared the way for a more rational use of pedigrees and of progeny tests and sib tests. Mendel's
law of segregation explained that the parent would usually transmit different things to different offspring. That made clear the necessity of having a moderately large number in a progeny test or a sib test if the test is to be dependable. It also made clear the necessity for these offspring or sibs being unselected samples if the tests are to be unbiased. That the same considerations apply to judging an individual’s phenotype in cases where repeated records are available, as in production by cows in different lactations, type ratings at different ages, the qualities of different litters from the same sow, etc., is still incompletely accepted, although understanding of it is gaining some ground each year.

In the third place, Mendel’s laws put a vastly different interpretation on the fact that the amount of individual variation within a population generally remains nearly constant from one generation to the next. Before Mendelism, breeders generally believed in blending inheritance and hence assumed that the continued interbreeding of a population would automatically lead rapidly toward perfect uniformity of that population. The fact that no such rapid increase in uniformity actually occurred was interpreted by them as evidence that something (mutations, we would say today) was pouring new hereditary variations into the population about as fast as the tremendous rate at which those were supposed to be extinguished by the blending nature of inheritance. Hence breeders imputed extraordinary importance to the source of new heredity; that is, to the causes and nature of mutations. At least as early as “Hardy’s law,” it began to be seen that the Mendelian mechanism did not of itself cause the population to become either more or less variable as the generations passed, although the full consequences of this appear first to have been stated clearly by Fisher (1930). In pre-Mendelian times the breeders speculated much about the causes of the supposedly enormous stream of new heredity being poured into their populations. They devoted part of their resources and energy to maintaining some conditions or treatments which they believed favorable for producing desirable mutations. The Mendelian discoveries freed them from the necessity of this and let them use the resources and energy thus re
leased for more efficient selection or for special tests and mating systems.

The first students of Mendelism in farm animals were much too optimistic about the simplicity and effectiveness of its direct applications. Some entertaining and often instructive information on this point can be had readily by browsing through the early volumes of the *Proceedings of the American Breeders Association*. The major difficulty was that the number of genes was vastly greater than the early workers anticipated and that the Mendelian identification of individual genes was usually made impossible by overlapping environmental effects, complex gene interactions, incomplete penetrance, thresholds for manifestation, etc. Little success was had in crossing breeds to produce new ones as simply as a child may change the position of the blocks in its playing set, as the early post-Mendelian writers sometimes inferred would be the case.

**EFFECTS OF GENETICS ON ANIMAL BREEDING PRACTICES**

Plans for testing and recording individual phenotypic merit by disinterested and impartial persons had been put into operation even before 1880 by the Holstein-Friesian breeders in the United States, although this was selective testing of only such animals as the breeder wished. Private records of production had been kept much earlier, of course. Attempts to record fleece production of sheep go back at least into the early 1800's in France, Germany and Vermont. Speed records for light horses were among the earliest of such measurements of individual merit, the *General Stud Book* for Thoroughbreds having been founded on those in the 1790's. Pulling tests for draft horses were devised and used, especially in the 1920's and 1930's, but did not have much influence on draft horse breeding.

The testing systems sponsored by the breed associations spread first through the dairy breeds, where they were common by 1910. Efforts to use them widely in the other classes of livestock were generally less successful, except in chickens where trapnests and egg-laying contests early became a prominent feature of poultry breeding but
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were either used privately or were sponsored by the Agricultural Experiment Stations. The swine registry associations always asked the breeder to state the number of pigs farrowed and weaned in each litter. Since the 1920's many of them have instituted "Production Registry" systems in which the numbers of pigs farrowed and weaned and the weaning weights of the litters are witnessed by disinterested parties. Some of this sow testing has been done, especially in other countries, by special sow testing associations. In Denmark, measuring phenotypic merit in swine has been developed to an elaborate progeny-testing system in which rate and economy of gain and many carcass qualities are measured at official testing stations, usually on something like three or four thousand pigs per year. Many other pigs are measured under somewhat less careful supervision at other local stations. A little of this is done also in Sweden, Norway and Holland and was being done in Germany in the 1930's. The dairy breed associations in the United States have had under way for some twenty years official type classification plans which are non-selective within herds.

The information from most of these plans was used by the breeder in whatever way he chose, although a few of the plans gave the association the right to deny registration to animals below a certain standard. Many of the breed registry systems in Europe have long had scoring or some other form of type inspection as a prerequisite to registry, but it is not clear that the results were any better than in breeds which gave no such authority to inspectors. Presumably the information about production or type was used mostly to guide in individual selections, but it was also used in pedigree selection and in progeny testing. Progeny testing was rather unsystematic at first but gained precision as experience grew and as the biometrical consequences of Mendelism and of environmental effects, which might be either random or biassed, became clearer. As it became clearer that the samples must be representative if they were to be used without bias, non-selective systems of testing were devised. Most of the dairy breed associations had these under way by 1930.

In some of the systems of measuring individual merit, the primary motive was cost-accounting and an immediate increase in net income
by culling the less productive individuals and by improving feeding
and management. For example, identifying and culling the low-
producing cows and improving the feeding, so as to increase immediate
profits, were the primary aims of the cow testing associations first
devised in Denmark in the early 1890's and introduced to the United
States in 1906. The increased emphasis on the genetic goal in the
United States was formally indicated twenty years ago by changing
the name from "Cow Testing Association" to "Dairy Herd Improve-
ment Association."

Some of this increase in measuring and recording individual merit
probably would have occurred, even if genetics had stood still, since
breeders have always had faith in the power of selection and since
the advantages of having production or type measured impartially
were obvious in a system where the practical needs of salesmanship
were always tempting the seller to make claims which were at least
a bit extravagant. Yet the movement could hardly have grown as
rapidly or as soundly without the parallel development of genetic
theory. The necessity of the sample of progeny or sibs being unse-
lected would not have been understood as soon or as well had not
the geneticists already been struggling with and at least partially
solving the parallel problem of how to test an individual for homozy-
gosis where dominance is complete.

Some breeders always were intrigued with the possible results of
various mating systems. Much empirical information concerning this
was acquired long before Mendel. An example was Bakewell’s dictum
in the eighteenth century that inbreeding produces prepotency and
refinement. The animal breeding literature of the late nineteenth
century is full of somewhat vague but persistent references to the
mysterious power of inbreeding to intensify and “fix” transmitting
ability to a degree far beyond what could be done merely by selecting
and mating together similar but unrelated individuals. It was ob-
served long ago that for really unifying a group and welding it into
a genuine breed, some inbreeding seemed well-nigh indispensable.
Inbreeding in order to test animals severely, prior to linebreeding to
them, had been practiced at least a little in founding most breeds,
although the principle appears first to have been expressed clearly in
post-Mendelian times—notably by Wriedt in the 1920’s.

Although Mendel himself had worked out the consequences of
selfing on single pairs of genes, the early students of Mendelism were
slow to extend this to many pairs of genes and to the milder systems
of inbreeding possible in animals. Pearl’s attempts, beginning in 1912,
and Jennings’ more complete Mendelian analysis a few years later
began to clarify the principles involved but genetic theory hardly
overtook animal breeding practice in this respect until the publica-
tion of East and Jones’ *Inbreeding and Outbreeding* (1919) and of
Wright’s monumental work on mating systems (1921). A more ra-
tional use of mating systems, especially of linebreeding and of cross-
breeding, then became possible and soon developed. The genetical
theory reconciled the apparently contradictory earlier empirical find-
ings that producers of seedstock could often use inbreeding methods
to advantage, while the producers of market stock could rarely if
ever do so, but usually found outbreeding advantageous.

Attempts to produce intensely inbred lines for commercial use did
not get under way with animals until the commercial success of
hybrid corn had begun to seem assured, but it is worth remembering
that inquires into the biological consequences of such inbreeding in
animals began much earlier. The U.S.D.A. experiments with the in-
breeding of guinea pigs, which ultimately led Wright to his explora-
tions of the general consequences of mating systems, were begun in
1906 and were carried out for more than a dozen years with little or
no change in plan. Many other smaller and shorter-lived experiments
on this topic were conducted. Experiments with irregular inbreeding
systems or with inbreeding milder than full-sibbing were exceedingly
sketchy until Wright (1922) had developed a measure of the prob-
able Mendelian consequences of such matings, so that results could
be compared with theory.

As of now it seems that making inbred lines and using them to
produce “hybrids” for commercial use can be successful in chickens,
where the hybrid corn pattern can be followed closely. Between thirty
and forty million such “hybrid chicks” are expected to be produced
in the United States in 1951. Possibly the production and use of in-
bred lines may be commercially successful in hogs where a rotational crossbreeding plan can approximate the hybrid corn plan to some degree, although rates of reproduction and other biological differences, as well as economic ones, make it impossible to follow the hybrid corn plan exactly. At any rate, something like ten thousand "hybrid boars" will be produced in 1951 by breeders, in addition to a few hundreds by the agricultural experiment stations which are exploring the possibilities and best methods of making this procedure genuinely useful. The ultimate commercial success of the method with hogs is still in doubt. It seems less likely with less prolific animals, such as sheep and cattle, but there is enough possibility of success that some of the agricultural experiment stations are making some pilot lines and crosses with sheep and with cattle to gain firsthand experience with the problem. Whatever may be the ultimate outcome concerning the use of inbred lines in animal breeding, it seems inconceivable that by trial and error alone we could have found our way through to methods as complicated as these. The way had first to be cleared considerably by the development of genetic theory.

Selection indexes for maximizing the improvement which can be made by a given amount of selection are an old idea in a general way. Indeed every formal description of a breeder's ideal animal is to some extent a selection index in which only the individual's own characteristics are considered. A score-card with varying numbers of points assigned to the different characters is an attempt to quantize that ideal. Several attempts to make selection more quantitative and objective, using multiple correlation methods, were made between 1910 and 1930, especially in plant breeding. These had little success. The major trouble appears to have been failure to discriminate between the genetic and the phenotypic correlations between two or more characters of the same individual. The first moderately successful attempts to surmount that difficulty and use this information appear to have been those of Smith in 1937 and of Hazel in 1943. Those, and the considerable number devised since, promise to increase the efficiency of selection by at least a few per cent, although they are just beginning to be used. Perhaps more important still, the devising of a selection index highlights the present gaps in genetic
knowledge which most urgently need filling to permit animal breeding improvement to be still more rapid.

The information needed most for constructing selection indexes is, first of all, a description of the ideal and a means of measuring each character included. Second is the heritability of each character; that is, the amount of genetic and environmental variation in it. Third are the genetic and environmental correlations between the various characters. These latter often yield some surprises, as when one character turns out to be more valuable as an indicator of the environment, which ought to be discounted for effects on other characters, than it is for its own sake. In extreme cases this may even reverse the sign of the selection coefficient for that character so that sometimes one would make fastest net progress by selecting against a desired but rather unimportant and only slightly heritable character.

When the characters of sibs, parents, progeny, or other relatives are included, as if they were additional characters of this individual itself, the selection index includes also all of progeny-testing, sib-testing, and other use of pedigree. A limited example of how this works is in my paper (Lush, 1947) on family and individual selection. It is still too soon to be sure how the use of selection indexes will actually affect animal breeding practice, but the indexes worked out have already reconciled several apparently conflicting ideas.

With this development of selection indexes and related aspects of population genetics, we are becoming able to compare the efficiencies of two or more breeding plans which in part may be mutually exclusive. Resources are always limited. Compromise between merit in one character and mediocrity or defect in another is eternally necessary. Characters are neither equally heritable nor equally important. They unfold at different times of the year or at different stages in the life cycle. Some are much more cheaply measured than others and, as Harland has emphasized (1944), if the more cheaply measured ones can be observed first and the defectives in those can be discarded early, the more expensive observations need be made only on those individuals which survived the early cullings. Thereby much money may be saved, or labor and other resources turned to more productive purposes. Keeping the labor force nearly constant through-
out the year may require doing some of the breeding work at a slack season, even though it could be done a bit more accurately at some other season. For example, I once thought to examine pullets in November for whether they laid eggs with blood spots. Thus I would do some mass selection on them before ever they went into the breeding pens. But I learned that at that season and that age the work and costs of examining the eggs were entirely extra, and heritability and the incidence of blood spots in that flock were so low that the genetic gain to be had by culling the few pullets who produced blood spots in October and November was simply not at all worth the cost. Eggs laid in the stud pens during the breeding season had to be candled anyhow, so that the net cost of candling them also for blood spots was almost zero. Also the winter incidence was a bit higher, so that the effectiveness of selection against them was higher. These circumstances compelled us to do our mass selection after the pullets went into the stud pens. We changed our plans and put about one fifth more pullets in each pen, so as to be able to cull the chicks from those who laid two or more blood-spotted eggs during the breeding season and yet usually have as many chicks from each pen as we wanted. Then a slack labor season occurred on this farm in May and June. Also the incidence of blood spots was then nearly maximum for the year. Consequently during May and June we trapped and candled the eggs on some sample days in those houses which contained the sisters of the hens which had been in the stud pens and had not yet been culled. On the basis of this sib-test of their dams, the chicks were culled from many hens who themselves had not been caught laying blood-spotted eggs but whose sisters laid more than the average percentage of those. Then in August and September we made a selection index for the surviving young cockerels, using what our data indicated to be at least roughly accurate emphasis on the performance of dams, of full and half sisters of dam and of sires, and (where they were from parents more than a year old) the cockerels' own half and full sisters. Had this been a brown-shelled breed, it would have been necessary to break the egg and thus destroy it for breeding purposes. Then we would have had to omit the mass selection or at least to do it on a sampling basis, presumably after the
breeding season had ended. The costs of destroying the eggs would have led us to reduce the size of our samples, at least a little.

I mention this case only to illustrate how biological and economic circumstances pertaining to a character and perhaps peculiar only to one breeding establishment or perhaps pertaining to a whole species, may modify the breeding plan. Such operational problems rise continually. To solve them correctly requires that one estimate the probable results of each procedure and its probable costs. We are now beginning to be able to do that, although for most characters and species the present state of knowledge leaves the accuracy of these estimates rather low. We need to know more about the heritability of the various characters and more accurately their genetic and environmental correlations with each other. Moreover, as in most statistical problems, we estimate only the most probable result, with perhaps some notion of the likely variation around that average. In our actual operations with finite samples we may, of course, get results either better or worse than we had expected, even when our formulas for prediction are correct.

But in any event the quantizing of breeding plans is beginning to replace fairy tales and wishful imaginings. Careful calculations are usually more tedious and less entertaining than day-dreaming but they yield more solid results in the long run! I suppose both types of thinking are needed, somewhat as a community may need optimistic salesmen to stir up enough enthusiasm to get the citizens to vote bonds for constructing a power plant, but they need pessimistic, or at least careful, engineers to design and build that plant! Especially is there room for enthusiastic optimists among the breeders and for pessimists among their bankers in a field like animal breeding, where the chance inherent in Mendel’s law leads one to expect that his calculations will rarely turn out to be exactly true in the finitely small samples with which he works!

The possibility that overdominance and epistasis may be important makes our calculations concerning the probable results of mass selection, progeny-testing, and sib-testing less accurate. In the main these phenomena shift our thinking and our actions away from the individual and more toward the family or inbred line or some special
cross as the unit with which we work and on whose characteristics we decide what to do. Not all of the implications are yet clear. Probably they will be clarified on plants, or on prolific and short-lived laboratory animals, before they have much effect on animal breeding practice. Yet already a few workers with animals are venturing to try some pilot testing with plausible plans even as complicated as reciprocal recurrent selection.

**ACTUAL CHANGES IN THE PRODUCTIVENESS OF ANIMALS**

Figures 1 to 12 show how the average productiveness per-animal has changed during recent decades for as many important characteristics and in as many different countries as I could find data which seem dependable. The taking of adequate and unbiased data on these subjects is a distinctly modern development. A few records on individual animals and occasionally on all or most of a herd for a short time have been preserved from much earlier times, but these are almost invariably exceptional or highly selected records. It seems impossible to discount the biases accurately enough to draw from them dependable conclusions about animal productiveness in earlier times.

![Fig. 1. Average fleece weights (in pounds), by five-year periods, for all sheep in New South Wales. (Data from Dalgety's Annual Wool Review.)](image-url)
Figure 1 shows the average weight of fleece per sheep in Australia since 1881. The upward trend is unmistakable although we may argue about the reasons for it and about whether the lesser rate in the last few years indicates that a genetic limit or ceiling is being approached, or was caused by emphasis in selection shifting from fleece weight to other characters perhaps negatively correlated with that, or by deterioration in pastures, increase in parasites, or some other environmental change perhaps connected with the disastrous droughts of 1943 and 1944. The numbers are so vast and the means of estimating the total weight of the clip good enough so that even small annual changes can hardly have been wholly accidental.

![Butterfat Production in New Zealand](image)

**Fig. 2.** Average annual production of butterfat by cows in New Zealand. (From 24th Annual Report of New Zealand Dairy Board.)

Figure 2 shows the average butterfat production per cow in New Zealand since 1910. The three different lines illustrate the difficulties of defining and measuring exactly what is meant by "average production per cow." The trend is unmistakably upward. The large yearto-year variations are to be expected, I suppose, in a country where
the dairy production is made almost wholly from pasture and where the weather varies considerably from year to year. A little of the upward trend may have been due to the partial replacement of Shorthorns by Jerseys.

![Graph of Fat Percent](image)

**Fig. 3.** Average test of milk from purebred Friesian cows in The Netherlands. (From "Erfelijkheid en Fokkenj" by Dr. E. T. Roelofs, published by Van Gorcum & Comp N.V., Assen, The Netherlands.)

Figure 3 shows the average fat per cent in the milk of Friesian cows in Holland since 1906. Fat per cent is more highly hereditary than most characteristics. Its upward trend appears not to have been affected even by such extreme nutritional changes as were caused by the feed shortages in World War I.

![Graph of Production](image)

**Fig. 4.** Average production of cows in the Danish cow testing associations.
Figure 4 shows the changes in average production per cow in Denmark since 1904. Besides the general upward trend, the most striking feature is the sharp decline in milk and fat production during World Wars I and II when almost no imported feedstuffs were available. The same feed shortages caused scarcely a ripple in the trend toward higher fat percentage.

![Graph showing average production of cows in Sweden](image)

**Fig. 5.** Average production of cows in Swedish cow testing associations.

Figure 5 shows the changes in dairy production in Sweden. As in Denmark, the two wars reduced milk and fat production but affected test scarcely if at all. That the irregular year-to-year variation was larger in Sweden may plausibly be ascribed to Swedish dairying being based a bit more on pastures and less on roots and concentrates than is the Danish.

Figure 6 shows changes in the average production of the Herd Improvement Registry cows of the Holstein-Friesian breed in the United States. This is a non-selective plan within each herd, for if a man tests any of his cows, he must test them all. However, the breeds which start testing for the first time in any year may be more or less productive than those which drop out that same year. It seems pertinent to note that since about 1920 the passage of the 4 per cent milk ordinances, especially in the eastern cities, has put this breed under especially heavy economic pressure to increase the test.
Icy L. Lush

many breeders under this stress were emphasizing test, and consequently were neglecting quantity of milk, to an extent beyond what was to the best interests of either the community or themselves.

Figure 7 shows the trend in average butterfat production among cows in Iowa dairy herd improvement associations. This is unselec-
tive testing within the herd but herds which enter into it may have lower or higher average than those which drop out. The upward trend may include environmental changes as knowledge of management improved or as changing economic circumstances made it profitable to reduce or to augment the labor and other resources used in the dairy enterprise on each farm. This last would be specially pertinent in Iowa, which is a region of diversified agriculture with several choices open to most farmers concerning the enterprises into which they will throw their resources. Thus the upsurge of production in 1934 and 1935 could have been due to drought and other circumstances which made dairying the most profitable enterprise into which the farmer could channel his resources, or it could have been due to the fact that the less intensive dairymen discontinued testing during the hard times. The upsurge in 1941 may have reflected the patriotic response to appeals to increase production when the war began; while the decline which followed was the result of the shortage of farm labor and the increasing higher profit of other farm enterprises not so rigidly shackled by price control. This will illustrate that the interpretation of these trends is far from what the Germans call "eindeutig!" Nevertheless the magnitude of the trend is striking. The mean has increased nearly a third in about five generations of cattle.

Figures 8 to 10 show changes in important characteristics among pigs at the Danish testing stations already mentioned. Because management was not wholly unified until 1926, the earlier figures may not be completely comparable with each other. About 1926 the economic conditions, especially competition to hold a large share of the British market, caused breeders to change strongly the emphasis in their selections, which had been on rate and economy of gain as the major items but changed toward carcass quality as the major goal. Increasing emphasis on carcass qualities, when making the selections, naturally meant that selection for other qualities had to become less intense. The improvement in economy of gain appears to have been large up until about 1929, but modest since then. Rate of gain, which is closely correlated with economy, reached its peak at the same time and has shown but little if any improvement since 1930. By contrast,
Figure 8. Feed requirements per unit gain by pigs at the official testing stations in Denmark. (Data for Figures 8 to 10 are from Bulletin 248 and earlier reports, Agricultural Research Laboratory, Copenhagen.)

Figure 10 shows marked improvement in the three most emphasized measurements of carcass quality. That back fat and thickness of belly went simultaneously in opposite directions, but that both of these were the directions in which selection was being exerted, comes perhaps the closest of any of these nation-wide sets of data to fulfilling the requirements of a scientific selection experiment in which selec-
tion is conducted in both directions at once. Body length can be seen in the live animal clearly enough so that mass selection might have made the changes in it, but direct selection for or against thickness of fat could have been practiced only through sib and progeny tests.

![Graph showing changes in body length and fat thickness over time.](image)

**Fig. 10.** Annual averages for three important measures of carcass quality for all pigs from the official testing in Denmark.

Figure 11 shows the average production of eggs per hen in the United States. It illustrates again, as the New Zealand dairy data did, the necessity for clarity in defining and measuring what is meant by "average production." It shows even more extremely than the others that the upward trend has been much steeper in recent years.
Fig. 11. Average annual egg production per hen in the United States.
(From Bureau of Agr. Economics, U.S.D.A., August-September, 1948 and
April, 1950.)

