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THE RELATION OF THE CHEMIST TO THE WINE INDUSTRY

CHARLES S. ASH

San Francisco, Cal.

I think that I am correct when I state that there are few, if any, industries where it is so difficult for the chemist to prove his necessity as in the manufacture of wine. This is due to the following facts:

The most common difficulty is that people connected with the wine industry have absolutely no idea of chemical science and cannot see any use for the chemist in their business.

The wine industry is not what we would call a true manufacturing industry, as for example, the sugar industry; therefore, the chemist has no value in a mathematical capacity. He does not trace out losses in manufacture as does the sugar chemist. It is apparent to any business man that if there are 10,000 lbs. of raw sugar put into a refinery in a day and if 9,500 lbs. are recovered in the refined product that he has a loss of 5 per cent. The chemist here is of value to him to ascertain definitely the loss of manufacture, to help him minimize that loss, trace up leaks, improve processes of refining and many other incidentals, which has made the sugar chemist renowned throughout the world. This example holds good in any manufacturing process where the raw material is converted into a finished article. Even in the kindred industry of grain distillation, we have the obvious necessity for the chemist; for it is a self-evident fact to the distiller that the man, who can increase the yield of alcohol from a given weight of cereal, is of value to him. In the yeast industry (another kindred industry) this example holds good. In fact an instance has come to my knowledge where a chemist has increased the yield of yeast for his employers over 30 per cent. All of these things mean profit to the merchant and profit is "Raison d'etre" of all enterprise. In other manufacturing industries, the chemist has proved his value by making

immense fortunes in by-products. In a strictly chemical line, as in the manufacture of chemicals, as well as in the manufacture of dyes and colors, the whole industry is absolutely dependent upon chemical laws hidden or obvious. The innumerable new dyes and colorings are the product of brains alone and would never have seen the light of day but for the master minds, who have built up these synthetical products. As grapes are very poor in by-products, and as wine is a natural product, the wine chemist was shut out from usefulness in this direction.

Compared to the above industries, the wine industry is the most venerable; wine having been made by the Egyptians many centuries before Christ. It is mentioned throughout the Old Testament repeatedly. As wine has made itself spontaneously long before such a thing as chemistry was ever dreamt of (that is our conception of exact chemical science) it was considered a natural product. About fifty or sixty years ago, when Pasteur made his classical researches, considerable light was thrown on the subject and for the next fifty years, his views made themselves slowly felt throughout the world. The first effect was in the beer industry where yeast was planted in sterile medium and the quality of the beer has depended greatly upon the quality of the yeast. In the wine industry this was not the case, as the process of making wine was roughly this: The grapes were crushed and allowed to ferment. They fermented spontaneously on their own yeast and made good or bad wine as the composition of the grape, climate, temperature during the fermenting period and other conditions allowed. We hear to this day the most abused phrase of "the especially fine vintage of 188—". This was simply that the grapes ripened well and that the temperature during the fermenting period was ideal. Such a thing as controlling the temperature was unthought and unheard of.

When the chemist looked for employment in the wine industry, he was asked what he could possibly do, what he could possibly find out that an expert taster did not know. How could he benefit the wine industry in any way? He replied that he could analyze the wines, determine the amount of alcohol, total acids, volatile acids, solids, tannin, glycerol, etc. This brought the reply, "Well, if I did know, what good is it to me? I am glad

to know that wine has, for example, 12 per cent alcohol, and has a total acid of 6 parts per thousand and volatile acid of one part per thousand but, after I do know it, what does it mean to me? I am very pleased to know it but it does not do me any good. It does not tell me whether the wine is good, bad or indifferent; I can tell from my taste all those things. There is nothing I cannot tell. I have been running this wine business for the last fifty years without a chemist and made money, and I should be able to run it a great many more years without one." What he said was, in a way, absolutely true. This was the unfortunate thing because a chemist, applying for a position in the wine industry, is not a wine man and knows nothing about the nature of wine. His value only starts to be of importance when he does become a wine man. The fallacy of the wine merchant at this time (fifteen or so years ago) was that he did depend on the chemist, that, in fact, all over the world preservatives were used; a chemical product itself, preservative values having been found by chemists. Therefore, no matter how troublesome a wine may have been, preservatives in a great or small quantity were added, and the wine kept indefinitely. Chemists, however, were finally employed in the wine industry. I think that the first wine laboratory of any corporation in the United States, and possibly one of the first in the world, was formed in 1895; Before long the chemist was able to tell the merchant what 12 per cent alcohol meant, what the total acidity of .5 per cent meant and what 2½ per cent of solids meant; and, furthermore, what the expert taster could taste, what he could not taste and could also prove the taster was correct or incorrect in his opinion of a wine. For example, if he considered a wine sound that had .150 grams volatile acid (as acetic) per 100 c.c.; then the taster was wrong, that the wine was sour that contained such a high percentage of volatile acid. This soon became apparent to the taster himself and a new crop of difficulties arose. He wanted to be checked in his work and expected a determination of volatile acid (or as we commonly call it, "volatile") could be done almost as fast as he could taste wine and if he had 200 or 300 samples, he would expect them to be done in one day. He wanted them running day and night, Sundays and holidays, not,

of course because they were of any value but simply to prove his opinion and the poor, lonely wine chemist, harassed as he was, and upon trial, had very little satisfaction in finding one test, besides that of alcohol, which was of some value to his employer. But now other difficulties arose for the wine merchant. Pure Food Laws were coming into effect in European countries. Salicylic Acid (the common preservative used) had to be abandoned and the chemist was instructed to look for a preservative that could not be detected (and he is still looking). Then benzoic acid was adopted as a preservative, as benzoates, at that time, were very difficult of detection. Chemical science soon, however, caught up to them so that they were of no value. However, during this period, the chemist had become acquainted with the wine business and learned a few relations between composition of the wine to its keeping qualities and, therefore, was able to interpret an analysis. He could anticipate, to a certain extent, whether or not a wine would blow up, turn sour or spoil in some way when it reached a warm climate and, instead of finding a new preservative, concentrated his thoughts on getting the wine into such a condition that there would be no need of a preservative, and that a preservative would simply increase the cost of the wine and do it little, if any, good. This I think holds good in all food industries; that the need for preservatives decreases as our knowledge of the product increases. Through the efforts of the writer, wine was shipped without preservatives as an experiment. The first car was shipped out with many misgivings. It was expected that every barrel would blow up, and the entire car be lost. This was a very trying time for the chemist, as his theories were on trial and a failure meant a return to the old régime. The car, however, gave perfect satisfaction and nothing was heard afterwards. From that time on, the amount of preservatives decreased and methods for improving the vintage increased; in fact, a chemist's knowledge took the place of preservatives in this business. This was sometime before our national Pure Food Law. The firms, not employing a chemist, were found to be at quite a disadvantage, as their goods with preservatives, were subject to seizure, both by municipal and state authorities. This gave them undue notoriety and they

were forced to hire a chemist to improve and control their methods of manufacture and preparation of wine for the market. So much for the troubles and difficulties of pioneer chemists in the wine industry.

The chemist, having proved his value, now reached out to other things. He now started to look into the many causes of trouble. First, why some wines would not clarify, why wines of some localities and some districts degenerated, why some wines, shipped from the cellar in excellent condition, spoilt. These were the problems that confronted and still confront the wine chemist. He found out, however, that wines would not clarify, usually either on account of disease or on account of the composition of the wine, usually insufficient tannin. Then he started to improve the clarifying medium and to look into the reason of the degeneracy of wine after shipment. He found certain diseases which had to be, and were, overcome (these diseases, of course, never took place when preservatives were used, as the antiseptic action was too great for the micro-organisms to overcome). He then started to look into reasons of locality to find out why some cellars and some vintages turned out very poor wine. This, of course, was not original in California alone, as it was found in every other place throughout the world, and a great deal of literature on the subject was being printed so, while this did not take a great amount of original research, he acted as a medium to distribute scientific knowledge to the cellar superintendents, so that their methods of handling the vintage would be improved. This, also, at times, meant an outlay of money and is always, in corporations, a difficult thing to obtain. It was proven that wines, fermented at a high temperature, spoilt while those, fermented at a low temperature, nearly always kept sound. His labors then took on methods of controlling the temperature of the vintage, improving and handling, and also to ferment on cultivated yeasts of known virility and not leave the fermentation to chance. The quality of the wine, as well as its keeping properties were improved and the amount of spoilt wine reduced to a minimum. It is apparent that a successful business must turn out a uniform product. There is no difficulty in turning out a uniform sugar for example, but with wine, every

vintage is slightly different and, if a brand of wine is established, it is necessary to supply your customer with wine of exactly the same type. Otherwise, the consumer, being used to one wine objects to any other. The wine chemist has to help in the production of uniform products. As the blending of wines is the final operation (and as these blends compose at times 100 different wines) this is perhaps one of the most important duties of a wine chemist. Blends are usually made up in sample, analyzed, blended as near as possible to the composition of the previous blend of this type, and the blends are then distributed to the winery, which is to make them up and, after blending, are sent back to the laboratory where the chemist analyzes them again to check up and see whether these blends have been properly and uniformly made.

The following samples show the method of checking the blending. The analyses of the sample blend (made in the laboratory) and the actual blend (made in the cellar) must agree, otherwise, the blend is not uniform and must be reblended:

	Per cent Alcohol by Volume	Grams per 100 Cubic Centimeters			
		Total Acidity	Volatile Acidity	Reducing Sugar	Tannin
Winehaven Claret Blend No. 485 (265,000 gallons).					
Sample Blend ...	12.29	.500	.060	.145	.135
Finished Blend ..	12.37	.510	.060	.150	.140
Wahtoke Port Blend No. 482 (144,500 gallons).					
Sample Blend ...	20.78	.390	.043	6.60	.070
Finished Blend ..	20.78	.390	.046	6.63	.079

Besides this the wine chemist has duties in common with all chemists. He must analyze the water and soils of all the vineyards owned by his company, analyze the supplies, such as used

either in wines or in the vineyards, advise as to fertilizers to be used and devise means to gather as many by-products as possible.

I may say in conclusion that the wine chemist, in spite of temporary discouragements, is having more intimate relations with the Wine Industry. He is, in fact, becoming quite friendly, and he has hopes of being on the same good terms as his brothers in the sugar, dyeing, oil, petroleum, gas, soap and other industries, which the chemist has made famous.

INTERPRETATION OF THE RESULTS OF WINE ANALYSIS

By Charles S. Ash

San Francisco, California

Introduction.

It is evident that the analysis of any product is useless unless it can be correctly interpreted; in other words, every analysis that is made must be interpreted to be of any value. What the diagnosis is to the physician, interpretation of analytical data is to the chemist. Some of these interpretations are purely mathematical. The value of sugar cane is dependent on sugar content; ores are valued by the amount of metal they contain, and, therefore, assays are interpreted with little or no difficulty. The interpretation of analytical data on some other products are, on the other hand, most difficult and it is only by a careful study of many analyses of these products of known origin that we are able to show the true meaning of their chemical composition. In food products, we are quite content, as a rule, to tell from analytical data whether the food in question is pure or adulterated. As this interests most chemists, we will confine ourselves almost entirely to this question.

The interpretation of wine analysis has been confined almost entirely to the judgment of its purity; in fact, the only result of these interpretations has been the formation of a set of standards to which a wine must conform in composition to be considered pure wine. These hard and fixed standards do not accomplish the object of detecting adulteration on one hand while, on the other, they often work real hardship on wines of pure origin. Grapes of the same variety, grown in different soils and in different climates, produce wines of absolutely different taste, and composition. If this is true of the same variety of grapes, under different conditions, what must be the difference in composition of hundreds of varieties of grapes grown in almost every condition of soil and climate in the world! This we will discuss later.

There has been a tendency in the past, in writing of interpretations of wine analysis, to quote the interpretations of previous authors and this has influenced our already meager literature on this subject to such an extent that we have had but little original thought for many years, the last interpretation being only a compilation of previous data. To avoid such a tendency, the present writer will avoid previous literature on the subject, preferring to treat it from an independent viewpoint. I think you will pardon the writer for his temerity in taking this stand when he explains that yearly, for the past fourteen years, there has been received in his laboratory from 15,000 to 30,000 samples of wine of *known origin*. All these wines are examined and, at least, one-half analyzed.

We will, therefore, consider that the interpretation of wine analysis has two objects,—one to judge the purity of wine and the other to judge its quality and condition. We will take, under these two heads, part 1, dealing with adulteration and part 2, with condition, soundness and disease.

Part 1.—The object of all adulteration is to cheapen the article in question or to increase its commercial value by artificial and false means. No one will adulterate unless it is profitable and no one will substitute an artificial product, which costs more than the natural one. This, of course, is plain. In wine then, as in every other product, adulteration aims to decrease the cost of production or to increase its selling value. We, therefore, will consider the various forms of adulteration which may be used.

1.—Increase of volume.....	Addition of water Artificial wines Wines of foreign fruits
2.—Increase of strength.....	Addition of spirits and sugar, or both
3.—Increase of stability.....	Preservatives
4.—Improvement of appearance ...	Artificial coloring
5.—Changing of taste.....	Artificial flavor, saccharine, etc.

6.—Modified or fixed spoilt wine.

7.—To these forms of premeditated adulteration, we may still have adulteration which will come under the head of

accidental adulteration. Under this head, will come the presence of heavy metals, as zinc, iron, copper or arsenic.

Dilution.

Of all forms of adulteration, the most common, the most profitable and the most difficult of detection is dilution: the simple addition of water. The first two facts are self-evident. Why dilution is so difficult of detection needs some study. Having already touched on the effect of climate, soil and variety on the composition of the grape, we will go into further details. Now, it hardly seems necessary to state that grapes, grown in warm climates or grapes ripened to perfection, have more sugar than unripened grapes. It is also obvious that grapes that are unripe have a greater acid content than ripe grapes.

The following example shows the effect of climate on the composition of the juice of the same variety of grape:

Variety Carignan

	(1) Grown in France (Midi)	(2) Grown in Fresno, Cal.
Density at 60F.....	1.076	.0997
Total Solids, Grams per 100 c.c.	18.81	24.2
Reducing Sugar " " " "	16.2	23.26
Total Acids as Tartaric " "	.840	.590
Potassium Bi-Tartrate " "	.580	.376

The next example illustrates the effect of ripening on the composition of the grape juice:

(3)
Variety Zinfandel

	Sugar (% Balling)	Acidity as Tartaric
July 10	6	1.12
" 20	9	1.10
Aug. 1	14	.980
" 15	20	.700
" 20	23	.620

Now, the essential point I wish to bring out is this: that grapes, high in sugar content must necessarily be low in acid content and vice versa. Both conditions, that is, high sugar content and high acid content rarely exist in the same grape. On the other hand, low sugar content and low acidity also do not exist in the same grape. This is a generality. *This is very important.* It will, therefore, be seen that the resulting wine from grapes of high sugar content, will be high in alcohol and low in acidity while wines, made from grapes low in sugar content, would give a wine low in alcohol and high in acidity. To repeat again, both high alcohol in a wine and high acidity, and low alcohol and low acidity, do not exist. We have, therefore, in cold countries the difficulty of obtaining grapes that ripen to a sufficient sweetness so as to give wine of high enough alcohol to preserve itself and a low enough acidity to be drinkable while, on the other hand, in warm regions, we have the reverse trouble of getting wines of high enough acidity and low enough in alcohol to have sufficient character and flavor to be considered desirable wine. These wines, as you see, are pure wines of absolute different composition and a hard and fixed standard made in either country to suit the conditions of the native wine may work hardship on the wines of the other country or, on the other hand, if the standard for example, happens to be based on the composition of the wine of the cold country, there would be little difficulty in diluting the wine from the warm country and still conform to the standard of pure wine.

I have spoken on the difficulty of a fixed standard. Let us take, for example, the American standard. This standard was meant to be, and is, a liberal standard, which tries to embrace all pure wines of the world. This standard is as follows:

	Red	White
Alcohol % by volume	7 to 16	7 to 16
Volatile Acid as Acetic, Grams per 100 cc	.14	.12
Reducing Sugar " " " "	.1%	1%
Ash	.16	.16
Sugar Free Solids " " " "	Not less than 1.60	1.40
Sodium Chloride " " " "	" more " .10	.10
Potassium Sulphate " " " "	" " " .20	.20

Let us say, for example, we have below an example of average, normal California Red Wine with the following composition:

Alcohol % by Volume	12%
Volatile Acid Grams per 100 cc	.100
Sugar Free Solids	2.50
Ash	.270
Sodium Chloride	.005
Potassium Sulphate	.015

This wine can safely be diluted one-half (2–3rds wine and 1–3rd water) and still be able to conform to these standards. The composition of the diluted wine would then be as follows:

Alcohol % by Volume	8%
Volatile Acid, Grams per 100 cc	.067
Sugar Free Solids	1.67
Ash	.180

This comes well within the standard of purity as laid down by our government. On the other hand, these standards will work hardship on some of the finest old wines produced. This we will discuss when we take up the question of Volatile Acid of the wine, as it is out of place under this heading.

To return; we have shown what can be done under the standards laid down by our government. There is nothing in these standards showing the relation of alcohol to total acidity, much less showing the composition of this acidity in natural wine.

In this case cited, the original acidity of the undiluted wine was .6 grams per hundred cubic centimeters. When this wine is diluted, we find an acidity of .4 grams per hundred cubic centimeters. *A natural wine with an acidity of .4 grams per 100 c.c. and an alcoholic strength of 8% by volume does not exist.*

The obvious question is then asked: why a diluted wine cannot be acidified to raise the acidity to that of normal wine of such an alcoholic strength. This certainly complicates matters. The only available acids are, as we know, Citric and Tartaric Acids. As Citric Acid is never present in grapes in quantitative amounts, its presence in most cases will be indicative of manipu-

lation. We have now only Tartaric Acid. The natural non-volatile acidity (fixed acidity) of wine is largely made up of Potassium Bi-tartrate and not Tartaric Acid. Free Tartaric Acid is only present in small quantities in natural wine. So, if Tartaric Acid was added to this diluted wine we will have a larger amount of free Tartaric Acid than Potassium Bi-Tartrate. We cannot use Bi-Tartrate to acidify the diluted wine, as it is only sparingly soluble. The following table is explanatory:

	Natural Wine	*Diluted Wine*	*Diluted Wine Acidified*
Alcohol % by volume	12.0	8.0	8.0
Total Acidity (as Tartaric) Grams per 100 c.c.	.600	.400	.800
Volatile " (as Acetic) " " " "	.100	.067	.067
Sugar Free Solids " " " "	2.50	1.67	1.67
Ash " " " "	.270	.180	.180
Potassium Bi-Tartrate " " " "	.300	.200	.2000
Free Tartaric Acid " " " "	Trace	Trace	.400

Here, in the natural wine, we have .3 grams Potassium Bi-Tartrate and a trace of Free Tartaric Acid, and in the diluted wine, we have .2 grams Bi-Tartrate, while in the diluted acidified wine, we have .2 grams Bi-Tartrate and .4 grams Free Tartaric Acid; the Free Tartaric Acid, being in excess of the Bi-Tartrate, while it should only be one-third at the most. Now, suppose we add the alcohol % by volume and the total acidity (grams per litre). We have in the natural wine a total of 18, in the diluted wine 12, and in the acidified wine 16. Whenever a Red Wine has a total acidity of less than 16, it should be investigated. All this data is relative to the effect of dilution on acidity. I know the difficulty of showing everything in one example, but we have already gone to greater length than I had wished to go. It is obvious to us all that dilution will lower the percentage of solids, the percentage of ash and other constituents of the wine. I might state that the alkalinity of ash, figured in terms of Bi-Tartrate should approximately equal the percentage of Bi-Tartrate found in the

original wine; in other words, the alkalinity is almost due entirely to the Bi-Tartrate which is converted into Potassium Bi-Carbonate by incineration. In conclusion, it would appear that California red wines, having a sugar free solids less than 2.30 in conjunction with an alcohol plus acid total of less than 16, is to be regarded with suspicion. With white wines, a sugar free solids of less than 1.60 and alcohol plus acid total of less than 15, should also be looked upon with suspicion. Wines, having a large amount of Free Tartaric Acid, in proportion to the Potassium Bi-Tartrate, and coupled with low sugar free solids would indicate that the wine had been diluted and acidified. The relation of Potassium Bi-Tartrate to the free Tartaric Acid is the same in white wines as it is in reds.

Artificial Wines

These wines are made by fermenting sugar, sucrose or glucose, either alone or thrown over grape pomace. They are, in consequence, very light in color or colorless, and have either to be blended with natural wines or colored artificially. They are also high in free Tartaric Acid and low in Bi-Tartrate and low in solids and ash. They often have no Potassium Bi-Tartrate at all, the acidity being due to free Citric or Tartaric Acid. Those made from glucose are always high in solids, due to dextrin or other unfermentable substances. Such substances are too easy of detection to be mentioned here. Those made from sucrose are always a superior article. They are, however, very expensive and in countries where grapes are cheap (as in California) cost more than the natural wine. For example, sucrose cost, say, 5c per lb.; grapes, having a sugar percentage of 22 per cent will have 440 lbs. of sugar per ton of grapes; 440 lbs. of sugar, in turn, at 5c. per lb. would cost $22 and, therefore, sugar would be equal to grapes costing $22 per ton. The average price of grapes in California, year in and year out, will be from $12 to $30, the average cost being somewhere around $18. Now, it is obvious that no one would use this form of adulteration unless the price of grapes were well over $30 per ton. In colder countries where the grapes do not ripen to a sufficient sugar

percentage, the sucrose is very often added to give the resulting wine sufficient alcohol to preserve it. When this is not done to excess, it is almost impossible (without a thorough knowledge of the composition of natural wines of such district) to show this form of adulteration. Sucrose is inverted by the action of the yeast and acid, before fermentation. The inverted sugar is very closely allied to that of the natural sugar of the grape. I might say in conclusion, all the artificial wines, that the writer has seen, have come, of course from localities where grapes were dear. Their acidity has been due almost entirely to free Tartaric or Citric Acid. They are often artificially colored and preserved with some preservative. Most of these have been made from glucose. Sometimes sweet wines, like Port, have been made. These wines contain about 12 per cent alcohol and as high as 20 per cent of total solids, the method of making being that the glucose solution has fermented until fermentation takes place no longer and the wines stick. Preservatives are then added and the wine is colored; sometimes synthetical flavors are also added. These wines never cloud and give no trouble, during changes of temperature in climates, that all natural wines do.

Foreign Fruit

This form of adulteration I hardly think exists in the state of California on account of the low price of grapes and the comparatively high price of other fruits. In Europe, apples and figs have been, at times, fermented and mixed with natural wines. Such wines, by themselves, would easily be detected, as very few other fruits, beside the grape, contain any Bi-Tartrate. The absence of Bi-Tartrate or a very low percentage of same in a comparatively normal composition otherwise, would tend to show this form of adulteration. When more light is shown on the acid composition of various fruits, no trouble will be experienced in detecting such adulteration.

Increase of Strength

This is done by the addition of sugar, as had already been described, or spirits, or possibly both. The addition of spirits

cannot take place in American dry wines, as wines must have 4 per cent of total solids to be eligible to fortification. Neither can taxpaid spirits be added to such wines, for it is against the revenue laws to mix taxpaid and free spirits. Such a form of adulteration would be criminal and liable to prosecution. Some countries allow spirits to be added to wines for exportation. This is readily detected on account of its abnormally high alcohol together with high acidities. The addition of spirits and then dilution by water afterwards, would make a double dilution and would be detected under means already described in the paragraph on dilution.

Increase of Stability

Whenever wines have been diluted or made from unsound material, preservatives are sometimes added to give them keeping qualities, without which they would get progressively worse, so as to be impossible to market them. In this connection the use of preservatives, irrespective of whether they are harmful to the human system or not, should be prohibited in the wines for the reason that poor or putrid articles could be marketed, which would be impossible to do without their use.

Improvement of Appearance

Whenever artificial or imitation wines are sold, either alone or mixed with natural wines, the color is insufficient to meet the popular demand. It is, therefore, imperative that these wines be given the appearance of normal wines. To do this, they must be colored. Aniline colors are used for this purpose almost entirely, as vegetable colorings are not fast and either fade or deposit when subjected to daylight. The usual clarifying methods (the addition of albumen) will very often deposit vegetable colors and, therefore, after wine has received a clarification, vegetable colorings are almost entirely removed from the wine. The above statement, in reference to the coloring of wines, is applicable to red wines entirely. White wines are rarely colored, though in isolated cases, the writer has seen wines given green tints by artificial means.

Change of Taste

Sometimes flavors are added to natural wines to improve the state, or give them either the appearance or taste of old wines but, in most cases, this is a waste of money. Such form of adulteration is very difficult of detection. However, a higher percentage of esters than is normal in a wine, especially volatile esters, will tend to show adulteration of this form. Saccharine has been at times added to white wines to imitate wines of the Sauterne type. This is very simple of detection, 1st—by the method of detecting saccharine itself, and, 2nd., that the total solids of such a sweetened wine is no higher than the normal solids of a dry white wine. Such sweetened wines are also very low in reducing sugar and, therefore, cannot derive their sweetness from natural sources.