Figure 12 summarizes the situation in the United States in the
units which the Bureau of Agricultural Economics uses for weighting
and combining the production of the various species of animals. In
30 years the production per animal unit has increased by a bit more
than 40 per cent of its initial level. Except for the drop in the early
1930's, the steadiness of that increase is impressive. Presumably that
drop was mainly caused by the nearly nation-wide and severe drought
of 1934 and its subsequent effects, although perhaps the preceding
economic conditions had lowered the farmer's incentive or ability to
care well for his animals.

We would like, of course, to be able to separate each of these
trends into its genetic and its environmental component. If these
were planned laboratory experiments, I suppose we would have tried
to do this by selecting simultaneously in opposite directions. But that
was not done in the cases shown. It seems impractical even to start
such lines now for use in similar inquiries 10, 20, or 50 years from
now. Even if we had such lines, the interpretation would offer diffi-
culties in measuring the selection differentials used and in determin-
ing the extent to which they represented direct selection for the
character concerned, or resulted indirectly from its correlation with some other, perhaps unidentified, character with which the character under observation was correlated. The best we can do in most cases is indirect and involves such things as measuring the parent-offspring regressions and the selection differentials actually achieved. It should

Fig. 12. Changes in number and productiveness of farm animal units in the United States. (From "The Agricultural Situation," U.S.D.A., May, 1950.)

be pointed out that even the undoubtedly large improvements in nutrition and sanitation which have been made may not have prevented a net decline in environment as we crowd our animals more and make other changes consequent on more intensive production.

For characters which are repeatable from one year to another, as is the production of sows or hens or dairy cows, a more promising method has been suggested recently. Net changes in environment from one year to another are measured by using animals which make
records in consecutive years. Statistically this consists of "fitting constants" for the intrinsic productivity of the groups born in various years and for the environment of each year. Precautions are necessary concerning age corrections and for discounting accurately the effects of continuing selection whereby some of the poorest producers are discarded each year. Figure 13 shows the results of this method applied to butterfat production in the Holstein-Friesian herd at Iowa State College. The straightline trend was upward at the rate of 5.9 pounds per year. The genetic component in this was 2.5 pounds or nearly half of the total.

![Graph](image)

**Fig. 13.** Average fat production (first 8 months of the lactation and corrected to mature age) of the Holstein-Friesian herd at Iowa State College, 1938 to 1949. The solid line connects the actual averages or is their straight-line trend of +5.9 lbs. per year. The broken line connects the annual averages corrected by "fitting constants" for year-to-year changes in net environment or shows the average upward straight-line trend of +2.5 lbs. per year in these corrected averages.

These particular figures do not warrant any generalizations, although we see no bias in the method by which they were derived. They are undependable because: (1) They are based on one herd, averaging about 50 to 60 milking cows, and hence may have considerable sampling error; (2) the time involved is only 12 years. This is only about two and one half generations in cattle and is much
too short an interval on which to base a dependable trend line. Yet they do agree with a conclusion drawn previously from considerations of heritability and the amount of selection reasonably possible—that a genetic improvement of about two or three pounds per year every year over long periods of time is a reasonable estimate of what one can expect a whole breed of dairy cattle to achieve. The case shown in Figure 13 is presented merely to call attention to this method which seems worth wider testing. Possibly it may contain fallacies which we do not see.

With due regard to all these qualifications, it is my opinion that a large part of these truly astounding changes made in animal productivity over the last 20 to 50 years is genetic. Also it seems that in general the rate of improvement has increased during the last 20 to 25 years. This is about the period during which genetic theory had reached the point where it could help animal breeders to make more extensive and sounder use of progeny and sib testing than they had done before. The widespread adoption of non-selective systems of impartially testing and measuring individual merit was, of course, a prerequisite. Its accomplishment is a monument to the faith of breeders in the possibilities of further breed improvement, although of course the individual breeder also usually hoped to profit financially by the advertising he would receive if his animals did in fact as well as he hoped they would.

These data give little indication that the populations concerned have come close to any biological or physical limit or ceiling for any of these characters. That explanation for the recent trend in Australian wool seems unlikely because the change in trend was so sudden. Also several other explanations are at least equally plausible and the time covered is too short to justify us in concluding that the upward trend has ceased permanently. Shifts in emphasis in selections seem adequate to explain the changes in trend for rate and economy of gain among the Danish pigs. Also the flattening of the trends for body length and for thickness of fat accord almost perfectly with the changes in selection emphasis which inevitably occurred when, in the late 1930's, the average approached the optimum for the British market and a few carcasses too long and with back fat too thin began
to appear. The other trends seem to show no real indication of slackening.

No genetic techniques are yet available for estimating the genetic limits for any characteristic in any population, unless one knows what genes are present and knows their effects and frequencies. Such knowledge we practically never have for any economically important characteristic, at least in naturally cross-fertilized species. This is in contrast to our considerable ability to forecast the rate of improvement possible in the immediate future, provided we know something about the resemblance between relatives, beyond that due to environmental similarity, and are not badly deceived by epistasis or overdominance. It is my considered judgment that in animal breeding the "endless frontier" is just as real as Vannevar Bush has expressed it to be for scientific research in general.

REQUIREMENTS FOR FUTURE RAPID PROGRESS

For progress to continue to be rapid in the future, certain things appear necessary. First among them is freedom to experiment and to exchange information. I see no prospect of that being denied us in the United States but it is a matter concerning which we should never relax our vigilance. In many other lands, it has already become illegal for a farmer or breeder to use a bull or boar which has not been approved by some official or inspecting committee. This is done, of course, in the name of "the public good," although we are not told explicitly how infallible is the judgment of those who decide what is for the public good. Phillips has explained (1945) the principle in terms clear to every biologist. None should know better than biologists that no inspector or committee of inspectors is infallible in such matters. The correlation between their judgment and the actual facts is certainly low, although probably usually positive. Yet even biologists have at times approved or even voted to extend to such inspectors authority which could be fully justified only by imputing infallibility to them. Some of the individual's freedom to experiment is gone. In some of these cases, although perhaps only in a minority, society has thus itself closed the door on a road which
would have led to a richer food supply for it. Fortunately in the United States we have had practically none of this, except in the cases of the stallion registry laws of some states. In some of these the camel's nose was already under the tent but the declining interest in horses had much the same effect as if the camel had died before it could get farther.

Secondly, wherever organized research is necessary we need truly independent replication of it. Here again we have been unusually fortunate in the United States. Our state agricultural experiment stations have been independent enough of the United States Department of Agriculture and the latter has made such wise use of its power over the state stations (for example the considerable influence of the Office of Experiment Stations) that rarely has there been a time or a case in which one or a few men with certain views could block the independent testing of those views by others who were perhaps eager to prove them wrong. Perhaps there is such a thing as waste of resources through too much replication of experiments, but the waste of resources can be even greater if we wrongly accept as fact conclusions which would have been rejected if the experiments had been repeated independently once or twice. In this day of coordination and organization we are in a bit more danger of losing this requisite for rapid further progress than we are of losing the first one, but we are not in nearly as much danger as are our fellow workers in many other countries where the national government is not so limited in power as ours, and where a single institution is sometimes designated to receive all the support for research in a given field. The legislators and administrators may have been so oversold on the magic of research that they underestimate the fallibility of the researchers!

A third requirement is that the research worker or his colleagues carry his findings through to their commercial utilization. A few people still imply that so-called "fundamental" research is in some way more honorable or more difficult than applied research. Most of this can written off as evidence of their unfamiliarity with applied research. Properly done, the two fields (if there really is any sense to a distinction between them) feed ideas and problems back and
forth to each other. Some contact with applications will give the 
worker on fundamentals a better balanced judgment about what 
problems really are important. After all, he cannot work on every-
thing. If other things are nearly equal, he would usually choose to 
work on the problem which seemed to have the most possibilities of 
expanding in importance. And some contact with fundamental 
research may help the men on the applied side remember that un-
expectedly useful results often turn up in surprising places and that 
you can rarely be sure of what is on the other side of a door until 
you open it!

Presumably most of the research in methods and principles of 
animal breeding will be done at public institutions, but private indi-
viduals or corporations will do most of the research on details of 
final applications. Public servants can rarely indulge in the enthusi-
astic salesmanship necessary to promote an enterprise commercially, 
and yet retain the judicial temperament and impartial scepticism 
necessary for doing sound research. Moreover, self-interest remains a 
powerful incentive to good work. Also a multitude of researchers, 
even when individually less efficient, may be more apt somewhere 
among them to hit the jackpot in applied research, where the alter-
natives are often so many, than a small number of better organized 
and trained research units. An intermediate stage which promises to 
become more important is the research done by the breed associa-
tions. Several of these already have their research committees and a 
few have even hired full-time men specifically for research on their 
problems.

CONTRIBUTIONS OF ANIMAL BREEDING TO GENETIC 
TECHNIQUES AND OUTLOOK

The techniques found useful in animal breeding research come 
mostly from the genetics laboratory. But designed experiments with 
farm animals are always expensive in money and are often frightfully 
expensive in the amount of time required to see them through. To the 
extent that this prevents such experiments, animal breeding re-
search and research in human genetics are much alike. The major
differences between them in analyzing non-experimental data are in the abundance of the material, the rarity or abundance of full sibs as compared with half sibs, the degree of prior selection (which has often been more intense with animal material and is a major pitfall), the completeness of the information on each individual, and the extent to which environmental differences may be confounded with genetic differences.

It was not until late in the 1930's that animal breeding had reached the point where there was much need for special adaptations of the standard methods in experimental designs. Dickerson's paper (1942) on designs for testing inbred lines of swine, or the paper by Dickerson and Hazel (1944) on balancing the increased accuracy of the progeny test against its undesirable effect in lengthening the generation interval may be cited as examples of this stage. As compared with plant breeders, animal breeders may need to design their experiments more intricately and some are already calculating their results more exhaustively in order to wring from them the last possible bit of information. No difference in principle is involved; it is simply a matter of deploying most efficiently the always limited resources for research. In plant breeding one can sometimes learn more by throwing a whole barrage of experiments around a question than by spending the same amount of money and time in refining the plans and in exhaustive analysis of the results. The greater costs per animal than per plant make this rarely true in animal breeding, although the situation in this respect varies widely with the species. For example, chickens or in some cases even pigs may conform more closely to what I have just said about plants than do tree crops such as apples, rubber, and tea.

Animal breeding research may have some far-reaching cultural effects on our thinking about human problems because the techniques and problems are partially similar. For example, the genetics of differences between breeds of animals has many points of similarity with the genetics of differences between races of man. The fantastic reverence for purity of breeding still found sometimes among animal breeders can be matched by equally fantastic notions concerning racial homozygosity in man. At the opposite extreme it is occasionally
said even yet that the breeds of farm animals differ only in unimportant and superficial details of color, shape of head, set of horns, etc. These assertions can be matched by many a casual implication or explicit assertion that differences between the races of man are only a matter of skin color, or shape of hair, or “accident of birth,” whatever that may mean! Because of the lower emotional voltage of the problem in animals, we can find the facts of the case more objectively there. Moreover, the real although limited possibilities of experimental work sometimes permit an experimental test in animals. The general occurrence of heterosis in breed crosses is of itself enough to prove that the breeds generally differ genetically in many other respects besides their superficial trademarks, whatever the true genetic explanation of that heterosis may be.

With the animals we have long since given up the idea that the breeds could be arranged in a single hierarchy from best to worst. Instead, each has its advantages and disadvantages which vary from one ecological niche to another. At a given moment this comes close to an experimental demonstration of what Haldane (1946) has deduced (on logical grounds and the currently known laws of genetics) must be the case in man. Likewise, when we look at the animal picture over periods of a half century or longer, we see unmistakable shifts in the numerical proportions of the various breeds, the occasional rise of a new breed out of a more or less complex cross, and the occasional splitting of an existing breed into two or more. Frequently we see signs that a breed is about to split into several fragments but these are eventually reconciled and united again into a somewhat freely interbreeding population so that the breeding structure is what Wright has called “reticulate.” In fact we see phenomena parallel with almost everything that the students of modern population trends or of physical anthropology have found about the races of man. Sometimes the causes of shifts in number are plain to see in changed ecological circumstances which have altered the relative economic advantages and disadvantages of the breeds; sometimes they appear to be wholly accidental. These latter are often accidents of salesmanship or conflicts of personality among the leading breeders or promoters. Accidents and genuine changes in
the selection coefficients both have their counterpart in man and in
nature.

In optimistic moments I think that this flood of parallel light
must soon clarify some of our social conflicts and dispel at least the
more extreme racial nonsense of both kinds. When more pessimistic,
I doubt that man is to any marked degree a logical animal, except
possibly in his abstract thinking, and I question whether we will let
ourselves learn anything about man from studying his animals, un-
less the conclusion be already personally pleasant to us.

A scientific discovery is not usually accepted, even by fellow
scientists, for several years. I suppose we should be glad that this is
so, because the first interpretations are so often wrong, at least in
part. Yet this lag in acceptance often seems wastefully and even
tragically long. For example, to anyone familiar with animals it is
well-nigh impossible to understand the reluctance of some psycholo-
gists to study or even to admit the role of heredity in innate mental
differences between individuals in the same family or strain. Even
our grandfathers knew well that such things played a large part in
animal behavior and that many of these mental differences were
hereditary in the sense that they "ran in families" more than could
possibly be explained as caused entirely by cultural transfer. What
possible reason can there be for our reluctance to accept the same
conclusion for man or even to study it in human beings, except that
we feel instinctively that some of the consequences would be per-
sonally repugnant to us? When optimistic, I think perhaps this
situation is our own fault and that if large numbers of us who know
animals had gone to work in earnest on animal psychology 30 or
more years ago, the mass of facts would have carried conviction long
ago, and the genetic aspects of psychology in man would not have
been as backward as they are. But when I think on how much was

known over 30 years ago about differences in individual animal
ability, in dogs and horses especially, and when I reflect on how little
effect such work on man as that of Terman and of Burks appears to
have had, or when I hear and read again and again nearly every one
of the common fallacies against which Jennings warned us so clearly
more than 20 years ago in his _Biological Basis of Human Nature_.

"
(1930), I grow pessimistic again and think that perhaps we never will adopt as valid any conclusion concerning man unless it already be personally pleasing to us! So perhaps I greatly exaggerate the net effect which research in animal breeding has had or ever can have on our ideas concerning man.

REFERENCES


THE purpose of this paper shall be to indicate the contributions of genetics to plant pathology during the past 50 years. As a basis for such an analysis it is pertinent at the outset to summarize the status of plant pathology at the turn of the twentieth century and to indicate briefly its points of contact with plant breeding during the previous century.

The first half of the nineteenth century was a period of turmoil in plant pathology in which two schools of thought on causation of disease in plants debated at length. The age old concept of spontaneous origin of microorganisms still held sway and parallel with it the autogenetic origin of disease was generally accepted. Environmental factors were the causal ones and the microorganisms which had been named and arranged by mycologists for decades were generally regarded as the products of disease which arise spontaneously in lesions of the host. Targioni-Tozzetti had suggested in 1767 that rust of grain and smut of wheat were instigated by parasitic microorganisms. No experimental proof was offered, however, until the publication of Prevost in 1807. This contribution, furthermore, lay refuted and generally unnoticed for another 45 years. The challenge of the autogenetic theory was not made again seriously until the work of De Bary (1853). Thus approximately 50 years before the founding of genetics, modern concepts of
plant disease began to receive more general acceptance and 1853 is often taken as the beginning of modern plant pathology.

The major point of contact between plant pathology and genetics has to do with resistance of plants to pathogenic agencies and the mode of inheritance of resistance and pathogenicity. The observation that cultivated varieties sometimes differ in their reaction to disease goes back as far as Theophrastus (371-286 B.C.) (Hort, 1916). We know little about what use was made of the fact in the amelioration of crop plants in succeeding centuries, but probably many varieties were discarded empirically because of extreme susceptibility to one or another disease. Occasional records indicate that plant breeders not uncommonly gave attention to disease resistance. Thomas Andrew Knight, well-known English plant breeder in the first half of the nineteenth century, noted differences in resistance of wheat varieties to rust. In 1853, Anderson, also in England, noted that certain varieties of turnip were more resistant than others to clubroot (Plasmodiophora brassicae Wor.) In 1851, Berkeley in the original description of the smudge disease [Colletotrichum circinans (Berk.) Vogl.] of onion recorded that white bulb varieties were seriously affected while colored varieties growing alongside were free from disease.

With the relatively rapid acceptance of the parasitic nature of many diseases after the middle of the nineteenth century it might be expected that more attention would have been given to resistance. However, modern plant pathology became engrossed primarily for its first 50 years in other interests. The discovery, naming, and classification of new pathogenic fungi were paramount. The role of bacteria as plant pathogens was not suggested until 1878 and its validity was debated until 1900. Not until 1900 were plant viruses recognized fully as distinct, transmissible, pathogenic agencies which did not fit in with the then recognized orbit of the microorganism.

The impact of the potato late blight [Phytophthora infestans (Mont.) De Bary] epidemics of the 1840's and the growing importance of the degeneration complex, now regarded as the accumulation of tuber-borne viruses within vegetative clones, stimulated a new interest in potato breeding beginning soon after 1850. It was Chauncey Goodrich of Utica, New York, who in 1851 obtained from South America
a variety named Rough Purple Chile. From it was derived Early Rose and Beauty of Hebron. In 1877, Charles Darwin became interested in the possibility of breeding for late-blight resistance and gave encouragement and some financial aid to private breeders in England and Ireland. In fact in 1876 there had been introduced in England a new potato variety, Magnum Bonum, derived from a cross between Goodrich's variety Early Rose and an English variety Victoria. Magnum Bonum had marked tolerance to late blight and it remained in high favor until about 1890, when its resistance appeared to decline.

In 1878 the downy mildew [Plasmopara viticola (Berk. & Curt.) Berl. & DeT.] of grape was first noted in Europe by Millardet (1885) who found it near Bordeaux in a nursery of seedlings which had been imported from the United States. This disease remains a relatively unimportant one on native American species but the wine grapes of Europe proved to be very susceptible and the environment very favorable. American seedlings were at that time being imported because they were known to be resistant to the Phylloxera root louse. Although the root gall could be controlled by using American seedlings as root stocks this did not take care of the mildew. Millardet and others initiated at this time hybridization programs for the transfer of mildew resistance to European varieties. Although this program has been carried on more or less continuously since, no horticulturally satisfactory highly resistant varieties are yet available. Perhaps the impetus for breeding of mildew resistant grapes and late blight resistant potatoes was slackened somewhat by the discovery of Bordeaux mixture and its prompt adoption between 1882 and 1885. This fungicide happened to be highly effective against both diseases. Forty years of frustration in the control of late blight now seemed to be over and the fear of another such struggle with the impending calamity in the grape industry was alleviated. It is a matter of historical significance to the subject we are discussing that what appears to have been a good start in the application of plant breeding to plant disease control slowed down. While it is customary to attribute the slow-down to the rise of Bordeaux mixture I shall attempt to bring out later that it is more likely that the chief reason was that neither plant pathology, nor plant breeding pos-
sessed the information upon which to build a program which was basically sound.

During the last decade of the nineteenth century the growing importance of the cereal rusts generally was responsible for the focusing of the attention of plant breeders upon differences in reaction of varieties to one or another rust. European breeders were giving especial attention to yellow rust [Puccinia glumarum (Schm.) Erikss. & Henn.]. In the United States and Canada black stem rust (Puccinia graminis Pers.) of wheat was the center of interest. The wheat crop migrated with the pioneers and Puccinia graminis and the dooryard barberry followed along. By 1890 black stem rust was serious in Australia where Cobb and Farrer (Cobb, 1890, 1892) evaluated many varieties for resistance and carried out the first basic studies on the interaction of resistant and susceptible varieties to this pathogen.

MENDELIAN SEGREGATION OF DISEASE RESISTANCE

With the rediscovery of Mendel's laws at the turn of the twentieth century genetics began to furnish a scientific basis for the interpretation of the inheritance of plant characters. It is a significant coincidence that at this very time Biffen at Cambridge University had made crosses between Rivet, a wheat variety long used because of its high resistance to yellow rust, and the varieties Michigan Bronze and Red King which were very susceptible. In 1905 Biffen published an epochal paper in which he applied Mendel's law to his results. He found that the $F_2$ progenies from Rivet x Michigan Bronze and Rivet x Red King segregated at the ratio of 3 susceptible to 1 resistant while the $F_2$ families could be grouped into approximately one fourth true-breeding resistant lines, one fourth true-breeding susceptible lines and one half segregating lines. He thus concluded that resistance to yellow rust appeared to behave as a simple Mendelian character.

It is interesting to note the scepticism with which this conclusion was received by plant pathologists. Butler (1905) in India immediately countered to state that resistance in wheat to a given rust applies only to a particular variety in a particular locality and does not necessarily hold when the variety is transferred to another locality. Evans (1911)
in South Africa carried on studies of resistance to black stem rust. He used as one parent Bob's Rust Proof, a variety from Australia which remained consistently free from black stem rust at Pretoria although it rusted badly in some other parts of the Transvaal. A local variety, Wal Koren, was used as the susceptible parent. Susceptibility was dominant in the F₁, and, in fact, the hybrid plants were more severely affected than those of the susceptible parent line. Furthermore, the rust fungus from the hybrid plants was infectious to the resistant parent while rust from plants of the susceptible parent plant was not. This was interpreted as showing that the hybrid plants acted as a bridging host between susceptible and resistant varieties in accordance with the bridging-host theory of Ward (1902) which carried considerable weight at that time but was later discarded for the most part by rust pathologists. As a result of his experiments Evans was inclined to disparage the practical value of breeding for resistance since he believed that resistance would decline in new varieties as rapidly as they could be synthesized. Bitten was not discouraged by Evans' data, for in his experience with varieties and breeding lines of wheat resistant to yellow rust in England the resistant character remained stable for numerous generations. He pointed to the fact that Rivet, one of the oldest varieties in cultivation, was still highly resistant to yellow rust (Bitten, 1912).

While scientists in the British Empire were feeling their way towards the all-important problems of cereal rusts, advances along somewhat different lines were being made in the United States. During the first dozen years of the twentieth century the Fusarium wilt diseases of several annual crop plants came into prominence. Erwin F. Smith had pointed out in 1899 that several hitherto unstudied diseases of such crops as cotton, cowpea, watermelon, and cabbage were the result of infection of the root system by soil-inhabiting fusaria which multiplied in the vascular system of the plant concerned, resulting in stunting, chlorosis, vascular discoloration, and wilt.

W. A. Orton was detailed to a study of the wilt diseases of cotton, cowpea, and watermelon in southeastern states in 1899. He found the sea-island cotton industry fading as a result of the accumulation of the wilt pathogen in the coastal sandy soils. He also noticed that occasional
individuals withstood the disease. By selection within agronomic vari-
eties he built up resistant lines relatively rapidly (Orton, 1909). The
work initiated by Orton at the dawn of modern genetics has continued
up to the present and many commercial varieties used throughout
the world carry some of the germ plasm assembled at that time. In the
case of watermelon commercial varieties were generally susceptible and
opportunity for selection within horticultural stocks was not afforded.
The African citron, a member of the same species, was, however, found
to be highly resistant and when used as a parent in hybridization it
furnished germ plasm which incorporated resistance into the variety
Conqueror introduced about 1907. Some currently used wilt-resistant
watermelons probably derive their resistance from this source of germ
plasm. In the self-pollinated crop, cowpea, Orton found that most
varieties were uniformly susceptible, while one, Iron, was uniformly
highly resistant, a fact which is not surprising in the light of present
day knowledge. Other important advances in wilt resistance followed
promptly with the development of wilt resistant flax by Bolley (1905)
in North Dakota, wilt resistant tomato by Essary (1912) in Tennessee,
and yellows resistant cabbage by Jones and Gilman (1915) in Wis-
consin. In each of these studies of resistance remarkably rapid improve-
ment was secured by selection of resistant survivors within horticul-
tural or agronomic varieties, except in watermelon where an outcross
was first made. Little or no attention was given by these workers to
the nature of the inheritance of the resistant character. The fact that
the character was inherited was not questioned but it was not claimed
that it behaved as a unit character in any of the cases. Furthermore it
was implied by direct inference or otherwise that resistance was not
completely fixed, that continuous selection was advisable, and that
environment might be expected to influence the degree of resistance
in any one situation as compared with another. It is worthy of note
that some 40 years later some of the varieties are still used com-
mercially as a means of disease control while the germ plasm of other
is to be found in more recent varieties which represent improvement
in horticultural or agronomic characteristics.

While by 1910 a distinct start in wilt resistance had been made and
confidence in stability of resistance was accepted by pathologists (but
not without the doubts of sceptics), much confusion remained as to
the stability and inheritance of rust resistance. The point was that
varieties resistant in one locality succumbed in another and resistance
varied from season to season. The reasons for this are now obvious 
but it is important to emphasize here that where geneticists had not 
entered the picture (that is, in wilt diseases) resistance worked, while 
where they had endeavored to give a genetic interpretation (that is, 
in rusts) their interpretations failed to hold water. Forty years ago 
little stock was placed in disease resistance in most quarters. Today 
it is known to do marvels and is casually expected to do many more 
and sometimes to accomplish what appears to be the impossible. 
Forty years ago resistance was regarded by many as the play of en-
vironment, a mirage which would appear and disappear as environ-
mental complexes do. What has brought about the change? I shall 
try to show that the infiltration of pure genetics has had most to do 
with the change.

The contributions of genetics to plant pathology and thereby to 
plant disease control since 1910 can be divided into three major 
categories: (1) the variability of microorganisms and particularly 
pathogenic organisms; (2) inheritance of host resistance to the path-
ogen; (3) the influence of environment on the expression of the re-
sistant character. I shall discuss the above topics in the order given.

VARIABILITY IN PATHOGENICITY OF MICROORGANISMS

The first clear evidence that there exists within a given morphologic 
species of microorganism strains which differ in pathogenicity was 
that presented by Eriksson (1894) in Sweden in a series of papers on 
Puccinia graminis Pers. and other cereal rusts. He showed, first with 
black stem rust, that the organism collected from wheat did not 
infect rye and oats and certain other hosts, while collections from 
other host plants showed other ranges of pathogenicity. These so-
called strains were practically identical in morphology and infected 
the same alternate host, but they differed in their physiological char-
acters as expressed in their specific pathogenic properties. Eriksson 
declared several subspecies of P. graminis (for example P. graminis
tritici) largely on the basis of the genera of the commercial cereals to which they were specialized.

A few years later Ward (1902) in England in a study of the brome rusts found a similar phenomenon in that strains of the brome rust fungus were specialized to certain species of Bromus or to groups of species. He recognized that there were certain host species which were infected by more than one strain, some strains being more virulent than others on such an intermediate host. He believed that he had sufficient evidence to show that, when a given strain was grown for a sufficient number of vegetative generations on a non-congenial host, its pathogenicity was changed to the extent that it would infect a third previously completely resistant host species. This intermediate host species was designated by Ward as a bridging host. This theory, known as the bridging-host theory, assumes a certain plasticity of the pathogen which is influenced by the host substrate in such a way as to change its selective pathogenicity. This theory was proposed by Ward at Cambridge University at approximately the same time as his colleague Biffen proposed that the rust resistance of Rivet wheat was a Mendelian unit character. Ward's theory was seized upon by his student Evans in South Africa to explain his results and to refute Biffen. The conflict in theories had a great deal to do in the next decade with retarding progress, with frightening young investigators into other lines of emphasis, and with encouraging the old school of breeders who resisted the inevitable inroads of Mendelism.

The first rigid test of the bridging-host theory was carried out by Stakman in the United States with Puccinia graminis. In 1914 he published the results of a painstaking study with the subspecies of Eriksson on congenial and uncongenial hosts without securing any tangible evidence whatsoever that the host substrate had any measurable effect upon the selective pathogenicity of the race concerned. Following this the bridging-host theory was discarded generally by rust workers, although the subject of the influence of the host on the pathogen is by no means a closed one.

Another step in the field of variability in pathogenic organisms was the distinction of strains of the bean anthracnose organism on the basis of selective pathogenicity on horticultural varieties within
Phaseolus vulgaris (Barus, 1911). This is the first instance on record in which distinct physiologic races within a morphologic species of a pathogen were defined on the basis of horticultural varieties of a single host species rather than on the basis of differences between host species or genera. In 1917 Stakman and Piemeisel and, in the following year, Melchers and Parker (1918) reported, from independent investigations that collections of Puccinia graminis tritici from Triticum durum L. and Triticum vulgare L. were not all alike pathogenetically. This was the first evidence that the subspecies of Eriksson were not pathogenetically homogeneous. Further work by Stakman and associates rapidly built up evidence that many strains exist within each of the subspecies of Puccinia graminis. Plant pathologists increasingly recognized variability in pathogenicity of microorganisms not only in the rusts but generally among those which can be grown in pure culture. This situation added more confusion to the problem of developing disease resistant varieties and cast more doubt on its permanence and validity. Eventually, although tardily, genetics stepped in to seek fundamental facts which might add stability.

For the most part pathogenic fungi in their parasitic stages are haploid. The thallus may be uninucleate or multinucleate. In the Basidiomycetes it is usually binucleate and in this class it is sometimes referred to as diploid although this is not strictly the case. I will discuss this matter in more detail later. While many fungi are self-compatible and inbreed regularly, many are strictly heterothallic and only intercompatible lines will mate. Heterothallic species offer a tool whereby the inheritance of characters can be studied by genetic methods. It is significant to point out, however, that although heterothallism was first reported in the fungi by Blakeslee in 1904 and in the Hymenomycetes by Bensaude in 1918, there was no attempt by either of these workers to study the inheritance of specific characters. Heterothallism in the Ascomycetes was reported by Dodge in 1927 and that in the rusts was established by Craigie (1927) in the same year. We are indebted to Lindegren (1932, 1934), working in T. H. Morgan’s laboratory, for the first intensive study of genetics in the fungi. His work with Neutospora set a pattern which made it clear that fungi may be used effectively in the study of theoretical
genetics. The expansion of this work by others need not be men-
tioned in detail here. The work of Johnson and associates (Johnson
and Newton, 1946) on black stem rust and by Stakman and asso-
ciates (Christensen and Rodenhiser, 1940) on the smut fungi had
more immediate interest for disease resistance. For the first time
genetic tools were used to delve into the fundamental nature of vari-
ability in pathogenicity of microorganisms.

The long-cycle, dioecious rust such as Puccinia graminis is a par-
ticularly interesting organism from the standpoint of the inheritance
of pathogenicity. Some 200 physiologic races of P. graminis tritici
have been described which differ in their effect on a dozen varieties
of Triticum. Each race is defined on the basis of the type of host-
parasitic reaction which follows when it penetrates cereal plants of
these 12 varieties. In other words races differ only in the resistance
or susceptibility of host varieties to them. When the fungus is attack-
ing the cereal host the thallus is dicaryotic, that is, each cell contains
two haploid nuclei which originated in distinct compatible haploid
uninucleate thalli in the alternate barberry host. The true diploid in
the rust consists only of the teliospore in which as it matures the two
conjugate nuclei fuse. Reduction occurs in the next division of the
nucleus as the teliospore germinates. Only when the thallus of the
black stem rust fungus is binucleate will it attack the monocots,
cereals and grasses; only when it is uninucleate will it attack the
dicots, barberry and mahonia. Other cases of heterocaryosis in fungi
have received much attention when discovered. This extremely im-
portant case has generally passed unnoticed.

The work of Johnson and Newton (1946) at Winnipeg has laid a
basis for an understanding of the extreme variation in pathogenicity
of the rusts. While segregation of genes in Puccinia graminis takes
place in germination of the teliospore and their recombination occurs
in the maturing teliospore of the next generation, the step important
to pathogenicity and to cereal host resistance is when haploids mate
in the barberry leaf. The combined action of the two haploid nuclei
in the dicaryotic thallus determines the type of pathogenicity. John-
son and Newton have shown that collections of a so-called physiologic
race are not necessarily homogeneous genotypically but fall within
the confines of a given race because they are similar phenotypically.
Thus a given race, as now defined, is a group of biotypes which
happen to resemble one another only in the manner in which 12
selected host varieties react to them.

The immediate importance of these findings has to do with the
breeding of rust-resistant cereals and grasses. It shows that physiologic
races are rather highly stable and that new races appear largely as a
result of recombination of haplonts on the barberry. It shows further
that while so-called races are in fact collections of biotypes which
react similarly on 12 selected varieties, the biotypes may be expected
to react differently from each other when a longer list of varieties is
used. Specifically the important difference between subrace 15A
and subrace 15B when inoculated on new varieties of wheat now has a
fundamental basis of explanation. The long range importance of John-
son and Newton's work is that pathogenicity is a well-defined Men-
delian character.

In the smut fungi, also Basidiomycetes, a similar general pattern
occurs. Heterocercism is absent but in many cases fusion of compatible
haploid thalli to form dicaryotic mycelia is essential before pathogenic
action proceeds. Pathogenicity is controlled by specific genes, and
physiologic races insofar as pathogenicity is concerned depend upon
segregation and recombination in the sexual phase. As in rust fungi,
definite physiologic races can therefore be defined on the basis of
host variety reaction. These genetic facts, while they do not reduce
the complexity of breeding for smut resistance, eliminate confusion
and provide a basis whereupon definite progress is being made.

In the Ascomycetes the parasitic thallus is usually haploid and
monocaryotic. It may be consistently uninucleate as in Venturia or
multinucleate as in Sclerotinia. In heterothallic species inheritance
of characters can be studied as shown by Lindegren (Lindegren,
1932, 1934). Many such characters have been defined in Neurospora,
Glomerella, and Venturia. Two pathogenic characters in Venturia
have been shown by Keitt and associates to be inherited as inde-
pendent Mendelian single-gene units (Keitt, Leben and Shay, 1948).
Inheritance of Host Resistance to the Pathogen

The interaction of host and parasite makes up an extremely diverse set of phenomena. Pathogens relate themselves to their hosts in a variety of ways. It is apparent, however, that microorganisms within species and groups of species, genera or families commonly follow a set pattern. For instance, most dicaryotic rust thalli invade by way of stomata and remain intercellular except for characteristic intracellular haustoria; most monocaryotic rust thalli penetrate the host directly through the cuticle and are primarily intracellular for some time following penetration. Smut thalli penetrate directly in young tissue and progress for the most part intercellularly after they become established. Many types of host reaction occur, but again widely different host species tend to react in a similar pattern to a closely related group of pathogens. For instance, the range of reaction of resistant and susceptible varieties of wheat to various races of Puccinia graminis tritici has a close resemblance to the range between bean varieties to the dicaryotic stage of Uromyces phaseoli typica Arth., or flax varieties to the dicaryotic stage of Melampsora lini (Pers.) Lév.

Various types of host resistance have been studied. Some are cases of exclusion of the pathogen by superficial morphological or chemical barriers. Most cases are those of various degrees and manifestations of incompatibility of the pathogen with its host substrate. The relation of a given pathogen to its natural host substrate in most cases, then, follows a definite pattern. The relation of the same pathogen to a resistant variety of the host species is usually a fairly definite departure from the pattern with a susceptible variety. When this interrelation is understood, it has been shown in many cases, since Biffen’s pronouncement of 1905, to be a definite Mendelian character. The expression of the character may be influenced by environment and host nutrition as are many other plant characters, a matter to be discussed in the next section.
Resistance due to single-gene effects

Soon after Barrus defined the two races, alpha and beta, of the bean anthracnose organism, McRostie (1919) demonstrated that resistance to each was inherited as a distinct unit character. When Burkholder (1923) described the gamma race of the same organism, he also showed that resistance to it was another unit character. In general, high resistance in wheat varieties to a given race of the black stem rust fungus often behaves as a unit character. There are numerous cases now well established in which resistance and susceptibility to a race or species of the pathogen are controlled by single-gene pairs. Among the list are:

1. One type of resistance to cabbage yellows [Fusarium oxysporum f. conglutinans (Wr.) Snyder & Hansen] (Walker, 1930);
2. Pea wilt [F. oxysporum f. pisi (Linford) race 1 Snyder and Hansen] (Wade, 1929);
3. Near-wilt of pea [F. oxysporum f. lini (Linford) race 2 Snyder & Hansen] (Hare, Walker and Delwiche, 1949);
4. One type of resistance to tomato wilt [F. oxysporum f. lycopersici (Sacc.) Snyder and Hansen] (Bohn and Tucker, 1940);
5. Cucumber scab [Cladosporium cucumerinum Ell. & Arth.] (Walker, 1950), and many others.

It is to be noted that Mendelian inheritance is most readily demonstrable when the strain of the pathogen concerned is stable pathogenetically. If the pathogen is a mixture of pathogenic races the Mendelian pattern is obscured because, as a rule, resistance to different races is controlled by different genes in the host. By careful analysis of inheritance of resistance to many races of a given pathogen, as in flax rust (Flor, 1946, 1947), it has been proved beyond doubt that what appears on the surface to be a very complex situation may be resolved into a true case of Mendelian inheritance.

When single gene pairs control resistance and a single race of the pathogen occurs, as is not uncommonly the case in nature, host populations commonly fall into two readily distinguished resistant and susceptible classes. The same phenotypic character may be controlled.
by distinct gene pairs. The best example is illustrated by the work of Briggs and associates (Briggs and Stanford, 1939; Favret, 1949; Stanford and Briggs, 1940) with powdery mildew [Erysiphe graminis hordei (DC.) Marchal] of barley. They found in various varieties nine distinct genes, located at distinct loci, each of which controls the same phenotypic expression of complete resistance to race 3 of the pathogen.

Resistance due to multiple genes

Resistance in some cases depends upon the cumulative action of two or more genes. Knight and Clouston (1939-1948) reported four gene pairs which contribute to resistance to angular leaf spot (Xanthomonas malvacearum E. F. Sm.) in cotton. Two of these, B1 and B2, are cumulative in effect; B3 is linked with B2 and is additive; B4 is independent and has an additive effect with B2 and B3.

Resistance to black wart [Synchytrium endobioticum (Schilb.) Perc.] of potato has been interpreted by Black (1935) as the result of cumulative interaction of three gene pairs. Resistance to late blight [Phytophthora infestans (Mont.) De Bary] as found in wild species of Solanum is according to the suggestion of Black (1935) dependent upon two major gene pairs supplemented by several modifying genes.

There are other cases in which resistance behaves as a quantitative character and undoubtedly large numbers of genes are concerned. In these cases discontinuous classes of resistant and susceptible plants do not occur. Examples are found in resistance to root rot [Thielaviopsis basicola (Berk.) Ferr.] of tobacco (Johnson, 1930); to cucumber mosaic (Shiffris, Meyers and Chupp, 1942); to Type B resistance to cabbage yellows (Anderson, 1933; Blank, 1937); and to watermelon wilt [Fusarium oxysporum f. niveum (E. F. Sm.) Snyder & Hansen] (Bennett, 1936). It is not unexpected that resistance of this type is less readily fixed than single-gene resistance and is as a rule more responsive to environment than the latter.

Gene interaction in host resistance

The interaction of genes in their effect upon the host-parasite relation has received some study but in the main this is largely an
unworked field. In this connection I will review two cases, that of onion smudge resistance and that of bean mosaic resistance.

Resistance to onion smudge is a case of chemical exclusion of the pathogen. Penetration normally occurs first on the dry, dead outer scale on the cheek of the onion bulb. In the scales of resistant (red or yellow) bulbs there occur catechol and its acid protocatechuic acid, both of which are toxic to the pathogen. They are water-soluble and diffuse into the infection drop on the surface and kill the spores or inhibit infection. White (susceptible) varieties do not contain these substances. While the phenols are colorless, they are closely associated with bulb pigments and their chemical structures show that they are related to quercetin, an insoluble yellow pigment found in red and yellow scales. They may be building blocks or degradation products of quercetin, which, being insoluble, is harmless to the fungus. (Angell, Walker, and Link, 1930; Link and Walker, 1933.)

Although the living fleshy scales of yellow and red bulbs contain pigment in the outer epidermal cells and possibly the phenols, there is no exclusion of the pathogen from these scales if the outer dry scales are removed. This shows that the host-parasite interaction, insofar as resistance is concerned, is primarily a surface phenomenon. Compared with most host-parasite interactions it is a very simple one and therefore should not be taken as representative of the usual cases. It is in fact unique in that resistance may be tied to two relatively simple toxic materials in the host. It should be emphasized that these materials are not specific and have been shown to contribute resistance of colored varieties to five other pathogens of Allium.

The studies of Rieman (1931) and of Clarke, Jones and Little (1944) have provided a genetic basis for explanation of resistance to smudge. Three distinct gene pairs are concerned. $R$ is the gene for red color and its recessive allele $r$ is the gene for yellow. When either of these is allowed to express color, resistance occurs. Either the same gene controls color, as well as the colorless phenols which are responsible for resistance, or the gene for color and that for phenols are closely linked. Breeders have so far failed to break the linkage. The epistatic gene $C$ is necessary for expression of color and
for formation of phenols. If the recessive allele c is present in homozygous condition no color forms and the plant is susceptible. When R or r is accompanied by CC or Cc a third gene pair II may interfere with resistance. I in the homozygous condition completely inhibits color. It likewise inhibits the phenols and prevents expression of resistance. As a matter of fact most white varieties in commerce contain the genes for red or yellow color, and thus the genes for resistance, but they are completely suppressed by I. However, I is incompletely dominant over its allele i and when in the heterozygous condition in the presence of R and C color is intermediate (pink), while in the presence of r and C color is cream yellow. A critical study of the effect of I on expression of resistance and susceptibility, during several seasons where the play of environment was such as to shift the disease index of resistant and susceptible parents, showed that the effect of the II pair in heterozygous plants was to maintain a level of resistance as measured by disease index, which was close to the mean between indices of resistant and susceptible parents (Jones, et al, 1946). In other words intensity of resistance followed closely intensity of color.

Study of the nature and inheritance of resistance has in some instances been used to delineate and evaluate the interaction of two types of host resistance. One of these has been recently brought out in the study of onion smudge. It has long been known that volatile sulfides in the fleshy onion scales are fungistatic as well as the phenols in the outer colored scales. They are not in any way related to color and are not easy to evaluate. As indicated above, resistance to smudge is primarily a surface phenomenon and the pathogen enters the fleshy scales in spite of the presence of fungistatic volatile sulfides. Until recently it has been impossible to evaluate this possible secondary resistant factor. In the study of intermediate color and intermediate resistant progenies, that is, rr CC II, it was noted that when both parents were strongly pungent the disease index deviated consistently from the mean in the direction of that of the colored parent. When both parents were mild the index deviated consistently in the direction of the white parent. This suggested that the volatile sulfides contributing to pungency were expressed sufficiently to supplement
the effect of the phenols. Pungency is a quantitative character and the
number of genes involved is not known. When one parent was mild
and the other strong the effect of pungency was lost in the F₂ and
the indices coincided closely with those from mild x mild (Jones, et al,
1946). However it has been definitely shown that the pungent factor
will affect the smudge disease index and modify the expression of re-
sistance resulting from the action of color-associated phenols. (Hat-
field, Walker and Owen, 1948; Owen, Walker and Stalnann, 1950.)