Modified or Fixed Spoilt Wines

This is the last form of premeditated adulteration. Spoilt wines or sour wines, which have either been made from spoilt grapes or have been spoilt by neglect or degenerated by some disease, are often marketed after manipulation. The common method of doing this is to neutralize the excess of acidity, either by potassium, calcium or magnesium salts—usually calcium carbonate is used to do this work. The resulting ash of such wines is very high in lime salts, the total ash going considerably over 1-10th of the sugar free extract. The fixed acidity is usually very low in comparison to the volatile acidity. This is due to the fact that such manipulation is rarely, if ever, successful, and wines, having been neutralized in this way, usually are only temporarily checked; the disease continues and more volatile-acid is generated; the neutralization only neutralizing the acids present at the time of neutralization.

We now come to the final form of adulteration, which we will call Accidental Adulteration. That is the presence of small amounts of heavy metals in wine. The presence of copper, tin or zinc is due to the pipe line, which the wine is run through. The amounts are very low indeed and it is almost impossible to avoid these contaminations. Carelessness, however,

in allowing wines to stand in pipes until they corrode will very often increase this small percentage to an enormous amount. The presence of arsenic in wine can rarely be considered accidental adulteration, as it is a natural constituent of grapes in some localities. The largest amount ever seen by the writer in California wines is one part in four million: usually one part in twenty to fifty million is normal. Arsenic occasionally is added to the wines, by the use of sulphur in fumigating casks. This, of course, would be considered accidental adulteration.

I wish to conclude, as I have begun, by wishing to avoid a fixed standard applicable to all wines in the world. There is only one way which we can tell with approximate certainty whether a wine is pure or adulterated and this is to have for our standard of pure wine the same standard as the country from which the wine originates; for example, standards set down by the Swiss government for Swiss wines, should be applicable to Swiss wines; those set down by the German government for their Rhine and Moselle wines, and other districts, should be applicable to wines of that district; for French wines, those set down by the French government as standards for their natural wines of different districts, should be used on such wines. For American wines, standards should be made by the United States government or by the Pure Food Authorities of the different states where the wine is grown. In this way, we will not demand the same composition for Algerian wines as we do for German wines, nor the same for Swiss wines as we do for California wines. This I believe will be a very simple matter and, in my opinion, is the only way of controlling this question. A unification of analytical methods would also be a big factor, and should be urged by such bodies as this Congress. If not, we must follow the analytical methods of the country in question, that is, if we are examining French wines, we must realize that these standards have been based upon methods of official French analysis, and results, using any other methods, will either work hardship or may defeat the enforcement of a Pure Food Law.

UNDER THE HEAD OF SOUNDNESS Part 2.

Just one word, before I close this already too lengthy paper, in regard to unsound and diseased wines. Such wines can be made from grapes and be pure in every way and still be unfit for human consumption. A parallel case would be in any putrid, decayed or deteriorated food product.

This may be due to the following reasons, as the manufacture of wine from rotten or diseased grapes, or the wines may be made from sound grapes, and become diseased from improper methods of fermentation or neglect. To be brief, we will suppose that the Must contains

Sugar	20 grams per 100 c.c.
Acidity as Tartaric	8–10 grams per 100 c.c.

The corresponding wine, fermented under normal conditions, would have a composition somewhat as follows:

Alcohol	11.5 per cent by volume
Total Acid	.600 per cent
Volatile Acid	.060 per cent
Reducing Sugar	.120 per cent

However, if this wine should be fermented badly, or the temperature rise too high, so that the yeast is either killed or lies dormant, secondary fermentation will set in, and we will have a wine of quite different composition, depending on the severity of conditions or neglect in handling. A typical composition of such a wine would be as follows:

Alcohol	11 per cent by Volume
Total Acid	.700 per cent
Volatile Acid	.200 per cent
Reducing Sugar	.500 per cent.

Notice the difference of these two results made from the same Must. Let us go backward and say that we have a spoilt wine with this composition and see what we can tell about it from analytical data. In the first place, notice that this wine has a high volatile acid. This shows that it has been attacked by

micro-organisms and that secondary fermentation, in consequence, has set in. The product of such fermentation (the high volatile acid) is present. We then look at the reducing sugar and find that it is high. We see that the conversion of sugar into alcohol has not been completed and this confirms our opinion of secondary fermentation. The wine is absolutely spoilt and should not be used under any conditions. This wine should not be blended with any other wine, as it would simply contaminate the entire blend. A great many mistakes are being made in trying to work off wine of this kind in small quantities. This is an absolutely bad practice, for nine times out of ten, the entire blend is ruined. It is always best to make your first loss at once. Wine of this type, of course, would make excellent wine vinegar, and could be used for such purpose. In well-controlled wineries, wine of this type is reduced to a minimum

We will now follow new, sound wine over a period of years and see what changes we may expect in the volatile acid content.

	Dry Red or White	Sherry	Port, Angelica Muscat, etc.
Sound new wine	.05 to .08	.04 to .08	.03 to .05
Sound wine 1 year old	.06 " .100	.06 " .08	.04 " .06
Sound wine 3 years old	.09 " .120	.08 " .100	.05 " .07
" " 5 " "	.100 " .140	.100 " .120	.07 " .09
" " 8 " "	.120 " .160	.120 " .160	.08 " .100
" " 10 " or over	.140 " .170	.140 " .180	.100 " .120

This represents the natural increase of acidity one would expect. This is not intended as a fixed standard, but rather a guide. From this it is seen that a young wine, which would be condemned as spoilt, may be a perfectly fine sound old wine.

EXPERIMENTS ON FEEDING GUINEA PIGS "SALTS OF TIN" IN MEASURED QUANTITIES FOR SEVERAL WEEKS

By H. A. Baker
New York

Seven normal young Guinea Pigs, of average weight of about 257 grams, were put in separate pens and was each fed daily, except Sundays, one Gelatin Capsule containing 12.6 milligrams of tin in the form of Hydrates mixed with Corn Meal.

The capsules were prepared in the following way:

Four grams of pure tin were dissolved in a small amount of Hydrochloric Acid and evaporated almost to dryness. This was then neutralized with Sodium Carbonate and dried, after which it was mixed with fine Corn Meal. 55,000 milligrams of the mixture contained 4000 milligrams of tin.

201 Gelatin Capsules, weighing 11.438 grams, were filled with this Corn Meal mixture and found to weigh 46.281 grams, so that each full capsule weighed 230.25 milligrams. Subtracting the weight of the empty capsule, 56.9 milligrams, we have as the contents of each capsule 173.35 milligrams of Corn Meal mixture, which contained 12.607 milligrams of tin.

These capsules were all standard size so that we consider the amount of tin in each capsule to be the same, especially since the amount of Tin Salts in the mixture was small.

The capsule was administered to the Guinea Pig by forcing it down his throat, in order to make sure that the proper dose had been given.

The Guinea Pigs were given an ordinary diet of Carrots, Lettuce and Crackers.

As will be observed from the table appended, the Guinea Pigs showed different resistance toward this chemical. Five of the Guinea Pigs were fed until death occurred. They lived the following number of days: 9, 10, 19, 19, 22, respectively. Two

other Guinea Pigs which had taken a capsule a day for twenty-five days lived, but were after that fed no more capsules. Twenty-three days after these two Guinea Pigs had been fed no more capsules, they suddenly died from exposure from an open window.

In addition to observing the doses which were fatal to these Guinea Pigs, their livers were analysed in order to see whether or not any tin had become stored up there. Examination showed the livers of the dead Guinea Pigs to be somewhat bleached on the outer edges.

It can be observed from the table that these Guinea Pigs accumulated in their livers a small amount of tin each day, as long as the dose was administered. This rate of accumulation was about $\frac{1}{10}$ of a milligram of tin per day; the average rate for five Guinea Pigs being .113 milligrams of tin per day.

Using this rate as a basis for calculating the amount of tin in the livers of the Guinea Pigs numbers six and seven, at the end of the twenty-fifth day, we would have 2.827 milligrams of tin present in each liver. At the end of the twenty-third day, during which period no more tin had been fed, we still found .3 and .5 milligrams of tin in the livers of Guinea Pigs numbered six and seven respectively, which means that the tin had been eliminated from their livers at the average rate of .1099 milligrams and .1011 milligrams per day respectively. From the experiment, of course, it cannot be known that this average rate of elimination was the actual rate, and it probably was not. It is probable that the rate of elimination varied according to the concentration of the amount of tin in the livers of the Guinea Pigs and fell off as the concentration decreased.

It may be observed from the table that the average absorption in the livers of the amount of tin fed to the Guinea Pigs was .898%.

These individual doses were very large, and no other experiments have been carried on to determine what the effect of smaller doses for longer periods of time would have been.

The exceedingly small amounts of tin involved in the determinations were estimated by a very delicate iodimetric titration method, using N–100 Iodine. We consider the accuracy of

this titration to be within 3-10 of a cc, which means that the determinations are within .2 milligrams of accuracy, probably always tending somewhat toward high results.

TABLE SHOWING RESULTS AND ANALYSES FROM EXPERIMENTS ON FEEDING GUINEA PIGS "SALTS OF TIN" IN MEASURED QUANTITIES

By H. A. Baker

	GUINEA PIGS						
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
No. days fed Salts of Tin	9	10	19	19	22	25	25
Amount in Milligrams of tin as "Salts of Tin" fed each day	12.6	12.6	12.6	12.6	12.6	12.6	12.6
Total Tin fed as "Salts of Tin" in Milligrams	113.4	126.0	239.4	239.4	277.2	315.0	315.0
RESULT	Death	Death	Death	Death	Death	Living	Living
Weight of Liver in Grams	12.7	9.8	10.0	8.9	10.0	9.5 (Determined 23 days later)	9.8
Weight of Pig in Grams	260	215	251	298	306	248.5	220
Weight of Liver as percentage weight of Pig	4.88	4.56	3.98	2.99	3.27	3.82	9.46
Tin found in Liver in Milligrams	1.05	1.015	2.30	1.80	2.90		
Rate of accumulation of tin in liver per day in Milligrams	.1166	.1015	.1210	.0947	.1318		
Percentage total amount of tin found in liver	.926	.805	.961	.752	1.045		

GUINEA PIGS NUMBERS SIX AND SEVEN

These were fed no more "Salts of Tin" after 25 days, but at the end of 23 days more they died one night from exposure to cold from an open window.

	Guinea Pig No. 6	Guinea Pig No. 7
Estimated milligrams of tin in liver at end of 25 days, calculated from average rate of accumulation of tin in livers of other five Guinea Pigs..	2.83	2.83
Milligrams of tin found in livers after 23 days rest from feeding "Salts of Tin".....	.30	.50
Average rate of elimination of tin per day in milligrams.....	.1099	.1015

No physiological examination was attempted, so that the conclusions from this work are somewhat limited. The following deductions, however, would appear to be patent:

First.—That daily doses of 12.6 milligrams of tin as Hydrates were fatal to the Guinea Pigs in from nine to twenty-five days upwards.

Second.—That tin was progressively accumulated in the livers of these Guinea Pigs when fed daily doses of 12.6 milligrams of tin as Hydrates.

Third.—That the tin accumulated in the livers of the Guinea Pigs by feeding, as above noted, was eliminated rather slowly after feeding was discontinued.

It is desired to give credit to W. S. Sellars who performed the major part of the Laboratory work in connection with this experiment.

SPECIAL ADAPTATION OF IODINE TITRATION METHODS FOR THE ESTIMATION OF TIN, ESPECIALLY IN CONNECTION WITH DETERMINATIONS OF "SALTS OF TIN" IN CANNED FOODS

By H. A. Baker
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This method being an adaptation of parts of several standard and well known methods, no particular reference will be made to the sources of the principles employed in the method.

The tin in the canned food products is obtained as a sulphide precipitate from wet combustion, with Nitric and Sulphuric Acids, of 100 grams food product.

The clear Sulphuric Acid residue is diluted, neutralized with ammonia, and then rendered about 2% acid with Hydrochloric Acid, after which it is thoroughly saturated with Hydrogen Sulphide Gas. This precipitate is then filtered on a Gooch Crucible with a false bottom. The precipitate may contain foreign substances, such as Lime, Phosphorus and Silica, some Lead, or even small amounts of Iron, but none of these will cause any trouble subsequently in the titration so that the labor of separating the tin completely from the precipitate is obviated.

After washing the precipitate three or four times in a Gooch Crucible, it is transferred to a small porcelain dish by simply forcing out the false bottom of the Gooch Crucible and its asbestos pad and rinsing off the crucible.

The precipitate, mixed with asbestos, is now transferred to a 300 cc. Erlenmeyer Flask and boiled with strong Hydrochloric Acid; Potassium Chlorate being added from time to time to insure the complete breaking up and solution of the tin sulphide, as well as the elimination of the sulphur. This is accomplished in a very few minutes. A few strips of pure aluminum foil, free

from tin, are then added to the flask until all of the Chlorine is eliminated. This flask is then attached to a large Kipp Apparatus, charged with pure marble and Hydrochloric Acid, delivering Carbon Dioxide. The Carbon Dioxide from the Kipp Apparatus is passed through a Scrubber, then led into the Erlenmeyer Flask through a bulbed tube in the rubber stopper of the Flask, delivering the Carbon Dioxide near the surface of the liquid in the flask. It is led out of the flask through a second bulbed tube, the opening of which is near the top of the flask, and the Carbon Dioxide gas escapes from the end of a glass tube about 10″ long, immersed in water about 8″ deep. This gives a water seal to the delivery tube and a pressure against which the Kipp Apparatus must work. This obviates any violent flow of the gas when not desired and permits a gas pressure in the Erlenmeyer Flask.

Pure seamless black rubber tubing and $\frac{3}{8}$″ glass are used to form the connections specified.

When the flask is thus attached to the Carbon Dioxide Insulating Apparatus, as above mentioned, the air from the flask and the tubing connections is first thoroughly dispelled by lifting the delivery tubes out of the water cylinder seal so that the Kipp Apparatus has practically no pressure to overcome. A large amount of Carbon Dioxide is thus forced through the system and air is completely distilled. The rubber stopper in the Erlenmeyer Flask is then raised, and about one gram of Aluminum Foil is dropped into the flask. This quickly reduces the tin to the metallic form and evolves a great deal of Hydrogen Gas.

The flask is then placed on a hot plate and heated to boiling. The aluminum disappears and the tin is changed into Stannous Chloride. After a few minutes boiling, the flask is set off the hot plate and then cooled in ice water, while still under Carbon Dioxide insulation.

When the flask is first attached to the Kipp Apparatus, enough water is added to dilute the Hydrochloric Acid so that its strength is approximately 30 to 40%. After the addition of aluminum foil and boiling, the acid strength will be approximately 25 to 30%.

After cooling, as above mentioned, the contents of the flask are ready for Iodine titration. This may be accomplished by two methods; an excess of Iodine may be run into the Erlenmeyer Flask directly by simply lifting out the rubber stopper and running in the Iodine solution while Carbon Dioxide is issuing from the flask. The excess of Iodine must then be titrated back with Sodium Thiosulphate. Usually, however, it is satisfactory to simply detach the flask from its rubber tubes, wash down the tubing, rubber stopper and sides of the flask with some air free water, add starch paste and titrate directly and quickly with N–100 Iodine solution until a faint blue color is obtained. The asbestos which is in the flask will not interfere with this titration.

The air free water is made by boiling distilled water, adding a small amount of Sodium Bicarbonate and then a slight excess of Hydrochloric Acid.

Only one sample has been mentioned so far, but duplicates are always run together, as the Kipp Apparatus is arranged to handle two flasks at a time, simply by dividing the Carbon Dioxide Gas by means of a "U" tube and connecting an arm to each Erlenmeyer Flask. It is not only desirable but necessary with this method, as with practically all titration methods, to run duplicate samples. The N–100 Iodine is standardized against pure tin solutions or food mixtures, such as Apple Butter, containing a known or added amount of salts of tin.

The advantages which may be found in this method are:

First:—Only one filtration is required and that can be performed very quickly on account of the fact that it is performed under suction.

Second:—No perfect separation of the tin from other metallic precipitates or impurities is necessary.

Third:—There is no delay at any point in the method, such as long washings, waiting for filter paper to dry, or the loss of time over very slow and careful burning of the precipitate.

Fourth:—The titration reading gives the amount of tin directly, as no corrections are involved.

Fifth:—In the hands of competent operators, many more

analyses can be performed in a given time than with a Gravimetric method.

Sixth:—The accuracy of the method is very satisfactory, being at least as accurate as any Gravimetric method.

Seventh:—This same method may be used directly for metals containing tin without previous separation. Metals, such as tin plate, solders, Babbitt Metals or Composition Metals may be dissolved up directly with Hydrochloric Acid in an Erlenmeyer Flask attached to the Kipp Apparatus and titrated precisely as above described, by either direct titration method or the excess method, using, of course, a strong Iodine solution. Aluminum, Zinc, Iron, Lead, Antimony, Bismuth or small quantities of Copper do not interfere with the method. When large quantities of metals are dissolved directly in the Erlenmeyer Flask, the addition of Aluminum foil is not necessary. If metals are dissolved up in contact with the air, or Potassium Chlorate is necessary for complete solution, they may be reduced with aluminum foil and then handled exactly as previously described.

"SPRINGERS" IN CANNED FOODS—CAUSES AND PREVENTION

By H. A. Baker
New York, N. Y.

"Springers" is a trade term given to cans with bulging ends which contain perfectly sound and sterile food products. They are undesirable because the easiest test for the Housewife to apply, to tell whether the container is sound, is to observe that the ends of the cans are flat or drawn in slightly. It is desirable that this test should always be applicable and sufficient. Therefore, canned foods should be so packed that no "Springers" will be formed.

When a can is a "Springer" there is too much gas in it or not enough space to hold the gas under negative pressure at all weather temperatures.

The gases in the head space of these "Springers" are never more than three; Carbon Dioxide, Nitrogen and Hydrogen. Very often no Hydrogen is found. Oxygen is practically never found.

The Carbon Dioxide is formed in practically all canned foods during the time of processing. It is also formed excessively if food products are not worked through quickly from the beginning of their preparation to their final sterilization. This is true particularly of fruit and vegetable products after they have been peeled or their cells have been broken in any way or have been subjected to heat.

If food products are allowed to stand in containers before sterilization, an excessive amount of Carbon Dioxide Gas is formed.

Nitrogen Gas is simply a residue from unremoved air. Hydrogen Gas, when formed, is the product of attack of fruit or vegetable acids on the metallic container.

Changes in temperature of these cans produce changes in gas

pressure. At 85° Fah. we may have a well puffed can, at 60° one in which there is practically no pressure and at 45° to 50° there will be a vacuum. These changes occur with a decrease of temperature because the gas itself contracts, the solid and liquid contents of the can contract, and the solubility of the gas is increased.

"Springers" are usually warm weather phenomena.

The general history of the formation of gas and its behavior in a can are as follows:

First:—A certain amount of air is left in the can at the time of sealing, even if the cans have been "exhausted."

Second:—A certain amount of Carbon Dioxide is formed during the processing or cooking of the food products. If they have been allowed to stand after being prepared, an excessive amount is formed. The Carbon Dioxide is usually 8 to 15% of the gas in the head space at ordinary temperatures. At higher temperatures, more of the gas comes out of solution and can be found in the head space.

Third:—The oxygen left in the can disappears, either by combination with some element of the food product, such as Butter Fat in Milk, etc., or by combinaton with metal, or by combination with Hydrogen Gas formed by the action of Organic Acids on metal.

All three of these reactions have been traced. This withdrawal of Oxygen in the can tends to give the can a temporary vacuum.

Fourth:—When organic acids are present, Hydrogen Gas is formed in plain tin cans and helps to make pressure.

Fifth:—The influence of the increase of heat on the expansion of the solid, liquid and gaseous contents of the can, and the decrease of solubility of the Carbon Dioxide Gas are usually responsible for the appearance of "Springers."

Sometimes Hydrogen Gas Springers are formed which are usually old samples and could not be classed as temperature springers.

If very small head space is left, it requires but a slight expansion of the contents to change the contour of the can from a flat to a bulging condition.

These difficulties can be obviated if the following points are observed:

First:—Sufficient space must be left in the can to receive the gases which will be formed. This means evenly filled cans in which the exact amount of head space has been determined for each food article and process.

Second:—This head space must be "exhausted" adequately so that enough vacuum is left to receive the gases that will be formed and still leave the ends of the containers drawn in or under vacuum.

Third:—Cans, after sealing, must be processed as soon as possible to minimize the formation of Carbon Dioxide Gas, and there should be no undue delay in working the food product through the factory from the beginning of its preparation until it is sterilized.

Fourth:—With highly acid food products, the metallic container should have a protective coating of enamel.

APPARATUS FOR QUANTITATIVE EXTRACTION OF THE GASES IN CANNED FOOD CONTAINERS

By H. A. Baker

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Figure # 1 shows the apparatus ready for use.

Figure # 2 shows the apparatus actually in use, with the gas being collected in a regular gas burette.

The apparatus consists of an extensible strap iron frame in which a can may be set and clamped down by means of a screw clamp.

Entering at the base of this steel frame is a hollow steel needle which is observable in figure # 1. The rubber stopper shown alongside of this needle, in use, is placed over the needle. It is of such height that the rubber stopper must be considerably compressed before the steel needle punctures the bottom of the can. The steel needle is connected with a water supply in the cylinder having air pressure in its top. A stream of water, under about fifteen pounds pressure, can thus be forced through the puncturing needle into the can. Adequate water pressure from any other source would be satisfactory.

The strap iron holder, which is either screwed or clamped on to a table, has a twisted iron shank so that it tips at an angle of approximately 45°. This places the can in such a position that it can be punctured and the gas drawn off from its highest point as is shown in figure # 2.

In this figure, a regular Doremus Gas Extracting Apparatus may be seen.

Any other kind of puncturing arrangement, based on the same principle, would answer satisfactorily.

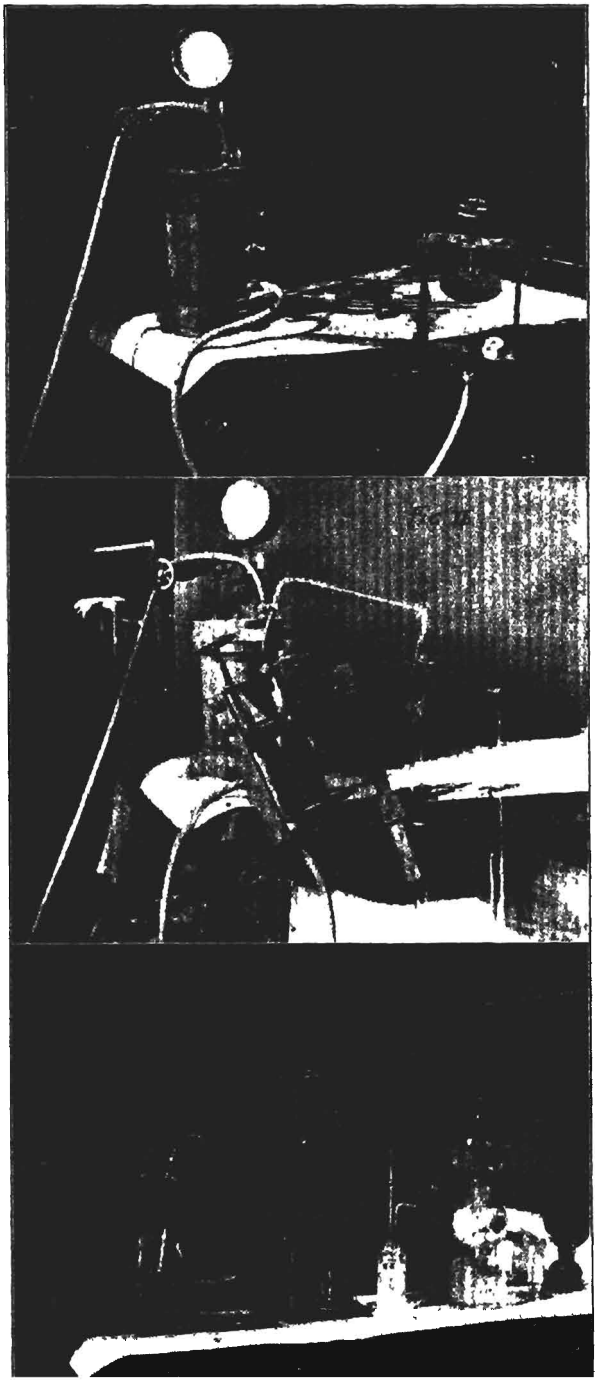
This clamp has a screw compression which works on the bottom of the can, and a hollow steel puncturing needle through which the gas is extracted from the top of the can. The puncturing needle is enclosed in a rubber stopper so that compression

and tight connection is necessary before the steel needle punctures the top of the can.

The top of the gas extractor is connected with a regular gas burette by means of capillary tubing with rubber connections.

The complete procedure for extracting the gas is as follows: 1st—After the can is in place, water is forced through the steel puncturing needle in the base, and while the water is still flowing through, the screw clamp from the top is turned down until the compression in the rubber stopper is great enough to allow the needle to puncture the base of the can. Water under pressure may then enter the can. The Doremus clamp is screwed down solidly, the hollow steel needle filled with water, the capillary connecting tubes between the gas burette and the extractor are filled with water and connected, as shown in figure # 2. The clamps on the rubber connections are then loosened, the gas burette opened and the Doremus clamp screwed down until the can is punctured. The gas then flows out through the capillary tubing, displacing the water in the gas burette.

When all of the gas has been removed, some of the liquid in the can will come over and sweep any gas in the capillary connections into the gas burette so that quick and complete extraction of the gas from the head space of the sample is obtained.



THE DISAPPEARANCE OF OXYGEN IN CANNED FOOD CONTAINERS

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There is always left a head space in the top of every can in which food is packed. In this head space or chamber there is always more or less air, even when an "exhaust" has been used to withdraw the air. The analyses of probably 100 samples of gas from these head spaces from sound cans containing nearly all kinds of food products have practically never shown any oxygen content. Slight traces have been found in cans containing foods of less than two months age.

The analyses usually show Carbon Dioxide 8 to 15% with the balance Nitrogen Gas. Hydrogen is also found to be present in some instances, particularly with acid fruits.

It might be presumed, in many instances, that the Oxygen combined directly with the food product in such oily foods as Salmon, Evaporated Milk, Pork and Beans, etc., but the absence of Oxygen in all classes of canned foods calls for another explanation, and in order to trace the disappearance of Oxygen and the appearance of Hydrogen Gas, sample cans were prepared containing distilled water, dilute solutions of Citric Acid and Salt and their gas changes were followed by analyses.

The analyses of many samples of gas, drawn from cans containing food products, have shown the presence of Hydrogen Gas, particularly where acid fruits were concerned. This gas does not usually appear until after the foods have been packed two or three months, and inasmuch as some corrosion has necessarily been taking place during this time, the question arises why Hydrogen Gas does not appear more quickly.

The following experimental pack and analyses were made in an attempt to find out the facts.

Ten regular No. 2 cans were filled as follows:

Two cans $\frac{5}{6}$ full with 500 cc plain distilled water.

Two cans $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}\%$ Citric Acid.

Two cans $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}\%$ Citric Acid and $1\frac{1}{2}\%$ Sodium Chloride.

Two cans $\frac{1}{2}$ full with 300 cc solution containing $\frac{1}{4}\%$ Citric Acid.

Two cans $\frac{1}{2}$ full with 300 cc solution containing $\frac{1}{4}\%$ Citric Acid and $1\frac{1}{2}\%$ Sodium Chloride.

These cans were sealed cold, leaving head spaces of approximately 100 cc and 300 cc of air at room pressure and temperature in the cans filled with 500 cc and 300 cc liquid respectively. They were cooked one hour at a temperature of 245° Fah. and then allowed to cool in the air. This was done on October 9th, 1911.

Analyses of the gas from five of the cans were made 42 hours afterwards; one analysis 21 days afterwards and four analyses $8\frac{1}{2}$ months afterwards.

The following are the gas analyses obtained:

1. Can $\frac{5}{6}$ full containing 500 cc plain distilled water, sealed cold, and processed one hour at 245° Fah.—after standing 42 hours had a gas content of the following analysis:

Carbon Dioxide	Trace
Oxygen	13.20%
Hydrogen	.00%
Nitrogen	86.80%

2. Duplicate of this sample, after standing $8\frac{1}{2}$ months, gave the following analysis:

Carbon Dioxide	Trace
Oxygen	.00%
Hydrogen	.00%
Nitrogen	100.00%

3. Can $\frac{5}{6}$ full containing 500 cc of $\frac{1}{4}\%$ Citric Acid solution, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content analyzing as follows:

Carbon Dioxide	.70%
Oxygen	9.65%
Hydrogen	.00%
Nitrogen	89.65%

4. Duplicate of this sample, after standing 21 days, gave the following gas analysis:

Carbon Dioxide	.85%
Oxygen	.40%
Hydrogen	.00%
Nitrogen	98.75%

5. Can $\frac{5}{6}$ full with 500 cc solution containing $\frac{1}{4}$% Citric Acid and $1\frac{1}{2}$% Sodium Chloride, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content of the following analysis:

Carbon Dioxide	.65%
Oxygen	9.05%
Hydrogen	.00%
Nitrogen	90.30%

6. Duplicate of this sample, after standing $8\frac{1}{2}$ months, had the following gas content:

Carbon Dioxide	1.20%
Oxygen	.00%
Hydrogen	.60%
Nitrogen	98.20%

7. Can $\frac{1}{2}$ full, containing 300 cc $\frac{1}{4}$ % Citric Acid solution, sealed cold, processed one hour at 245° Fah.—after standing 42 hours had a gas content analyzing:

Carbon Dioxide	.40%
Oxygen	11.60%
Hydrogen	.00%
Nitrogen	88.00%

8. Duplicate of this sample, after standing $8\frac{1}{2}$ months, had a gas content analyzing:

Carbon Dioxide	.20%
Oxygen	.00%
Hydrogen	.30%
Nitrogen	99.50%

9. Can $\frac{1}{2}$ full, with 300 cc solution, $\frac{1}{4}$ % Citric Acid and $1\frac{1}{2}$% Sodium Chloride, sealed cold, processed one hour at 245° Fah.—after standing 42 hours, had the following gas content:

Carbon Dioxide	.40%
Oxygen	12.00%
Hydrogen	.00%
Nitrogen	87.60%

10. Duplicate of this sample, after standing 8½ months, gave the following gas analysis:

Carbon Dioxide	.30%
Oxygen	.00%
Hydrogen	.80%
Nitrogen	98.90%

The following points in connection with these analyses are significant:

1st. Oxygen disappeared in all cans in the course of time, although the amount originally left in the cans was very excessive.

2nd. Oxygen disappeared in cans containing plain water, also in cans containing acid and acid and salt solutions.

3rd. Hydrogen was not found in any gas analysis until all of the Oxygen had disappeared, although steady acid corrosion had been going on.

If a stronger solution of Citric Acid had been used, much more Hydrogen would have been formed and probably the Oxygen would have disappeared more quickly.

The following analyses were obtained on commercial samples of food products:

Can, eighteen months old, containing Red Raspberries, furnished gas of the following analysis:

Carbon Dioxide	8.40%
Oxygen	.00%
Hydrogen	65.50%
Nitrogen	26.10%

Can, nine months old, containing Red Raspberries, furnished gas of the following analysis:

Carbon Dioxide	10.90%
Oxygen	.00%
Hydrogen	16.50%
Nitrogen	72.60%

Can, eighteen months old, containing Strawberries, furnished gas of the following analysis:

Carbon Dioxide	12.60%
Oxygen	.00%
Hydrogen	72.40%
Nitrogen	15.00%

Can, one year old, containing Strawberries, furnished gas of the following analysis:

Carbon Dioxide	13.20%
Oxygen	.00%
Hydrogen	27.20%
Nitrogen	69.60%

These containers were not properly protected by means of enamel, so that corrosion had been very excessive.

No analyses of gases from canned food containers have ever shown Hydrogen and Oxygen gas together, and inasmuch as Hydrogen Gas must necessarily be formed continuously from the beginning, when acid fruits are present, it would appear that the Hydrogen, under the conditions obtaining in a tin container, combines with Oxygen, and consequently cannot be found until all of the Oxygen has disappeared.

It would appear, therefore, that Oxygen disappears in tin food containers in at least the three following manners:

1st. By combining with the metals tin and iron, forming oxides.

2nd. By oxidizing tin or iron salts.

3rd. By combination with Nascent Hydrogen, when organic acids act on the metallic container.

It is also probable, in some instances, that Oxygen combines directly with the food product, during processing, particularly with such foods as Evaporated Milk, canned Salmon, Pork and Beans, etc. in which there are oily substances. In evaporated Milk and Pork and Beans, there is some caramelization which would also take up some Oxygen.

The analytical work reported in this paper was done by W. S. Sellars.

EGGS PRESERVED WITH SILICATE OF SODA

By J. M. Bartlett

Orono, Maine

For several years water glass or silicate of soda has been used for preserving eggs and has been endorsed by experiment Stations,* the Department of Agriculture** and some foreign scientists. With whom the idea originated the writer is unable to learn, but it was probably first used in Germany.† J. H. Thieriot in 1897 reports testing 20 methods of preserving eggs and obtaining the most satisfactory results with water glass. This method has not been used or recommended for commercial purposes, as cold storage is probably superior for that purpose; but for the family or home use, a supply of eggs can be carried from summer, when they are cheap, for use during the winter months when prices for fresh goods are beyond the means of ordinary incomes. Good eggs properly handled and preserved in water glass are much superior to most cold storage stock and for many purposes equal to fresh eggs.

To obtain the best results with this process it is necessary:

First, to have good fresh eggs.

Second, to have silicate of the right composition which does not contain free soda.

Third, the eggs should be kept completely covered with the solution in a galvanized iron or earthern vessel in a cool place.

The container should be covered to prevent evaporation. The composition of the silicate is a most important factor. Sodium oxide combines with silicon oxide in many different proportions and we find given in the chemical dictionary†† compounds containing from two parts Na_2O to one part of SiO_2 up to one

* Rhode Island Exp. Sta. Report 1900–1901 pp. 304.

* North Dakota Exp. Sta. Bulletin, 35, pp. 330–332.

** U. S. Dept. of Agriculture, Farmers' Bulletin 103.

† Experiment Station Record, Vol. 9, 1897–8, p. 981.

†† Nem's Handworterbuch der Chem., Vol. VI, p. 770.

part Na_2O to four parts SiO_2. The silicates containing such large proportions of Na_2O as the first mentioned would be too strongly alkaline for keeping eggs.* It has been found that eggs kept in a strongly alkaline solution absorbed some of the alkali and produce a jelly like condition of the whites. It is probable that the SiO_2 is not readily deposited from such solutions and the pores of the shell are not closed immediately, consequently some of the solution finds its way through to the interior and the property to which water glass owes its efficiency as a preservative is lost. The writer has obtained very satisfactory results with a silicate containing 24.2 parts of SiO_2 to 8.89 parts Na_2O made

	Six eggs weighed when put in solution	Six eggs weighed when taken out of solution
No. 1	57.18 gms.	57.12 gms.
No. 2	55.85 "	55.85 "
No. 3	62.40 "	62.35 "
No. 4	55.76 "	55.75 "
No. 5	56.67 "	56.62 "
No. 6	53.77 "	53.75 "
	341.63 "	341.44 "

into the glass by the dry process then dissolved by superheated steam and made up to a syrup testing 38 degrees B. One part of this syrup to nine parts of water makes a solution of about 0.045 specific gravity, in which fresh eggs readily sink and will remain submerged. Eggs kept in this way are of better flavor than cold storage stock. They never have the musty taste so often found in the storage goods and about the only difference between them and fresh eggs is a little lack of flavor. The shells are hermetically sealed and no bacteria can get through them, neither can oxygen, consequently if they contain any life when put in the solution it is very soon destroyed. Six fertile eggs put in a jar of water glass were kept in an incubator for 6 days at a temperature of 103 degrees F. and an examination

* Borntraeger (Oeslem. Chem. Ztg. 3, 1900, No. 12, p. 295.

at the end of that time was made, showing that the embryo had made no growth. There is very little change in the moisture content, and, unlike eggs in cold storage, the weight remains practically constant.

The writing of this paper and the limited investigation which is here given was suggested by a newspaper article which contained the statement that eggs preserved in water glass were unfit for food because they contained quite a quantity of soluble silica which if taken into the system was very dangerous and liable to cause coagulation of the blood. Notwithstanding the ridiculousness of the statement many people were alarmed and ceased to use eggs preserved in the silicate, and often inquiries were made to learn if any investigations had been made to determine if silica passed into the egg content. The work, therefore, was undertaken to determine principally whether eggs kept in water glass contained any more silica than fresh eggs. Some work was also done to determine if any marked changes take place in the nitrogen compounds. The most noticeable physical change in the eggs is a thinning out of the white which after the egg is kept 10 or 12 months does not coagulate so firmly as does that of a fresh egg, and the white appears much more watery.

The results of the investigation are given in the tables which follow. To separate the yolks from the whites completely, particularly in the preserved eggs, it was found necessary to boil the eggs before breaking them which, of course, caused some loss of moisture. The methods of analysis used in the experiments were those employed by the Bureau of Chemistry, U. S. Department of Agriculture,* in the work on cold storage eggs. The preserved eggs used were put down by the writer in a 10 per cent solution of 38 degrees B. water glass syrup, containing one part Na_2O to 2.7 parts SiO_2, in April 1911. They were, consequently, when examined about 11 months old. The other lot was put in the same kind of a silicate solution in February 1912 and examined in April, consequently were about two months in the solution.

* Bulletin No. 115, Bureau of Chemistry, U. S. Dept. of Agric.

WEIGHT OF EGGS, WHITES AND YOLKS AND LOSS IN BOILING

	Weight of Eggs Raw	Weight of Eggs Boiled	Weight of Shells	Weight of White	Weight of Yolks	Loss in Boiling	Total loss of moisture in boiling and shelling
	Grams	Grams	Grams	Grams	Grams	Per cent	Per cent
3 fresh eggs.....	171.272	166.838	17.93	88.51	59.301	2.59	3.81
3 w. g. eggs, one year.....	193.353	190.199	22.835	97.86	63.115	1.63	4.93
3 fresh eggs.....	175.102	170.304	18.071	98.683	52.738	2.74	3.20
3 w. g. eggs, 2 months.....	175.320	169.550	16.91	96.81	51.92	3.29	5.52

Partial Analysis of Fresh and Preserved Eggs

Wet Basis

	Moisture	Ash	Silica	Ether Extract (Petroleum)	Nitrogen present as				
					Total	Coagulated by boiling	Uncoagulated by boiling	Coagulated with salt and tannin	Uncoagulated
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Fresh Eggs									
White	87.40	0.785	0.005		1.78	1.585	0.195	0.115	0.08
Yolk	48.05	1.35	0.062	32.15	2.655	2.540	0.115	0.015	0.10
Preserved eggs. 11 months..									
White	85.15	0.70	0.006	0.02	2.05	1.675	0.375	0.365	0.11
Yolk	52.80	1.30	0.060	29.70	2.37	2.21	0.160	0.008	0.152
2 months in water glass....									
White	86.43	0.73	0.012	0.054	2.018	1.783	0.235	0.172	0.063
Yolk	50.25	1.47	0.040	30.68	2.53	2.405	0.125	0.015	0.110

Partial Analysis of Fresh and Preserved Eggs

Dry Basis

	Ash	Silica	Ether Extract (Petroleum)	Nitrogen present as				
				Total	Coagulated by boiling	Uncoagulated by boiling	Coagulated with salt and tannin	Uncoagulated
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Fresh Eggs.								
White.....	6.23	0.04		14.12	12.58	1.54	0.912	0.64
Yolk.....	2.64	0.119	62.98	5.20	4.98	0.225	0.029	0.195
Preserved eggs in water glass. 11 months.								
White.....	4.71	0.04	0.134	13.80	11.29	2.58	2.46	0.74
Yolk.....	2.46	0.113	56.25	4.49	4.19	0.30	0.15	0.288
Preserved eggs in water glass 2 months								
White.....	5.37	0.087	0.398	14.88	13.14	1.732	1.267	0.464
Yolk.....	2.93	0.080	61.04	5.04	4.81	0.23	0.03	0.22

In addition to the results given in the tables some work was done in coagulating the albumen of the white with different reagents. Three eggs of each kind were taken and the whites separated as completely as possible from the yolks in the raw condition. 10 gram samples of the whites were treated with acidulated water and boiled. The whites yielded copious flocculent precipitates which were thrown on tared filters, washed with hot water, dried and weighed.

The fresh eggs yielded dried albumen 10.0 per cent.

The 11 months old preserved eggs yielded dried albumen 10.28 per cent.

Samples treated with alcohol and allowed to stand several hours yielded:

Fresh eggs, dried albumen.....12.00 per cent.
Preserved eggs, dried albumen.....12.39 per cent.

Calculated from nitrogen content (n. x 6.25)

Fresh eggs	= 11.37 per cent.
Preserved eggs	= 11.62 per cent.

It is obvious from the figures here obtained and those given in the table that there is practically no difference in the total coaguable proteid matter of the fresh or preserved eggs. There seems to be a slight difference in the amount of nitrogen or protein coagulated by heat alone and is probably due to the presence of albumoses and peptones which are absent in fresh eggs but appear to develop as the esgg age.* This same change was noted in the case of cold storage eggs, and reported in a paper published by Dr. H. W. Wiley and others.†

CONCLUSIONS

1. Eggs packed in the right kind of water glass (silicate of soda) contain no more silica or other ash materials than fresh eggs.

2. The moisture content remains constant and a preserved egg weighs practically the same as when put in the solution.

3. The nutritive value as far as one can judge from the chemical analysis is the same as that of a fresh egg. The quality is superior to most cold storage eggs, as the pores of the shell are closed and no bad odors or flavors are absorbed.

* Allen's Commercial Organic Analyses, Vol. IV, p. 41.

† Bulletin 115, Bureau of Chemistry, U. S. Dept. of Agric., p. 32.

SOME OF THE RESULTS OF THE FOOD AND DRUGS ACT

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The complete study of this question would involve the principles governing the manufacture and sale of each particular kind of food and drugs. It would necessitate the discussion of the history and development of a large number of articles and of the varied influences which lead to the adoption and subsequent discontinuance of individual practices, in each. This would lead us into a maze of details which would require a considerable volume for their adequate presentation. In a paper of this scope, therefore, it is only possible to consider some of the general principles involved and a few of the fundamental changes that have resulted from the enforcement of the Act of June 30, 1906. At the time of the passage of the Food and Drugs Act, notwithstanding the creditable work that had been done by 27 States in the enforcement of their laws, labels on foods were very frequently so written as to deceive the consumer with respect to the character, value or origin of the product.

When the labels purported to give the weight of the product their statement was commonly exaggerated, sometimes being the gross weight of the product and package and sometimes having no relation to the weight of the product at all. Cans known technically as No. 1, 2, 3, etc., were sometimes designated as "1 pound," "2 pound," "3 pound," etc., though holding perhaps only two-thirds of that amount. Bottles of wine, oil and other products measuring five to a gallon,—sometimes a smaller amount,—were often labeled as quarts. Canned food and bottled goods are still sometimes referred to in grocers' lists and restaurants as "Pounds" and "Quarts," respectively, but the practice of designating them in that manner on the label and on the shipping case has been discontinued.

There are many difficulties encountered in marking the weight accurately upon a package of some kinds of food and it is probable that the misstatements that now exist on labels of food with respect to weight are often unintentional. The fraudulent statements which were prevalent five years ago have almost disappeared.

Closely related to misbranding with respect to weight is the practice in packing of canned food usually known as "slack filling." In such case the can was only partially filled with the food in question, the deficiency usually being made up with water to prevent the collapsing of the can. For instance, peas or beans were filled to within one-half inch, or possibly sometimes an inch, of the point to which they should be filled and then water or weak brine added to make up the proper volume. In packing cove oysters—in the extreme illustration of slack filling—only one and one-half ounces of oysters were sometimes placed in cans capable of holding over five ounces and the cans were then filled with brine. In tomatoes the cans were filled probably to within an inch of the top or sometimes only half or two-thirds full and the deficiency made up with water or perhaps weak brine. Steps were taken to correct this abuse and during the last year successful prosecutions have been maintained against packers of slack filled cans. The Department has announced publicly that this practice is fraudulent and it is believed that it has now been entirely discontinued.

Foods have been misbranded commonly with respect to the name of the place, (i. e. the country or region) in which they were produced or manufactured. This practice has obtained for two reasons: first, because of the desire of the packer in one locality to take advantage of a favorable reputation of another locality and so misbrand his goods as to the place of their manufacture; second, because of a certain glamor which a foreign name possesses for many consumers. As an illustration of the first class of abuses with respect to geographical name may be mentioned the packing of "Maine Sweet Corn" in Maryland, of "Michigan Apples" in Arkansas, of "California Canned Fruit" near the Atlantic Coast, of "Minnesota Flour" in the mills of Iowa and Missouri.

This form of misbranding gradually shades into the class where the misuse of a geographical name causes a false impression with respect to the material of which the food is made; for instance, the term "Vermont Sirup" or "Ohio Syrup" means maple sirup to the consumer because that is the only sirup made in Vermont and Ohio. These terms have been used frequently on the label of a cane sugar sirup colored and sometimes flavored in imitation of maple sirup. This form of misbranding has been corrected generally with reference to the staple articles of the United States.

Material progress has been made in correcting this form of misbranding in the case of foreign products and of foods manufactured in the United States in imitation of foreign products. Some of the brands of coffee which were formerly labeled "Mocha and Java," for instance, are now merely called "Coffee." Importers have been required to discontinue, on imported foods, the language of another country than that of their manufacture. Progress has been made in the correction of the label of imported wines which are commonly misbranded with respect to their character or class. Progress has also been made in the correction of the labels of certain products manufactured in the United States in imitation of foreign products, such as macaroni and tomato paste. Several kinds of cheese manufactured in imitation of well known foreign varieties are now labeled with the place of their manufacture. Rice grown in this country from Japan seed is labeled as grown in the United States. A product formerly called "Holland Rusk" with the label embellished with Dutch scenery is now labeled as made in Holland, Michigan, and the Dutch windmill has been removed from the label. This form of misbranding again merges into adulteration as, for instance, when cottonseed oil grown in the United States is placed in decorated tins so labeled as to represent the product to be an Italian olive oil. These practices still obtain to a certain extent, though to a much less degree than formerly.

One of the prevalent forms of misbranding is the use on the label of exaggerated claims regarding the strength and the nutritive value of the product. This form of misbranding is especially applicable to proprietary remedies but has also been practiced

largely with foods. Flavoring extracts were often labeled "Double Strength," or "Triple Strength," although the products so labeled were rarely beyond standard strength, and not infrequently were entirely fictitious. Breakfast foods and infant foods carried on their labels a glowing description of their miraculous nutritive value and sometimes curative properties. Cereal preparations of ordinary composition without any of the starch having been removed were sold under labels representing them to be diabetic foods. These practices have largely passed away as far as the labels are concerned. Unfortunately, the law does not reach posters and advertising matter sent through the mails and by such means fraudulent statements regarding the quality and nutritive value of some preparations is conveyed to the consumer. The labels themselves, however, have been greatly improved.

The addition to foods of substances of lower value to serve as a make weight and thus cheapen the food has largely been discontinued. At the time of the passage of the Food and Drugs Act such practices had been made away with in a number of the States but in other States and indeed in interstate commerce they were still quite prevalent. As illustrations of this practice may be cited rye flour and buckwheat flour, both of which contained a substantial amount of wheat flour; spices which were commonly loaded with cereal preparations, ground olive stones, cocoanut shells, etc., a line of preparations being manufactured and sold for the purpose of adulterating spices and pepper shells and olive stones being imported into this country for that purpose.

At the time of the passage of the Food and Drugs Act there was little pure maple syrup manufactured commercially and sold in interstate commerce. Immediately after the law went into effect there was practically no maple syrup to be had but brands of so-called maple syrup which had formerly borne on the label an offer of a large reward to anyone who would prove the presence of any adulterant appeared under a new label, declaring the contents of the package to be a mixture of maple syrup and cane sugar syrup.

Cider vinegar was commonly diluted with water to reduce its strength in acetic acid to the desired percentage and since this dilution brought the solids content down to a lower figure than

that in commonly accepted standards a quantity of boiled cider was added. It only remained to add a larger quantity of boiled cider and, of water and then to strengthen with distilled vinegar to obtain much larger yields and this practice merged gradually into the preparation of an entirely fictitious product manufactured from distilled vinegar, with color added and solids in the form of boiled cider. The detection of practices of this kind by analytical means offered many difficult problems which have been partly solved and maple sirup and cider vinegar may be cited as types of a large number of products which are now sold in the pure state to a very much larger degree than was true at the time of the passage of the Food and Drugs Act.

The two classes of substances relied on chiefly by the manufacturer in the preparation of fictitious products are colors and flavors. It was a difficult matter to handle either of these classes of substances in such a way as to imitate a natural food. The manufacturer who uses them is likely to go to extremes and the fictitious products he puts on the market are frequently of a hue that is scarcely to te found in nature, whereas the flavors are also commonly in excess. The improvement in natural products that has attended the work of the last five years, accompanied by the better information of the public regarding such matters, has resulted in a growing aversion for fictitious colors and flavors and many lines of products which were formerly in demand are now regarded by the public with disfavor. Moreover the wholesomeness of the colors employed has been considered. The Department has authorized the use in foods of a list of 7 coal tar colors and these must be manufactured in such a way as to be free from arsenic and other deleterious substances. Manufacturers have to a large extent complied with this regulation.

To a much greater extent than ever before manufacturers are giving attention to the question of the wholesomeness of substances used in the preparation of foods. Formerly this was not the case. When it was desired to begin the use of a preparation in the manufacture of foods the ordinary article of commerce was frequently employed without any thought of its possible injurious properties. When acid phosphate was employed, for instance, in the preparation of a food or drug the acid phosphate of com-

merce was used and it was not known that it contained a considerable amount of arsenic. Notwithstanding the fact that lead pipes have been known for generations to be improper for conducting water for household purposes, they were employed for tartaric and citric acid which were intended to be used as foods and a relatively large amount of lead thus found its way into the product placed on the market for ordinary consumption.

When a confectioner desired to give a gloss to some of his wares it occured to him that the product used by the painter would meet his requirements and he took ordinary shellac without considering whether the lac itself was injurious to health and without thinking of the fact that the shellac of commerce contains a considerable amount of arsenic. We even found a large shipper of green coffee who desiring to polish his wares and give them a faint yellowish shade, used the first yellow powder which came to his attention and this happened to be chromate of lead. When it was desired to prolong the life of certain foods and at the same time make unnecessary the care in handling which would otherwise be necessary among the preservatives suggested and largely employed are some substances whose toxicity was universally admitted; e.g.—formaldehyde and ammonium fluorid.