Another example which illustrates the importance of genetic anal-
ysis of resistance is concerned with bean mosaic, incited by bean virus
1. For more than 20 years this disease has been controlled by selec-
tion of resistant strains. Three main sources of resistance have been
used. In each case resistance was derived by selection within horti-
cultural varieties. I will discuss resistance from two sources; that is,
that from Corbett Refugee and that from Robust varieties. While
these varieties and varieties derived from them are highly resistant in
the field, early workers found that they reacted differently when
crossed with a common susceptible variety. Moreover when the two
resistant lines were crossed susceptible segregates appeared in the
F₂. Zaumeyer and Thomas (1948) and Grogan and Walker (1948)
independently noted that occasional plants of Corbett Refugee type
showed extreme necrosis when inoculated with the virus. Grogan
and Walker showed that inoculation by the approach-graft technique
resulted in top necrosis of all plants of Corbett Refugee type while no
effect whatever resulted when this was applied to Robust. With the
aid of this technique Ali (1950) cleared up the genetic basis of resis-
tance. Stringless Green Refugee was used as a susceptible parent.
The reactions of the three parental stocks are as follows:

<table>
<thead>
<tr>
<th>Parent</th>
<th>Response to rubbing inoculation</th>
<th>Response to approach-graft inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Str. Gr. Refugee</td>
<td>systemic mottle</td>
<td>systemic mottle</td>
</tr>
<tr>
<td>Corbett type</td>
<td>none</td>
<td>top necrosis</td>
</tr>
<tr>
<td>Robust type</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

If the second column in the above table is examined it will be
seen that Corbett and Robust are phenotypically similar by the
usual inoculation technique. In column three it is shown that by
the approach-graft technique each variety has a distinct phenotypic
expression. When progenies from Robust x SGR are rub-inoculated,
the F₁ plants all develop systemic mottle, the F₂ progeny segregates
into 3 systemic mottle to 1 healthy. When progenies from Corbett
type x SGR are used the F₁ plants are all healthy and the F₂ progenies
segregate into 3 healthy to 1 systemic mottle. When the two re-
sistant parents are crossed and the progenies are rub-inoculated, all
F₁'s are healthy and the F₂'s segregate into 13 healthy to 3 systemic
mottle. However if the F₂'s from this last cross are approach-graft
inoculated they segregate at the rate of 9 top necrotic to 4 healthy
to 3 systemic mottle. Two gene pairs are involved:

A is the dominant gene for systemic mottle.
a is the recessive gene for complete resistance.
I is a dominant inhibitor gene which prevents the expression of A as
systemic mottle but permits the entrance of the virus through the
phloem by way of approach graft, resulting in top necrosis.
i is the recessive allele of I.
Stringless Green Refugee is represented by AA ii.
Corbett type resistant lines are represented by AA II.
Robust type resistant lines are represented by aa ii.

Resistant varieties of the Robust type are therefore homozygous
for the recessive gene a and are completely resistant irrespective of
the condition of the II allele. Resistant varieties of the Corbett type are
homozygous for complete susceptibility and are resistant only because
of the dominant inhibitor gene. This gene in homozygous or in
heterozygous condition prevents entrance of the virus by the usual
wound or insect inoculation and there is no formation of systemic
mottle. The virus is therefore ordinarily checked and inactivated at
the threshold by the influence of the inhibitor gene. If phloem in-
vasion is assured by the approach-graft technique the threshold is
passed and virus increase in the vascular system leads to top necrosis
and usually to death. Fortunately it is not common in most regions
for invasion of the phloem and severe necrosis to take place in nature.
The importance to bean breeders of the distinction of these two types
of resistance, and the genetic pattern by which they are inherited
need not be elaborated here. It is an outstanding example in which pathological and genetic techniques have combined to unravel a problem which hitherto had baffled pathologists, geneticists, and breeders.

**INFLUENCE OF ENVIRONMENT ON EXPRESSION OF RESISTANCE**

The relation of environment to disease development is a subject which has received a large amount of attention in plant disease research since about 1910. It is now accepted as axiomatic that various environmental factors are bound to have more or less influence on the development of both the pathogen and the host. In consideration of the expression of disease resistance we are concerned more particularly with the influence of environment on the host-parasite interaction, whether the latter be a surface phenomenon as in onion smudge or one which gets under way after penetration. As a matter of fact, in a large proportion of cases, resistance to disease in plants consists of interactions which take place after penetration. Many cases of penetration, direct or through stomata or other openings, occur without any resulting infection. There does not appear to be any connection between the attraction or stimulus to penetrate and the state of affairs which permits pathogenicity. What it is that determines that certain parasites or viruses select certain hosts and not others, while other parasites have little specialization and are essentially omnivorous, no one has as yet discovered. Whether so-called resistance is more often actually a matter of retardation of the pathogen by the host or certain of its contents, or whether it is merely a lack of attraction for the organism on the part of the host, is still a moot question. However in the main, if a given pathogenic species is adapted or accustomed to attacking a host species, it usually goes through the same initial stages of penetration with all varieties or individuals of the host. In resistant individuals and varieties the usual chain of events is upset in some manner and degree. The manifestations of resistant reactions are many. For the most part we know about them by a study of the histological and cytological pictures.
While morphological reactions are sometimes specific and suggestive they do not reveal the physiological reactions which underlie them. The biochemical and physiological reactions are still more elusive, and here particularly we find the basic questions still largely in the realm of the unknown. In the main it is not easy, using the disease syndrome as a measuring stick, to distinguish between the direct effect of environment on the suppression of disease development and the effect of inherited resistance characteristics, which are also, to be sure, subjected to the play of environment. It is only by a critical study of numerous strains of the host over a range of controlled environmental levels that a satisfactory distinction can be made. We may as well state here that it is precisely at this juncture that some will seize upon this situation as an excuse to throw Mendelism to the winds and explain all the vagaries of disease expression on the basis of environment. Others will call any direct suppression of the pathogen by environment an increase in resistance of the host and conversely a direct enhancement of pathogenic action by environment an increase in susceptibility. It is in this area that both pathologists and geneticists need to polish their methods and be more discreet in their expression.

In a comprehensive study of nutrition in relation to the development of yellow rust of wheat, Gassner and Straub (1929) found that, in varieties which were extremely resistant and in those which were extremely susceptible to rust, variation in nutrition had little effect on their reaction to the pathogen. In varieties which had normally an intermediate reaction to rust the influence of nutrition was marked, and a shift in balance of mineral elements resulted in increase or decrease of resistance. As the inheritance of resistance in different varieties has been studied by others in this rust and in other rusts it has become increasingly evident that as a rule high resistance to a given race is monogenic while intermediate resistance is polygenic. This does not mean that monogenic resistance is stable under all environments. In fact Johnson and Newton (1941) have shown that varieties highly susceptible to a given race of black stem rust at the usual range of environment may be induced to show a highly resistant reaction at unusually high temperatures.
A not dissimilar situation prevails in vascular wilt resistance as illustrated by cabbage yellows. The early work on resistance yielded results rapidly by mass selection, which was a natural method in this highly self-incompatible plant. Resistance in these mass-selected lines was unstable under the play of environment. It was expressed meagerly and erratically in seedlings. It was suppressed in proportion to increase in temperature. It was incompletely dominant over susceptibility. When inbreeding was practiced by means of bud pollination, lines were eventually secured which were phenotypically similar to old lines under most natural field conditions, but when unusual environment or nutrition was employed, phenotypic dissimilarities became increasingly evident (Blank, 1937; Walker and Smith, 1930; Walker and Hooker, 1945). The second type of resistance (Type A) is monogenic; it appears early in seedling development; it is stable over a wide range of temperature. It is of interest to note, however, that in thorough histological studies of the host-parasite relation of the two genotypes, no evidence of any difference in kind of relation could be found (Anderson and Walker, 1933; Smith and Walker, 1930). They can be differentiated only by regulation of environment. By the use of controlled environment the two types of resistance are separated readily in the seedling stage and in cabbage breeding the desirable monogenic resistance is retained. Without this basic knowledge, however, confusion between the two types would continue to exist. An analogous situation has since been shown with respect to Fusarium wilt of tomato (Bohn and Tucker, 1940).

Many more instances of environmental effects upon the host-parasite relation might be elaborated. Suffice it to say here that only within the last two decades has the place of this phase come into full appreciation. Its importance is threefold. It is essential to the theoretical interpretation of Mendelism in relation to disease resistance. It is essential to serve as a basis for the study of variability in pathogenicity of microorganisms. It is vital in developing techniques for the most effective and most efficient means of screening and evaluating plant materials with reference to disease resistance in practical breeding programs with crop plants.
DISEASE RESISTANCE IN RELATION TO FOOD SUPPLY

I have related in some detail examples of the types of advances which have gone on chiefly in the last four decades in the application of genetics to plant disease problems. It is often stated that in discussions of this type we are inclined to leave the impression that theoretical bases are essential before applied progress is made. Of course history belies this assumption and it is no less true in the development of resistant varieties of crop plants. However, having been in the midst of this subject for more than three decades I am ready to defend the premise that through the application of facts derived from theoretical genetics the program as a whole has become more stable and the confidence of scientists and the support of the public have increased almost in proportion to the accumulation of basic facts.

There are many reasons why this nation went into and through World War II with more confidence and less fear as to food supply than it entered and struggled through World War I. I am bold enough to say that one major factor was the increasing application of genetics to plant pathology in the interim. Specifically the reasons are found in such facts as these: our sugar supply rests upon mosaic resistant canes and curly-top resistant beets; our cereal supply rests upon a range of varieties resistant to important races of rusts and smuts; our vegetable canning industry rests upon wilt-resistant peas, wilt-resistant tomatoes, wilt-resistant sweet corn, mosaic-resistant snap beans, and yellows-resistant cabbage. There is no need of lengthening the list to bring out the point.

UNSOLVED PROBLEMS

What of the future? An equally long list of diseases of food plants could be listed which can and will in the future be controlled through disease resistance. We should fail in our duty if we left the impression that the job is now all laid out and that from now on it is merely a matter of turning the grindstone. We are still on the threshold of understanding variability in pathogenicity of microorganisms. We
know little about mutations, their nature, their frequency, and their relation to the host substrate. Why does the tomato-leaf mold fungus (Cladosporium fulvum Cooke) turn out a new pathogenic race as rapidly and just at the same rate as the breeder synthesizes a new host line resistant to all previous known races? Is this the ghost of Ward's bridging-host theory haunting us? Why does the sister species (Cladosporium cucumerinum Ell. & Arth.) parasitic on cucurbits disappear in the other direction and leave the breeder, frequently at a critical time, with nonpathogenic cultures on his hand, only to reappear in a highly virulent form in the cucumber fields just after the pathologist has gone on his vacation? Why did the late blight fungus turn up in the form of an unusual race to smite down our tomato crop in 1946 to the tune of some millions of dollars in loss precisely 100 years after western Europe was smarting from crop losses and famine brought on by successive epidemics incited by the same fungus on potato. Mills and Reddick (Mills, 1940; Reddick and Mills, 1938) have shown this fungus to build up virulence in successive vegetative generations on host substrates arranged at increasing levels of resistance. In fact although heterothallism, the tool for the study of genetics in the fungi, was first found in the Phycomycetes in 1904 there is still not a single report on the genetics of pathogenicity in this class of fungi.

Progress in knowledge of the host-parasite interaction is dependent upon our knowledge of cell metabolism. When we know more about the physiology and biochemistry of the plant cell we can learn more about host-parasite interaction. There are undoubtedly many cases of gene-controlled resistance of potential use to the breeder which have not been analyzed because the symptom-syndrome relation to environment has not been worked out. Some of them have escaped notice but most of them await finer techniques based upon more fundamental knowledge in pathology and genetics.
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INTRODUCTION

The spectacular advances which man has made during the past century in controlling his physical environment have been largely the product of research in the physical sciences. Man has been far more adept at understanding and manipulating the non-living components of the universe than he has in controlling the living. But revolutionary changes are imminent. Many scientists, including physicists and chemists, now predict that the second half of the twentieth century will belong, not to physics and chemistry but to the biological sciences. I believe that these predictions are quite sound.

The trend is already foreshadowed by the important advances which have been made during the past 25 years in the field of applied biology. The discovery of insulin, penicillin, streptomycin, cortisone, and similar disease-destroying chemicals which are elaborated by living things are familiar to the man on the street. Less well known to the general public are the advances, perhaps even more far-reaching in their importance, which have been made in improving our basic food plants through the application of the principles of genetics. One of these—hybrid corn—is the subject of my paper. In my opinion hybrid corn is the most far-reaching development in applied biology of this quarter century. It has already affected more lives, I venture to guess, than any of the epoch-making discoveries in medical biology of the same period. Insulin and penicillin have saved thousands of
lives in the past 25 years, but the new abundance of foodstuffs which hybrid corn has created has saved millions of lives in this period of the world’s history. Hybrid corn may even prove, in future historical perspective, to have been one of the most important factors in saving our American culture and the European civilization from which it was born.

Two dramatic examples of the importance of hybrid corn are found in the role which it played during World War II and immediately thereafter. During the three war years—1942, 1943, and 1944—the American farmer, suffering from acute labor shortages on the one hand and from unfavorable weather on the other, produced 90 percent as much corn as he had during the previous four years of peace. Hybrid corn made it possible for him to step up, by approximately 20 percent, an already unprecedented production at a time when maximum production was desperately needed. Because of hybrid corn we not only suffered no real food shortages during the war but, on the contrary, we were able to ship food to our allies and to employ surplus grain as raw material for the manufacture of alcohol, synthetic rubber, explosives, and other materials of war.

At the conclusion of the war our corn surplus immediately fulfilled another and more peaceful but no less important role. In the year ending June 30, 1947, the United States shipped to the hungry peoples of war-torn Europe 18 million long tons of food. Very little of this was corn, but food is food and 18 million long tons of food is the equivalent of 720 million bushels of corn. In this same year the increase in yield of the corn crop of the United States resulting from the use of hybrid corn is conservatively estimated to have been more than 800 million bushels.1 Thus Europe’s food deficits during the

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1 It is difficult to determine precisely how much the American corn crop has been increased by the use of hybrid corn. McCall (1944) estimated that the increased yield in 1943 resulting from the planting of 49,428,000 acres of hybrid corn was 669,480,000 bushels, an average increase of approximately 13.5 bushels per acre. At this same rate the 1946 acreage of 61,614,000 acres would have resulted in an increase of 831,789,000 bushels. Data presented in this paper suggest that the increased yield accompanying the use of hybrid corn (but not necessarily resulting wholly from the superior productiveness of hybrid corn) is approximately 15 bushels per acre. At this rate the increased yield attributable to hybrid corn in 1946 was 924,210,000 bushels.
first post-war years were made up, with some to spare, by the surplus accruing from the use of hybrid corn. Western Europe became less receptive to communism because hybrid corn had made it possible for the New World to come to its aid in a time of great need. Thus the principles of heredity discovered by Gregor Mendel in 1865 and rediscovered in 1900 came to play an important, if not immediately obvious, part in stemming the tide of communism in Europe. Perhaps Russian antipathy to Mendel’s laws and to modern genetic theory is not unfounded.

METHODS OF PRODUCING HYBRID CORN

What is hybrid corn and how has it achieved such an epoch-making contribution to the world’s food supply? In a sense all corn is hybrid corn since corn is a cross-fertilized species in which hybridization between individual plants and between varieties and races is constantly occurring. Corn has had a long history of repeated hybridization and the accompanying hybrid vigor or heterosis has played one of the major roles in its evolution under domestication. What the modern corn breeder has done is merely to exploit more fully than is possible in nature a tendency characteristic of the species.

As a geneticist concerned with the problem of evolution under domestication, I would (taking a leaf from Dobzhansky’s book) define hybrid corn as a type of adaptive polymorphism in which natural selection, acting in a man-made environment, preserves certain chromosomes not primarily because of their intrinsic worth, but because they interact effectively with other chromosomes, similarly preserved, to produce a highly successful Mendelian population. As a practical corn breeder, I would define hybrid corn as any corn artificially hybridized in order to utilize the phenomenon of hybrid vigor or heterosis.

As most commonly used in the United States at the present time, hybrid corn involves as a first step the isolation of inbred strains. This is accomplished by self-pollination, an intensive form of inbreeding, which in the case of corn is accompanied by three principal effects: (1) the elimination of numerous deleterious recessives, (2)
a marked loss in vigor and productiveness, (3) the establishment of homozygous strains of great uniformity. These uniform homozygous inbred strains usually yield less than half as much as the open-pollinated varieties from which they were derived. Their only value is as potential parents of productive hybrids.

**Fig. 1.** The effect of inbreeding upon vigor in corn. The plants represent an F₁ hybrid and seven subsequent generations of self-pollination. Corn becomes homozygous and uniform after five or six generations of inbreeding.

When these unproductive inbred strains are crossed they give rise to vigorous hybrids, some of which are appreciably more prolific than the original open-pollinated corn from which they came and it is this phenomenon which is exploited in the modern commercial production of hybrid corn. The problem of hybrid seed production is substantially simplified by combining four inbred strains instead of two into a combination known as a double cross and this is now the common commercial procedure. It was the invention of the double-cross method by Jones in 1917 which made hybrid corn at once a practical reality instead of a future possibility.
Paul C. Mangelendorf

Fig. 2. Diagram illustrating how commercial hybrid corn is usually produced.

Fig. 3. Ears illustrating how four uniform inbred strains are first combined into two single crosses and subsequently into two double crosses. Single crossed seed, since it is produced on inbred strains is expensive. Double crossed seed borne on vigorous single crosses is much cheaper to produce.
Hybrid Corn

There are many variations of this prevailing double-cross method. Single crosses, the product of crossing two inbred strains, are employed when maximum uniformity is required as in the case of sweet corn used for canning. Three-way crosses produced by hybridizing a single cross with a third inbred strain are useful for testing the "combining ability" of new inbreds. This is also true of "top crosses" which are hybrids of inbred strains and open-pollinated varieties. When two or more inbred strains are combined with an open-pollinated variety, the product becomes a "multiple top cross," a type of hybrid which has been very useful in the early stages of the corn-breeding program in Mexico. "Synthetic" varieties represent advanced generations of hybrids produced by combining a number of inbred strains, usually four or more. Other modifications of the common method have resulted from the discovery that inbred strains used in hybrids need not be inbred to the point of uniformity and homozygosity but can be used after one generation of self-pollination.

THE GENETIC BASIS OF HYBRID CORN

The basis for all types of hybrid corn is the phenomenon known as hybrid vigor or heterosis which often accompanies the hybridization of unrelated varieties, races, or species. There is a divergence of opinion on how heterosis should be defined. In Webster's International Dictionary heterosis is, "the greater vigor or capacity for growth frequently displayed by crossed animals or plants as compared with those resulting from inbreeding." This is approximately the meaning which Shull (1948), who coined the term, intended that it should have. This is the definition to which the majority of geneticists would probably subscribe. To include as manifestations of "vigor" such unrelated characteristics as the capacity of insects and birds to produce more and better eggs or of excised roots to elaborate or utilize essential metabolites creates no fundamental conflict with this definition. Many geneticists, however, feel the need of more precise terms for particular types of heterosis. There is no reason why special terms with restricted definitions should not be coined to meet such needs.

On the question of the genetic basis of heterosis in corn there is
even greater divergence of opinion than on its definition. There are at present two principal hypotheses. The one, foreshadowed by Bruce's speculation, first clearly expressed by Keeble and Pellew, and later elaborated upon by Jones in terms of the chromosome theory of heredity, holds that heterosis is caused by bringing together in the hybrid the dominant favorable genes of both parents. This theory says, in essence, that the hybrid is better than its parents because it exhibits the best qualities of both while concealing their defects. The second theory, actually the earlier, holds that heterozygosity *per se* is responsible for heterosis. Both Shull and East attributed heterosis to the physiological stimulus of heterozygosity. More recently East (1936) explained heterosis in terms of the complementary action of alleles at the same locus, a phenomenon to which Hull has given the term "overdominance" because the effect of the complementary action is supposedly greater than that resulting from dominance alone. This theory paraphrases an old axiom to say in essence that two alleles are usually better than one.

Fundamentally the two theories are not completely conflicting. Both involve orthodox genetic interpretations in terms of Mendelian principles. Both postulate the complementary action of genes. The principal difference between the theories is that one supposes that genes at different loci complement each other, while the other holds that different alleles at the same locus produce this effect. Since it is difficult, if not impossible, to distinguish between alleles and completely linked genes, there is at least one situation in which the two theories are identical. From the standpoint of the practical breeder the most important difference between the two theories is that if the first is correct, it should be possible to gain maximum improvement by accumulating the maximum number of dominant favorable genes in the homozygous condition since dominance is assumed to be usually incomplete. If the second theory is correct, maximum productivity demands maximum heterozygosity. Perhaps it will be shown in the final analysis that both mechanisms are operating. Indeed, it would be surprising if this were not true. In the meantime corn breeders continue to exploit the phenomenon of heterosis for
the improvement of corn without being certain of its cause or in agreement on how to define it.

THE HISTORY OF HYBRID CORN

The history of hybrid corn is important in demonstrating how future advances in biology may be expected to occur. Most of the developments in science are the product of many men and many minds and in this respect hybrid corn is typical. Scientific achievements, like royalty and pure-bred livestock, often have long and complex genealogies. Lines of descent often trace back to more than one distinguished progenitor and the same progenitor sometimes appears more than once in the pedigree. Hybrid corn is no exception to this general rule. Two distinct lines of descent have converged to make hybrid corn an accomplished fact in our time and a third line has had a marked influence. If we trace these lines back to their recognizable sources they lead us to three famous biological scientists of the nineteenth century, Charles Darwin, Gregor Mendel, and Francis Galton.

One line of descent in the genealogy of hybrid corn, represented by the pioneering research of George H. Shull (1908, 1909), traces back through Johannsen, the Danish botanist, to Galton and from Galton to Darwin. Shull had no thought of improving the corn plant when he began his studies. His objective was to analyze the inheritance of quantitative characters and he chose corn as an appropriate experimental subject for this purpose. He inbred his lines to “fix” their characteristics and he crossed lines which had been thus inbred to study the inheritance of kernel-row-number.