The important point is not that some practices of this nature have been corrected and others are being corrected at this time, but that there is a rapidly growing tendency on the part of manufacturers when considering the use of a new or unusual substance or preparation in the manufacture of food to consider whether it is injurious to health, either because of its nature or composition or because of certain impurities and whether for any reason its addition to food is objectionable.

The removal of the manufacture of prepared foods from the home to the factory has made great changes in our civilization and made necessary precautions which were before unthought of. One of the most prominent characteristics of civilization is the increased emphasis placed on cleanliness and sanitary conditions. It is only this fact which has made possible in the home during the last century the preparation of many of the domestic preserved foods which are now most prevalent. The manufacture of these foods in the factory, however, has not been confined to men who

were qualified to enforce sanitary conditions such as are necessary for the successful preparation of many articles of food. The result has been that we have had placed on the market on the one hand preparations in a more or less advanced state of decay and on the other hand substances contaminated with pathogenic organisms. Thus there have been cases of contaminated water being bottled and sold as spring water and being used for the preparation of soft drinks and for serving from soda fountains.

Tomato catsup has been prepared from the peelings and cores of unwashed tomatoes, including a considerable part of rotting material, and by a process and amid surroundings which caused additional decomposition to take place during the course of manufacture. Ripe olives and figs were often imported into the United States in a wormy and decomposed condition. So little attention was given to the matter that it was the custom of railroads to sell unclaimed food products resulting from wrecks to the highest bidder with a knowledge that they would be placed on the market indiscriminately.

Badly contaminated water has been used for cleansing milk cans in dairies and together with contaminated ice has not infrequently been added to the milk. The sanitary condition of dairy stables has frequently been bad. Eggs so far advanced in decomposition that they would not be used by a housekeeper have been broken in large quantities and placed on the market either dried or frozen. Oysters and clams have been taken from contaminated water and placed on the market with the inevitable result of spreading typhoid fever.

These sanitary problems offer difficulties which cannot be overcome in a day but in all of them material progress has been made. A number of the States, realizing the importance of sanitary requirements, have enacted special sanitary laws whose enforcement has done much to improve the conditions formerly existing. Of still greater importance, however, is the fact that manufacturers as a whole have become interested in the desiderata of a food manufacturing establishment from a sanitary standpoint and the changes resulting in the cleanliness of their establishments, as well as in their utensils and the character of the raw material they employ, are among the most satisfactory

results of the recent enforcement of food legislation. Whereas formerly only rule of thumb methods were employed we now meet chemists, bacteriologists and microscopists in many general food manufacturing establishments. It is frequently made the duty of some special officer to study and be responsible for the sanitary condition of the factory. The health of the employees is considered with reference to the influence it may have upon the food. Cleanliness is more frequently required, as well as uniforms or special factory clothes, and in some establishments manicurists are employed.

The number of prosecutions that have been successfully maintained for the violation of the law is of minor importance compared with this change in the attitude of the manufacturers.

SUR L'ANALYSE DU PHOSPHORE DANS LES CENDRES DU LAIT

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L'expérience nous a démontré que l'acide phosphorique existant à l'état de phosphates dans les cendres d'un lait correspond à la totalité du Ph contenu dans ce liquide, c'est-à-dire au Ph minéral et au Ph organique: lécithine, nucléine, etc. . . . Cette particularité est très importante à connaitre pour éviter certaines erreurs d'appréciation sur la valeur alimentaire du lait.

Il s'ensuit donc, pour le cas particulier du lait de vache, lorsqu'on fait les cendres de ce liquide on ne provoque aucune disparition de phosphore par l'action du charbon sur les phosphates, et la matière grasse n'entraine aucune partie du phosphore à l'état de combinaison volatile. Il est inutile d'ajouter des sels de chaux, de baryte, de magnésie, etc., comme le préconisent plusieurs auteurs, pour éviter des pertes en phosphore par calcination.

Le phosphore total d'un lait peut donc être dosé directement sur ses cendres. D'autre part, en précipitant le lait par l'acide trichloracétique on détermine le Ph minéral dans le lactoserum et le Ph organique dans le coagulum.

L'ACIDITE ORIGINELLE DU LAIT

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Les auteurs ne sont pas tous d'accord sur la réaction à attribuer au lait, pour les uns ce liquide, à l'état frais, serait acide, pour les autres, il serait amphotère, c'est-à-dire possèderait une réaction alcaline et une réaction acide.

En étudiant cette question nous avons constaté que ces divergences d'opinions résidaient uniquement dans l'emploi d'indicateurs qui ne répondaient pas aux conditions expérimentales.

Nous avons établi que la phtaléine du phénol est l'indicateur de choix pour étudier la réaction du lait. Lorsque ce liquide est précipité par notre réactif alcool 65° acétique à 1-1000 nous constatons, en tenant compte de l'acidité du réactif, que l'acidité totale d'un lait frais se retrouve dans le coagulum et qu'elle est due exclusivement à la caséine libre.

L'expérience nous a démontré également qu'il existe dans un lait frais aucun acide libre, lactique, citrique, ni aucun sel à fonction acide, que l'augmentation de l'acidité d'un lait provient tout d'abord de la caséine déplacée, de sa combinaison calcique, par l'action de l'acide lactique formé aux dépens du lactose et que l'acidité lactique n'apparait ensuite que lorsque cet acide a réagi sur les sels de chaux du lait.

DE L'ACTION DU LAIT SUR CERTAINS RÉACTIFS

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Pour expliquer les phénomènes péroxydasiques obtenus au sein d'un liquide on s'appuie en général sur l'existence de substances diastasiques que certain considère comme une individualité définie. Or, jusqu'ici, il n'a pas été possible d'isoler à l'état de pureté les matières diastasiques actives et toujours nous les retrovons à côté d'éléments minéraux. Il existe donc une relation étroite entre tous ces éléments et leur ensemble constitue un système péroxydasique que nous retrouvons dans l'étude des diastases du lait.

Le but que nous poursuivons consiste à rechercher le mécanisme qui préside aux réactions colorées obtenues dans le lait avec certains réactifs.

Nous allons nous occuper, en particulier de l'action de la paraphénylènediamine sur le lait, ce corps formant le réactif le plus sensible pour la recherche des péroxydases du lait.

Dans cette étude nous avons été amenés à reproduire artificiellement des phénomènes péroxydasiques à l'aide de substances prises souvent en dehors des matériaux existant dans le lait.

On sait que la paraphénylènediamine par oxydation forme de la quinone. Cette oxydation se produit déja en laissant exposer à l'air une solution aqueuse de cette base qui devient *plus ou moins brune* suivant le temps d'exposition. On arrive également à ce résultat par l'action d'un courant d'O ou par la décomposition de l'H^2O^2 au sein d'une solution aqueuse de cette diamine, mais on obtient aucune coloration bleue comme celle qui se produit dans un lait frais additionné d'H^2O^2 et de paraphénylènediamine. Cette coloration n'est donc pas le résultat d'une simple oxydation, il est en effet nécessaire de faire intervenir une autre cause pour expliquer la coloration bleue.

Nous avons constaté qu'elle était due à l'action d'un produit intermédiaire entre la paraphénylènediamine et la quinone sur les sels de chaux.

Il nous suffit par exemple, de verser une goutte d'une solution de paraphénylènediamine sur un bâton de craie pour obtenir immédiatement une coloration bleue.

Ceci nous conduit à obtenir directement cette coloration avec certains sels de chaux en même temps que ces sels nous servent de catalyseurs de H^2O^2 pour l'oxydation de la paraphénylènediamine.

Prenons, en effet, du citrate de chaux on du phosphate tricalcique purs et secs, ajoutons 2 ou 3 gouttes d' H^2O^2 et mélangeons, si on ajoute ensuite une goutte ou deux d'une solution fraiche de paraphénylènediamine à 2% on obtient immédiatement la coloration bleue en question qui se fixe sur le sel de chaux. Si avant d'ajouter le réactif on délaye dans l'eau les sels de chaux insolubles et oxygènés on constate encore la coloration bleue qui reste toujours fixée sur la chaux.

Cette réaction se divise donc en deux phrases:

1°—Oxydation de la base par un phénomène catalytique.

2°—Coloration bleue produite par la chaux du sel sur le réactif oxyde.

Nous avons toujours obtenu ces résultats avec des poudres sèches imprégnées d'H^2O^2 et renfermant de la chaux, et toutes les fois que la chaux n'était plus en présence la réaction était négative.

Les expériences suivantes sont en effet très concluantes.

Si dans deux petites capsules on place dans l'une de la pierre ponce ordinaire et dans l'autre la même pierre ponce traitée par l'eau régale, lavée à l'eau et sèchée à 100°, puis qu'on répète l'expérience précédente, on constate que les grains de pierre ponce ordinaire sont seuls colorés. Les mêmes résultats sont également obtenus avec de la caséine privée ou non de sa chaux comme nous l'avons signalé dans un travail antérieur. Nous ferons remarquer en outre, que cette coloration bleue ne se produit que dans un milieu très légèrement acide.

En résumé, nous pouvons admettre, d'après ces expériences,

que l'oxydation de la paraphénylènediamine produit un laque bleu-indigo en présence d'un sel de chaux.

Voyons maintenant ce qui se passe lorsqu'on recherche les péroxydases du lait au moyen de la paraphénylenediamine.

D'après les théories actuelles il existerait dans le lait frais-des diastases capables de décomposer l'H^2O^2 et l'oxygène mis en liberté provoquerait la coloration bleu-indigo de la paraphénylènediamine, d'autre part, on sait qu'un lait porté à 80° perd la propriété de décomposer l'eau oxygénée.

La modification apportée par la chaleur dans un lait frais est due à la coagulation d'une partie de la matière protéique qui empêche la décomposition de l'H^2O^2 ajoutée au lait, mais nous sommes parvenus à isoler néanmoins le catalyseur d'un lait bouilli au moyen de la centrifugation comme nous l'avons démontré antérieurement, c'est-à-dire, en recueillant le dépôt formé au fond du tube du centrifugeur et la crème qui surnage. Ces deux parties du lait bouilli ou stérilisé sont capables de décomposer l'H^2O^2 et oxyder la paraphénylènediamine donnant la coloration bleue en présence de la chaux.

Une expérience nouvelle plus concluante encore nous a permis d'obtenir une réaction positive avec la paraphénylènediamine sur le lait entier chauffé à plus de 80° mais homogénéisé à l'aide d'une machine pulvérisant le lait à une pression de 200 atmosphères. Cette opération ayant pour effet de donner à toutes les molécules, des corps insolubles contenues dans le lait une même dimension. On rétablit ainsi, dans une certaine mesure, l'état colloïdal primitif du lait et son catalyseur a pu agir à nouveau sur l'H^2O^2 et donner en présence de la paraphénylènediamine la coloration bleue.

Nous écartons dans cette expérience comme dans toutes celles que nous avons faites sur les laits bouillis les causes d'erreur dues à la présence de bactéries ou de muscédinées qui peuvent exister lorsqu'on opère avec des laits altérés.

Nous avons constaté que la réaction avec du lait homogénéisé est moins intense qu'avec du lait frais mais il n'en reste pas moins établi que nous pouvons redonner par un procédé mécanique à du lait chauffé à plus de 80° son pouvoir péroxydasique.

Nous avons encore démontré avec ces laits que la coloration

bleue du réactif employé est bien due à la présence des sels de chaux du lait. En effet, en prenant un lait fixé et stérilisé, nous pouvons lui rendre son maximum d'action en introduisant dans ce lait un catalyseur artificiel, soit une poudre pulvérulente insoluble sans action chimique comme la Sio^2 pure par exemple, soit des traces d'une solution d'oxalate de fer. Dans ces conditions la coloration bleue avec la paraphénylènediamine devient très intense.

Toutes ces expériences démontrent bien que les réactions négatives avec le réactif à la paraphénylènediamine dans un lait chauffé à 80° ne sont dues qu'à un changement d'état physique du lait et que les péroxydases ou les catalases qui ont été signalées, et qu'aucun auteur n'a pu isoler à l'état de pureté doivent être considérées jusqu'à présent comme des combinaisons organo-métalliques jouant un rôle chimique et non biologique.

A CHEMICAL INVESTIGATION OF ASIATIC RICE

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Introduction.

The following paper contains a description and the results of a complete chemical investigation of twenty-seven samples of Asiatic rice, which was recently carried out at the instance of the Siamese Government. The samples were collected in the open market at Singapore and Shanghai and no effort was made to prepare them in any way differently from those rices which are ordinarily exposed for sale in the Asiatic market. The relation of an exclusive rice diet on the etiology of beri-beri disease has been much discussed for a number of years past. This paper does not pretend to decide this controversy but is offered as a contribution to the general knowledge of the chemical constitution of rice. As far as the authors are aware the results on the phosphate content of eastern rices is the most complete as yet published.

Description of Samples.

The samples reached the Institute on October 30th, 1911, and the box containing them was opened on October 31st. The samples were contained in twenty-seven 10 pound cotton bags numbered serially 1 to 27. No other distinguishing marks or information was found.

The cotton bags were found to be frail and rotten and in some cases were broken through, so that the contents had partially escaped. All the samples contained living weevils, and a few worms and beetles were also found. The condition of the samples made it necessary to hand pick them to remove insects. They were then immediately packed in glass bottles, stoppered and labeled.

The appearance of the samples indicated that they represented a medium grade of white or milled rices. On the trip from the

Far East the samples had evidently suffered desiccation with the result that some of the grains had become abraded and broken. As it was not believed, however, that the grain had suffered in such a way as to affect the chemical analysis except in regard to moisture content and the weight per 100 grains, it was decided to be unnecessary to delay the investigation by awaiting a new importation of samples from the Far East.

The Analytical Work.

The analytical work was carried out by the methods recommended by the Association of Official Agricultural Chemists of the United States, and comprised the following elements usually sought: Moisture, Ash, Proteids, Ether Extract (mainly Fat), Fibre, Starch and other Carbohydrates, Weight per 100 Grains.

The above determinations have usually been accounted sufficient to fix the nutrition value of a given cereal. In view, however, of a recently published claim that milled rices are deficient in organically combined phosphorous, phosphate determinations were carried out on each sample. The results have been carefully checked and may be taken as accurate for the samples worked on.

Tabulation of Results.

The results of the analytical work on the twenty-seven samples submitted are given in Table I, with the exception of the phosphate contents which are tabulated separately in Table III. Table II gives the results of analysis of two fresh samples of South Carolina (U. S. A.) rices bought at a prominent grocery house in Washington, D. C. These samples are denominated Numbers 29 and 30. Sample 29 is the ordinary very white large grained rice as sold in the United States at about ten cents a pound. Sample 30 was sold for a slightly higher price and purported to be a "natural uncoated special pure rice." Table III gives the phosphate content of all samples, reported as phosphoric anhydride, P_2O_5. In Appendix A are given the results of an examination of various rices exhibited at the World's Columbian Exhibition, at Chicago, in 1893, the analyses made by

the Division of Chemistry, U. S. Department of Agriculture. Appendix A is preceded by an extract from Bulletin No. 13, and is followed by a summing up of the results.

TABLE I

RESULTS OF ANALYSIS OF TWENTY-SEVEN SAMPLES OF RICE SUBMITTED TO THE INSTITUTE OF INDUSTRIAL RESEARCH BY THE SIAMESE LEGATION, WASHINGTON, D. C.

Sample No.	Weight of 100 Grains	Moisture	Ash	Ether Extract	Crude Fibre	Proteids	Starch and Carbohydrates
1	1.565 grams	11.02%	0.46%	0.31%	0.40%	8.13%	79.68%
2	1.39 "	10.99%	0.51%	0.29%	0.60%	8.25%	79.36%
3	1.181 "	11.11%	0.56%	0.20%	0.29%	7.38%	80.46%
4	1.036 "	10.82%	0.46%	0.15%	0.20%	8.44%	79.93%
5	1.708 "	11.54%	0.40%	0.13%	0.82%	8.44%	78.67%
6	1.651 "	10.51%	0.49%	0.28%	0.83%	7.56%	80.33%
7	1.498 "	11.14%	0.50%	0.20%	0.72%	7.81%	79.63%
8	1.244 "	11.31%	0.48%	0.15%	0.47%	7.75%	79.84%
9	1.481 "	11.10%	0.55%	0.68%	0.66%	8.31%	78.70%
10	1.409 "	11.30%	0.41%	0.63%	0.43%	7.81%	79.42%
11	1.329 "	10.60%	0.49%	0.20%	0.21%	7.63%	80.87%
12	1.725 "	11.28%	0.47%	0.31%	0.27%	7.56%	80.11%
13	1.723 "	10.45%	0.45%	0.17%	0.60%	8.06%	80.23%
14	1.541 "	10.94%	0.44%	0.53%	0.76%	7.56%	79.77%
15	1.141 "	10.44%	0.54%	0.10%	0.31%	7.81%	80.80%
16		11.08%	0.85%	0.28%	0.44%	8.25%	79.10%
17	0.958 "	10.51%	0.74%	0.12%	0.16%	7.81%	80.66%
18	0.892 "	10.49%	0.60%	0.30%	0.32%	8.00%	80.29%
19	0.788 "	9.99%	0.48%	0.94%	0.33%	8.06%	80.20%
20		10.06%	0.55%	0.71%	0.51%	8.13%	80.04%
21	1.238 "	9.21%	1.23%	0.80%	0.77%	8.44%	79.55%
22	1.175 "	9.19%	0.72%	0.87%	0.56%	8.94%	79.72%
23	1.533 "	9.32%	0.57%	0.52%	0.45%	8.75%	80.39%
24	1.179 "	9.55%	0.77%	0.91%	0.47%	8.38%	79.92%
25	1.429 "	10.37%	0.58%	0.16%	0.23%	8.38%	80.28%
26	1.413 "	10.04%	0.72%	0.59%	0.45%	7.63%	80.57%
27	1.581 "	10.81%	0.51%	0.44%	0.31%	8.63%	79.30%

TABLE II

RESULT OF ANALYSIS OF TWO SAMPLES OF SOUTH CAROLINA RICE

Sample No.	Weight of 100 grains	Moisture	Ash	Ether Extract	Crude Fibre	Proteids	Starch and Carbohydrates
29	2.241 grams	10.23%	0.47%	0.42%	0.29%	9.00%	79.59%
30	2.238 "	9.01%	0.37%	0.21%	0.36%	8.13%	81.92%

TABLE III

RESULTS OF PHOSPHATE DETERMINATIONS ON TWENTY-SEVEN SAMPLES OF RICE SUBMITTED TO THE INSTITUTE OF INDUSTRIAL RESEARCH BY THE SIAMESE LEGATION, WASHINGTON, D. C.

Sample No.	$\%P_2O_5$	Sample No.	$\%P_2O_5$	Sample No.	$\%P_2O_5$
1	0.22	10	0.31	19	0.31
2	0.39	11	0.32	20	0.30
3	0.30	12	0.23	21	0.41
4	0.20	13	0.21	22	0.39
5	0.28	14	0.21	23	0.42
6	0.26	15	0.30	24	0.58
7	0.31	16	0.49	25	0.24
8	0.26	17	0.35	26	0.22
9	0.30	18	0.35	27	0.34
South Carolina rice.....		29	0.29		
		30	0.24		

Interpretation of Results.

A careful inspection of the results shows, that all of the analyses of the samples submitted compare favorably in respect to nutrition value with the samples given under the World's Fair report which includes typical rice analyses as quoted by various authorities (see Appendix A). The results also for the most part compare well with the analyses of the South Carolina rices given in Table II. The phosphorous content of the imported

samples (Table III) shows considerable variation; in some cases it corresponds to the average for milled white rice which is reported to be about 0.25%; in other cases it is as high as is usually shown in rices treated by the parboiling process. It would appear that the white rices as represented in the twenty-seven imported samples show on the average as high a nutrition value as the white rices from other sources. The moisture content and weight per 100 grains is somewhat low in the imported samples, for the reason stated above.

Interpretation of the Analytical Results in Relation to the Etiology of Beri-Beri.

It has recently been claimed by Doctors Fraser and Stanton of the Institute for Medical Research, Kuala Lumpor, that the low phosphorous content of white milled rices is a predisposing cause of beri-beri. (See "The Lancet" London) Vol. 176, p. 451 (1909). It is further stated by Doctors Fraser and Stanton that: "From epidemilogical considerations and from experimental evidence it appears that Siam rice is considerably more potent in its beri-beri producing powers than Rangoon rice."

Opposed to the conclusions of Doctors Fraser and Stanton stands the opinion of Dr. Hamilton Wright, former Director of the Institute for Medical Research, Federated Malay States, an eminent investigator of the Etiology and Pathology of Beri-beri. Dr. Wright's published opinion, based on years of study and clinical experimentation is quoted below:

(An inquiry into the Etiology and Pathology of Beri-beri. Hamilton Wright, M. D., Studies from Institute for Medical Research, Federated Malay States, Vol. 2, No. 1, p. 58 (363).

"The theory of the causation of beri-beri that fits the above facts and all others observed in British Malaya is that beri-beri is due to a specific organism which gains entrance to the body via the mouth, that it develops and produces a toxin chiefly in the pyloric end of the stomach and duodenum, and that the toxin, being absorbed, acts atrophically on the peripheral terminations of the afferent and efferent neurones. Further, that the specific organism escapes in the fæces and lodges in confined places through accident or the careless personal habits of those

affected by the disorder, and that in the presence of congenial meteorological, climatic and artificial conditions of close association from overcrowding, the organism becomes virulent and, gaining entrance to the healthy body in food, etc., contaminated by it, gives rise to an attack of the disease. The fact that the germ remains so closely focal can, I think, be explained by its being at once destroyed by the action of direct sunlight or that the presence of CO_2 or some other gas is necessary for its virile development. It seems from my observations here that the active stage of the organism in the body is between three and four weeks. I base this estimation on the facts that the preliminary feeling of oppression in the epigastrium ceases at the end of about three weeks, and that it is rare to find the lesion of the gastric and intestinal mucose in cases of only six weeks' standing."

Conclusion.

As far as the results of analysis can be interpreted in the light of the information at hand, there would appear to be no reason why the white milled rices from one section of the world should be held more responsible for mal-nutrition than similar rices from other sections.

APPENDIX A.

EXTRACT FROM BULLETIN NO. 13, U. S. DEPARTMENT OF AGRICULTURE. DIVISION OF CHEMISTRY

Foods and Food Adulterants. Investigations made under direction of H. W. WILEY, Chief Chemist. Part 9. Cereals and Cereal Products, Washington, D. C., 1898.

Rice may reach the analyst in three different states, viz.: unhulled, hulled, and polished. He may also have occasion to examine the broken fragments used in polishing and hulling, the waste in manufacturing rice bran and other products. The most important of these products in the present connection is the polished rice as it is found in commerce, ready for preparation as food. Rice is a cereal in which the starchy matters predominate, and in which there is a marked deficiency of proteids and

oils as compared with other standard cereals. The composition of rice, as determined by the analysis of samples exhibited at the World's Columbian Exposition, and by standard authorities, is best shown in the table of maxima, minima, and means, as in the case of the other cereals which have been mentioned. In the following table the items marked I, II, and III, represent data obtained at the World's Columbian Exposition, while the means of all the samples there analyzed are given in another part of the table.

Table of Maxima, Minima, and Means of Constituents of Rice

Kinds and Nos. of samples	Wt. of 100 kernels	Moisture	Proteids	Ether extract	Crude fiber	Ash	Carbohydrates, excluding fiber
	Grams	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1. Rice in the hull (foreign):							
Maxima	a3.250	b11.52	b8.40	b2.04	b11.47	a4.66	a65.70
Minima	b2.842	a9.03	a8.23	a1.44	b9.45	b3.26	a65.01
Means	2.979	9.88	8.32	1.71	10.62	4.12	65.35
2. Unpolished rice (foreign):							
Maxima	c2.826	c12.57	cI0.50	c2.26	c1.00	c1.22	c77.34
Minima	c2.260	c10.92	c7.27	c1.62	c0.87	c1.04	c73.35
Means	2.466	11.88	8.02	1.96	0.93	1.15	76.05
3. Polished rice (foreign):							
Maxima	b2.633	b13.15	b10.33	c0.54	a0.56	a0.65	c81.66
Minima	a1.560	c11.82	c5.42	c0.04	a0.27	a0.28	b75.62
Means	2.132	12.34	7.18	0.26	0.40	0.46	79.36
Mean composition of polished rice, etc., as given by Jenkins and Winton.							
Polished rice (10 analyses)	.....	12.40	7.40	0.40	0.20	0.40	79.20
Rice bran (5 analyses)	.....	9.70	12.10	10.90	9.50	10.00	49.90
Rice hulls (3 analyses)	.....	8.20	3.60	0.70	35.70	13.20	38.60
Rice polished (4 analyses)	.....	10.00	11.70	7.30	6.30	6.70	58.00

a Guatemala. b Johore. c Japan.

Table of Maxima, Minima, and Means of Constituents of Rice.—*Continued*

Kinds and Nos. of samples	Wt. of 100 kernels	Moisture	Proteids	Ether extract	Crude fiber	Ash	Carbohydrates, exclusive fiber
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Mean composition of rice, etc., as given by Konig.							
Unhulled rice (3 analyses)	.....	11.99	6.48	1.65	6.48	3.33	70.07
Hulled rice (41 analyses)	.....	12.58	6.73	1.88	1.53	0.82	76.46
Polished rice (9 analyses)	.....	12.52	7.52	0.84	0.48	0.64	78.00
Means of World Fair samples.							
Unhulled rice (4 analyses)	2.929	10.28	7.95	1.65	10.42	4.09	65.60
Unpolished rice (6 analyses)	2.466	11.88	8.02	1.96	0.93	1.15	76.05
Polished rice (14 analyses)	2.132	12.34	7.18	0.26	0.40	0.46	79.36

The mean composition of the different classes of rice as shown by the analyses of the World's Fair samples is almost the same as that shown by the work of other analysts collated as indicated above. A typical unhulled rice has about the following composition:

Weight of 100 kernels, grams	3.00	Crude fiber, per cent	9.00
Moisture, per cent	10.50	Ash, per cent	4.00
Proteids, per cent	7.50	Carbohydrates, other than crude fiber, per cent	67.40
Ether extract, per cent	1.60		

A typical hulled rice, but unpolished, has about the following compositions:

Weight of 100 kernels, grams	2.50	Crude fiber, per cent	1.00
Moisture, per cent	12.00	Ash, per cent	1.00
Proteids, per cent	8.00	Carbohydrates, other than crude fiber, per cent	76.00
Ether extract, per cent	2.00		

A typical polished rice has a composition represented by the following numbers:

Weight of 100 kernels, grams	2.20	Crude fiber, per cent	0.40
Moisture, per cent	12.40	Ash, per cent	0.50
Proteids, per cent	7.50	Carbohydrates, other than crude fiber, per cent	78.80
Ether extract, per cent	0.40		

SULLA MATURAZIONE DEL FORMAGGIO PECORINO

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Il pecorino è un formaggio grasso costituito di sostanze azotate e grasse; si ottiene dal latte di pecora, che ne può dare fino al 22 % (fresco) = la cagliata si cuoce e poi si foggia in pani cilindrici che vengono salati e si fanno stagionare almeno per nove mesi.

La sua pasta è bianco-gialliccia, omogenea o con piccoli e scarsi occhi = ha sapore ed odore piccanti, come i latticini pecorini in genere e tanto più pronunziati quanto più il formaggio è stagionato.

Il costituente principale del formaggio è la caseina, la quale, durante il periodo della maturazione, per effetto di speciali fermentazioni, origina materie albuminoidi solubili, ammidi prodotti ammoniacali, altre sostanze di natura ancora non ben definita e forse anche sostanze grasse.

Il formaggio contiene, oltre la caseina, acqua, sostanze grasse, lattosio, sali minerali (NaCl) in proporzioni molto variabili a secondo della provenienza dei modi di fabbricazione e dell'età.

Lo studio delle trasformazioni che il formaggio subisce quando è abbandonato all'azione delle diastasi e dei microrganismi, i quali fanno ad esso subire un cambiamento completo, fornisce uno dei più importanti capitoli della chimica del formaggio.

Questo prodotto durante la maturazione subisce a poco a poco diverse modificazioni. La massa caseosa prende un aspetto untuoso, nello stesso tempo che si sviluppano l'odore e il sapore che caratterizzano il formaggio maturo, nel quale l'analisi indica piccole quantità di ammoniaca, di acidi grassi e di leucina.

In principio, nel formaggio fresco vi sono necessariamente gli elementi del latte = caseina, burro ed anche del lattosio che la premitura non ha completamente eliminato.

Era interessante ricercare ciò che divengono questi principi durante la fermentazione casica. La perdita di peso che la materia subisce in seguito a questa fermentazione, perfettamente

constatata dall'esperienza è dovuta alla distruzione totale o parziale dell'uno o dell'altro di questi principi? Quali sono quelli che resistono, quali quelli che spariscono? La materia grassa è realmente aumentata nell'invecchiamento del formaggio?

In conseguenza di uno studio sul formaggio di Roquefort il Blondeau (1) affermò che nella maturazione del formaggio si formavano-dei principî grassi a spesa della caseina, la qual cosa fu poi confermata da Kemmerick (2) e da Fleischer (3) nonchè da Musso e Menozzi (4), i quali credono appunto che la formazione del grasso nelle stracchino abbia origine indirettamente dalla caseina.

Il Brassier (5) intanto poco dopo del Blondeau, in uno studio sulla trasformazione della caseina nella maturazione dello stesso formaggio Roquefort, criticando fortemente le esperienze del Blondeau, che egli dice "ben lontane d'essere al riparo di ogni critica" afferma che non vi è formazione di grassi a spesè della caseina; e gli studi di Manetti e Musso sul parmigiano confermano questa opinione. Anche le ricerche del Kellner (6) sulla quantità di burro in confronto di quella dell'acido fosforiéo e della calce confermano la stessa cosa, la quale provano pure le determinazioni di E. Schulze ed U. Weidmann (7).

Tali quistioni io ho voluto riesaminare analizzando il formaggio in diverse epoche, cominciando dal momento in cui fu coagulato e pressato.

Ho esaminato il pecorino da un punto di vista chimico, seguendo le trasformazioni che la caseina subisce dal momento della coagulazione fino a quello nel quale il formaggio maturo è messo in commercio per la consumazione.

Si comprende facilmente che la maggiore difficoltà consisteva nel preparare un formaggio fresco di costituzione sufficientemente omogenea ed ecco come ho proceduto:

Del latte di pecora fu lasciato per 24 h. in cantina, indi, separatane la crema, fu fatto agire il presame a 35°. Dopo la presa del

(1) Ann. de Chim. et de phys (4), I. 208.
(2) Pflüger's Archiv. f. d. gesammte Physiologie (1869), 409.
(3) Virchow's Archiv. f. pathol. Anatomie u. phys. (1871), LI. 40.
(4) Le stazioni sperimentali agrarie italiane (1877), VI. 201.
(5) Ann. de Chim. et de phys (4), V, 270; C. (1865), 888.
(6) Landw. Versuchsst (1880), XXV. 39.
(7) Landw. Jahrbücher (1882), XI. 587.

caglio fu favorito lo scolamento del siero mettendo a sgocciolare il siero sopra una tela e sottomettendolo poi ad una forte pressione.

Il formaggio aveva così una consistenza ferma e secca e fu diviso in 15 parti che furono gettate sotto la pressa in apposite forme.

Il n. I esaminato immediatamente, ha dato la composizione del formaggio appena coagulato e pressato, e gli altri, che hanno ricevuto la salatura con sale in polvere uniformemente ripartito nella pasta prima di essere gittata in forma, furono esaminati susseguentemente alla distanza di un mese l'uno dall'altro. Al nono mese esaminando il formaggio n. 10 ho ottenuto risultati uguali a quelli avuti per il n. 9 esaminato nell'ottavo mese, il che mi indica che la maturazione si era completata all'ottavo mese appunto e la prima parte del lavoro è così esaurita.

Resta ora a vedere se questa maturazione che all'ottavo mese sembra completa, sia veramente tale o giunta a questo punto continua molto più lentamente, tanto che l'esame del formaggio non dà sensibili variazioni di composizione nel breve periodo di un mese. Inoltre, dato che il processo continui, esso ha un arresto definitivo o si collega con altro processo che trasforma diversamente i prodotti formati nella maturazione? Mi riservo di rispondere a ciò con altra nota poichè ho in corso esperienze in proposito.

Ho creduto opportuno esaminare, con le norme ordinarie, il latte che è servito alla preparazione del formaggio nonchè il prodotto secondario della preparazione stessa, il siero. Riporto quindi qui appresso i risultati ottenuti rispettivamente dall'uno e dall'altro esame.

COMPOSIZIONE CENTESIMALE DEL LATTE

Acqua	gr:	81.2817
Grasso	"	6.8107
Caseina	"	5.2716
Lattalbumina	"	1.0433
Lattosio	"	4.7106
Ceneri	"	0.8220
	"	99.9399

COMPOSIZIONE CENTESIMALE DEL SIERRO

Acqua	gr:	89.2449
Grasso	"	2.9703
Sost. proteiche	"	1.7645
Lattosio	"	4.1619
Ceneri	"	0.1670
Acidità (espressa in acido lattico)	"	0.0359
	"	98.9945

ESAME DEL FORMAGGIO N. I.

(fresco = appena coagulato e pressato)

CARATTERI FISICI = Massa bianca, secca e fragile, senza odore nè sapore sensibili. Messo su carta bibula non lascia traccia di corpo grasso.

La carta di tornasole indica reazione leggermente acida.

DETERMINAZIONE DI ACQUA = La determinazione dell'acqua fu fatta pesando esattamente una certa quantità di formaggio e seccandola prima nel vuoto su H_2SO_4 e poi in stufa a 110° fino a peso costante. Poichè il pecorino ha una pasta abbastanza dura, ho creduto conveniente operare la determinazione direttamente su di esso, senza aggiunta di sabbia, come generalmente si usa fare con altri formaggi.

Per quanto, sia nel vuoto su H_2SO_4, che in stufa a 110°, vadano via altre sostanze volatili, come NH_3, ed altri prodotti di decomposizione eventualmente presenti, pure la determinazione si può ritenere esatta, perchè questi sono in tale piccola quantità che si possono trascurare.

I risultati finali delle determinazioni sono riuniti nel seguente quadro:

Prove	H_2O %	
I	gr:	40.7518
II	"	41.4391
III	"	41.0574
Media	"	41.0828

DETERMINAZIONE DELLE SOSTANZE GRASSE E DEGLI ACIDI GRASSI = Questa determinazione fu fatta estraendo con etere puro ed anidro il formaggio ridotto in piccoli pezzi e mantenuto in sacchetto di carta in apparecchio Soxlet.

Poichè l'etere estrae dal formaggio, insieme al grasso, l'acido lattico e gli acidi grassi eventualmente presenti, così l'estratto etereo, prima seccato e pesato, fu poi ripreso con etere, neutralizzando l'acidità con soluzione di carbonato sodico.—In tal modo i gliceridi restano nella soluzione eterea, mentre passano in quella acquosa, i saponi alcalini degli acidi grassi e l'acido lattico allo stato di lattato.

Separata la soluzione eterea, e ripetutamente lavata con acqua, fu determinato esattamente il contenuto in grasso neutro, distillando l'etere dalla soluzione eterea e pesando il residuo dopo averlo seccato a 110°.

Per differenza dall'estratto etereo si ha la somma degli acidi grassi liberi e dell'acido lattico (formati nella maturazione), dalla quale somma togliendo la quantità di acido lattico, che si determina a parte, si ha anche il contenuto in acidi grassi.

RISULTADO DELLE DETERMINAZIONI

Prove	Nel prodotto naturale			Grasso neutro per 100 nel prodotto seccato a 110°
	Estratto etereo %	Grasso neutro %	Ac. latt. acidi grassi %	
I	2.7391	1.8583	0.8808	3.1540
II	2.7902	1.8824	0.9078	3.1949
III	2.7454	1.8750	0.8704	3.1824
Media	2.7582	1.8719	0.8863	3.1771

La materia grassa, ottenuta per evaporazione dell'etere, aveva la più grande analogia col burro per il suo sapore e per la temperatura di fusione che era 28°—Credetti opportuno trattarla con soluzione alcoolica di KOH bollente per esaminare i prodotti

della sua saponificazione: ottenni, dopo saturazione dell'alcale con HCl diluito, delle laminette cristalline che, disseccate in carta bibula, abbandonarono a questa una certa quantità di sostanza oleosa, restando una materia che io potetti far cristallizzare sciogliendola in alcool. Le laminstte brillanti, micacee, che così ottenni, ricordavano le lamine dell'acido margarico. La sostanza oleosa, della quale s'era impregnata la carta non poteva essere altro che acido oleico; ma la quantità di materia sulla quale operavo, era troppo piccola per permettermi d'acquistare una nozione dompleta sulla natura della materia che presenta tutti i caratteri del burro. Era importante constatare che la quantità di grasso che si trova nel formaggio appena preparato non oltrepassa il 2 %, e che esso non può essere che del burro meccanicamente, trasportato mella preparazione.

DETERMINAZIONE DELL'ACIDITA RIFERITA IN ACIDO LATTICO = La determinazione fu fatta nel prodotto naturale adoperando circa gr: 10 di campione per ogni prova. 1 gr: 10 di sostanza venivano scaldati con acqua a più riprese, decantando ogni volta il liquido: i liquidi riuniti e filtrati furon portati a 200 cc., e sopra 100 cc. (circa gr: 5 di sostanza) fu titolata l'acidita con soluzione N—10 di KOH, indicatore il tornasole

I risultati sono riferiti nell'unito quadro:

Prove	Nel prodotto naturale			Acidita riferita ad acido lattico % nel prodotto seccato a 110°
	Estratto etereo %	Grasso neutro %	Ac. latt. ac. gras %	
I	0.7930	.....	.....	1.3459
II	0.9106	.....	.....	1.5455
Media	0.8518	0.8863	0.0345	1.4457

DETERMINAZIONE DEL LATTOSIO = Questa determinazione non ha grande importanza e non si esegue che nel formaggio freschissimo. Poichè è difficile estrarre completamente il lattosio con acqua leggermente scaldata, a circa gr: 50 di formaggio furono

aggiunti cc. 60 di una soluzione di NaOH diluitissima, leggermente scaldata, e in adatto recipiente fu continuato il mite riscaldamento per qualche ora.

Freddata la massa grassa fu forata e se ne separò la soluzione acquosa, ripetendo a più riprese il trattamento.

I liquidi acquosi separati si acidificano con acido citrico per precipitare la caseina e si filtrano. Il filtrato si porta a volume noto e su una parte aliquota di esso si procede direttamente al dosamento del lattosio col liquido di Fehling.

Riporto nel seguente quadro i risultati ottenuti:

Prove	Lattosio %	
	Nel prodotto naturale	Nel prodotto seccato a 110
I	2.1913	3.7192
II	2.2100	3.7510
III	2.1752	3.6919
Media	2.1921	3.7207

DETERMINAZIONE DELL'AZOTO TOTALE = L'azoto totale fu determinato col metodo Kyeldahl adoperando come ossidante, insieme all'H_2SO_4 conc., un miscuglio di p.1 di ossido di mercurio giallo, p.1 di solfato di rame e p.8 di solfato potassico. Distrutta la materia organica, furon precipitati allo stato di solfuro il mercurio ed il rame, prima della distillazione con ossido di magnesio, mediante apposita soluzione di solfuro sodico. Anche questa determinazione fu fatta sul prodotto naturale con i seguenti risultati:

Prove	Nel prodotto naturale		Nel prodotto seccato a 110°	
	N totale %	Sostanze azotate %	N totale %	Sostanze azotate %
I	8.5198	53.2487	14.2909	89.3181
II	8.4913	53.0706	14.4122	90.0762
III	8.5027	53.1418	14.4316	90.1975
Media	8.5046	53.1537	14.3782	89.8639

DETERMINAZIONE DELLE CENERI = La determinazione fu fatta sul prodotto naturale. Riporto i risultati nel seguente quadro:

Prove	Ceneri %	
	Nel prodotto natur.	Nel prodotto seccato a 110°
I	0.7618	1.2930
II	0.7392	1.2546
Media	0.7505	1.2738

Il pecorino dunque, appena coagulato e pressato ha la seguente composizione centesimale media:

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	41.0828	.....
Sostanze grasse	1.8719	3.1771
Acido lattico	0.8518	1.4457
Altri acidi grassi	0.0345	0.0585
Lattosio	2.1921	3.7207
Sostanze azotate	53.1537	89.8639
Ceneri	0.7505	1.2738
	99.9372	99.5397

Le varie determinazioni sui diversi formaggi, corrispondenti ai n. 2, 3, 4, 5, 6, 7, 8, 9 e 10 furon fatte (due o tre prove per ogni determinazione) seguendo i metodi precedentemente descritti per il n. 1, aggiungendo l'esame delle sostanze azotate, nonchè quello sulla natura del grasso formato dopo che il formaggio (n. 6) aveva acquistato tutti i caratteri esteriori che doveva comunicare ad esso la stagionatura—

Come si sa, le sostanze azotate del formaggio sono costituite da sostanze proteiche solubili ed insolubili, dai loro prodotti di decomposizione, da saponi e sali ammoniacali.

Questi diversi prodotti sono stati valutati nel modo seguente:

Ho pesato esattamente circa gr: 20 di formaggio, e, dopo averli pestati in un mortaio, vi ho aggiunto acqua a poco a poco, fino ad avere un volume uguale a circa il doppio di quello del campione—Ottenuta così una pasta omogenea, ho lasciato riposare per le ½ h. perchè si fossero imbevute bene di acqua tutte le particelle solide, indi ho aggiunto ancora dell'acqua agitando—Separata così la materia grassa, seguitai ad aggiungere acqua e versai il tutto in palloncino tarato da 250 cc. fino a raggiungere il volume = Agitai e lasciai riposare per 15 h. Chiarificatosi il liquido fu filtrato, raccogliendone cc. 200.

Su cc. 25 di questo liquido fu fatta la determinazione di azoto col solito metodo di Kyeldahl = Quest'azoto costituisce quello dell'estratto acquoso, cioè quello delle sostanze proteiche solubili e dei prodotti di decomposizione (basi amidiche e ammoniaca), che son pure solubili. Per differenza dall'azoto totale si ha quello delle sostanze proteiche insolubili.

Questi due dati hanno una grande importanza in quanto danno il peso ed il corrispondente valore in azoto della sostanza organica resa solubile dai processi fermentativi, ed il loro rapporto dà il *coefficiente di maturazione*—

Le sostanze proteiche sono state separate dai prodotti di decomposizione (basi ammidiche) impiegando l'acido fosfo-wolframico. La presenza di quest'acido non impedisce la determinazione di azoto col metodo Kyeldahl nel precipitato e nel liquido filtrato.

Cc. 50 del liquido precedentemente preparato furono portati a cc. 70–80 aggiungendo cc. 15 di HCl al 20 % e precipitando con fosfo-wolframato sodico. Raccolto sul filtro e lavato il precipitato con soluzione d'H_2SO_4 (5 %), poi con alcool assoluto, fu seccato, determinando l'azoto sul prodotto secco. Dalla quantità d'azoto trovata fu tolta quella corrispondente all'ammoniaca contenuta nella soluzione acquosa del formaggio, la quale è pure precipitata dall'acido fosfo-wolframico, e la differenza rappresentava quindi *l'azoto delle sostanze proteiche solubili nell'acqua*—

L'ammoniaca si determina a parte, su altri cc. 50 della soluzione primitiva, spostandola, all'ebollizione, con ossido di magnesio.

Il rapporto fra l'azoto delle sostanze proteiche dell'estratto acquoso a freddo e quello totale del formaggio rappresenta il *coefficiente di solubilizzazione*, cioè l'azione dei fermenti diastasici.—

La differenza fra l'azoto totale dell'estratto acquoso e quello delle sostanze proteiche precipitate con l'acido fosfo-wolframico costituisce *l'azoto dei prodotti di decomposizione.*

Il rapporto fra l'azoto non proteico dell'estratto acquoso e quello totale del formaggio rappresenta il *coefficiente di decomposizione*, cicè l'azione diretta dei microrganismi.

L'*azoto ammidico* è quello che risulta dall'azoto totale dell estratto acquoso detratto della somma dell'azoto delle sostanze proteiche solubili e dell'azoto ammoniacale.

Le basi ammidiche, provenienti dalla decomposizione della caseina, sono essenzialmente costituite di leucina e contengono solo in piccolissima quantità tirosina, butilammina, amilammina, ecc. fino ad arrivare all'ammoniaca. Per questo nel fare il calcolo delle basi ammidiche dall'azoto trovato, io ho potuto considerar questo come proveniente integralmente dalla leucina, senza tema di grave errore.

Sperimentalmente infatti ho potuto osservare il seguente fatto:

Il residuo del trattamento con etere per l'estrazione delle sostanze grasse e degli acidi grassi, ripreso con alcol a 36°, mi fornì, dopo evaporazione del solvente, dei cristalli madraperlacei di leucina, misti a piccolissime quantità di altri corpi colorati in giallo, che non ho esaminato sia per la scarsezza del materiale, sia perchè, allo scopo del lavoro, la loro conoscenza non importava gran che, potendosi dedurre approssimativamente la loro natura da quanto finora si sa sulla maturazione.

Ciò che resta del formaggio, dopo l'estrazione con etere e relativos eccamento è costituito da tutte le sostanze azotate organiche; ammoniaca, lattosio e sali minerali, naturali ed aggiunti con la salatura. Il residuo così costituito cede all'alcool a 36°, quando venga trattato con questo solvente, tutti i suoi costituenti ad eccezione della caseina: conoscendo quindi il % di azoto totale, nonchè quello ammoniacale, ed i % di lattosio e di sali minerali naturali ed aggiunti nel formaggio, ho determinato l'azoto totale dell'estratto alcoolco (36°), e sottraendo da esso l'azoto ammoniacale, determinato a parte, ho potuto conoscere

l'azoto ammidico, il quale corrisponde quasi esattamente a quello contenuto nella quantità trovata di leucina.

ESAME DEL FORMAGGIO N. 2

(fresco di un mese che ha subito la salatura)

CARATTERI FISICI = Il formaggio che ha subito la salatura, coagulato e pressato da un mese, ha completamente cambiato d'aspetto. Esso ha già preso l'aspetto d'un corpo grasso e macchia la carta bibula sulla quale è deposto. Il suo sapore comincia ad esser dolce e piacevole, il suo odore appena sensibile.

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	37.5080	.....
Sostanze grasse	7.9349	12.7398
Acido lattico	0.8649	1.3887
Altri acidi grassi	0.7899	1.2683
Lattosio	2.1949	3.5177
Sostanze azotate	44.5937	71.5966
Ceneri e cloruro sodico	5.8099	9.3280
	99.6962	99.8391

ESME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.2529	1.5806	0.4060	2.5375
	Insolubili	6.5037	40.6481	10.4418	65.2612
Non proteiche sol	Ammoniacali	0.1436	0.1743	0.2305	0.3198
	Ammidiche Leucina	0.2348	2.970	0.3769	3.5266
Totali		7.1350	44.6000	11.4552	71.6451

I diversi coefficienti di solubilizzazione, decomposizione e maturazione, calcolati da questi dati, come è precedentemente detto, sono i seguenti:

COEFFICIENTE DI SOLUBILIZZAZIONE (dovuta all'azione delle disastasi)	0.0354
COEFFICIENTE DI DECOMPOSIZIONE (dovuta alla azione dei microrganismi)	0.0530
COEFFICIENTE DI MATURAZIONE	0.0970

Le sostanze azotate di questo formaggio hanno, secondo i dati analitici, la seguente composizione centesimale:

Caseina solubile	gr	3.5439
" insolubile	"	91.2289
Ammoniaca	"	0.3908
Basi ammidiche (leucina)	"	4.9260
	"	100.0896

Da questi risultati pare dimostrato che il soggiorno all'aria (per la maturazione) del pecorino ha per effetto di aumentare la quantità di materia grassa, che esso contiene in proporzione abbastanza considerevole; ma prima di esaminare la natura del grasso formato in queste condizioni, trovo conveniente attendere che il formaggio abbia acquistato tutte le qualità che un soggiorno più prolungato all'aria deve comunicargli.

La quantità di caseina invece, che prima era tutta insolubile, è considerevolmente diminuita, e di essa una parte, per azione delle diastasi, si è resa solubile, porzione della quale, per azione dei microrganismi, si è decomposta in basi ammidiche fino ad arrivare all'ammoniaca.

ESAME DEL FORMAGGIO N. 3

(fresco di due mesi che ha subito la salatura)

CARATTERI FISICI = Questo formaggio ha l'aspetto di un corpo grasso come il precedente: ma il suo sapore è più dolce e piacevole,

il suo odore più sensibile, proprietà che vanno sempre più accentuandosi nei formaggi corrispondenti ai successivi numeri 4, 5, 6, 7, 8, 9 e 10.