That Shull should have been able from the limited experiments then completed and from unreplicated yield tests, not only to draw valid conclusions regarding the effects of inbreeding and crossbreeding, but also to design a new method of corn breeding based upon the exploitation of heterosis, is one of the most fortunate achievements of our time in the field of applied biology. Shull’s idea of maintaining otherwise useless inbred lines of corn solely for the purpose of utilizing the heterosis resulting from their hybridization was
revolutionary as a method of corn breeding. It is still the basic principle which underlies almost the entire hybrid corn enterprise. Hybrid corn did not come into being, however, until the first line of descent, represented by Shull, converged with the second line, represented by East, Hayes, and Jones, to produce a kind of genealogical “heterosis” whose results have been nothing short of explosive.

The second line of descent in the pedigree of hybrid corn, like the first, traces back to Charles Darwin. Darwin was interested in the effects of inbreeding and crossbreeding as factors in evolution and he conducted numerous experiments on self- and cross-fertilization in plants, including corn as one of his subjects. Darwin’s results were known before their publication to Asa Gray with whom he carried on an extensive correspondence and who had visited him in England. One of Gray’s students of that period was William Beal, who subsequently at Michigan State College wrote a review of Darwin’s book and carried on experiments in corn with the direct purpose of utilizing hybrid vigor to increase yield, the first controlled experiments of their kind. Beal’s work was the progenitor of the corn-breeding program at the University of Illinois under Holden, Shamel, Hopkins, and East (Holden, 1948) and this in turn was the parent of the long-term experiments at the Connecticut Agricultural Experiment Station on the effects of inbreeding and crossbreeding under East, Hayes, and Jones (Crab, 1947). The Connecticut program reached fruition in 1917 in the invention of the double cross, a device for simplifying hybrid seed production, and in Jones’ theory of heterosis which interpreted the phenomenon in terms of the chromosome theory of heredity and thus brought into the genealogy a third line of descent tracing back to Mendel through Morgan and his students. His theory undoubtedly gave as much stimulus as his double cross, if not more, to practical hybrid corn breeding. Historically, then, hybrid corn became transformed from a magnificent design into a practical reality when Jones’ method of seed production made it feasible and his theory of heterosis made it plausible, a combination difficult for even the most conservative agronomist to resist. The impact of Jones’ two contributions upon practical hybrid corn breeding is easily demonstrated by the sudden expansion of corn-breeding programs in vari-
ous parts of the United States following the publication of his several papers (Mangelsdorf, 1948).

Edward Murray East is usually mentioned as one of the participants in the development of hybrid corn. East's part is not as specific as that of Shull and Jones. He made an important contribution, however, in initiating and maintaining the long-range experiments on inbreeding and crossbreeding which finally furnished two important keys to the problem of practical hybrid corn breeding. Without East's work we would not have had hybrid corn as soon as we did; indeed we might not have had it now, although it would inevitably have come sooner or later.

By 1933 hybrid corn was in commercial production on a substantial scale, and the United States Department of Agriculture began to gather statistics on it. By 1949, 77.6 percent of the total United States acreage was in hybrid corn.

The roster of corn breeders who have produced this unprecedented accomplishment is a long one, including many distinguished names—Richey, Hayes, Wallace—and a score or more of others. Yet in the light of their huge accomplishment—65 million acres of hybrid corn in 1950—the number of men who have participated in the development is almost unbelievably small. Some 25 men, not more than 50 at the most, have played a major part in an agricultural revolution affecting more than 150 million Americans. One man in each six millions of the population has made an important contribution toward determining the prosperity and destiny of the entire group. These men have been catalyzing agents effective in concentrations of approximately 0.2 parts per million.

SIGNIFICANCE OF HYBRID CORN

The significance of hybrid corn in human affairs is manifold. It is an excellent example of scientific research at its best. It illustrates how the scholars of one generation reach new heights by almost literally standing upon the shoulders of those who have preceded them. It shows how painstaking experimentation and brilliant speculation complement each other to produce a near-miracle which neither
would have accomplished alone. Hybrid corn illustrates especially well the necessity and importance of the free interplay of theory and practice which flourishes most successfully in a non-totalitarian society. Marxian ideology emphasizes the integration of theory and practice but modern exponents of Marx seem not to realize that this interaction cannot be achieved by edict or decree. It is something which, in the words of the popular song, "comes naturally" and it does so only in a free society where there is no neglect of the untammeled search for truth on the one hand and no overemphasis on utilitarian aspects on the other.

The importance of the theoretical background would be difficult to overestimate. It would, for example, have been virtually impossible for so-called "practical" plant breeders, living in an authoritarian country and working under duress to improve the corn plant, ever to have discovered or invented hybrid corn. The reason is obvious. The first step in hybrid corn production is inbreeding and inbreeding leads not to immediate improvement but to a drastic reduction in yield. It would take a very brave corn breeder, indeed, to report to his superiors in the party hierarchy that he had, after two generations of inbreeding, succeeded in reducing yield by 50 percent. Indeed, even in our own democracy it has not always been easy for corn breeders to defend this paradoxical procedure of "advancing backwards." I still have vivid memories of some of my own experiences in the early days of the corn-breeding program with which I was associated in Texas and I am sure that other corn breeders, like me, have been the target, if not of outright criticism, at least of good-natured ridicule at the hands of practical farmers.

HYBRID CORN AND THE FOOD PROBLEM

But the real significance of hybrid corn lies, of course, in the contribution which it has made and can make to the world's food supply and here it can contribute more than is, at first glance, apparent.

Hybrid corn is much more than a method of increasing the productiveness of corn. In biological terms it is a kind of enzyme or catalyst which leavens the entire agricultural economy. In terms of
anthropology it is a wedge which splits wide-open an entire cultural complex of long standing allowing a new cultural pattern to be formed.

The average acre yield of corn in the United States has increased from approximately 22 bushels per acre in the early 30's, when hybrid corn first began to be used commercially, to approximately 33 bushels in the late 40's when it occupied some 75 percent of the total corn acreage. An increase of 50 percent, when only three acres out of every four were planted to hybrid corn, suggests that if all were planted to hybrid corn the increase would be about 66 2/3 percent. Since in-

![Graph](image-url)

Fig. 4. Graphs showing how the average yield of corn in the United States has risen in almost exact proportion to the increase in percentage of the total acreage planted to hybrid corn. With three acres out of every four now planted to hybrid corn, the average yield has increased from approximately 22 bushels per acre to approximately 33 bushels.
creases of this magnitude are seldom met in controlled experiments, it follows that the use of hybrid corn has brought in its wake other improved agricultural practices including crop rotation, the use of fertilizers, and the growing of soil improvement crops. The successful utilization of hybrid corn has made the American farmer receptive to an entire complex of new and improved methods.

What has happened in the United States can be expected to happen eventually in other parts of the world where corn is an important food plant. Already corn production in Italy and other parts of Southern and Eastern Europe has been greatly increased by the importation of American hybrid seed corn which fortunately has proven to be well adapted. In 1949, 2000 tons of sixteen different American hybrids were planted in Italian fields on about 150,000 acres. In 1950, at least 3000 tons of hybrid seed were exported to Italy. Yields of the best American hybrids are substantially greater than those of the best native varieties. Increases of 25 to 30 percent are produced in experimental plots under controlled conditions, but the Italian farmer, like his American counterpart, reports much greater increases on his own farm.

In the countries of Latin America where corn is the basic cereal, new hybrids especially adapted to local conditions will usually have to be developed since hybrids from the United States are generally not adapted. Corn-breeding programs aimed at this objective are in progress in Mexico, Guatemala, Salvador, Costa Rica, Colombia, Venezuela, Brazil, Uruguay, Chile, and Argentina.

The corn-breeding program in Mexico which has been conducted under the auspices of the Rockefeller Foundation, in complete cooperation with the Government of Mexico, is a splendid example of exporting a technical skill for the benefit of a friendly neighbor without sacrifice to our own capital assets. The Mexican hybrid corn program, under the leadership of J. George Harter and the immediate direction of Edwin J. Wellhausen and Lewis M. Roberts, has succeeded in seven years in revolutionizing corn production in Mexico. Begun in 1943, it had by 1948 affected Mexico's corn crop so that, for the first time since the Revolution in 1912, Mexico had no need to import corn. The Mexican corn crop of 1950 is estimated to be the largest in its
history. Here two men, in a population of some 22 million people, have shaped the nutritional destiny of the entire mass. The catalyst in this case has been effective in a concentration of approximately 0.1 parts per million. Since this represents approximately the same order of magnitude as the effectiveness of corn breeders in the United States, there is reason to believe that the figure may have a general validity.

To appreciate the full significance of what has been done in Mexico one must understand what corn means to the Mexican people. There is probably no other country in the world in which the population has become so dependent upon a single food plant. To millions of Mexicans, as to their ancestors before them for centuries past, corn is literally the staff of life; the daily bread which, eaten three times a day, 365 days a year, provides the fuel that keeps the human mechanism functioning. A Mexican laborer, when he can get it, will consume as much as two pounds of corn a day. When corn is plentiful the Mexican is happy and relatively prosperous. When it is scarce there is unrest and danger to stable government.

In Mexico, as in the United States, hybrid corn has brought other important innovations in its wake. A drastic change in his basic food plant has "softened up" the Mexican farmer, traditionally one of the world's most conservative individuals, making him receptive to other changes. As recently as 1943, for example, Mexico's most competent agricultural experts expressed the conviction that it would be impossible, because of Mexico's traditional reliance on corn, to introduce grain sorghums into Mexico in regions where rainfall is inadequate for corn and where the more drought-resistant sorghums might be expected to flourish. Today—seven years later, a short period in terms of agricultural progress—two agencies of the Mexican Government are each claiming the prerogative of distributing sorghum seed to the Mexican farmer. New wheat varieties resistant to rust, soy beans, sweet clover, and other new soil-improving crops are meeting the same warm reception that has greeted the introduction of hybrid corn. The entire agricultural enterprise of Mexico is in a state of flux as a consequence of the labors of a small band of well-trained geneticists.

What has been done in Mexico can presumably be done in other
Latin-American countries where corn is the basic food plant. A second experiment under the auspices of the Rockefeller Foundation has been started in Colombia and it will soon be known whether the successful Mexican experiment can be duplicated in other countries. What can be done with corn in America can perhaps also be done with rice in China, with wheat in parts of India, and elsewhere in the world where the economy is strongly dependent upon a single crop. Experience in Mexico has shown that the most effective way to start an agricultural revolution is to improve the basic crop plant by the application of genetic principles. Soil improvement and the control of soil erosion are in the long run perhaps even more important objectives than crop improvement, but it is a drastic change in his basic crop plant which first brings new hope to the tradition-bound farmers of undeveloped countries and prepares the ground for other even more far-reaching changes.

HYBRID CORN AND THE FUTURE

The problem of population and the food supply has now become one of the world’s most acute and pressing problems and is directly or indirectly the cause of much of the world’s present unrest. It is difficult for Americans, living in a land of agricultural surpluses, and taxed to maintain scarcity prices in the face of plenty, to realize that two-thirds of the world’s people are, by modern nutritional standards, inadequately fed and are suffering from chronic malnutrition (Boyd-Orr, 1950). What can be done to solve the problem of the world’s underfed people while the world’s population is increasing at the unprecedented rate of 22 million people per year, a rate which promises to accelerate still further before it begins to decline?

There is no simple answer to this question. There are those, probably unduly pessimistic, who regard the problem as insoluble and who fear that the world must resign itself to the inexorable consequences of the Malthusian Law: ever-recurring famine and ever-increasing poverty. There are others, undoubtedly too optimistic or too sentimentally humanitarian, who believe that the United States alone can feed the world. They would have us continue indefinitely
to produce and export surpluses to underfed peoples, not realizing that this measure is only a short-term palliative at best and that to export food is in reality to export a part of our permanent soil fertility. Between these two extremes lies the solution. The most hopeful circumstance at present lies in the fact that food is, after all, only one form of energy and man's greatest source of energy, solar radiation, is still almost untapped and virtually inexhaustible. The amount of solar radiation which falls upon all parts of the world exceeds by far in energy equivalents man's needs for food, fuel, and power. Man is an ingenious animal who has, sooner or later, always found a way to exploit to his own satisfaction or benefit the natural resources at his command. It is almost inconceivable that he will not also learn to utilize solar energy, especially since he has now in parts of the world accustomed himself to the luxury of unlimited power resulting from the exploitation of fossil fuels which represent the stored solar energy of the past. Probably he will also learn to control universally, as he already has in some parts of the world, the unrestricted growth of population which is the basic cause of the present problem. The great danger of this century is that he will not do so in time and that global chaos will prevail before he has succeeded either in harnessing solar energy for new methods of food production or in stabilizing his own reproductive rate. It is during this period that the more prosperous nations of the world must, in their own enlightened self-interest and not motivated merely by generosity or by sentimentalism, make every possible contribution toward developing the agricultural resources of the world. It is during this period that the improvement of our cultivated plants and domestic animals can make their most vital contribution. It is during this period that hybrid corn will be of even greater significance to human affairs than it has been in the past. Hybrid corn cannot solve the problem of the world's food supply, but combined with other effective methods it can surely help.
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MENDELIAN POPULATIONS AND THEIR EVOLUTION

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THE best view of a mountain range is usually obtained from a distance; at close quarters minor peaks, and even hills, obstruct the vista of the whole range. To understand man and his universe is the goal of the scientific movement; this goal must always be kept in view, in order that we do not mistake means for the end. Well authenticated facts are the life blood of science, and gathering them will always remain the principal function of scientific research. But science is more than a mass of facts; it is a meaningful system of significant facts. Facts taken out of their context, showy methods to solve meaningless problems, and learned terminologies for conceptual trivialities are often amusing to play with, but they stultify the work of a scientist. Integration of the results obtained by individual scientists and by various disciplines is therefore an important function which should be performed. The stones should be fitted to form an intelligible mosaic. The general view of the world unfolded by science should be kept before our eyes in order that scientific work be directed purposefully and effectively. Now, experience has shown that, at least in biology, generalization and integration can best be made by scientists who are also fact-gatherers, rather than by specialists in biological speculation. The chief aim of the American Society of Naturalists is to promote this generalization and integration.

Because it ties together the greater number of most diverse facts

1 Address of the President of the American Society of Naturalists.
into a simple and meaningful system, the theory of organic evolution is by far the most significant generalization which has yet emerged from the biological sciences. In the light of this theory, living beings are no longer considered as products of fortuitous accidents, or the caprice of some deity. The living world as we see it today is the outcome of a long historical development enacted during approximately one billion years of earth's existence, and still continuing to occur before our eyes.

Furthermore, the causes of this evolutionary development happen to appeal to our reasoning faculties. They seemed to us to make sense. Darwin was the first to suppose, and his view has been borne out by the subsequent developments of the evolutionary thought, that organisms exhibit evolutionary changes because they become adapted, through natural selection, to live in different environments. The existence of environments unoccupied by life, or of inefficiently exploited ecological niches, constitutes a challenge to which the protoplasm may respond by evolutionary inventions of new kinds of organisms adapted to the demands of these environments. The alga *Sphaerella nivalis*, which lives on patches of permanent snow in high mountains, or *Phormidium bijahense* and *Oscillatoria filiformis*, which occur in the hot springs of Yellowstone at 85° C (Copeland, 1936), are among the more spectacular responses to apparently impossibly difficult challenges. But organic diversity as a whole must also be regarded as an answer of living matter to the diversity of environments which exist in the world.

Darwin's theory of natural selection has pointed the way to explanation of adaptive evolutionary changes. But a causal analysis of such changes became possible only in our day, because of the great discovery made by Mendel some 85 years ago, to the rediscovery of Mendel's work 50 years ago, and to the subsequent development of Mendel's principles. This causal analysis is still far from complete; even so, it represents one of the signal achievements of biology during the first half of the twentieth century.

Darwin pointed out that differential survival of the better-adapted variants, and elimination of the less well-adapted ones, should lead to a gradual rise of the level of adaptedness of species in which such
differential reproduction takes place. But Darwin realized that the process which he postulated can occur only in species which possess a supply of heritable variations in fitness to survive in existing environments. He concluded, quite correctly as we know, that most existing species do possess such a supply. But he realized that the origins of this supply had to be elucidated by further work. Mendel’s discovery supplied the key to such elucidation. Hardy and Weinberg in 1908 used it to open the first lock. Investigation of the mutation process in Drosophila by Morgan and his followers created a solid base for further advance. The next step was taken by Chetverikov in 1926. His paper was published only in Russian, and it remained unknown to Fisher (1930) and to Wright (1931), who, together with Chetverikov, may be considered founders of the modern analysis of evolutionary phenomena.

The pre-Mendelian view, which was of necessity accepted by Darwin, was that the heredity of the offspring is a blend, a fusion product, of the heredities of the parents. If this were correct, sexual reproduction would devour and consume the heritable variation present in panmictic populations at a prodigious rate, namely, it would reduce the genotypic variance by one-half in every generation. At this pace of expenditure, a population would soon come to consist of genotypically uniform individuals. It would become a pure race, in which selection would cease to operate, unless, of course, genotypic variance were to arise anew at a rate at least comparable to its destruction.

It is perhaps fortunate that Darwin did not make the calculations which would have revealed to him this most serious of objections against his theory. For the difficulty is now known to be spurious, because of Mendel’s demonstration that the heredity transmitted from parents to offspring is an aggregate of genes, which do not blend but which segregate. The importance of this fact for evolution is immense. Sexual reproduction does not erode and level off, but on the contrary conserves, hereditary variability. Every sexual species accordingly possesses a gene pool, in which each gene may be represented by a certain number of alleles, and each chromosome by one or more structural variants. The frequencies of each allele and each
chromosome variant in the gene pool remain constant from generation to generation, unless mutation, selection, or genetic drift intervene to alter them.

The classic way to study a species, or a part of it, is to determine the modes or averages for as many traits as possible, in as large a sample of its representatives as practicable. The resulting system of averages is taken to be a common property of the species as a whole, and is believed, at least by implication, to characterize the ideal species type. This conception of species type was logical so long as the heredity of the offspring was supposed to be an alloy of the heredities of the parents. The species type would then have been the limiting condition towards which the species would gravitate owing to sexual reproduction. But with gene heredity, a system of character averages has no real meaning, in the sense that it is not a property of any spatio-temporal object. Such a system of averages is a statistical fiction which, to be sure, may be very useful for purposes of description and cataloguing. Building a convenient catalogue of organisms is one of the tasks of biological systematics. On the other hand, a biologically realistic description of a species or a race should, theoretically, indicate the frequencies in its gene pool of gene alleles and chromosomal variants. At the present state of knowledge, such descriptions are admittedly too difficult to make to be useful in practical systematics. It must, however, be established as a principle that, in living things, diversity and variability are more fundamental than types and averages. It is a habit of thought fostered by the exigencies of catalogue making, to regard individuals of a species as more or less perfect incarnations of a species ideal. This habit stems ultimately from Platonic philosophy and from scholastic theology. It conflicts with Mendel's findings, is basically anti-evolutionist, and is responsible for much confusion in biological thinking.

Species and races of practical systematics are categories of man-made classification. But it happens to be convenient to delimit species and races in such a way that they usually coincide with certain spatio-temporal entities, which are integrated systems of genotypes bound together by having access to common gene pools. It is important, then, to distinguish between systematic categories, set up for prac-
tical purposes of catalogue making, and the underlying spatio-temporal entities. These latter may be referred to as Mendelian populations. A Mendelian population is a reproductive community of sexual and cross-fertilizing individuals which share in a common gene pool.

The apprehension of Mendelian populations is made difficult by the compound nature of many of them. The biological species is the largest and most inclusive Mendelian population. Supraspecific groupings, such as subgenera, genera, etc., do not possess common gene pools, and consequently do not have the biological reality of Mendelian populations. But species are differentiated into complexes of subordinate Mendelian populations, which may be referred to as subspecies, races, or local populations. Each of these subordinate gene pools may, like the gene pool of the species, be uniquely characterized in terms of frequencies of gene alleles and chromosome variants. The smallest Mendelian populations are panmictic units (Wright 1943), which are groups of individuals any two of which have equal probability of mating and producing offspring (provided, of course, that they are of opposite sex and are sexually mature). Panmictic units are integrated into more or less complex systems of Mendelian populations, which culminate in species.

Mendelian populations can be recognized as separate entities even if they are quite similar genetically. Genetically similar Mendelian populations may have separate gene pools because these populations are isolated from one another on different islands or by other means. By contrast, a systematist recognizes the existence of two or more taxonomic groups only if he finds genetic difference between them. The first question asked about taxonomic groups is: What traits are common to individuals within a group but differ in individuals of different groups? The first question asked about a Mendelian population is: What is its breeding structure?

The distinction between Mendelian populations and taxonomic groups can be illustrated even more vividly by considering the situation in organisms which reproduce asexually, by parthenogenesis, or by self-fertilization. If these methods of reproduction are not facultative (as they often are), but are established to the exclusion of cross-
fertilization, the organisms concerned can not form Mendelian populations in the above-defined sense. For example, a clone of bacteria in which no sexual fusions occur is not a reproductive community and has no common gene pool. Individuals of a clone are genotypically alike, barring mutation; yet, a clone has no biological unity, except retrospectively by virtue of common descent from a single individual. By contrast, members of a Mendelian population have a continuous biological bond, because of the occurrence of matings in the reproductive community. Considered biologically, such a species as *Homo sapiens* or species of *Drosophila* flies or of birds are quite different phenomena from the clusters of clones which are referred to as "species" in bacteria, fungi imperfecti, or in obligatory apogamic plants. Nevertheless, systematists find it convenient to use the same taxonomic categories and the same descriptive techniques for all organisms.

By far the most complex system of Mendelian populations exists in the human species. Because of this complexity, anthropologists and geneticists are only beginning to learn how to disentangle and study these populations, or isolates, as they are often referred to in man. Like many other biological species, man is geographically polytypic, that is, composed of major and minor geographic populations, or races, which differ in frequencies of many genes. But in addition to the geographic races, man has evolved national, linguistic, religious, economic, and other cultural isolates. Furthermore, human isolates do not form hierarchies of inclusive and subordinate isolates, as animal and plant populations usually do. For example, economic isolates may cut across linguistic boundaries, and religious isolates may overlap geographic ones. The lack of a clear idea of what constitutes a Mendelian population, or a race, has caused a great deal of confusion in anthropology. For example, people with blue eyes, or with round or with oblong heads, or with heads shaped like some prehistoric skull, or fat people, or people convicted for crime, or suffers from cancer or other diseases do not form Mendelian populations. It is meaningless to call such collections of individuals races, as they have sometimes been called.

Another difficulty in the study of Mendelian populations arises from the fact that they are not fixed and static, but are dynamic
entities which undergo evolutionary changes. A Mendelian population may split in the course of time into two or more derived ones; conversely, once distinct populations may fuse into one. Excellent examples of genesis, divergence, convergence, and fusion of Mendelian populations can be observed in the human species. Thus, the fluidity of social isolates is quite apparent, and development of culture has led to gene exchange between geographic races. Considered biologically, the rise of tribes, nations, and castes leads to the appearance of new gene pools. Wars, migrations, and social upheavals lead usually to the breakdown of barriers between existing gene pools. A fascinating and yet almost untouched problem is to consider and to evaluate social forces from the standpoint of their evolutionary significance.

Now, the differentiation and fusion of Mendelian populations takes place gradually and continuously. Only in exceptional cases, such as the formation of polyploids, can a new reproductively isolated population become established within a generation. Situations are, therefore, quite common in all sorts of organisms in which the boundaries between populations are not sharp or are barely indicated. This unavoidably leads to a certain degree of arbitrariness in the delimitation of populations, and of races and species to which they correspond. Hence, the disagreements among authorities as to how many races a certain species consists of, or whether certain populations should or should not be considered distinct species.

The operational difficulties encountered in the delimitation of races, species, and other Mendelian populations are, thus, an inevitable result of the continuity of the evolutionary process. These difficulties have, however, led many biologists to the view that the only objective units in biology are individuals, while all supraindividual complexes are descriptive devices created by the investigator for his own ends. The antithesis to this attitude, which may, I think, be properly labelled defeatist, is the contention that complexes of individuals often reach levels of integration so advanced that they become “supraorganisms.” The idea of supraorganism has been very ably developed, for example, in the recent book of Allee, Emerson, Park, Park, and Schmidt (1949). These authors are inclined to re-
Mendelian Populations and Their Evolution

gard not only colonies of social insects and various intraspecific aggregations, but also biotic communities consisting of many species, as supraorganisms.

The validity and usefulness of the concept of supraorganism will probably depend on the implications which the term is made to carry. But it must be admitted that one of the striking facts disclosed by modern ecology is that individuals are rarely, if ever, independent of other living individuals; they are nearly always members of more or less highly integrated systems. From the evolutionary standpoint, the individual cannot be considered apart from the Mendelian population of which he is a member. Mendelian populations are the most fundamental of the integrational forms which such systems take among sexually reproducing organisms. The integrative agent is in this instance the process of reproduction itself. The fundamental nature of this cohesive force needs no emphasis, although it is a matter of opinion whether the reproductive bond in a Mendelian population can profitably be likened to the organismic integration of cells and tissues in an individual. However that may be, Mendelian populations are spatio-temporal entities. It is interesting that Mendel's discovery implicitly reaffirmed the reality of both individuals and populations in sexually reproducing organisms. An individual is not a reflection of an ideal species type, because every individual possesses a unique genotype; in sexual species with many unixed genes the probability of two or more individuals having by chance the same combination of genes is remote. On the other hand, the sexual unions and the gene segregations which occur in every generation condition both the continuity and the changeability of the gene pool of a Mendelian population.

It is less evident, and therefore worth greater emphasis, that Mendelian populations are, to an even greater extent than individuals, units of natural selection, and therefore of adaptation and of evolutionary change. Elimination of ill-adapted individuals within a population, exemplified by the destruction of deleterious mutants, is only one of the many forms of natural selection, and this particular form, as pointed out especially by Schmalhausen (1949), is largely a conservative rather than a creative agent. On the other hand, differ-
ential reproduction of populations appears to be very important in adaptive evolution. Most interesting for the present discussion are selective processes in populations with balanced polymorphism. Populations of many species of Drosophila are mixtures of chromosomal variants differing in the gene arrangement of certain chromosomes. Flies with different gene arrangements interbreed freely, so that individuals are formed having two chromosomes of a pair with similar and with different gene arrangements (structural homozygotes and heterozygotes).

Now, the heterozygotes are, with few exceptions, adaptively superior to the corresponding homozygotes. Adaptively inferior homozygotes are nevertheless produced in natural populations in every generation. Poorly adapted genotypes are, consequently, normal components of the species. This seemingly strange situation is, however, explained very simply. If two gene alleles, or chromosomal variants, A¹ and A², form a heterozygote, A¹A², which is adaptively superior to both homozygotes, A¹A¹ and A²A², natural selection will tend to establish an equilibrium state, at which both A¹ and A² will be present with certain definite frequencies. The crux of the matter is that the average fitness of an individual in the population will be greatest when A¹ and A² reach equilibrium frequencies. In other words, natural selection enhances the adaptedness of the Mendelian population as a whole, at the price of continuous production of some less well adapted individuals.

The study of Drosophila populations has disclosed not only that balanced polymorphism is a very common phenomenon in that genus but that the intensity of the selective processes involved is greater than most geneticists suspected might be the case in natural populations. How widely similar phenomena are spread in organisms other than Drosophila is for the time being an open question. Crow (1948), Dobzhansky (1950), and Brieger (1950) have inferred that heterosis in maize must be due to a kind of balanced polymorphism, and it is possible that this is also the case in other sexually reproducing species which have large effective breeding populations. It would be unprofitable at present to speculate as to the extent to which recessive hereditary diseases and genotypic inferiorities that plague human
populations may represent the genetic chaff unavoidable in the production of highly fit heterozygous types. But, assuredly, this possibility must be kept in mind in studies on the genetics of human populations.

The relationships between the adaptedness of individuals and of populations are evidently complex. It is just as platitudinous to assert that the welfare of a population depends upon that of its members as it is to say that the health of the body is determined by the soundness of its parts. Life has evolved a hierarchy of integrative levels: genes, chromosomes, cells, individuals, several orders of Mendelian populations, and of biotic communities. The existence of all levels is based ultimately on some patterns of physico-chemical reactions, as yet unknown, which result in the self-reproduction of certain molecules, or molecular aggregates, called genes. Self-reproduction is accordingly the basic phenomenon of life, because its consequence is the process of natural selection, and hence of evolution. In turn, natural selection increases the efficiency of self-reproduction and develops a diversity of self-reproducing entities capable of functioning in a variety of environments.

The structure of genes and of all products of their integration is an outcome of a long evolutionary development controlled by natural selection. Moreover, and this is fundamental, the different methods of integration are themselves a result of adaptive evolution. This is fairly generally recognized for levels up to and including the individual; for example, the formation of multicellular organisms from colonies of only loosely associated cells is clearly an adaptive step. But the same principle applies with equal force to supraindividual levels, namely, to Mendelian populations and biotic communities.

Mendelian populations owe their existence to sexual reproduction. Darwin and Weismann realized that sex is an evolutionary adaptation, but the situation could not be understood except on the basis of Mendel's discovery. It remained for Wright, Muller, Darlington, and others to develop a cogent theory. Sexual reproduction, with its accompanying mechanisms of meiosis and contrivances that promote cross-fertilization, brings forth innumerable gene combinations which are tested for fitness by natural selection. A Mendelian population
is, therefore, a laboratory for experimentation with genetic materials. From this experimentation come evolutionary inventions. To be sure, such inventions can also be made without sex, by a lucky concatenation of mutational steps. But the probability of success is vastly increased by sexual reproduction. This is why sex has become established as the prevalent method of reproduction in higher organisms. The deterioration of sexuality which has taken place in some groups, chiefly among plants, does not contradict this view. Natural selection is not a spirit endowed with foresight but a mechanism which is basically opportunistic; it favors changes that are immediately useful, regardless of their eventual harmfulness. The advantages of an assured seed set, and other temporary benefits discussed by Stebbins (1950), account for losses of sexuality in evolution.

The integration of individuals into Mendelian populations, into sexual supraorganisms if you will, is an evolutionary adaptation. The further integration of elementary Mendelian populations into populations of higher orders, such as races and species, is likewise adaptive. It can be shown that formation of races and species has become necessary owing to the great diversity of environments found on our planet. In an absolutely homogeneous and constant environment, two or more genetically different groups of organisms could not coexist indefinitely. As pointed out by Gause, this is because one kind would in the long run prove more efficient, and would outbreed and crowd out the others. A single genotype, or at most a single Mendelian population, could exist in an absolutely homogeneous universe. In such a single population, new genotypes of superior fitness might emerge from time to time, and displace the ancestral genotypes. In principle, evolution is, therefore, compatible with the existence of a single kind of organism in the universe, instead of the immense diversity of organisms which actually prevails.

However that may be, the world in which we live is far from homogeneous. The diversity in space is most obvious: different climates and biotic conditions are encountered in different parts of the world. Adaptation to this geographic diversity of environments necessitates a corresponding genetic divergence of populations that are allopatric, that is, inhabit different territories. It is this divergence
which gives rise to allopatric Mendelian populations which differ in frequencies of genetic variants and which are termed races. If evolution consisted of allopatric differentiation alone, its outcome would be a single species, but one which would be more or less highly polytypic, that is, split into numerous geographic races.

A multiplicity of environments occurs, however, also within the ambit of activity of a living individual, or within the distribution range of its sex cells, spores, or seeds. Different foods, different microclimates, several predators, parasites, etc., may occur within small areas. Adaptation to the variety of ecological niches which therefore exist in close proximity to each other, engenders in its turn the diverse life forms which are sympatric, that is, live within territories of the order of magnitude of the distribution means of an individual (see Mayr, 1947, for further discussion of allopatric and sympatric differentiation).

Adaptive diversity of sympatric organisms takes two forms: polymorphism within a population and speciation. Sympatric organisms must, by definition, meet each other, and, if they reproduce sexually and by cross-fertilization, they may also mate and produce offspring. In the absence of genetically conditioned barriers to crossing, sexual sympatric individuals will form, then, a single Mendelian population. Such a population would comprise a variety of genotypes each of which possesses high adaptive value in some of the different locally available environments. Such a population is called adaptively polymorphic.

Polymorphism is, in fact, observed in populations of most species. It is doubtful if any sexual species is adaptively quite monomorphic. Some species and some local populations are, however, strikingly more polymorphic than others. An attractive working hypothesis is therefore that the amount of polymorphism present in a species or a population is a function of the variety of adaptive niches which this population is able to conquer and exploit; polymorphic species would accordingly be adaptively more versatile, and the relatively uniform ones more specialized. This hypothesis has been found to give a satisfactory account, for example, of the different degrees of polymorphism observed in local populations and species of the willistoni
Adaptive systems based on intrapopulational polymorphism have, however, a serious limitation. Adaptive types within a Mendelian population interbreed, and gene segregation takes place in the progeny. So long as the forms crossed differ in only a single or in a few genes (or in balanced polygenic complexes, such as those guarded by inversions in chromosomes of Drosophila populations), the segregation products can fit into one or another of the adaptive niches which the whole population occupies. But, with the progress of evolution of life, adaptive systems have appeared which involve integrated systems of numerous genes. Suppose, for example, that the genotype {A'B'C'D'} enables its possessors to be water-dwelling, and the genotype {A'B'C'D'} to be terrestrial animals. The recombination products {A'B'C'D'}, {A'B'C'D'}, etc., may be altogether disharmonious and unable to exist either in water or on land. Using Wright's very apt metaphor, the original genotypes occupy "adaptive peaks," and the recombinations fall down into "adaptive valleys."

The well-adapted genotypes, {A'B'C'D'} and {A'B'C'D'}, could maintain themselves in allopatric populations, and could live in different territories separated by a geographic barrier. However, if the ecological niches to which each genotype is suited should exist in both territories, then only one of them would be occupied, leaving the other niche empty, in both territories. On the other hand, should the two populations become sympatric, their members would hybridize. The resulting gene exchange and recombination would give rise to a mass of disharmonious genotypes, which would pull down the level of adaptedness of both populations concerned. In reality, this dilemma is avoided quite simply. The populations become sympatric and occupy all the adaptive niches for which they are suited, but only because hybridization and gene exchange between them are prevented, or made rare enough, so that the adaptedness of the populations remains high. This is exactly what is observed in nature: gene exchange between sympatric Mendelian populations is reduced...
or suppressed by a great variety of reproductive isolating mechanisms.

The writer has suggested, as early as 1933, that species are reproductively isolated Mendelian populations. The process of speciation must, then, be regarded as an evolutionary adaptation which permits the development of immense organic diversity, particularly the diversity of sympatric species. Between one and two million species of animals and plants have developed on the earth neither to please nor to plague biologists and collectors. This diversity of species is a device which enables life to exploit the multiform opportunities offered by the environment. Speciation is accordingly a form of integration of Mendelian populations engendered by natural selection in response to the challenge of the diversity of sympatric environments.

In view of the more than century-long, and notoriously inconclusive, debate about what species are, it is gratifying that a fairly general agreement about the matter seems at last in the offing among biologists. It is recognized, except by a few conservatives, that the attainment of reproductive isolation between genetically diverging Mendelian populations is the essence of biological speciation. Reproductive isolation between populations is, accordingly, the criterion for the recognition of the specific entities which have biological meaning. Of course, it does not follow that different species are always "inter-sterile." It can not be too strongly emphasized that hybrid inviability and hybrid sterility are only two of the many kinds of reproductive isolating mechanisms. One could cite numerous instances in which fertile hybrids between species are known in the laboratory or in nature, or in both. What matters is not whether hybrids can be obtained but whether the Mendelian populations do or do not exchange genes, and if they do whether at a rate which destroys the adaptive equilibrium of the populations concerned. Seasons of sexual maturity or of flowering, attraction of different species of insects for pollen transport, being members of different biotic communities in the same geographic region, weakness or lack of sexual attraction between members of different populations, are some of the reproductive isolating mechanisms which may keep species genetically apart, despite the possibility of production of fertile hybrids under some circumstances. Moreover, it is a very common situation that
species are kept apart not by one but by several reproductive isolating mechanisms, none of which may be absolutely effective when taken alone, but which eliminate all gene exchange when combined. An example of this situation are the sibling species *Drosophila pseudoobscura* and *Drosophila persimilis*. Hybrids between them are rather easily produced in the laboratory, and yet no hybrids have ever been found in nature, despite the side by side occurrence of the parental species in many habitats.

Not only the presence but also the degree of reproductive isolation between species will be determined by the exigencies of adaptive evolution. Gene exchange between Mendelian populations is adaptively favorable or unfavorable, depending upon the fitness of the recombination products in relation to the environment. If some of the recombination products are valuable in some environments, it is advantageous for species populations to keep open a more or less narrow channel for gene exchange. Hence the occurrence of introgressive hybridization between some species, and its absence between others. The evolutionary importance and the prevalence of introgressive hybridization have been, in this writer's opinion, greatly exaggerated by some authors. This controversial problem cannot not be discussed here in detail. Nevertheless, it should be pointed out that no matter how common such hybridization may prove to be, the formation of reproductive isolating barriers between Mendelian populations must be considered one of the basic phenomena of evolution. In the last analysis, speciation is an adaptive accompaniment of sexual reproduction, just as sexual reproduction is a corrective to the relative stability of the gene.

Evolution, then, is a creative process, but not in the sense of being directed by some supernatural force, as has often been explicitly or implicitly supposed by vitalists and their modern successors, the finalists. Such a direction would still amount to an inexplicable caprice of a Creator or Director, but a caprice lasting a billion years instead of the six biblical days. Evolution is creative because it involves the formation of previously non-existent coherent entities, Mendelian populations culminating in species; because these entities enable life to spread into and to exploit new environments more and
more efficiently; and because evolution, like all creative processes, involves risk of failure or miscreation, in other words, partakes of the quality which, in application to human affairs, is called freedom. This gives to organic evolution a character which can be described again only by an anthropomorphous term, namely, a character of dignity.

ACKNOWLEDGMENTS

The writer is indebted to Drs. A. B. da Cunha, J. Clausen, L. C. Dunn, C. Epling, J. Gregg, E. Mayr, C. Pavan, and G. L. Stebbins for many discussions, which helped greatly to arrive at clear statements of the problems dealt with in this address. These colleagues do not, however, necessarily share some of the opinions expressed therein.

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Theodosius Dobzhansky


IN celebrating the jubilee of genetics, we celebrate at the same time the culmination and triumph of the biological revolution inaugurated during the nineteenth century, which is destined, I believe, to have even more profound results than the physical revolution inaugurated during the seventeenth.

The geneticists of the world are grateful to the Genetics Society of America for organizing these celebrations. In the 50 years since Mendel's Laws were so dramatically rediscovered, genetics has been transformed from a groping incertitude to a rigorous and many-sided discipline, the only branch of biology in which induction and deduction, theory and experiment, observation and comparison have come to interlock, in the same sort of way that they have for many years done in physics.

I myself, in my own person, have seen genetics grow from the elementary facts of Mendel's two laws, as exemplified by the factorial inheritance (as it was then called) of a few characters of a very few organisms, which were presented to my schoolboy mind 46 years ago in Punnett's little book, to its present position, both central and comprehensive, among the biological sciences. Today, as Goldschmidt has long emphasized, genetics is inextricably entangled with the science of individual development. Furthermore, at one extremity it has become biochemistry, at the other evolution, thus drawing some of the veils from two major secrets of life—the secret of its essence or distinguishing property, and the secret of the variety and strange-
ness of its manifestations. Largely as a result of genetic research, we can today comprehend biological reality as a process, with genetics in the modern extended sense providing the basic machinery of that process.

These 50 years of genetics represent a triumph of intellectual comprehension. Thanks to them, we can now in principle grasp the bewildering multiplicity of the life process—the million and more existing kinds of plants and animals, the succession of other types now extinct, the variety of human groups and individuals, as the consequences of a single basic fact: the existence of complex self-copying and self-varying organic units, the possession by living matter of the dual property of multiplying both its constancy and its variation. The gene-complex in the chromosomes provides the material basis for this dual function, and is therefore the organ not only of heredity but of evolution.

The property of self-copying appears to be due to the cooperation of proteins of a very complex type with a certain kind of nucleic acid. The capacity of genes for variation can be looked on as a result of the complexity of this chemical partnership. The gene is capable of being structurally altered, by certain types of radiation and subatomic particles, by certain chemical reactions, and by "spontaneous" re-arrangements, and yet retaining its essential property of producing a copy of itself. All gene-variations which do not interfere with the power of self-copying are automatically multiplied and transmitted: today we call them mutations. It is now clear that the essential distinguishing property of life is this double capacity for varying within quite wide limits while retaining the capacity for self-reproduction.

**NATURAL SELECTION**

The products of the self-reproducing units constitute the individual organism; and organisms, at all stages of their cycle of development, are subject to natural selection. Natural selection is Darwin's convenient term to denote the results of the differential reproduction of self-reproducing variations in relation to the conditions of the environment. Once we remember that it comes automatically into opera-
tion whenever there is self-reproduction and self-reproducing variation—in other words in all living organisms—we can discount its metaphorical connotations.

There was a time when an influential school of thought denied natural selection any “creative” effects. This view has now had to be abandoned. Natural selection is creative, in the sense that it can and does operate to produce evolutionary novelty, and that without it evolutionary novelty would not and could not have been produced. But it will only be creative in certain conditions of the evolutionary environment; in others it will operate to discourage novelty and to produce and maintain stability.

One of the greatest changes of biological outlook during my active life has been the re-erection of natural selection as the main and perhaps the sole agency of significant evolutionary change and adjustment. The early Mendelians would have found it very paradoxical to be told that their science was destined to lead to this result, but so it was. The recognition of the greater importance of small than of large quanta of variation in heredity and adaptation; the discovery of the prime source of evolutionary variation, in the shape of gene-mutation; the rigorous thinking of Mendelian genetics, which led rapidly to the final discrediting of Lamarckism; and finally the demonstration by R. A. Fisher and others that evolution by natural selection could not be fully effective with any form of inheritance involving either blending, as postulated by Darwin, or with the inheritance of acquired characters, as postulated by the neo-Lamarckians; whereas it could and would work on the basis of particulate inheritance—it was such developments of Mendelian genetics which paved the way for the resurgence of Darwinism. Since the 1930’s, the study of evolution has been revivified and has become more scientific with the discovery of its material basis. It is largely through this revivified study of evolution that genetics has exerted its influence on modern thought.

I may perhaps recall with some personal satisfaction that, perhaps owing to a certain familiarity with field natural history, I clung firmly to a belief in the principle of natural selection and its efficacy during
the period, up to the early or middle 1920's, when it was being neglected or attacked by most of the biologists who thought of themselves as advanced, and when even eminent geneticists could seriously assert that without natural selection all existing types could have come into being, and a vast number of others as well!

I cannot refrain from one personal memory. When T. H. Morgan visited Oxford, I think about 1923, I took him up to the Hope Department of Entomology to see some of the wonderful series illustrating mimicry in butterflies which Poulton has amassed there. When I went back to fetch him for a luncheon, I could hardly prevail on him to move. "This is extraordinary!" he said, with his vivid enthusiasm; and then, "I just didn't know that things like this existed!"

I mention this incident not only for its intrinsic interest, but as a symptom of another great change that has come over the biological scene in the last 30 years—the linking-up of intensive and extensive biology, of laboratory and museum, of experimental research and fieldwork. Each subspecies, each adaptation, each palaeontological trend, is the result of a natural experiment in genetics, on a far larger scale, especially of time, than anything that we biologists can attempt. Now that we are beginning to understand the conditions of such natural genetic experiments, and the forces at work in them, they can yield us results of great value, unobtainable otherwise, and complementary to the results of laboratory research.

ADAPTATION

Natural selection will be constantly adjusting the organism to its environment, biological as well as physical—in other words, tending to produce more and more complete adaptations. Granted the genetic facts of particulate inheritance, mutation, and sexual re-combination, the adaptations produced can be of the most astonishing delicacy and complexity. Thus Muller has recently brought specific proofs of the capacity of natural selection to act effectively on differences beyond the power of human discrimination.