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	34.1019	.....
Sostanze grasse	13.9999	21.4644
Acido lattico	0.8799	1.3491
Altri acidi grassi	1.5399	2.3610
Lattosio	2.1949	3.3653
Sostanze azotate	40.3287	61.8284
Ceneri e cloruro sodico	6.2819	9.6314
	99.3271	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.4888	3.0550	0.7494	4.6837
	Insolub.	5.2244	32.6525	8.0099	50.0618
Non prot. sol.	Ammoniacali	0.2799	0.3399	0.4291	0.5210
	Ammidiche (Leucina)	0.4595	4.2340	0.7044	6.5910
Totali		6.4526	40.2814	9.8928	61.8575

COEFFICIENTI =

di solubilizzazione	0.0757
di decomposizione	0.1145
di maturazione	0.2351

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	7.5841
" insolubile	"	81.0609
Ammoniaca	"	0.8438
Basi ammidiche (leucina)	"	10.5110
		99.9998

ESAME DEL FORMAGGIO N. 4

(salato di tre mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	34.0178	.....
Sostanze grasse	21.4021	32.6375
Acido lattico	1.9968	3.0451
Altri acidi grassi	1.9331	2.9480
Lattosio	1.1198	1.7077
Sostanze azotate	33.0625	50.1889
Ceneri e cloruro sodico	6.2115	9.4724
	99.7136	99.9996

ESAME SOSTANZE AZOTATE

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostazne azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	0.8823	5.5143	1.3393	8.3706
	Insolubili	3.4827	21.7668	5.2868	33.0425
Non prot. sol.	Ammoniacali	0.3530	0.4286	0.5358	0.6506
	Ammidiche (leucina)	0.5720	5.3522	0.8683	8.1246
Totali		5.2900	33.0619	8.0902	50.1883

COEFFICIENTI

di solubilizzazione	0.1667
di decomposizione	0.1748
di maturazione	0.3415

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	16.7089
" insolubile	"	65.8365
Ammoniaca	"	1.2963
Basi ammidiche (leucina)	"	16.1884
	"	100.0301

ESAME DEL FORMAGGIO N. 5

(salato di quattro mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9765	.....
Sostanze grasse	25.1742	38.1293
Acido lattico	2.5612	3.8793
Altri acidi grassi	2.1375	3.2375
Lattosio	0.5882	0.8909
Sostanze azotate	29.3750	44.3583
Ceneri e cloruro sodico	6.2750	9.5043
	100.1276	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0180	6.3625	1.5372	9.6075
	Insolub.	2.6925	16.8281	4.0658	25.4112
Non prot. sol.	Ammoniacali	0.3897	0.4732	0.5884	0.7144
	Ammidiche (leucina)	0.5998	5.6187	0.9057	8.4746
Totali		4.7000	29.2825	7.0971	44.2077

COEFFICIENTI =

di solubilizzazione	0.2166
di decomposizione	0.2105
di maturazione	0.7455

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	21.7279
" insolubile	"	57.4681
Ammoniaca	"	1.6159
Basi ammidiche (leucina)	"	19.1879
		99.9998

ESAME DEL FORMAGGIO N. 6

(salato di cinque mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9551	.....
Sostanze grasse	27.2007	41.1852
Acido lattico	2.8578	4.3272
Altri acidi grassi	2.2502	3.4071
Lattosio	0.3238	0.4904
Sostanze azotate	27.0625	41.0895
Ceneri e cloruro sodico	6.2744	9.5003
	99.9245	99.9997

ESAME SOSTANZE AZOTATE

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0165	6.3531	1.5434	9.6462
	Insolub.	2.2456	14.0350	3.4096	21.3100
Non prot. sol.	Ammoniacali	0.4079	0.4953	0.6193	0.7520
	Ammidiche (leucina)	0.6600	6.1756	1.0021	9.3766
Totali		4.3300	27.0590	6.5744	41.0848

COEFFICIENTI =

di solubilizzazione	0.2347
di decomposizione	0.2466
di maturazione	0.9282

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	23.4786
" insolubile	"	51.8681
Ammoniaca	"	1.8304
Basi ammidiche (leucina)	"	22.8227
		99.9998

ESAME DEL FORMAGGIO N. 7

(salato di sei mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9884	.....
Sostanze grasse	28.1554	42.6265
Acido lattico	2.9998	4.5417
Altri acidi grassi	2.3025	3.4860
Lattosio	0.1906	0.2886
Sostanze azotate	25.9875	39.4741
Ceneri e cloruro sodico	6.3289	9.5818
	99.9131	99.9987

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 100° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0234	6.3962	1.5545	9.7156
	Insolubili	2.0395	12.7468	3.0979	19.2223
Non prot. sol.	Ammoniaca	0.4172	0.5066	0.6337	0.7695
	Ammidiche (leucina)	0.6779	6.3431	1.0297	9.6349
Totali		4.1580	25.9927	6.3158	39.3423

COEFFICIENTI =

di solubilizzazione	0.2461
di decomposizione	0.2633
di maturazione	1.0386

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	24.6076
" insolubile	"	49.0339
Ammoniaca	"	1.9490
Basi ammidiche (leucina)	"	24.4033
		99.9938

ESAME DEL FORMAGGIO N. 8

(salato di sette mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9432	.....
Sostanze grasse	28.5344	43.1968
Acido lattico	3.0729	4.6520
Altri acidi grassi	2.3287	3.5253
Lattosio	0.1245	0.1885
Sostanze azotate	25.8062	39.2319
Ceneri e cloruro sodico	6.2814	9.5092
	100.0913	100.3037

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina	Solubili	1.0294	6.4337	1.5649	10.0797
	Insolub	1.9948	12.4675	3.0376	18.9537
Non prot. sol.	Ammoniacali	0.4216	0.5119	0.6409	0.7782
	Ammidiche (leucina)	0.6832	6.3927	1.0386	9.7181
Totali		4.1290	25.8058	6.2780	39.5297

COEFFICIENTI =

di solubilizzazione	0.2493
di decomposizione	0.2675
di maturazione	1.0698

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	24.9312
" insolubile	"	48.3127
Ammoniaca	"	1.9836
Basi ammidiche (leucina)	"	24.7723
		99.9998

ESAME DEL FORMAGGIO N. 9

(salato di otto mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9415	.....
Sostanze grasse	29.1974	44.0852
Acido lattico	3.1319	4.7579
Altri acidi grassi	2.3399	3.5547
Lattosio	0.0529	0.0805
Sostanze azotate	25.0000	37.9783
Ceneri e cloruro sodico	6.2820	9.5433
	99.9456	99.9999

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0297	6.4356	1.5642	9.7762
	Insolub.	1.8586	11.6162	2.8234	17.6462
Non prot. sol.	Ammoniacali	0.4249	0.5159	0.6439	0.7818
	Ammidiche (leucina)	0.6868	6.4263	1.0433	9.7621
Totali		4.0000	24.9940	6.0748	37.9663

COEFFICIENTI =

di solubilizzazione	0.2574
di decomposizione	0.2779
di maturazione	1.1521

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	25.7485
" insolubile	"	46.4759
Ammoniaca	"	2.0640
Basi ammidiche (leucina)	"	25.7113
		99.9997

ESAME DEL FORMAGGIO N. 10

(salato di nove mesi)

COMPOSIZIONE CENTESIMALE =

	Nel prodotto naturale	Nel prodotto seccato a 110°
Acqua	33.9421	.....
Sostanze grasse	29.1810	44.1749
Acido lattico	3.1504	4.7693
Altri acidi grassi	2.3595	3.5720
Lattosio	0.0573	0.0868
Sostanze azotate	25.0000	38.0818
Ceneri e cloruro sodico	6.1531	9.3148
	99.8434	99.9996

ESAME SOSTANZE AZOTATE =

		Nel prodotto naturale %		Nel prodotto seccato a 110° %	
		Azoto	Sostanze azotate	Azoto	Sostanze azotate
Proteiche (caseina)	Solubili	1.0346	6.4662	1.5759	9.8493
	Insolub.	1.8401	11.5006	2.8029	17.5181
Non prot. sol.	Ammoniacali	0.4264	0.5177	0.6495	0.7885
	Ammidiche (leucina)	0.6989	6.5396	1.0646	9.9614
Totali		4.0000	25.0929	6.0929	38.1174

COEFFICIENTI =

di solubilizzazione	0.2586
di decomposizione	0.2813
di maturazione	1.1737

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE =

Caseina solubile	gr.	25.8398
" insolubile	"	45.9580
Ammoniaca	"	2.0688
Basi ammidiche (leucina)	"	26.1332
		99.9998

Riassumo nei seguenti quadri i risultati analitici di tutti i formaggi esaminati sostituendo alle sostanze azotate totali, calcolate dall'azoto trovato, quelle risultanti dall'esame di esse.

COMPOSIZIONE CENTESIMALE DEL PRODOTTO NATURALE=

Numeri corrispondenti ai campioni	1	2	3	4	5	6	7	8	9	10
Acqua	41.0828	37.5080	34.1019	34.0178	33 9765	33.9551	33.9484	33.9432	33.9415	33 9421
Sostanze grasse neutre	1.8719	7.0349	13.9999	21.4021	25 1742	27.2007	28.1554	28.5344	29.1074	29.1810
Acido lattico	0.8518	0.8649	0.8799	1.9068	2 5012	2.8578	2.9098	3.0720	3.1319	3.1504
Altri acidi grassi	0.0345	0.7899	1.5399	1 9331	2.1375	2.2502	2.3025	2.3287	2.3399	2.3595
Lattosio	2.1921	2.1949	2.1949	1.1198	0.5882	0.3238	0.1906	0.1245	0.0529	0.0573
Sost. azotate — Cascina solubile	.....	1.5806	3.0550	5.5143	6 3625	6.3531	6.3962	6.4337	6.4356	6.4602
Sost. azotate — " insolubile	.....	40.6481	32.0525	21.7068	16.8281	14.0350	12.7408	12.4075	11.6102	11.5000
Sost. azotate — Ammoniaca	53.1537	0.1743	0.3399	0.4286	0 4732	0.4953	0.5066	0.5119	0.5159	0.5177
Sost. azotate — Basi ammidiche (leucina)	.....	2.1970	4.2340	5.3522	5.6187	6.1756	6.3431	6.3927	6.4263	6.5396
Sost. minerali (Ceneri e NaCl)	0.7505	5.8099	6.2819	6.2115	6.2750	6.2744	6.3289	6.2814	6.2820	6.1531
	99.9383	99.7025	99.2798	99.7430	99 9951	99.9210	99.9183	100.0909	99.9396	99.8675

COEFFICIENTI

Di solubilizzazione	.....	0.0354	0.05757	0.4667	0.2166	0.2347	0.2461	0.2493	0.2574	0.2585
Di decomposizione	.....	0.0530	0.1145	0.1748	0.2105	0.2466	0.2633	0.2675	0.2779	0.2813
Di maturazione	.....	0.0970	0.2351	0.3415	0.7455	0.9282	1.0386	1.0698	1.1521	1.1737

COMPOSIZIONE CENTESIMALE DEL SECCATOA A 110°=

N. corrispondenti ai campioni	1	2	3	4	5	6	7	8	9	10
Acqua	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Sostanze grasse neutre	3.1771	12.7398	21.4044	32.6375	38 1293	41.1852	42.6265	43.1968	44.0852	44.1749
Acido lattico	1.4457	1.3887	1.3491	3.0451	3.8703	4.3272	4.5417	4.6520	4.7579	4.7693
Altri acidi grassi	0.0585	1.2683	2.3610	2 9480	3.2375	3.4071	3.4860	3.5253	3.5547	3.5720
Lattosio	3.7207	3 5177	3.3653	1.7077	0.8909	0.4904	0.2086	0.1885	0.0805	0.0868
Sost. azotate — Caseina solubile	.....	2.5375	4.6837	8.3706	9.6075	9.6462	9.7156	10.0797	9.7762	9.8493
Sost. azotate — " insolubile	.....	65.2612	50.0618	33.0425	25.4112	21.3100	19.2233	18.9537	17.6462	17.5181
Sost. azotate — Ammoniaca	89.8639	0.3198	0.5210	0.6506	0.7144	0.7520	0.7695	0.7782	0.7818	0.7886
Sost. azotate — Basi ammidiche (leuc.)	.....	3.5266	6.5910	8.1246	8.4746	9,3766	9.6349	9.7181	9.7621	9.9614
Sost. minerali (Ceneri e NaCl)	1.2738	9.3280	9.6314	9.4724	9.5043	9.5003	9.5818	9 5092	9.5433	9.3148
	99.5397	99.8876	100.0287	99.9990	99.8490	99.9950	99.8669	100.0015	99.9879	100.0352

COMPOSIZIONE CENTESIMALE DELLE SOSTANZE AZOTATE=

Caseina solubile	.....	3.5439	7.5841	16.7089	21.7279	23.4786	24.6076	24.9312	25.7485	25.8398
" insolubile	.....	91.2289	81.0609	65.8365	57.4681	51.8681	49.0339	48.3127	46.4759	45.9580
Ammoniaca	.....	0.3908	0.8438	1.2963	1.6159	1.8304	1.9490	1.9836	2.0640	2.0688
Basi ammidiche (leucina)	.....	4 9260	10.5110	16.1884	19.1879	22.8227	24.4033	24.7723	25.7113	26.1332
	.....	100.0896	99.9998	100.0301	99.9998	99.9998	99.9938	99.9998	99.9997	99.9998

Dal confronto delle precedenti analisi si vede chiaramente che nella maturazione la sostanza grassa aumenta, rapidamente nei primi mesi e molto più lentamente in seguito con una percentuale che dal 2 % circa nel formaggio fresco si eleva fino ad oltre il 29 % nel prodotto maturo. In relazione a quest'aumento è la diminuzione, per quanto non proporzionale, delle sostanze azotate, le quali, diminuendo, mutano anche natura, poichè si solubilizzano e si decompongono fino ad arrivare all'ammoniaca, prodotto che viene in parte fissato dagli acidi grassi provenienti dall'ossidazione dell'oleina, costituente, come dimostrerò in seguito, della sostanza grassa contenuta nel formaggio.

In diminuzione, per la stagionatura, è pure il lattosio; ma proporzionalmente in aumento è l'acido lattico, il che mostra la trasformazione del primo nel secondo prodotto.

Osservando poi la composizione centesimale delle sostanze azotate all'inizio ed a fine maturazione si vede come queste, essenzialmente costituite in principio di caseina insolubile, vadano gradualmente trasformandosi, tanto che a fine maturazione la caseina insolubile non costituisce che il 46 % circa delle sostanze azotate totali, mentre il resto risulta trasformato parte in caseina solubile, parte in ammoniaca e basi ammidiche (leucina) con preponderanza di queste ultime.

I coefficienti di solubilizzazione e di decomposizione, in relazione con le trasformazione che man mano subiscono le sostanze azotate, crescono entrambi, ma non proporzionatamente l'uno all'altro, e mentre all'inizio della maturazione il primo ha un valore numerico di poco superiore alla metà del secondo, alla fine diventano quasi uguali mantenendosi sempre però una lievissima prevalenza del secondo sul primo; La loro somma inoltre, che a principio rappresenta quasi il coefficiente di maturazione, nel prodotto stagionato è inferiore alla metà del valore numerico di quest'ultimo coefficiente—

Da quest'osservazione si può trarre un'utilissima conclusione circa il giudizio sul grado di maturazione di un formaggio, e cioè UN PRODOTTO E'TANTO PIU'MATURO QUANTO PIU'VICINI FRA LORO SONO I VALORI DEI COEFFICIENTI DI SOLUBILIZZAZIONE E DI DECOMPOSIZIONE (il primo deve sempre essere inferio-

re al secondo) E QUANTO PIU'LA LORO SOMMA E'INFERIORE ALLA META'DEL COEFFICIENTE DI MATURAZIONE.

Dietro i suddetti risultati dunque non si può avere più dubbio: della materia grassa si è formata a spesa della caseina, durante la maturazione.

Prima di discutere il modo come, io credo, questa materia abbia potuto formarsi ho cercato di precisarne bene la natura.

Il formaggio n. 6 (salato di cinque mesi) possedeva tutte le qualità che esso era suscettibile di acquistare. Untuoso al gusto, presentava, tagliato col coltello, un aspetto grasso. Esso non si divideva più in frammenti e macchiava la carta sulla quale si deponeva. Possedeva inoltre un debole odore non notato nei formaggi precedenti, e credetti quindi opportuno fare l'esame del grasso in esso contenuto.

Per preparare la materia grassa da servire per l'esame chimico ho adottato il metodo del Laboratorio Chimico di Washington, che qui appresso descrivo:

Gr. 300 di formaggio, ridotti in frammenti della grandezza di un pisello, si trattano con 700 cc. di potassa (50 %) a 20° in una bottiglia a bocca larga, promuovendo la dissoluzione della caseina con una forte agitazione.

In dieci minuti la caseina è sciolta, ed il grasso viene alla superficie in piccole masse. Scuotendo il recipiente si fa in modo che il grasso si raduni in una massa sola, e con l'aggiunta, nel recipient di acqua fresca, il grasso raggiunge il collo del vaso, dal quale si può togliere con un cucchiaio. In tale operazione esso non viene attaccato, e, lavato con molta acqua per asportare tutto il residuo non grasso che vi si può trovare, in poco tempo è completamente separato, e si può preparare, filtrandolo ed asciugandolo, come è prescritto per l'esame delle materie grasse—

Questo grasso, che si trovava presente nel formaggio, nella proporzione del 27, 2007 %, sciolto in alcool bollente, ha abbandonato, per raffreddamento, dei piccoli cristalli di una sostanza bianca, che, purificata per diversi trattamenti con alcool, ha fornito dei cristalli di aspetto madraperlaceo, costituiti di margarina pura: fondono infatti a 41°, e sottoposti all'azione di una temperatura elevata si decompongono dando luogo ad acroleina.

Per la sua caratterizzazione ho eseguito sulla sostanza le determinazioni seguenti coi risultatti che qui sotto trascrivo:

Indice di saponificazione	198.0
Indice di acidità	0.0
Numero degli eteri	198.0
" di jodio	0.0
" degli acidi volatili	0.0
" degli acidi fissi	95.51

Questi caratteri sono abbastanza precisi per provare che la sostanza grassa, formata a spese della caseina, contiene della margarina; ma per maggiore sicurezza, ed a conferma di ciò, ho sottoposto ad analisi la sostanza che ero riuscito ad isolare, nonchè l'acido grasso proveniente dalla sua saponificazione.

ANALISI DELLA SOSTANZA GRASSA FUSIBILE A 41°

I. Gr. O. 2962 di sostanza danno gr. O. 8284 di CO_2 e gr. O. 3269 di H_2O.

II. Gr. O. 3062 di sostanza danno gr. O. 8562 di CO_2 e gr. O. 3387 di H_2O.

E calcolando il %:

Trovato		Calcolato per $C_{54}H_{104}O_6$
I	II	
C=76.26	76.25	76.41
H=12.26	12.28	12.26
O= ...	.....	11.33
		100.00

Per ottenere l'acido grasso corrispondente ho saponificato con KOH ed ho poi decomposta l'emulsione saponosa con H_2SO_4 diluito. L'acido grasso, così messo in libertà, si riunisce alla superficie del liquido e comincia ad ammassarsi verso 40°.— Questa sostanza, sciolta in alcool bollente, dà, per raffreddamento, delle pagliette madraperlacee, che fondono a 58°—60°.

ANALISI DELL'ACIDO FUSIBILE A 59°

I. Gr. 0.3292 di sostanza danno gr. 0.9100 di CO_2 e gr. 0.3736 di H_2O.

II. Gr. 0.1905 di sostanza danno gr. 0.5270 di CO_2 e gr. 0.2159 di H_2O.

Calcolando il %:

Trovato		Calcolato per $C_{17}H_{34}O_2$
I	II	
C=75.38	75.43	75.55
H=12.60	12.58	12.59
O= ...	.....	11.86
		100.00

La sostanza grassa contenuta nel pecorino, dietro questi risultati, non è che margarina, accompagnata da un altro corpo grasso, maggiormente solubile in alcool, che si ritrova nel solvente dopo che si è deposta, per raffreddamento, la margarina.

L'alcool evaporato a h.m. ha lasciato, come residuo, un olio leggermente giallastro, di sapore dolce e untuoso, liquido alla temperatura ordinaria. Quest'olio, scaldato, si decompone a 260° dando luogo a vapori di acroleina.

Su di esso ho eseguito, come sul precedente grasso solido, le seguenti determinazioni coi risultati che trascrivo:

Indice di saponificazione	190.0
" di acidità	0.0
Numero degli eteri	190.0
" di jodio	83.93
" degli acidi volatili	169.68
" " " fissi	0.0

Sottoposto ad analisi tha dato i seguenti risultati:

I. Gr. 0.2341 di sostanza danno gr. 0.6621 di CO_2 e gr. 0.2453 di H_2O.

II. Gr. 0.2785 di sostanza danno gr. 0.7901 di CO_2 e gr. 0.2914 di H_2O.

III. Gr. 0.1975 di sostanza danno gr. 0.5602 di CO_2 e gr. 0.2074 di H_2O.

Calcolando il %:

Trovato			Calcolato per $C_{57}H_{104}O_6$
I	II	III	
C=77.12	77.36	77.35	77.37
H=11.64	11.62	11.66	11.76
O= ...	.....	.....	10.87
			100.00

Quantunque la proprietà e l'analisi dimostrino che la sostanza liquida che accompagna la margarina non è che oleina, ho voluto saponificarla, e fare l'analisi dell'acido grasso proveniente da questa saponificazione.

L'olio ottenuto dall'evaporazione dell'alcool, dal quale si era separata la margarina, è stato saponificato con soda e l'emulsione decomposta con H_2SO_4 diluito—Si è così ottenuta una sostanza bianca leggermente giallastra, di sapore dolce e untuoso, che resta liquida fino a—10°.

Sottoposta ad analisi ha dato i seguenti risultati:

I. Gr. 0.2202 di sostanza danno gr. 0.6170 di CO_2 e gr. 0.2405 di H_2O—

II. Gr. 0.2005 di sostanza danno gr. 0.5620 di CO_2 e gr. 0.2182 di H_2O.

Calcolando il %:

Trovato		Calcolato per $C_{18}H_{34}O_2$
I	II	
C=76.41	76.43	76.59
H=12.11	12.08	12.05
O= ...	.....	11.36
		100.00

Questi risultati dimostrano che la sostanza grassa contenuta nel pecorino è un miscuglio di margarina ed oleina con prevalenza della prima, giacchè dei gr. 27, 2007 di grasso contenuto in gr. 100 di formaggio, gr. 15, 4337 sono di margarina e gr—11,7570 di oleina.

Dalle analisi precedenti si deduce che la caseina, nella maturazione, si è in parte trasformata in materia grassa, e ciò che è evidente è la singolare coincidenza che la sostanza grassa del burro è formata egualmente di margarina ed oleina presso a poco nelle stesse proporzioni nelle quali queste due sostanze costituiscono il grasso del formaggio, il che fa pensare che la materia grassa del burro si sia formata, nell'economia, a spese della caseina per reazioni analoghe a quelle che nel formaggio han dato luogo alla sostanza grassa che ivi si trova.

I formaggi n. 7, 8, 9 e 10, specie questi ultimi—(9 e 10), conservati oltre il quinto mese, mostrano nei loro caratteri esteriori delle profonde modificazioni. Il colore non tarda molto ad alterarsi, passa dal bianco al bruno, ed il formaggio prende un odore sempre più forte. Cambia anche il suo sapore e finisce per acquistare un gusto forte e piccante.

Importava conoscere le cause di queste modificazioni, ed ho voluto quindi esaminare anche il grasso del n.10, come avevo fatto per il n.6, poichè avevo notato che dopo il quinto mese la produzione di esso non aumentava più sensibilmente, come nei primi cinque mesi, pur rendendosi la percentuale delle sostanze azotate sempre più piccola.

La sostanza grassa nel formaggio n.10 è presente nella proporzione del 29 %. Di essa ho preparato un campione con lo stesso metodo avanti descritto, e l'ho sottoposto allo stesso trattamento. 1 gr. 29 di sostanza grassa, di color giallo-carico, li ho trovati costituiti di gr. 26.657 di margarina e di gr: 2,343 di oleina.

Questi risultati dimostrano che una parte del grasso che entra nella costituzione del pecorino si è decomposta, mentre si è formato del grasso nouvo, ed è sopratutto l'oleina che ha subito una profonda modificazione a contatto dell'aria.

Bisognava quindi andare alla ricerca, separazione e dosamento

di questi prodotti di ossidazione dell'oleina e per far ciò ho operato nel modo seguente:

Ho scaldato gr. 500 di formaggio, ridotto a piccoli pezzi, con circa 5 litri di acqua, a più riprese, decantando ogni volta il liquido=i liquidi riuniti e concentrati a un litro furon fatti freddara e filtrati. Il filtrato aveva preso una tinta giallastra e possedeva inoltre un odore forte ed un sapore piccante che ricordava quello del formaggio. Ho aggiunto al liquido acqua di barite ed ho poi distillato: si è formato un abbondante precipitato con sviluppo contemporaneo di odore ammoniacale. I vapori sviluppati condensati erano alcalini e contenevano dell'ammoniaca, della quale ho constatato la reazione alcalina e l'effetto su una bacchetta di vetro gabnata d'HCl.

L'ammoniaca era stata già determinata nell'esame delle sostanze azotate e non mi son quindi curato di determinarla ancora quì: essa fu trovata in quantità sufficiente per saturare tutti gli acidi grassi presenti, pure a parte complessivamente determinati.

Ho evaporato dunque il liquido a piccolo volume, e durante l'evaporazione si son depositati parte dei sali di bario che ho cercato di separare gli uni dagli altri impiegando il metodo Lerch, profittando cioè della loro differente solubilità in acqua calda—

Quando il liquido fu ridotto ad 1/10 circa del volume primitivo l'ho filtrato per separare i sali di bario depositati durante l'evaporazione, e nel filtrato si son prodotti, per il raffreddamento, degli aghetti riuniti in fasci assai voluminosi del peso di gr: 5,9192; i quali sottomessi ad analisi han dato i seguenti risultati:

I. Grm: 0.4834 di sostanza danno gr. 0.6931 di CO_2 e grm. 0.2663 di H_2O
II. Grm: 0.4396 di sostanza danno gr. 0.6309 di CO_2 e grm. 0.2430 di H_2O
III. Grm: 0.9106 di sostanza fanno gr. 0.5773 di $BaSO_4$ corrispondente a gr. 0.33939 di Ba
IV. Gr: 0.8996 di sostanza danno gr: 0.5708 di $BaSO_4$ corrispondente a gr. 0.33557 di Ba

Calcolando il %:

Trovato				Calcolato per Ba $(C_6H_{11}O_2)_2$
I	II	III	IV	
C =39.09	39.13	.....	....	39.23
H = 6.12	6.14	.....	.....	5.99
O =.....	.....	.....	.....	17.46
Ba=.....	.....	37.27	37.30	37.32
				100.00

Dai risultati di quest'analisi la sostanza solubile nell'acqua bollente ridotta a piccolo volume era il sale di bario dell'acido caproico, acido trovato nei prodotti d'ossidazione dell'acido oleico.