The changed attitude to the problem of adaptation was well
summed up by R. A. Fisher's aphorism, that natural selection is a mechanism for generating a high degree of improbability. The enormous degree of that improbability was, I think, first clearly demonstrated in a general way by Muller in 1929. It is, of course, due to the capacity of natural selection, acting in a Mendelian universe, to combine over a series of generations a number of mutational steps, each of which is itself an improbable or rare event, the separate improbabilities are not merely added up, but multiplied by each other, at each new step. What this involves may be clearly pictured when we recall that the number of generations available for the evolution of the human eye, for instance, is of the order of $10^8$.

Muller gave us a vivid picture of what we may call the improbability-generating capacity of natural selection, by calculating the number of organisms which would be necessary, on the basis of our knowledge of the frequency of mutation and other biological processes, to throw up a single organism as complex as ourselves on the basis of chance alone, without the intervention of selection. An extremely conservative estimate is $10^{3000}$, which much transcends any numerical properties of the entire Einsteinian universe. An improbability-generating capacity of this order would be quite sufficient to account for the most elaborate adaptations.

Thus the old arguments about the impossibility of imagining that "chance" could "create" a hand or eye or other complex adaptive organ, no longer carry any weight. In fact, the "argument from improbability" has recoiled on the heads of its users, and the apparently incredible complication of a organ must now be taken as additional evidence for the power of natural selection. Tertullian, fortified by faith, wrote "credo quia impossibile"; the geneticist, fortified by a quantitative grasp of natural selection, can say "credo quia improbabile."

In general, the argument from design, after originally bringing the very idea of adaptation into bad odor, has been reborn, but radically

1 I once asked Professor Fisher where he had published this, to which he replied that he had merely stated it verbally at a meeting. However, it sums up the situation so pithily that I feel it should be recorded in print.

2 I use this common misquotation, in place of his actual phrase, "certum quia impossible est," because it is familiar, and also here more apt.
transformed. The organization of animals and plants is bound to look purposeful, for it has come into existence by virtue of functionally subserving an end. Indeed, the correlation between visible structure and functional end is so strong that it is usually possible to deduce the end from the structure.

On the other hand, all that natural selection can do, since it is in essence merely differential survival, is to produce something which, in the circumstances, is capable of surviving. There is no universal or ideal recipe for survival in a given habitat or way of life, any more than there is only one way of painting a picture of the Annunciation or the Siege of Troy. A bird breathes in a different way from a bee; but both are well adapted to aerial life. Of two closely related forms, one shows highly detailed special adaptations, the other remains generalized and relies on plasticity of behavior or on high reproductive potential, but both may be able to survive.

This, with the fact that the evolution of any organ is a long historical process, accounts for many of the disharmonies and imperfections shown by organic structures. The eye is a marvel of adaptive coordination; but its construction involves what seem to us extraordinary stupidities, such as the fact that the light has to penetrate a considerable layer of nerve-fibres before it reaches the light-sensitive apparatus. There is an historical reason for this fact: but it seems stupid to us, because we can understand the principles on which a form-registering organ must work, and could design something to meet the specifications (as indeed we have done in the photographic camera). But natural selection cannot design anything as a finished product. It can merely ensure that its product works, here and now.

This becomes important when we consider evolution in its long-range aspects. Shorter-term success through exceedingly close or detailed adaptation may lead to extinction if conditions change, as in plants which have cut down outbreeding, or in animals which have become restricted to one species for their food. A resounding success in one field may prevent later progress: thus, as Krogh long ago pointed out, the insects were—happily for us!—barred from ever achieving even moderate size, and therefore the large brain needed for a plastic intelligence, through their adoption of tracheal respira-
tion, besides being committed to an exo-instead of an endo-skeleton. And any high degree of specialization for a particular mode of life eventually leads to an evolutionary dead end, after which no major improvement or radical novelty is possible.

NON-ADAPTIVE CHARACTERS

I return to the all-pervading influence of natural selection, and the consequent omnipresence of adaptation, both of characters and gene combinations. The establishment of non-adaptive (that is, selectively neutral or even disadvantageous) characters by "drift" in small-population seems, as Fisher has demonstrated, to happen much less often than its discoverer, Sewall Wright, imagined. As Ford, Muller, and others have shown, truly non-adaptive genes and gene combinations are exceedingly rare as part of the normal genetic outfit of organisms. Many characters previously thought to be selectively neutral, such as the chromosome rearrangements found by Dobzhansky or Dubinin in local populations of Drosophila, turn out to have marked survival value, positive or negative, in different conditions; and the occasional "correlated characters," as Darwin called them, which appear as non-adaptive by-products of a potentially adaptive change (such as the tan body-color associated with a genetic absence of phototropism in Drosophila, or the red color of vertebrate blood), will themselves be exposed to the adjusting action of natural selection if they are in the least degree either deleterious or beneficial.

Sometimes indeed, some invisible physiological effect of a gene may turn out to be as or more important than the more obvious but still adaptive visible effect with which it is correlated. Thus, as Ford has finally shown, the spread of dominant melanic forms of moths in industrial districts is due more to their greater general viability than to any protection from predators afforded by their dark color in the sooty surroundings, although the cryptic coloring of the non-melanics is enough to outbalance the melanics' vigor in the normal countryside. Finally, there are many more genes than previously suspected which exert a powerful selective action through their purely physiological results, while their visible effects, though sometimes
striking, appear to be selectively more or less neutral. Thus the white
form of the dimorphic female of the clouded yellow butterfly Colias
philodice, as Hovanitz has demonstrated, enjoys a marked advantage
in certain climates through its propensity to be active at lower tem-
peratures than the "normal" yellow form.

As a human illustration of the power of selection we may mention
the age-incidence of human carcinoma. It is no longer in doubt that
there is a genetic basis for the propensity to form malignant tumors,
including the time of life at which they tend to develop. In women,
reproduction ceases after about 45; and in all human societies up to
quite recent times, the expectation of life was so low that very few
men reproduced themselves at higher ages. Accordingly, since natural
selection operates through differential reproduction, it can work on the
possession of early-occurring but not on those of late-occurring (post-
reproductive) tumors. The result is visible in the fact that the incidence
of carcinoma is very low in youth and early middle age, but shows an
enormous rise between 40 and 50. This rise does not occur with sar-
comas, which are so comparatively rare that selection cannot act power-
fully upon them.

The converse of the delicacy of adaptation produced by natural
selection is the degeneration that occurs when selection is absent.
This, we now realize, occurs inevitably in sexually reproducing species,
through the recombination and accumulation of deleterious recessive
mutant genes which would be weeded out if selection were oper-
ating. In apogamous or apomictic forms, however, the process of
genetic degeneration is immensely slower; and as a result we may
find what I have called "relict adaptations," such as the floral mech-
anism of dandelions, or the copulatory mechanism of obligatory
parthenogenetic Psychid moths—structures which were built up as
elaborate adaptations to cross-fertilization, but now persist uselessly
because they are unable to degenerate.

EFFECT OF SELECTION ON THE GENETIC MECHANISM

One of the most interesting developments of genetical theory in
recent years is the recognition that natural selection will operate to
produce adaptive improvement in the genetic mechanism itself. The need for complexity in the genetic mechanism has led to an increase in the number of different kinds of self-reproducing units or genes; the need for close adjustment of the processes of individual development and maintenance has led to the stabilization of the quantitative relations of the great majority of genes, by means of their union into chromosomes and the evolution of mitosis and meiosis; the need for evolutionary flexibility has, among other things, led to the development of balanced arrangements of multiple factors within chromosomes; including the elaborate special mechanisms that Mather has described as polygene systems. The conflicting advantages of high adaptation in the present and of evolutionary plasticity in relation to possible changes in the future, have given rise to a wide spread in the balance between outbreeding and inbreeding in reproductive mechanisms.

RATE OF MUTATION

Even the rate of mutation itself has been controlled by selection. In general, its rate has been markedly kept down. Left to themselves, molecules of such extraordinary complexity as the genes would be rather unstable, undergoing alterations and rearrangements—in other words, mutating—with such frequency that the genetic stability of the organism, both individual and species, would be endangered. Selection has raised the energy-barriers of genes, and so brought down this instability to manageable proportions. Precisely how this has been effected, we do not know, though clearly the method of reducing the instability of a protein molecule is of immense biochemical interest and importance, and we should carefully study the mode of action of those genes, for instance in Drosophila and maize, which affect the mutation rate of other genes. We can only draw attention to the remarkable fact that, through natural selection, evolving life has controlled its fundamental chemistry, tamed its own basis.

Natural selection has now been studied comparatively, just as has mutation, or chromosomal organization; and we are now beginning to build up a natural history of selection. Selection operates differently in different circumstances. Intraspecific selection may produce results
disadvantageous to the species as a whole. This Haldane pointed out, as also the fact that genetic altruism could be evolved only in forms like social insects where a neuter caste exists. I myself have drawn attention to the fact that intra-sexual selection will be several hundred per cent more intense in certain polygamous types of birds than in monogamous species—with striking visible results in the exaggerated development of male display characters. Sewall Wright has demonstrated that selection will be much more effective in obtaining new adjustments to new conditions in wide-ranging species which consist of a number of partially discontinuous subspecies, than in those, whether small or large, which are continuous throughout.

Most important is the fact, particularly well brought out by Simpson, that the different rates and directions of evolutionary change which we find when we study the actual course of evolution, can in principle be explained by differences in the strength of selection, and in the way it acts in different phases of an evolutionary cycle, or in relation to different degrees of ecological opportunity, or on types with different reproductive mechanisms. Thus we no longer need appeal to imaginary changes in mutation rate, to orthogenetic mutation, or to any mythical élan vital or entelechy. This confirms and extends R. A. Fisher's previous conclusion, reached on theoretical grounds, that selection and not mutation pressure must be the primary cause of evolutionary change.

Wholesale extinction will occur among established dominant groups of animals when environmental conditions change markedly, as they do during a geological revolution for instance, and especially when another type able to take advantage of the new conditions is in existence. So-called explosive evolution (explosive in the sense that it is to be measured in hundreds of thousands rather than in millions of generations, which for higher animals means million-year rather than ten-million-year units of time) occurs whenever a new and as yet unspecialized type finds itself in a situation with great ecological opportunities, as when the early marsupials colonized the mammal-free Australian region, or the early placentals could take over all the ecological niches rendered vacant by the extinction or reduction of reptiles.
In such conditions of wide ecological opportunity selection causes a generalized form to radiate rapidly into a number of basic types, each at the beginning of adaptation to a particular way of life. This "explosive" phase is followed by one of specialization, in which the group of species constituting each type is pushed on by selection at a more or less steady rate and in a more or less common direction, towards a higher degree of adaptation. This steadying of the rate of evolutionary change will automatically occur when all the main ecological niches have been occupied; selection then can do no more than ensure that they are occupied more efficiently. Furthermore, once a certain kind of specialization has begun, selection can only act so as to produce a further degree of the same specialization. But what is at work is orthoselection, not orthogenesis.

Eventually, however, specialization reaches a point at which further change in the same direction is impossible, or is not of biological advantage, or would even be disadvantageous. Selection then acts as a stabilizing force on major change, though it is often still capable of making minor adjustments such as the production of new species.

Even such minor evolution, however, will vary in mode and rate according to the nature of the type on which selection is acting. Thus James Small has shown that the rate of formation of new species in diatoms from the Upper Cretaceous to the present is much more rapid in the Pennales, which possess allogamous sexual reproduction, than in the Centrales, which lack it.

There exists finally what might be termed all-round specialization, for it consists in the evolution of a new general type, not specialized for any particular way of life, but embodying an all-round improvement for dealing with the environment—including, of course, the biological as well as the physical environment—and with the business of living in general—temperature-regulation, for instance, or a placenta, or a brain conferring greater capacity of profiting by experience. But it also has the property of not leading up an evolutionary blind alley, as one-sided specialization always does, so that it is only through such lines that continuing advance to higher levels of possibility is assured. For this reason, accordingly, one may properly give such tendencies the name of evolutionary progress.
Genetics, Evolution and Human Destiny

Whatever we call it, the fact remains that among the thousands of dead-end and often vanished specializations, and the millions of species, evanescent variations on a theme, to which those specializations have given rise, a small and constantly diminishing number of trends have assured the longer-range continuity of life from one level of its achievement to the next.

All this is relevant for our commemoration of the progress of genetics, since we can no longer separate genetics in the restricted sense of heredity—inheritance and variation from one generation to the next—from genetics in the extended sense of evolution, that is, inheritance and variation over periods of geological time. The one is an extension of the other.

Today, thanks largely to the past 50 years of genetics, we possess new ways of looking at both these processes. Like so many other new viewpoints imposed by scientific discovery, they are at first difficult to adopt, and indeed may seem unnatural. But once they become established, they sweep away huge fields of error and superstition.

I well remember the difficulty I had as a boy of weaning myself from the idea that an animal must transmit its visible characters in heredity, and of thinking in terms of the transmissions of unit-particles which then interact with their environment to produce the visible characters. Even today Lysenko and his followers find this so difficult that they call it nonsense, and cling to the Lamarckian view; and this, however natural it appears to the naive mind, is, luckily for the human race, incorrect.

Teleology, maternal impressions, and the peculiar beliefs of various peoples and epochs as to the role of the two sexes in heredity—all these and much else have also fallen into the intellectual discard as a result of the progress of genetical science.

Natural selection appears at first an equally topsy-turvy way of looking at things. It seems at first so obvious that the animals we know are what they are because of some conscious desire or purpose, that we recoil from the notion of their having been produced by the blind and automatic agency of natural selection. And it is difficult to realize that, even though some organisms may utilize purpose and other mental or psychological functions during their individual lives, pur-
Pose can have played no part whatever in their evolutionary origin. One of the major features of evolution thus appears as the generation of apparent purpose by means of the blind and unconscious siting of non-purposeful variations. But once the difficulty is overcome, the selectionist approach turns out to provide new illumination as well as a new angle of view. Fancies and superstitions drop away, and order reigns—an order producing results stranger than any human imaginings, but vast and monolithic, revealing the whole of life as one unitary process. Life in its entirety is seen as another name for the process of biological evolution. That process automatically results from self-reproducing but self-varying living matter interacting with its physical and biological environment, through the indirect mediation of natural selection. It is irreversible, and constantly produces novelty and increasing variety. Like other processes, it takes a definite time to run its course—rather over a thousand million years, to be as precise as is at present possible. As a generator of major progressive change evolution in its original form now seems to have ceased. Where major progressive change is still occurring, it has become quite subsidiary to the new process of human evolution, different both in methods and results, by which it has been superseded.

In transforming itself, life also transforms its environment, even its inorganic environment. The rocks of the earth's crust, its covering of soil, the composition of its atmosphere, the distribution of temperature and pressure and moisture over its surface—these and much else are different because of biological evolution.

If asked to name the most significant characteristic of the process of evolution by natural selection, I would say the fact that it steadily reveals new possibilities inherent in living matter. The upper limit attained by life in respect of complexity, of knowledge of and control over environment, of independence of external change, of individuality (or more correctly individuation), of internal harmony of parts, and many other properties, have constantly risen during geological time.
MIND

It would have been more correct to speak of the possibilities inherent in the world-stuff; for the most startling potentiality revealed by evolution is mind, and mind cannot be said to be contained, even as a potentiality, in matter. In most organisms—all plants, and all animal types produced in the early stages of evolution—there is no direct evidence of mind at work, no need to postulate mental properties. But higher animals are clearly the seat of mental processes akin to ours, processes of perception, cognition, emotion, will, and even insight.

We must conclude that the world-stuff possesses not only material properties, but rudimentary potentialities of mental properties as well, and that these properties, when specialized out of their latent state into actuality, are of advantage to their possessors.

We have a rough analogy in electricity. The electric eel and the electric ray are capable of giving a powerful shock. This has been known empirically for thousands of years, and is said to have been utilized by Galen, as a cure for persistent headache. But no one knew what shock was. Much later it was equated with other large-scale electrical phenomena; and later still it was discovered that all material changes, including those in the nerves, muscles and glands of our bodies, are accompanied by minute electrical changes. These have no special biological function; they are an automatic by-product of the fact that all matter has an electrical aspect, or, if you prefer, that matter is under one aspect electricity. But in these two groups of fish, special mechanisms have been evolved for accumulating, reinforcing and intensifying these minute changes until the resultant electrical phenomena have become of specific biological value to their possessors in the struggle for existence.

The same sort of thing, mutatis mutandis, would seem to apply to mind. The world-stuff has two aspects, material and mental, the material being what is detectable and operative externally in a given process, the mental being the same process as experienced from the inside. (The one real difference from my previous example is, of
course, that the word aspect is used somewhat differently in the two cases, in that the electrical and other aspects of matter are commensurable, while the material and mental aspects of the world-stuff are not.)

In most processes, the mind-aspects of the world-stuff are still as undetectable as were the electrical aspects of material processes up to the late nineteenth century. It would indeed be best not to call them mental at all, but mind-like, or mentoid, if such a hybrid term be permitted. And in most organisms they remain as biologically functionless as are the electrical accompaniments of the processes of life. But in higher animals, notably in vertebrates, but presumably in arthropods and cephalopods as well, the special mechanisms we call brains were evolved, one of whose functions is the intensification of the mentoid properties of the stuff of which they are made to a level at which they can properly be called mental properties, and at which they become of biological service to their possessors. Looked at objectively and materially, the biological value of a brain is that of being an organ for general coordination of all information about significant changes in the outer environment and the animal’s own organism.

We do not know why this intensification of the mental aspects of the brain’s activity should be of biological value—why it should be directly useful to an animal to perceive an image of the outer world, to experience the impact of quantitatively different wave-lengths of light as qualitatively different sensations of color, to feel an emotional reaction towards an event, to have a sense of purpose with regard to an action. We can only take the evidence of the colossal natural experiment of evolution that they are. For we can be quite certain that unless acute perception, for instance, did play an indispensable part in utilizing sensory data, unless highly developed cognition and other mental properties were not somehow necessary to secure the success of the brain as a coordinating organ, they could not possibly have been brought into being by the utilitarian mechanism of selection that operates in biological evolution.

Whatever the mode of their origin, eventually the mental properties generated by the process of biological evolution came to have an effect upon the course of that process. If it had not been for the
existence of the mental property of perception—the perception of a complex shape or color-pattern for instance—there would not exist any cryptic, warning or threat coloration, any mimicry, Batesian or Mullerian, any bright flowers, any display-plumage in birds, any recognition marks. This emerges most clearly from the fact that color is well developed in the patterns evolved in biological relation to organisms which are known to possess color-vision, but is much less prominent (and is apparently utilized merely to effect contrasts in intensity) when the sifting organism possesses only black-and-white vision.

The transmission of individual mental experience or of its results, however, does not occur in animals, save for a few cases among the higher vertebrates in which there is some sort of training or education of the offspring by the parents. But even here, it only bridges the space between one generation and the next, so that it is never cumulative, and there is nothing that we could call an organ of experience common to the entire species. Essentially, then, the mental functions of life were generated by the material process of natural selection, and are transmitted indirectly by the material vehicle of the gene-complex. Below the human level, mental functions and activities have not succeeded in invading the evolutionary process itself.

The discovery of the nature of the genetic process has led at one and the same time to a realization of the vast scale of its field of operation, and also of its limitations. In its short-range aspects, the genetic mechanism, by its nature, cannot transmit experience or knowledge acquired by the individual organism, or the effects of the environment upon the organism, or those of its individual will or purpose. It can indeed transmit no mental experience, nor any result of mental experience, but merely the capacity for having a certain kind of experience, including in some animals the capacity for learning by experience. It is a purely material mechanism and accordingly cannot be either operated or transformed except by the tedious, difficult, and often wasteful material process of selection, natural or artificial.

In its long-range aspects, the genetic mechanism is also limited in the range of results which it can produce. Sometimes the limita-
tions inhere in the nature of the material on which it has to work. For instance, all higher organisms depend on the energy of muscular contraction for their locomotion; and accordingly no flying animal could ever approach the size of even the smallest airplane. Again, it is probably impossible for a living organism ever to develop a wheel mechanism, since, at least in the early stages of its evolution, a rotating part could not be supplied with nourishment. Again, as I have already noted, the mechanism can never act by foresight or even as if by foresight: it always operates ad hoc, by a constant patching towards better performance in existing circumstances.

In the most general terms, its results are always utilitarian and always relative. They are utilitarian because they come into existence only in virtue of helping their possessor to survive and reproduce; they are relative in being related to their utility, to the material in which they have to be expressed, and above all to the environment in which they have to demonstrate their utility.

Thus, as Darwin long ago pointed out, natural selection can never generate anything which is primarily for the advantage of another species. Nor can it generate anything except in relation to some definite factor in the environment. Why, for instance, do we not possess an electric sense, that could save us from getting killed or damaged by live rails and high-tension wires? For the simple reason that no such objects, nor indeed any electric currents capable of inflicting damage, and also capable of being avoided, existed in the world before the present century of our era: the only dangerous electrical phenomenon in nature, apart from the rare electric fish, is lightning, and no sensory equipment would enable us to avoid that. Why do we not possess a radiation sense? Because, on the negative side, the radiations which reach us from outer space are not intense enough to cause any appreciable physiological damage; and, on the positive side, because they are not vehicles of any immediately useful information, as are light-waves and sound-waves. Why do we not possess telescopic eyes? Once more, because the advantage given to our animal ancestors by such organs would have been negligible or nil. On the other hand, why is our vision restricted to about one octave of light-waves, the main part of which is common to all seeing ani-
mals, though there are slight variations in its limits at either end? Partly at least, because the light that reaches the earth and is reflected from the objects which it is useful for animals to see and to be aware of, has its maximum energy concentrated in this region.