Le acque madri non più suscettibili di cristallizzazione le ho trattate con H_2SO_4 ed ho ottenuto, per distillazione, un acido incoloro che ricorda, per l'odore, il burro rancido, per cui ho pensato che potesse essere dell'ossido butirrico, opinione che mi è stata confermata dall'analisi del suo sale di argento che ho ottenuto precipitando una soluzione alcolica di $AgNO_3$ col liquido acido raccolto nella distillazione.

Il sale d'argento così ottenuto, lavato e seccato, pesava gr. 7.4432, il che corrisponde a gr. 3,3590 d'acido butirrico, acido che entra nella composizione di questo sale così come lo dimostra la seguente analisi.

I. Gr. 0.6292 di sostanza danno gr. 0.5665 di CO_2, gr. 0.2056 di H_2O e gr. 0.3480 di Ag.

II. Gr. 0.4851 di sostanza danno gr. 0.4365 di CO_2, gr. 0.1599 di H_2O e gr. 0.2684 di Ag.

Calcolando il %:

Trovato		Calcolato per $AgC_4H_7O_2$
I	II	
C =24.55	24.53	24.61
H = 3.63	3.66	3.58
O =.....	.....	16.43
Ag=55.30	55.32	55.38
		100.00

Per questi dati sperimentali posso concludere che l'acido butirrico è nel numero dei prodotti contenuti nel liquido filtrato.

I prodotti rimasti nel filtro l'ho addizionati con una certa quantità di H_2O e li ho messi a bollire, filtrando poi il liquido bollente. La mescolanza sulla quale operavo venne così divisa in due parti = una solubile in H_2O bollente, dalla quale la separai per evaporazione a b.m., l'altra insolubile. Quest'ultima parte, seccata e pesata, era gr. 3.2672 corrispondenti a gr. 2.3530 di acido caprico, poichè il sale di bario sul quale operavo era del caprato di bario come dimostra l'analisi:

I. Gr. 0.3564 di sostanza danno gr. 0.6527 di CO_2 e gr. 0.2564 di H_2O.

II. Gr. 0.3118 di sostanza danno gr. 0.5712 di CO_2 e gr. 0.2246 di H_2O.

III. Gr. 0.7115 di sostanza danno gr. 0.3456 di $BaSO_4$ corrispondenti a gr. 0.203178 di Ba.

IV. Gr. 0.7985 di sostanza danno gr. 0.3889 di $BaSO_4$ corrispondenti a gr. 0.2286 di Ba.

Calcolando il %:

Trovato				Calcolato per $Ba(C_{10}H_{19}O_2)_2$
I	II	III	IV	
C = 49.94	49.95	.....	.....	50.10
H = 7.99	8.00	.....	.....	7.93
O = ...	.....	.....	.....	13.37
Ba = ...	.....	28.55	28.63	28.60
				100.00

Anche dell'acido caprico è stata notata da Redtenbacher la presenza nei prodotti d'ossidazione, per NHO_3, dell'acido oleico:

La parte solubile in H_2O bollente fu ottenuta per evaporazione del liquido: il suo peso era di gr: 3.2047.

Sottomessa ad analisi ha condotto ai seguenti risultati:

I. Gr. 0.3386 di sostanza danno gr. 0.5618 di CO_2 e gr. 0.2186 di H_2O.

II. Gr. 0.3058 di sostanza danno gr. 0.5079 di CO_2 e gr. 0.1970 di H_2O.

III. Gr. 0.7984 di sostanza danno gr. 0.4390 di $BaSO_4$ corrispondenti a gr. 0.25808 di Ba.

IV. Gr. 0.8125 di sostanza danno gr. 0.4472 di $BaSO_4$ corrispondenti a gr. 0.2629 di Ba.

Calcolando il %:

Trovato				Calcolato per $Ba(C_8H_{11}O_2)_2$
I	II	III	IV	
C =45.24	45.29	.....	.....	45.39
H = 7.17	7.15	.....	.....	7.09
O =.....	.....	.....	.....	15.14
Ba=.....	.....	32.32	32.35	32.38
				100.00

I risultati di quest'analisi mi condussero ad ammettere che la sostanza analizzata era il sale di bario dell'acido caprilico anch'esso trovato nei prodotti d'ossidazione dell'acido oleico.

Riassumendo, i sali di bario che son riuscito a separare gli uni dagli altri profittando della loro differente solubilità in H_2O bollente, sono: Butirrato, Caproato, Caprato e Caprilato di Bario. Ho inoltre pesato questi differenti sali, i quali sono presenti nelle seguenti proporzioni in gr. 500 di formaggio:

Butirrato d'argento gr. 7.4432 corrispondenti a gr. 3.3590 di ac. butirrico

Caproato di bario gr. 5.9192 corrispondenti a gr. 3.7515 di ac. caproico

Caprato di bario gr. 3.2672 corrispondenti a gr. 2.3530 di ac. caproico

Caprilato di bario gr. 3.2047 corrispondenti a gr. 2.1820 di ac: caprilico.

Calcolando il % in acidi liberi si ha:

Acido butirrico	gr.	0.6718
" caproico	"	0.7503
" caprico	"	0.4706
" caprilico	"	0.4364
	"	2.3291

Dopo ciò, poichè nel formaggio (maturo) questi acidi sono saturati dall'ammoniaca, si può rappresentare la composizione del formaggio n. 10 nella seguente maniera:

Acqua	gr.	33.9421
Margarina	"	26.6570
Oleina	"	2.3430
Butirrato d'ammonio	"	0.8015
Caproato "	"	0.8602
Caprilato "	"	0.4879
Caprato "	"	0.5752
Acido lattico	"	3.1504
Lattosio	"	0.0573
Caseina solubile	"	6.4662
" insolubile	"	11.5006
Basi ammidiche (leucina)	"	6.5396
Sostanze minerali (ceneri e Nacl)	"	6.1581
		99.5391

Sono dunque autorizzato a dire che nel pecorino conservato per nove mesi a contatto dell'aria si trovano, indipendentemente dalla margarina e dalla oleina, tutti i prodotti d'ossidazione di quest'ultima sostanza, e poichè l'oleina che si trova nel formaggio di cinque mesi è in buona parte sparita, bisogna concludere che gli acidi butirrico, caprico, caprilico e caproico si originano dall'ossidazione di questa sostanza.

Tutti questi acidi si trovano ugualmente nel burro invecchiato con la differenza che nel formaggio essi sono saturati dall'ammoniaca, e sono appunto questi sali ammoniacali che danno al formaggio un sapore differente da quello del burro rancido, nel quale

gli acidi sono gli stessi, ma allo stato libero, non saturati da alcuna base.

Nell'epoca in cui Chevreul intraprese il suo celebre lavoro sui corpi grassi, egli si occupò dello studio del burro e trovò in questa sostanza, divenuta rancida, gli acidi butirrico, caprico e caproico. Malgrado lo stato imperfetto nel quale si trovava in quell'epoca l'analisi organica egli seppe perfettamente distinguere questi acidi e ne fece anche uno studio abbastanza completo. Dopo d'allora un gran numero di scienziati si è occupato dello stesso argomento e ricordo specialmente Lerch, il quale dopo aver saponificato il burro rancido lo distillò con un eccesso d'H_2SO_4 diluito, guingendo così ad ottenere fino a cinque acidi volatili (butirrico, caproico, caprico, caprilico e vaccinico).

M. Bromeis[1] ha studiato egualmente la costituzione del burro e vi ha trovato, indipendentemente dalla margarina e dall'oleina, dell'acido butirrico.

Dall'accordo di questi risultati con quelli che io stesso ho ottenuto si vede chiaramente che bisogna attribuire all'oleina l'irrancidimento del burro, come indubbiamente è all'ossidazione di questa sostanza che bisogna attribuire la produzione dei differenti acidi dei quali ho potuto constatare la presenza nel pecorino. La sola differenza che sembra esistere è che nel burro questi acidi sono allo stato libero, mentre nel formaggio essi sono combinati all'ammoniaca.

Credo così d'aver dimostrato che la caseina si trasforma, nella maturazione del formaggio, in una sostanza grassa avente la più grande analogia col burro, poichè si compone di margarina ed oleina, e queste sostanze entrano nella sua costituzione presso a poco nelle stesse proporzioni nelle quali esse si trovano nel burro.

Resta ora a stabilire come questa trasformazione avviene, ciò che mi propongo di fare in una prossima nota.

Napoli, Istituto di Chimica Generale della R. Università—Maggio del 1912.

[1] Ann. der Chem. und Pharm. XLII, 46.

THE GRINDING OF CORN-MEAL FOR BREAD

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At the present time, when the high prices of food occasion so much concern, and a conservation of all the resources of this country awakens so much interest, it is somewhat amazing that the United States produces such an enormous crop of Indian Corn and yet in the larger portion of this Country it is consumed in the form of bread to a very small extent.

Dr. Charles D. Woods, Director of the Maine Agricultural Experiment Station, has compiled an excellent treatise on the Food Value of Corn and Corn Products, published as Farmers' Bulletin No. 298 by the U. S. Department of Agriculture 1907; and in this he sets forth most plainly the advantages of composition, digestibility, wholesomeness, convenience and pecuniary economy of corn as a food for man.

The Encyclopedia Britannica 11th Ed. p. 449 states: "As an article of food maize is one of the most extensively used grains of the world. Although rich in nitrogenous matter and fat it does not make good bread."

It is generally understood, and so far as I have been able to obtain reports, it appears that in the U. S. the considerable use of corn as a bread is confined to the southern States and there largely to the population of the country, and smaller towns. In many of these localities it is more largely used than is wheat.

The readiness with which it may be prepared and the rapid and simple methods by which it may be cooked, as well as the pleasant and satisfying character of the food, its composition approaching to that of a complete ration, have much effect in determining its uses as a staple food.

On the other hand, a considerable amount of corn meal is made throughout the Central and Western States, especially at the

larger business centres—but it is used only sparingly;—often in admixture with wheat flour and largely in the form of mush (or hasty pudding). A few estimate its use as one fiftieth, and more at one hundredth of that of wheat flour.

In 1890 the U. S. Secretary of Agriculture, Hon. J. M. Rusk, endeavored to induce a larger use of corn a as bread stuff in Europe and made some expenditures under the efficient management of Mr. Chas. J. Murphy as a Special Agent, but does not seem to have succeeded, and there is probably even a smaller proportional consumption now in the United States than there was at that date in the form of bread, while the manufacture of grits and other corn products has been largely increased.

It is my endeavor in this study to ascertain, why this apparent inconsistency; that this cheaper, healthy food is so sparingly consumed, where economy in living is of so great import.

The question seems narrowed down to the manner of making the meal as being the factor which determines the extent of its use; and hence this, the narrow range of the discussion in this paper.

As to the grain itself, the comparatively high fat content, viz., 5 per cent. constitutes a considerable portion of its food value, while its presence adds to the care required in curing and keeping the grain, and also to the difficulty of keeping the meal for more than three or four weeks in most climates.

Hence it is that those supplying distant trade and most of the larger mills, find it best to kiln-dry the grain, so destroying any bacteria which may have infected it, and in grinding it, to remove the germ, so as to obtain a product freed in large measure from these drawbacks, thus treating both yellow and white corn.

The grinding is conducted by water, steam, or electricity for power, and we might judge their use as entirely a matter of indifference, yet that is not wholly so, as I shall have occasion to mention later on.

The mills employed formerly were only burr-stones (or occasionally made of some local sandstone), but in recent years, most of the larger mills use steel rollers, similar to those used for wheat flour.

Samples of meal have been secured from most of the corn pro-

ducing states, and to avoid advertising or embarassments, these are designated by numbers, stating only the states from which they come. In the endeavor to ascertain the different forms of meal made, this collection of samples must not be considered as representing the relative production, inasmuch as the very numerous small "custom mills" scattered throughout the rural districts generally make corn meal in one very simple manner, with a pair of burr-stones, driven slowly by water power.

The examination of these meals has been made as follows: and since in bread making, any husk is always removed, all was first passed through a sieve of sixteen meshes to the inch.

1st. *Volume*: 50 grms. of meal were jarred vertically, for 5 minutes, in a glass cylinder, 100 c.c. measuring 7 inches in height, and from the volume read off, the weight in pounds, of a bushel of meal was obtained.

2nd. *Size*: 100 grms. of meal was shaken uniformly for 10 minutes in a nest of eight brass sieves, which time was longer than necessary for most samples, but in a few instances, where the sample was more oily, the smaller sizes were not sharply separated. These sieves, to one inch, had meshes: 16, 20, 24, 30, 36, 40, 50. Any finer sizes would have been of no use. On Table I, the per cent passing each number of mesh is indicated, omitting fractions.

3rd. *Fats:* In some typical samples only, as indicated, a determination of the fat was made. Sudan III was used as a staining in examining the meal under the microscope and proved very satisfactory in bringing out the fat globules, but it afforded no quantitative estimation. A comparative estimate of the amount of freed fat was obtained by noting the length of time respectively taken by each specimen to attain the same (dark brown) color when soaking in a water solution of Osmic acid. About .25 grms of meal in a watch glass was moistened by 3 c.c. of a 0.2 per cent solution of OsO_5 in from 10 to 30 minutes or more, the uniform color was obtained. The comparison was made with three pieces of cardboard giving near shades of a brownish black color and the time was noted as the darkening sample passed each of these shades of color; the average of these three periods is the figure given on Table I.

And we may take the reciprocals of these figures as expressing the amounts of free fat present.

4th. *Cooking:* While it may be that for each variety of meal there is a mode of cooking to which it is specially adapted—in order to obtain a comparison of the meals, the method of cooking selected is of the simplest kind and one which conspicuously brings out any imperfections or flavors.

Each sample was treated approximately as follows:

A.	Meal, sifted	100 grms.
	Salt	2 "
	Water—about	90 "

Mixed quickly to a soft dough, formed to 3 or 4 small rolls, placed on a pan and baked in a hot oven, at 440 Fahr. for 30 minutes.

B.	Meal, sifted	100 grms.
	Salt	2 "
	Lard	10 "
	Water—about	85 "

Mix with salt, "lightly" mix with lard—then with water and bake as above.

One can expect little satisfaction from single tests of this kind not only because of the special difficulty of making ordinarily good bread upon so small a scale, and of baking it uniformly from day to day, but still more, in observing and expressing the slight differences of taste that are presented.

In describing the character of the bread, the following abbreviations are employed in the Table:

g, good; p, poor; f, flavor; n, no flavor; s, sweet;
c, coarse; t, tough; d, dry; v, very;
w, white; W, exceptionally white; y, yellow; Y brilliant yellow.

TABLE I.

Whole grain burr ground	Kilned or Raw	Bushel weighs lbs.	Sized by sieves mesh to inch							Required for color min.	Cooked	
			16	20	24	30	36	40	50		Color	Taste, etc.
(1) Va.	R	57	2	6	8	15	20	30	19	19	w	gd
(2) "	K	55	.3	2.	4.7	11	21	17	44	14	w	gd
(3) "	K	52	0	.1	.5	7.4	27	48	17	30	w	g
(4) "	K	59	0	1	13	20	19	36	11	14	w	gt
(5) "	R	55	1	3	6	12	22	32	24	17	w	vgfs
fat: 4.71												
(6) Tenn.	R	57	1.6	4.4	9	13	27	26	19	20	w	gf
(7) N. C.	R	56	.1	.6	3.3	14	20	35	27	22	w	vgf
(8) "	R	62	1	10	20	16	29	14	10	12	w	gfs
(9) Ala.	R	57	2	6	11	16	18	20	27	12	w	gst
fat: 4.39												
(10) Del.	R	60	0	0	.4	3.6	31	40	25	10	w	pt
(11) Ark.	R	60	1	5	16	17	29	17	15	7	w	gf
(12) Miss.	R	62	2	6	19	17	14	33	9	9	w	gs
Roller ground												
(13) Md.	K	55	0	1	2	19	20	41	17	25	w	gf
(14) "	K	54	0	0	1	14	22	42	21	29	w	gft
(15) Ky.	R	56	1	7	14	19	15	26	18	14	w	gcs
(16) "	R	52	1.3	4.4	7.3	16	18	34	19	40	w	gcs
Fat: 2.20												
(17) Mich.	R	59	0	3	13	18	26	21	19	29	y	gsf
(18) Iowa	R	54	.6	7.4	24.	27	26	11	4	48	y	scf
(19) Kan.	K	58	0	.1	.2	3.7	31	40	25	77	W	vgf
Fat: 1.78												
(20) Conn.	K	56	.2	8.6	36	27	16	9	3.2	108	Y	gcf
Fat: 1.75												
Degerminated roller ground												
(21) Pa.	R	55	1	1	6	18	22	29	23	45	y	gcf
(22) Ky.	K	52	0	0	1	13	42	28	16	15	w	vgn
(23) Mo.	K	54	.1	.1	5.8	17	30	27	20	33	W	gcn
(24) Mo.	K	59	0	0	6	23	36	19	16	29	W	gt
Fat: 1.25												

TABLE I.—*Continued*

Whole grain burr ground	Kilned or Raw	Bushel weighs lbs.	Sized by sieves mesh to inch							Required for color min.	Cooked	
			16	20	24	30	36	40	50		Color	Taste, etc.
(25) Iowa		59	0	5	17	20	23	19	16	28	W	gt
(26) Neb.		56	0	0	3	19	38	19	21	18	W	gtc
(27) Ill.	K	61	0	2	10	22	28	22	16	78	w	gn
(28) Mich.	R	53	0	.3	4.7	21	21	30	23	72	Y	gscf
Whole grain ground at Univ. Va.												
(29) Ill. Agr. Dept. fat: 5.66			1	4	14	11	37	23	10	46	w	gn
(30) Ill. Agr. Dept. fat: low ground			1	5	17	15	29	15	18	31	w	gnd
(31) Ill. Boone Co. once:			10	15	16	12	12	18	17	25	w	gc
(32) Ill. Boone Co. twice:			7	14	17	12	13	15	22	21	w	
(33) Ill. Boone Co. thrice:										20	w	

In order to compare the weights per bushel, it seemed well to employ a uniform method of settling the meal, hence it was jarred in the measure until it would settle no further. Specimens containing much of fine powder settle very compact, therefore in this respect these results cannot be compared with those usually observed.

It may be noted that some of the granular meals are compact but others as (16) and (22) which appear to be "cut meals" are more bulky.

Some years ago, it was the custom of many steam driven mills to grind corn by burr to a fine powder, in order to make a whiter meal, resembling wheat flour; this made a very compact bread. But no such meal is found among the samples examined, and the making of such meal seems to be now generally discontinued and replaced by the manufacture of a meal which is chiefly coarse and contains but little of powder. The superior whiteness of

some of these specimens is attained by selection of well-matured white corn, "scouring" the grain when shelled, and removing all husk and germ as soon as they are set free by the rollers employed—as seen in Nos. (19), (23), (25). Similarly from yellow grain very clean specimens are Nos. (20) and (21).

It is to be noted that the nutty flavor of white corn (somewhat like that of a chestnut), as well as the peculiar flavor of yellow corn are volatile bodies (i. e. odours) and are largely retained by the fat. The flavor is therefore to a large extent removed with the germ and is diminished by too much heating in the grinding or by Kiln drying. It is also removed by too long repeated grinding as is shown in No. 31 made by a single grinding of good grain in a metal hand grist mill, while the same grain was ground twice in making No. 32.

Nos. (29) and (30) were made of good corn and were run through the metal grist mill three times, thereby losing all "flavor." All the degerminated meals are found to be without the nutty flavor although No. (22) is certainly excellent in all other respects.

As to the kiln-dried meal of white corn, they too, generally retain little flavor, but Nos. (19) and (22) make excellent bread and have a pleasant sweet taste.

In examining this lot of selected samples, generally donated by the makers, it is natural that in tasting the raw meal I found but one that possessed any mustiness, and in this instance the sample was obtained from a retail grocery store without any inquiry as to its freshness.

The removal of the germ presents an important economical feature. Taking the whole meal as containing 5 per cent. fat, the degerminated meal will have about 1.5 per cent. fat, thus 100 lbs. of meal would so lose about 3.5 lbs. of edible digestible fat, which will correspond, allowing for the increase in starch, to a loss in food value of 30 cents or more. The reality of this loss is brought out by the fact that when cooking the meal, or other material to be eaten with it, one will ordinarily add this much additional fat, at a cost of 10 cents or more per pound, to replace that which has been removed.

As to the effect upon cooking.

The making of a proper dough largely depends upon the fine particles retaining water sufficient to fully soften the enclosed larger grains when heated in the oven, hot from the first. If the meal is too largely coarse it can hardly be made to stick together, and the dough presents a rough surface, from which the water may escape (dry out) before the starch and protein are properly softened, so producing a very hard bread, insufficiently cooked. An application of this principle is presented in a favorite method of cooking an "ash cake." The plain corn meal dough is wrapped in a cabbage leaf and buried in hot ashes until done. In this the flavor of the meal is peculiarly well preserved and we may obtain a very appetising food. Hence it is that some portion of the corn must be finely ground to obtain a meal for general use.

If a meal is too fine, it may become too compact when made into a dough, in such case the addition of a very little baking powder, say 3 grms. to the foregoing receipt will sufficiently open the dough to give good bread.

On the other hand very coarse and gritty meal is, in this respect, better adapted to making mush or a batter bread in which, while being cooked, it necessarily remains in contact with an excess of water.

All of the above samples of meal were also cooked according to receipt B. and there was little variation in the good bread obtained—tender, porous and appetising but varying with the peculiarities of the grain as to texture, color, and flavor of the meal.

The meal thus mixed with the fat should be but lightly pressed, with no pressing or "working" (as must also be done in making pastry with wheat flour), so subdividing the dough; and the resulting bread will be excellent.

We have in corn a natural mixture of starch, protein, fibre and fat, which, when simply ground and moistened by water, gives a dough all ready for baking, the oil serving to separate the mass and prevent it from becoming too compact or hard; hence it is to be treated in a wholly different manner from wheat and, to the writer, is not to be cooked with wheat flour with any advantage.

It seems therefore that the work done in refining corn meal is, so far as its use for bread is concerned, not well directed, in that the portion of the grain so removed is the very portion in which rests one of its chief advantages. It is true that meal from whole corn will not keep, under most conditions, more than 3 or 4 weeks, but is it not profitable to supply this fresh meal to obtain its several advantages?

In recent years there has been much and successful endeavor to free wheat flour from all bran and husk, so securing its pure whiteness while losing the advantages of the rougher fibre and ash content. But I think I have shown that similar processes do not improve corn meal, and that there is by this process loss of some of its most valuable constituents.

It appears from the favor with which oatmeal and many of the numerous breakfast foods are received (so much so, that in some cases these are sold at from 3 to 5 fold of the price of the grain from which they are made), that there is a craving of many stomachs for rougher food such as stimulates the processes of digestion; and this is certainly in many instances the explanation of the satisfaction with which corn bread is preferred by many as a staple diet.

But when corn is well matured, kept to thoroughly dry on the ear, and then, as it is needed, ground without heating, by burr stones, slowly turned by a water wheel, it furnishes a sweet, nutty flavored meal, which combines the most valuable of nutrients, and when cooked in the simplest manner, furnishes a food which is to many of mankind very acceptable, and to some, the staple of life.

(*Abstract:*)

FOOD STANDARDS, THEIR NATURE, HISTORY, AND FUNCTIONS

By William Frear
State College, Pa.

Nearly all civilized lands have enacted general food laws covering all foods. They prohibit the sale, as normal, of products that depart in certain, very generally defined ways from the normals corresponding to the food names used; but do not define these normals. In the absence of sufficiently complete, accurate, and concise definitions of these normals, the executive officers of these laws have been obliged to judge for themselves what the respective normals are, subject to the confirmation of the Courts.

A food standard is the expression of a food normal, and may include chemical and physical limits indicative of kind and quality. The existing systems of standards, British, Bavarian, German, Austrian, American, Swiss, Italian and Holland, are the work of experts representing the executive branches of the respective governments.

Nature: Food standards should correspond to the people's concepts corresponding to the several food names, and, since the laws with which they are to be used are quasi-criminal, should correspond to the lowest quality, within the kind, acceptable without notice of inferiority. They should represent present usage, the concepts of the public, where they differ from those of the trade, and those of home, instead of foreign countries. Since foods are chiefly of domestic, rather than of factory production, domestic usage should determine the normals, but due consideration should be given to the requirements of commercial distribution, as contrasted with immediate, domestic consumption.

Matter: As their material, food standards should contain what is necessary, (1) to distinguish the various normals from

each other; (2) to distinguish each normal from its substitutes, imitations, and adulterated modifications. The data should be both authentic and broadly representative. A standard may be useful, even though incomplete; but incompleteness of chemical and physical data should not lead to a definition of species broader than the public concept therefor. Such data should represent the products of the country in which the standard is to be used.