Why do we like the sweet taste of sugar? Doubtless because sugars are both common and nutritious. Lead acetate also has a sweet taste, but is poisonous; we may safely say that if it had been common in the environment, and sugar scarce, we should find sweetness unpleasant. The fact that in man (and apes) some individuals are genetically equipped to be able to taste phenylthiourea and others are not, show how readily selection could get to work in such matters.

In a way most disconcerting of all, there seems no escape from the conclusion, drawn forty years ago by Bergson, that our intelligence, the main mode and organ of our cognition, is enmeshed in this web of relativism—a utility article, distorted and limited in comparison with what cognition could be if developed as a pure activity, for its own sake. Since our environment is for practical and immediate purposes cut up into separate pieces—objects—with different properties, and with different biological significance for the animals with which they come into relation, our main organ of knowledge has been constructed so as to emphasize this discontinuity, and to be able to handle the continuous flux of reality as a series of separate objects. The other type of cognition—synoptic cognition by insight or intuition, whether aesthetic or intellectual, or by comprehension of a complex situation as a whole, especially when that situation is a process in time—is still for most practical purposes quite subsidiary. The works of the great artists, the insights of the great scientific discoverers, and the recorded experiences of the great mystics give us a dim idea of what synoptic cognition could have achieved if it could have been adequately developed during evolution.

It seems at first sight very surprising, though very significant for general thought, that natural selection, operating on the self-varying material mechanism of heredity, can take life so far and no farther. Up to a certain point, it seems, natural selection can continue to generate real novelty, to reveal new possibilities inherent in the
world-stuff: beyond that point it cannot go unaided, but needs supplementing by a new method.

Earlier I spoke of the diminishing number of "progressive" lines leading to new levels of possibility. By the Pliocene, these seem to have been reduced to one only, that leading to man. Elsewhere, in all other animals and in all plants, major progressive evolution, it appears, had come to an end. In our present knowledge of evolutionary genetics, it can never start again so long as man remains in existence. It is indeed probable that it could not do so even if man were to become extinct. It is, of course, difficult to prove a negative, and possibly a generalized and intelligent type like the raccoon might be able to take advantage of the situation: but we can be fairly certain that most mammalian lines, including the monkeys and apes, are now too specialized.

Genetics and evolution have thus provided man with a new and scientifically grounded picture of his destiny. It is to continue the evolutionary process onto new levels of achievement and progress. Man is the spearhead of evolution, the sole agent or instrument of the cosmic process for securing further evolutionary advance. The general implications of this fact are tremendous—much too vast for me to deal with here. I can only mention some of its mere narrowly biological implications.

We have seen that natural selection cannot push specialization beyond a certain limit. It would seem that, about 10 million years ago, the specialization of life as a primarily material organization had reached its limit, beyond which it could not be driven by the material methods of natural selection.

TRADITION

But what of the one progressive line that did produce a new dominant type—our own—and in so doing introduced life to new possibilities? This confirms my argument, for it was only able to do so by adopting quite new methods. In human affairs, both continuity and change are secured primarily through a new method of transmission, usually called tradition. While biological genetics rests upon
a material basis of genes and chromosomes, tradition—the genetics of human societies—rests upon a mental or psychological basis of socially transmissible ideas, emotions, or attitudes. Instead of the self-reproduction and self-variation of matter we have the self-reproduction and self-variation of mind. But whereas, in a thousand million years of biological evolution, the self-transmitting matter of the gene-complex has become organized into a beautiful and delicately ordered system, there has been no time for anything of the sort to happen with self-transmitting mind. There is as yet no single over-all system of what is to be transmitted; transmission is still crude and haphazard, and its effects are often the reverse of what was expected.

The new method of securing continuity and change, however, enjoys two enormous advantages over the old. The first is that it involves consciousness, and accordingly, unlike the method of biological evolution, can utilize foresight and planning. Thus the individual steps in social variation, unlike the random steps of gene-mutation, will on the whole show an adaptive bias, and the whole process of social evolution has the possibility of becoming purposeful.

The second advantage is that it enables and indeed necessitates the pooling of individual mental experiences; it is therefore essentially cumulative, and may cause change to occur in a geometrical ratio, instead of by the arithmetical steps of slow addition of which alone natural selection is capable. Accordingly, we find that the rate of human social evolution has throughout been of a different order of speed from that of biological, that it has shown and is still showing an acceleration, and that in the last twenty thousand years it has introduced the world-stuff to more new possibilities than biological evolution had been able to do in the last twenty million.

It is worth noting that in the one field where deliberate attention has been given to the technical problem of how to acquire, transmit, and accumulate experience—the field of natural science—the advance has been most extraordinary. Here we can say that the mechanism of progress itself has been improved, and rapidly so. This at least gives us hope that similar disinterested (but not uninterested!) attention to the techniques for efficient transmission of artistic experience, of a sense of participation, of a common background of thought and a
general attitude of pooled purpose, would result in equally desirable and equally spectacular improvements.

This is certainly not the place to pursue the subject of man’s new method of evolution. I will merely point out that it is impossible to transfer the ideas and principles of biological genetics and evolution directly and apply them wholesale to man’s social genetics and evolution. They either do not apply, or apply only in a limited and often misleading way. Each time that evolution passes a critical point—from inorganic to biological, and from biological to human—new principles are introduced, and the old ones, though they continue to operate, do so only as second-order factors.

However, there is one point on which the experience, if not the principles, of biology can be of service in regard to man’s new method of evolution, and that is in relation to the method of studying it. The triumphs of genetics which we are here celebrating have been made possible through concentrating on the mechanism of transmission instead of on the visible characters of organisms. Genetics, long-range as well as short-range, made negligible progress so long as the fundamental distinctions between phenotype and genotype, and between environmental and genetic influences, were not recognized. This recognition, elementary though it seems today, was not easy. It is natural to assume that what we have to account for is the transmission of the effective characters of the animals and plants about us: but it was precisely this assumption that made the early work of the biometricians sterile and led the Lamarckians so far astray.

We can be sure that, in spite of many differences in detail, the same holds true in principle for social genetics and evolution. The transmission and transformation of ideas and attitudes, of understanding and of directive thought and feeling, can never be immediate or direct; it proceeds indirectly, through vehicles and mechanisms of one sort or another. We need to know what vehicle is needed to convey the essence of an idea to the average man and woman, to the bulk of the community as opposed to its leaders. We need a fuller appreciation of the stages and mechanisms of development of the individual mind, including its repressions as well as its transcendences,
its tendencies to projection and other false simplifications as well as its pure intellectual capacities, if we are to make an effective bridge between the minds of successive generations. We need much more study of the material bases of mental transmission—ritual, language, art, shared actions. We need a study of the barriers to participation, whether barriers within the individual mind, between minds of different types and different degrees of development, or between different groups, before we can secure a more fruitful participation. We need to know more about the material and mental brakes on change, and also about the dangers of too rapid change, before we can evaluate the transmissive mechanisms of past human history, much less begin to think of planning improved mechanisms for the future.

You may say that this is obvious. I do not think so, and I reiterate, as the main piece of advice which geneticists can properly give to their colleagues in the human sciences, the need to concentrate on the mechanism of transmission, including the transmission of change, in human societies.

Furthermore, geneticists should be more active in the field of education, to ensure that the facts and principles of genetics and evolution—genetics in the broadest sense—find their rightful place and are universally and adequately taught. Genetics is the most rigorous of all biological disciplines, and of the utmost importance for an understanding of social affairs. And as for evolution, it is certainly the greatest clarifying and unifying concept in biology: yet even today, nearly a century after the publication of the Origin of Species, it is scarcely even mentioned in many schools, and very inadequately utilized—at least in my country—in most university teaching.

Evolutionary biology is the best example of the historical approach in science, as opposed to the observational or the experimental; and thus it provides the best link between physico-chemical science and human history. Furthermore, it is the only bridge between the purely material world of lifeless matter and the human world of mind, thus enabling every aspect of reality to be seen as part of one comprehensive process. The best of all ways of celebrating the jubilee of genetics would be, I suggest, to launch a large-scale campaign to se-
care for genetics in this broad sense its due place in general education.

I said earlier that in man the biological mechanism of heredity and evolution had on the human level become increasingly subsidiary to the new social method of transmission of tradition. This is true at present; but it does not mean that it is no longer important, nor that it might not later become equally essential.

In the first place, the genetic diversity, both of human individuals, and of human groups, is greater in extent and has had more important effects than is now customarily admitted. The enormous phenotypic differences, in individual and social or group achievement, are of course obvious. At the moment, it is socially and intellectually fashionable to minimize or even to deny such genetic differences. This is sometimes done in the name of democracy, or because of the hypnotic effects of the ideas of the American and French revolutions concerning the equality of man, or as a misinterpretation of Christian doctrine, or in natural reaction against the errors of racism, and of eugenics when treated as a dogma and not as an applied science. The Russian Communists have adopted a slightly different attitude, involving a belief in Larmarckism and therefore in the possible equalization of men as a result of equalizing social conditions; it is this, among other things, which has led them to repudiate Mendelian genetics on ideological grounds. Some of them indeed are now maintaining that man is no longer at all subject to purely biological laws, but only to social laws! In addition there has been the realization that man is the most plastic of organisms, and that differences in environment and opportunity can account for enormous differences in achievement. This, however, is far from equivalent to saying that differences in achievement are always due to differences in environment and opportunity. They certainly are not, and I see very large openings for genetic studies in human affairs.

In the field of individual genetics, such studies are already well advanced, and we can look forward with confidence to being able to map the distribution in the population of the genetic bases of various important properties—intelligence, resistance to various diseases, longevity, special aptitudes, temperament, and so forth.
Again, we may see how certain characteristics are unexpectedly favored by certain changes in the social sphere. For instance, as Muller was the first to point out, a genetic tendency to short sight can be useful to its possessors in a society which has come to have need of fine workmanship, but has not yet discovered optical methods of correcting myopia or adjusting our vision to very short distances.

When it comes to differences between groups, the distinction between what is due to genetic difference and what to differences in environment is much more difficult. We can no longer take advantage of the varying degrees of genetic relationship provided by human mating and measure the correlations between identical twins, sibs, cousins and the like so as to disentangle nature from nurture.

However, now that the general principles of genetics and selection have been thoroughly established, we can apply them to particular social circumstances and see what conclusions can be drawn from them. While such deductive method falls short of experimental proof, and while, in the welter of multiple causation which envelopes all social phenomena, it cannot give the quantitative accuracy which it achieves in astronomy, for example, yet it can provide qualitative indications of great importance, and help us considerably in our understanding of human history. If it cannot provide proof, it can often provide strong presumption.

On the basis of our established knowledge about (1) genetic variability in general and that of the human species in particular, (2) the efficacy of selection and the detailed adjustments it can produce, and (3) the evolutionary effects of different kinds of mating systems, we can make deductions about quite a number of kinds of human groups. All I can do here is to mention a few examples of the sort of conclusions which may be drawn, and to hope that this branch of applied genetics will receive the attention that it deserves.

We may, for instance, safely presume that the visible and measurable differences between the major racial groups are in the main adaptive, as are those of any animal or plant populations which have evolved in relative isolation in climatically and ecologically different regions. Then it is to be presumed on general grounds, though it has not yet been proved, that some at least of the racial groups will, if
proper techniques and methods can be devised, be found to differ in average level of intelligence. Although we can be sure that any such average difference would be only moderate, and that populations of different racial origin would always be found to overlap in genetic intelligence except for a small sector at either extreme, yet it must be pointed out that where intelligence is, as with the method of social transmission found in man, a major factor in progressive change, a quite small excess of individuals of very high intelligence will have a disproportionately large effect.

It is also true that, in many kinds of human social system, a small minority, if possessed of the right genetic qualities as well as of appropriate traditions and of social or political power, can effectively dominate a much larger group. It seems fairly clear that many conquering minorities, like the Moguls, or the Normans, or the Watusi, were selected for genetic qualities conducive to what is broadly called leadership before conquering the people over whom they later ruled—who in their turn may often have been pre-selected for patience or docility. When the dominance of a minority has continued for long periods, it will generally be found that either inbreeding or some form of mate selection has been at work to perpetuate the genetic differences between dominant and subordinate groups. But when there has been random outcrossing, and still more when, as after the early conquests of Islam, there has been polygyny together with mate selection that has not been concerned with qualities making for leadership, the genetic differential can be, and has been, rapidly dissipated.

The best example of mating systems which perpetuate or even accentuate genetic differences in human groups are, of course, those operating in castes like those of India. A really scientific analysis of the environmental and genetic contributions to the differences between a few selected castes would be extremely illuminating.

Then it must be pointed out that way of life must have had a strong selective influence on human groups. The most obvious example is the difference between nomadism and agriculture. Success in the two ways of life demands very different qualities: and we can be sure that, through differential survival and also perhaps more so
through defection to the opposite way of life, nomads and agriculturists early came to differ considerably in many temperamental qualities.

Migration, of course, often has strong differential effects. Thus for instance the presumption is overwhelming that the Irish who emigrated to the United States in the mid-nineteenth century were significantly different in their genetic qualities from those who stayed in Ireland. The rural exodus from country to town, as for example in nineteenth century Britain, also presumably exerted a sifting action.

Wholesale migrations of entire communities will also often have genetic effects, though of a rather different kind. The migrations forced on Central Asiatic tribes, for instance, by climatic change or by pressure from other peoples, must have weeded out weaklings and favored both physical and temperamental vigor. Although it is exceedingly difficult to disentangle genetic and environmental factors, general theory assures us that both must have been operative.

To sum up, though of course communications and outcrossing of all sorts are constantly tending to break down the genetic differences which isolation and selection, migration and inbreeding have established, many such differences still persist, and an understanding of them will aid materially in the interpretation of history.

On general genetic principles, the product of crossing between two distinct geographical races is likely to be less well adapted than either parent strain, as is well seen in the unfavorable results of crossing certain breeds of dogs, like St. Bernard with Great Dane, as shown by Stockard, or in natural examples like that of the hoodie and cor- rion crows, which interbreed freely along the 5000 km. boundary where their ranges come into contact, to produce a hybrid zone, whose narrowness proves that the hybrids must be at a selective disadvantage. In man, the probable production of a certain number of "disharmonic" types through wide crossing will certainly be offset, and may well be outweighed, by that of valuable new recombinations: but we must not imagine that all the effects of "racial" crossing in man are necessarily good, and may not include certain undesirable genetic results.
One of the social implications of genetics is all too obvious: the human species is faced in the biologically immediate future with the possibility of genetic degeneration. We know that in any normal species mutations are constantly occurring, that most mutants are deleterious, and that selection is constantly eliminating them: there is thus a balance between new mutations and their elimination. With complete recessives, the manifestation of the mutant in double dose must precede its selective elimination: if selective elimination is reduced, deleterious recessive mutations will accumulate, and will be manifested in higher frequency.

This must certainly be happening now as the result of civilization. Selection of many sorts is being reduced—by medical care, by social services, by diminution of selective competition, both intra-group and inter-group. The inevitable result, unless steps are taken to prevent it, will be a gradual lowering of the average level of the genetic basis of all desirable human qualities.

In some civilizations there is a further tendency at work—the differential reproduction of different types and of different social and economic classes—the effects of which are also probably on balance deleterious. In the United States one-sixth of the population is producing one-half of the next generation: it is most unlikely that this fact has no differential genetic consequences. Both in Communist Russia and in most capitalist countries, those with higher genetic intelligence have, on the whole, a lower reproductive rate than the less intelligent, and this must be dysgenic. The higher reproductive rate of the economically lower levels in many capitalist countries probably means a slight differential multiplication of the more shiftless and less enterprising, and in any case cannot possibly be favorable in its results. The hopeful fact that in one or two countries, such as Sweden, the differential has been reversed, so that the economically higher classes now have the higher reproductive rate, should be carefully studied and its lessons applied.

If we remember how small a degree of differential survival can produce a major result over a series of generations, we have every reason to take these questions seriously. What to do about them is another matter. Personally, it seems to me that in this case the best
defence is attack, and that the best point of attack at the moment is in the field of ideas.

In this field there are various things which the geneticist can do. He can point out the present dangers of degeneration as inescapable deductions from the established facts and principles of his science: and, more important because more positive, he can indicate the future possibilities of improvement, by means of a rational eugenics.

The geneticist is naturally well aware of the fact that for full expression of a character you need both genetic basis and suitable environment, and will only disagree with the social reformer if he is told that social reform can do everything that is required. Here the geneticist has a solemn duty to perform, in warning against false science and unjustified optimism. He can point out that eugenics does not necessarily connote ideas of racial or class superiority, or wholesale sterilization, or an impossible reversion to the wasteful and inhuman, or rather pre-human methods of natural selection by means of disease, famine, and other agents of high mortality.

Nor does eugenics mean entrusting "the authorities" with some arbitrary power of deciding what are good and what are bad hereditary qualities. It is extremely simple, I should have thought, to obtain agreement on the view that certain heritable characteristics are generally desirable—notably greater physical vigor, less liability to specific defects and diseases, greater intelligence, and various special aptitudes. If we could raise the average of these characteristics by even five or ten per cent, an almost incalculable burden of suffering, frustration, and inefficiency would be lifted from the shoulders of man.

What is more, the geneticist knows that by appropriate methods, such a result could be perfectly well achieved, not in a few years or a few decades, but over a measurable series of generations. Admittedly, this could not happen without somewhat radical changes in our laws and customs and above all in our general outlook towards various basic questions involving sex and reproduction. But this is not to say that it is impossible. One outlook can be overthrown by another. Once the fact is grasped that we men are agents of further evolution, and that there can be no action higher or more noble than the raising of the inherent possibilities of life as represented by the
human species, then we shall somehow find ways and means for overcoming the resistances which stand in the way of our performing that part of our destiny and our duty. Here, again, it is knowledge and understanding which can liberate us and make action possible.

There is one point of method which we ought not to forget. And that is that the scientific eugenist, the geneticist who wants to apply his genetics in the form of eugenics, is bound to advocate the fullest possible equalization of opportunity, not only on moral or political grounds, but for technical genetic reasons—because it is the simplest way of finding out the degree of genetic inequality present, and therefore of ensuring the rapidity and effectiveness of any eugenic measures that may be adopted. The only caveats concern the speed with which it is effected, too great a speed being likely to upset the nation’s economy and its stability; and the methods used, to ensure that they do not involve levelling down as well as levelling up. The Russians, with their mystical belief about equality, are still shrinking from this apparent paradox; and so, for quite other reasons, are various groups in the West, groups both left and right in their political complexion. But the apparent social paradox is a methodological truism, and one which it is the responsibility of the geneticist to make clear.

There is one related point, where the geneticist and the human scientist can profitably join forces. It appears that in evolution in general, the upper level attained by life is as valuable a measure of evolutionary progress as the average level—in some ways more so: and the historian and anthropologist will tell us the same sort of thing about human history (though in modern conditions the average level is coming to play an increasingly important part). Further, in human evolution, due both to its new method of variation and transmission, the exceptional individual can play a much more important role than in any animal species, and the genetically gifted minority will of necessity be the most important agency of any change deserving the name of progress. But it will not be as efficient as it ought to be unless it is provided with the most favorable social environment for developing its genetic potentialities.

Here we have a further paradox. It is obviously both necessary and desirable to raise the level of nutrition, health, education and oppor-
tunity of the average, the bulk of the community, the Common Man; but it is also necessary and desirable to raise the level of opportunity and achievement of the genetically gifted minority, the élite in the proper sense of that much-abused word, as meaning the people chosen to do the work which only they can do as it should be done. And the opportunity should include not merely educational facilities and opportunity to devote the maximum effort to their real work, as little as possible hampered by material cares and social frictions, but also the possibility of perpetuating both the stock and the tradition of an élite. How to manage to do this, instead of dragging the best down towards an ineffective mediocrity (even if the mean level of that mediocrity is rising), or on the other hand of encouraging the best at the expense of the mass, and therefore exploiting the mass and preventing it from participating fully in the social adventure—that is the problem, and by no means an easy one. Perhaps the solution for the time being is to aim at a basic minimum of opportunity for all, together with a maximum of opportunity for those whose talents enable them to grasp it. In any case, it can at least be posed as a real problem of how to get the best out of the limited amount of exceptional talent available; and to pose a problem in concrete terms is a necessary first step toward its solution.

In conclusion, I would say only this. First, that geneticists may well be proud of the part they have played in making possible the new conception of human destiny which I earlier outlined. They have provided the intellectual and scientific foundations on which alone the idea of man as the spearhead or agent of evolution can rest and can develop in stability and strength.

Secondly, that one of the clearest conclusions to be drawn from a genetic and evolutionary approach to human affairs is that ignorance is one of the chief obstacles to human advance, and that the acquisition of new knowledge is probably the most important of all man's evolutionary tools. Genetics is a basic and fundamental field of knowledge, and geneticists, if they are ever assailed by doubts as to the value of their specialized work, or attacked on account of its alleged lack of utility, can be reassured of the high value of their professional efforts for the progress of their species.
Thirdly, that a study of evolutionary progress, and of the directions and trends that have proved desirable in the past, helps to indicate the aims of the consciously-directed evolution of the future; and that one of those aims, as ultimate as anything can be in this relativist and imperfect world, is the production of more fully-developed and well-integrated human personalities, with the capacity for entering into more fruitful personal relationships, and for enjoying higher and more complete satisfactions. And this, in addition to providing valuable guidance to geneticists when consulted on human genetics and eugenics, can be an assurance and encouragement to them in their other capacity, as human individuals.

And finally, I would say that a realization of the truly astonishing advance made during a mere 30 years in this difficult and obscure subject, which demanded new methods and new attitudes as well as an unusual degree of cooperation between different specialities, is extremely encouraging, and one of the few grounds for optimism in this difficult world of today. In particular, when I think of the violence of the disputes on this subject between various sects of science a mere 30 or 40 years back, and how these have now been reconciled by the development of genetics into a single corporate body of scientific knowledge and principle, with its own field, its own momentum, and its own power of adaptability and progressive change, I wonder whether we may not look forward rather more optimistically than is now the fashion to a reconciliation of the world's present warring political and social ideologies in a similar higher synthesis.
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