Consistency: Systems of food standards should be consistent; but the criterion of consistency is external, not internal; consistency with the people's concepts, not etymological consistency is the aim. Even a given word varies in its meaning with the context. Moreover, there is no simple mathematical formula that can serve safely to fix the relation of the minimum to the average of quality.

Form: A clear, concise definition, closely knit with the most serviceable limits, will better endure legal analysis than the encyclopediac form of description with loosely appended chemical limits. The latter form is, however, superior educationally.

Function: Standards are practically essential as bases of reference in the enforcement of general food laws, unify executive and judicial decisions, relieve the trade and the public from confusion and uncertainty. They do not interfere with variety in production, nor should minimum standards tend to lower the general average of excellence; since, however, such standards do not in reality represent the absolute minima of quality, their limits should be set with caution and common sense, and be widely published before application, lest honest, but unskilled or ignorant producers be injured.

International standards are practicable only for a limited number of products, but are desirable for these.

Provision should be made for the continuous addition of new limits and standards as the need and data therefor appear; but radical changes, made with frequency in existing standards, are gravely unsettling to producing interests.

THE PACKING OF AMERICAN SARDINES

By H. H. Hanson

Scattered along the Maine coast from Portland to Eastport there are about fifty-five different factories, whose combined annual output is usually somewhere between 125 and 200 millions of cans of sardines, valued at from five to seven million dollars according to the season. A large proportion of this industry is located around Eastport and Lubec, in Passamaquoddy Bay, where a majority of the inhabitants are dependent upon it, either directly or indirectly, for their livelihood.

A study, not yet entirely completed, was undertaken for the purpose of obtaining information on certain points of this industry which were perplexing alike to the packers themselves and to the food officials of the country. A comparison of the American and Foreign packed sardines and a description of the packing process is at once interesting and instructive as throwing light on some of the points under consideration.

The name sardine, which comes from the island of Sardinia, around which sardines abound, is not the name of a particular species, but is applied to fish of the genus *Clupea*, various species of which are canned in different parts of the world.

There are three important respects in which the Maine sardines differ from the foreign sardines, of which the French pack is generally recognized in this country as the most desirable. First, the fish packed in France under the name sardine is the *Clupea pilchardus*, while the fish packed in Maine under that name is the *Clupea harengus*, two distinct species of the same family which differ somewhat from each other both in appearance and flavor. Second, French sardines are packed in olive oil while the Maine sardines are put up in cottonseed oil. Third, in handling the French pack the single fish is the unit and quality is at all times considered of paramount importance; while in handling the Maine pack the hogshead is the unit and quantity is always sought. In America the French sardine retails for from

thirty-five to sixty cents per can, while the Maine sardine retails for the most part for five cents. The markets for these two general grades seem to be well established and although some fancy goods, which bring a high price, are put out it is quite certain that the Maine packers cannot be brought to the point of adopting French methods of handling even though the product might thus be improved in quality.

The fish are caught almost entirely in weirs, large circular or oval traps having long wings extending out from the one opening and arranged so as to guide the schools into it. These weirs are large enclosures built mainly of brush topped with marlin and are so located that there will be perhaps twenty feet of water in them at low tide. Fish may be taken coming in on the flood tide or going out on the ebb. It is not uncommon to take 100 hogsheads in a single weir, and even larger catches have at times been reported.

When a catch has been made the weir-man closes the gate of his trap and awaits the coming of the sardine boat. The competition between the boats running for the different factories is often very keen. Not many years ago there was sharp bidding between the skippers of these boats so that the prices paid for the fish were often ridiculously out of proportion to their value. As high as $30 per hogshead has been given. During the last few years a more or less fixed price has been paid for the fish and the boat which arrives first at the weir has first claim. At the beginning of the season about $12 per hogshead may be offered and later, when the fish become more plenty, the price is dropped to about $6.

When the fish have been purchased a purse seine or net is carried around the entire catch in the weir, drawn together at the ends, and closed at the bottom, so that the sardines are brougth into a compact mass, and may be scooped with hand nets into dories and conveyed to the sardine boats lying just outside. These boats range in size from remodelled life boats holding perhaps ten hogsheads to trim two-masters holding eighty hogsheads. As the fish are scooped into the holds of these boats salt is sprinkled over them, at the rate of about 100 pounds to the hogshead, and when the hold is full a race

for the factory is begun, each skipper striving to land his catch first. Arriving at the wharf of the factory the boat is drawn under a windlass from which a tub is let down to the hold and the fish are scooped into the tub, drawn up, and dumped into a sluice as fast as it is possible to work. Along this sluice, flushed with water, the fish run, sometimes through a contrivance which separates the large from the small, into large wooden pickling tanks where they are kept in salt brine long enough to give them a flavor and aid in preserving and hardening the flesh. Time allowed in the pickle varies with the condition of the fish and amount of salt they have already had in the boat.

From the tank most of the fish run to the flaking machine, a mechanical arrangement for distributing the fish evenly upon heavy wire screens upon which they go to the steam boxes for cooking. In some factories this flaking is done by hand, but if the machine is well made and properly run it will do the work much faster, although it is inevitable, especially when the fish are fat and tender, that many are broken. From the steam boxes the fish are run into a drier where they are kept long enough to thoroughly dry and, upon emerging from this process, and sufficient time having elapsed for them to cool, they are taken at once to the packing tables. The packers are girls and women of all ages who cut off the heads with shears and pack the fish in cans so rapidly that although they are paid only about sixteen cents per case of one hundred cans, they earn at times three or four dollars a day.

The small fish are for the most part packed in oil in small cans holding four ounces. These are called "quarter oils." The larger fish are usually packed in mustard sauce in cans holding eleven ounces. These are called "three-quarter mustards."

Usually the oil is placed in the can after the fish but the mustard sauce is put into the cans first. After the cans are filled they are taken to the sealing machine and when the covers are in position they are processed in a tank of boiling water from one and a half to two and a half hours. This process is called bathing. They are then cooled, cleaned, examined for leaks, "fats" and "slacks," and finally packed in cases for shipment.

"Fats" are cans which have been carelessly filled too full and, for that reason might afterwards be mistaken for "swells," which have spoiled by fermentation. Such cans are punched and oil drawn out to reduce the swelling, after which they are again sealed and bathed. "Slacks" are those which do not contain enough and these are punched, heated and oil drawn in to fill them out.

There are a number of modifications of this general process in use in various places. A few packers behead all fish before cooking. A few fish are fried or baked instead of being steamed. Some of the quarter-oils contain a bay leaf or a clove and there are a few fish put up in tomato sauce. Several different styles of ovens and drying machines are in use and there are many different types of sealing machines, a new one now being perfected apparently being a great improvement over any other previously made. Whereas all other machines now in use seal the cans by rolling the edges of the can and cover tightly together, the new machine seals them hermetically. This machine also automatically introduces into the cans the proper amount of oil, fluxes the edges, places the covers and, after tightly sealing, turns them out at the end ready for the bath at nearly double the rate of the old machines. An endless belt is arranged to bring the cans to this machine directly from the packing tables.

There is always great waste of fish during the process. A twenty per cent loss of the catch is always reckoned upon and it sometimes runs as high as fifty per cent. When fish break in large numbers during the process of preparation for the cans as happens under certain conditions, the waste is large. When large fish which would ordinarily go as "mustards" are cut to the size of "quarter oils" the waste is again large for the fish are sometimes cut in two in the middle in order to make them short enough for the purpose. This latter practice is resorted to, however, only when particular orders for "quarter oils" must be filled regardless of the size of the fish. An attempt to utilize some of this waste is being made in some factories by canning it as "deviled fish," but most of the waste is sold at a dollar and a half per hogshead to the fertilizer factories where the oil is pressed out and the pomace used in fertilizer.

The two most important questions which have been studied are the cause of swells, that is, cans swelled out by inside pressure caused by fermentation, and the breaking of the fish during the process of preparation for the cans.

By correspondence with packers and by investigation in the factories it seems evident that the cause of the swells is imperfect sealing rather than imperfect or incomplete processing or bathing. Experiments with four-ounce cans ("quarter oils") and with eleven ounce cans ("three-quarter mustards") were made at several factories and it was found that in from twenty-five to thirty minutes after the cans were immersed in the boiling bath the temperature had risen at the middle of the can to 100 degrees C. The smaller cans are bathed in the various factories from one and a quarter to two hours, and the larger cans from one and a half to three hours, the time varying in the different factories and with different conditions of the different catches. This would seem to be long enough to thoroughly sterilize the contents of the can. That this time is sufficient for such sterilization is indicated by the fact that in all cases where the hermetically sealed cans are now put out there is practically no complaint from swelled cans. This fact, taken in connection with the other fact that with the ordinary roll sealed cans leaks often occur and are found even before the goods have left the factory, would seem to place the blame for the swelled cans almost entirely upon the sealing machines which do not thoroughly close the cans. Corroborative evidence is furnished by the further fact that practically no living organisms were found on opening thoroughly sealed cans.

There are at least six different causes contributing to the breaking of the fish during the process of preparation for the market.

First. Fish that have lain in a shallow bay or over mud flats for several days will have softer flesh than those taken from deeper, cooler water, and are, of course, much more easily broken.

Second. Fish which are very fat are naturally more tender than fish which are lean and will break more easily, especially if they are not properly salted.

Third. The rough, careless handling which many of the fish receive would break even the firmest fish before they were finally placed in the cans.

Fourth. If fish are over salted they tend to break transversely across the side after steaming and drying.

Fifth. When the fish have undigested food in them softening and breaking commences quicker than otherwise.

Sixth. When the fish are kept too long out of water without sufficient salt, as is sometimes the case, they naturally begin to soften and decompose.

It has appeared to some that the breaking of sardines indicates that they are unfit for food. That this is not so is evident from the above. Of the six reasons given for the breaking only the two last could be considered as evidence of unfitness for food, and that the first of these is sufficient evidence of unfitness is questioned. That those which have burst from the last cause are unfit for food is, of course, unquestioned and, as their unfitness is at once made evident by the odor, they should be sent to the fertilizer factory. The breaks which occur in these fish are somewhat characteristic of the cause of the breaking. For example, as above stated, over salted fish after steaming and drying tend to break transversely along one side; fat fish tend to break along the backbone where the fat is deposited thickly and the skin is tender; feedy fish, that is fish with undigested food in them, soften very rapidly and tend to break along the under side where the flesh is thinnest. Rough and careless handling increases the tendency to break in all the other cases. That the breaking along the under side of feedy fish does not necessarily indicate unfitness for food would appear from the fact that regardless of thorough salting and careful handling the breaks begin to appear in about three hours after the fish are taken out of the water, but fish so breaking give no evidence whatever of either decomposition or decay, are as sweet and palatable, and, aside from appearance seem as good as those not broken.

Feedy fish, above mentioned, are usually full of either one or the other of two kinds of food known to the fishermen as "shrimp" and "red feed." This latter is the bane of the sardine industry

as it has long been known that fish containing it deteriorate much more rapidly than fish in any other condition. They often begin to break open before reaching the factory, and, if they contain much of this material, by the time they are ready for the can they are broken so badly that a large percentage of the catch is entirely unfit in appearance for packing. The study of the subject of breaking involved the questions: "What is 'red feed'? Why does it cause this deterioration?"

This "red feed" we have identified as one of the copepods, *Temora longicornis*, a microscopic crustacean of the family *Centropagidæ*. It is quite abundant in the region of Woods Hole, Mass., during the winter months and is described by Dr. William Morton Wheeler in a bulletin of the United States Fish Commission for 1900. Dr. Wheeler speaks of it as an essentially boreal form rarely seen in the above locality during the months of July and August. These are the months in which it is most abundant along the coast of Maine. Dr. F. H. Moore of the United States Fish Commission in a report of his investigations in the herring fisheries of Maine some years ago speaks of this crustacean as one of the copepods but apparently the species was not determined. He calls it in his bulletin "red seed" but this is evidently an error either in printing or in information.

It has long been recognized by sardine canners that fish containing "red feed" deteriorate much more rapidly than those containing any other kind of food but why this is so no one to our knowledge has ever before attempted to determine.

Althougth the sardines were never more abundant on our coast than during the season just passed, "red feed" was not particularly troublesome, so that not enough was obtained upon which to make thorough investigations, but two possible answers to the second question, the cause of the rapid deterioration, have suggested themselves. First, that the deterioration is due to auto-digestion induced by some particular enzyme. Second, and this seems more likely in the light of present knowledge, that a methylamine is responsible for the trouble. This compound has been identified in various fish and crustaceans. It was reported many years ago as being present in the roe of herring and it is not impossible that in this case "red feed" was

mistaken for roe. It has been reported very recently by Bigelow and Bacon as being present in considerable amount in the shrimps which are canned so extensively in Mississippi. In their investigation of this problem which was reported in the Journal of Industrial and Engineering Chemistry for November 1911, it was noted that this compound affected the hands of the workmen and had a corrosive effect upon materials which came in contact with the shrimp containing it. A parallel case seems to be found in the sardine industry, for at times when "red feed" is abundant the hands of the operatives who work upon the fish are made sore and at times also a strong odor of ammonia is noted during some of the processes. Either auto-digestion produced by enzymic action, or the presence of a methylamine, would probably cause the rapid breaking down of the tissues and the consequent softening and breaking of the sardines containing "red feed" and it is hoped soon to further investigate the question.

In closing it should be stated that the credit for a large part of the foregoing should be given to A. M. Buswell, Instructor in Industrial Chemistry in the University of Maine, who acted as field agent during the summer of 1911, and thanks are also due Dr. O. A. Johannsen of the Maine Experiment Station for aid in identifying the "red feed."

Maine Agricultural Experiment Station,
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ETUDE CHIMIQUE DES FRUITS DE SORINDEIA OLEOSA

PAR M. ALEXANDRE HÉBERT
Paris, France

I.—La matière première de cette étude consistait en fruits séchés au soleil de Sorindeia Oleosa A. Chev. qui nous avaient été adressés par M. Auguste Chevalier et qui provenaient d'un arbre commun au Soudan. Ce sont des fruits à noyau entouré de pulpe et de la grosseur d'une cerise; ils ont deux usages et ont été examinés à deux points de vue:

1°—La pulpe ou péricarpe du fruit est très sucrée; dans le pays d'origine, on fait fermenter ces fruits pour en obtenir une boisson analogue au cidre; 2° l'amande de la graine proprement dite, qui forme le noyau du fruit, est très oléagineuse; on en extrait de l'huile et on en prépare du savon.

Il convenait donc de vérifier, d'une part, la nature et la proportion du sucre existant dans la pulpe du fruit; d'autre part, la quantité et les propriétés de la matière grasse contenue dans les amandes.

II.—Pour effectuer l'étude chimique de ces fruits aux points de vue qui nous intéressaient, nous avons commencé par séparer les pulpes et les noyaux. A cet effet, 500 grammes de ces fruits séchés ont été mis en contact avec une quantité d'eau froide suffisante pour les recouvrir, après 24 heures de séjour, ils s'étaient gonflés et étaient d'une consistance telle qu'ils pouvaient être malaxés dans l'eau sans risquer d'écraser les noyaux. Ceux-ci, séparés ainsi des pulpes, ont été desséchés à l'air et mis de côté pour un examen ultérieur. On les a trouvés en proportion de 40 pour 100 des fruits secs accusant ainsi 60 pour 100 de pulpes.

Les pulpes gonflées ont été épuisées à trois reprises par l'eau froide pour dissoudre toutes les matiéres solubles et notamment les sucres qui s'y trouvaient. Finalement le résidu a été pressé

et le liquide provenant de ce pressurage a été joint aux liqueurs d'épuisement. Celles-ci ont été déféquées par le sous-acétate de plomb et le liquide filtré a été débarrassé de l'excès de plomb par l'hydrogène sulfuré. La solution incolore ainsi obtenue a été concentrée dans le vide au bain-marie à très basse température jusqu'a consistance sirupeuse, puis abandonnée à elle-même. Elle a refusé de cristalliser, malgré tous les subterfuges habituels employés dans ce but: concentrations diverses, reprises par l'alcool traitement au noir animal, etc. Le sirop réduisait énergiquement la liqueur de Fehling, donnait avec l'acétate de phényl-hydrajine une osazone cristallisée en aiguilles groupées en forme d'éventail, fusibles à 200° et correspondant aux propriétés de la phényl-glucosazone, déviait enfin à gauche le plan de polarisation de la lumiére, mais cette déviation correspondait à une quantité de sucre réducteur bien plus faible que celle indiquée par le titrage à la liqueur de Fehling. Somme toute, ces caractères répondaient au sucre interverti.

D'autre part, on a trouvé dans une quantité donnée des fruits secs, épuisés par l'eau froide comme nous l'avons indiqué, et par titrage à la liqueur de Fehling, une proportion de 22 pour 100 de sucres réducteurs et une quantité nulle de sucres non réducteurs. Si nous admettons dans ces fruits, à l'état frais, une teneur en eau égale à 90 ou 95 pour 100, teneur qu'on retrouve généralment dans les fruits de ce genre, la proportion de sucres réducteurs correspondrait à 1:10 ou 2:20 pour 100 des mêmes fruits à l'état frais.

Il résulterait de ces expériences que les matières sucrées des fruits de *Sorindeia Oleosa* A Chev. seraient constituées par du sucre interverti, mélange de glucose et de lévulose, ce qui justifierait leur emploi indigène pour la préparation d'une boisson plus ou moins alcoolique, et du genre du cidre, mais qui, en tous cas, ne peut certainment être que très peu riche en alcool.

III.—Les noyaux, obtenus comme nous l'avons dit, et qui constituaient 40 pour 100 des fruits secs, renferment 24 pour 100 de ces mêmes fruits secs en amandes. Celles-ci, après broyage et extraction à la benzine, lui abandonment une matière grasse dont la proportion atteient 25 pour 100 des fruits secs.

La matière grasse obtenue est solide à la température ordinaire, de couleur brunâtre et présente les constantes suivantes:

Densité à 17°	0.889
Point de fusion	16–17°
Point de congélation	12–13°
Indice d'acidité	4.90
Indice de saponification	185.00
Indice de Reichert	7.92
Indice d'Hehner	91.75
Indice d'iode	132.00

La graisse de *Sorindeia Oleosa* A. Chev., saponifiée par la soude alcoolique et acidifiée, fournit 92 pour 100 environ d'acides gras, jaunâtres, solides à la température ordinaire, fusibles à 39–40°. La séparation des acides gras saturés et incomplets effectuée par l'épuisement à l'éther des sels de plomb, a donné 24 pour 100 d'acides incomplets, liquides, de couleur jaune brunâtre, et 76 pour 100 d'acides saturés, solides, colorés en jaune brun, fondant à 44–45°. Ce point de fusion assez bas indique l'existence, dans la graisse étudiée, d'acides gras relativement inférieurs. L'usage de la graisse de *Sorindeia Oleosa* A. Chev. pour la préparation du savon se comprend ainsi parfaitement, cette substance grasse d'une part, ne paraissant pas comestible, et d'autre part, donnant des acides gras à point de fusion trop bas pour servir à la fabrication de bougies ou même de chandelles.

SUR LA COMPOSITION DE DIVERS PRODUITS, GRAINES OU TUBERCULES AMYLACES OU FECULENTS DE L'AFRIQUE OCCIDENTALE FRANÇAISE

PAR M. ALEXANDRE HÉBERT
Paris, France

Au cours de sa dernière mission en Afrique occidentale française, M. Aug. Chevalier a rapporté un certain nombre de produits, graines ou tubercules, de nature amylacée ou féculente, qu'il nous a remis pour en déterminer la composition chimique, en apprécier la valeur nutritive et en fixer l'emploi industriel possible. Ce sont ces diverses recherches que nous résumons ici.

GRAINES.—Maïs blanc du Dahomey.—Ce maïs nous a été remis sous forme d'épis dont une certaine quantité étaient malheureusement charançonnés; nous avons pu cependant en trouver quelques-uns intacts sur lesquels nous avons effectué l'analyse. Nous avons séparé dans les épis les glumes et glumelles, les rachis et les graines dont nous avons déterminé la composition. Nos dosages nous ont conduit aux résultats suivants:

	Séchés à l'air	*Séchés à 110°*
Poids moyen d'un épi entier	120 gr.	110 gr.
Décomposable en glumes et glumelles	16.6	16.6
graines	88.3	78.3
rachis	15.0	15.0

Analyse de la graine séchée et moulue

Humidité restant	1.63%	
Matières minérales	1.96	dont 0.49 solubles dans l'eau.
Matières grasses	3.70	
Matières azotées	11.55	dont 0.98 solubles dans l'eau.
Sucres réducteurs	0.36	
Sucres non réducteurs	0.95	
Gommes, tannins, acides végétaux	0.24	
Amidon	76.30	
Cellulose	1.36	
Vasculose	1.90	
Total	99.95	

Cette graine peut donc être comparée, au point de vue de sa valeur, à nos produits indigènes. Elle est d'autant plus intéressante qu'il s'en exporte d'Afrique des quantités importantes dont l'introduction pourrait rendre service à diverses industries.

Voandzeia Poissonni A. Chev.—Ces graines qui proviennent d'Ouaga dougou (Mossi), présentaient la composition ci-dessous.

Humidité	10.38
Matières minérales	4.34
Matières grasses	1.91
Matières azotées	21.41
Sucres réducteurs	traces
Sucres non réducteurs	0.41
Amidon	48.77
Cellulose	12.74
Total	99.96

Cette graine, riche en matières azotées, renferme moins d'amidon que les graines amylacées de nos pays; elle peut néanmoins être employée au point de vue alimentaire au moins dans les contrées d'origine.

TUBERCULES.—Ignames.—Ces tubercules qui nous ont été envoyés à l'état desséché, provenaient de la cote d'Ivoire; ils ont donné à l'analyse les résultats suivants:

Humidité	13.80
Matières minérales	2.40
Matières grasses	0.40
Matières azotées	5.75
Sucres réducteurs	1.00
Sucres non réducteurs	1.00
Amidon	73.80
Cellulose	1.25
Vasculose	0.60
Total	100.00

Ces tubercules sont assez comparables comme composition à la pomme de terre. Ceux qui ont été expédiés en Europe ont

été trouvés de valeur au moins égale au manioc sec. Le commerce des ignames africains pourrait donc prendre de l'extension comme produit alimentaire sous une forme quelconque.

DIEGEMTENGUERE (Vulg. Mossi).—Les tubercules de cette plante qui nous ont été remis provenaient d'Ouagadougou dans le Soudan français. Leur poids avait été déterminé à l'état frais ce qui nous a permis de fixer leur composition exacte à l'état frais et à l'état sec:

	Etat frais	Etat sec
Eau	57.90%	0.00%
Matières minérales	2.02	4.80
Matières grasses	0.21	0.50
Matières azotées	4.47	10.62
Sucres réducteurs	Néant	Néant
Sucres non réducteurs	2.69	6.40
Amidon	28.80	68.40
Cellulose	3.85	9.15
	99.94	99.87

Cette composition ratifie parfaitement l'emploi de cette plante qui est cultivée au Mossi, dans la boucle du Niger, pour ses tubercules alimentaires.

MOELLE d'ENCEPHALARTOS BARTERI.—Ce produit est extrait d'une plante de la famille des Cycadacées, dont la tige est pourvue d'une moelle abondante qui possède la composition suivante:

Humidité	12.80
Matières minérales	2.80
Matières grasses	0.60
Matières azotées	6.43
Sucres réducteurs	10.00
Sucres non réducteurs	1.10
Amidon	60.52
Cellulose	4.25
Vasculose	1.50
Total	100.00

Cette moelle est, comme on le voit, riche surtout en hydrates de carbone: sucres et amidon; cette richesse justifie parfaitement l'emploi indigène que l'on fait de cette moelle en fabriquant une sorte de pain avec la fécule qu'on en extrait.

(*Abstract*)

ON THE TASTE OF THE SALT OF GLUTAMIC ACID

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The glutamates having the general formula $C_5H_8NO_4M'$ are mostly soluble in water and all of them have a very distinct peculiar taste, the quality of which differs from all other well defined taste qualities hitherto known. Numerous food materials present this taste, but so much overshadowed by others, that no clear conception of this quality has hitherto been formed, although it contributes largely to the flavor. For this taste quality the name "glutamic taste" is proposed.

This taste is then demonstrated to be that of the monovalent glutamate ion $C_5H_8NO'_4$. For this purpose the threshold value of the taste has been carefully measured for the salts of sodium, potassium, magnesium, calcium and barium. The value has been found to be $\frac{1}{2500}$ normal for all the five salts. The taste-imparting power of the glutamates is very great.

The author was led to the discovery of the taste of glutamates by his investigation on the constituents of a certain sea-weed, which is used in Japan as a flavoring. He isolated glutamic acid from it and found that it is the salts of this acid that give the weed its peculiar flavor.

There are numerous flavoring substances which give glutamic taste, and among them meat-extract and allied preparations. But from obvious reasons a pure glutamate is much to be preferred over them. Of all the glutamates of non-poisonous metallic radicals the sodium salt is the most suitable. Within the last three years the manufacture of this salt has arisen in Japan and it is now rapidly becoming an article of general consumption.

There is hardly any doubt that the glutamate will come to be manufactured in a large scale in Europe and America. As the raw material for the manufacture is the hydrolytic products of proteins, there is a prospect that the chemical industry of these products will be greatly developed, bringing in its train numerous problems of great interest.

PROGRESS REPORT OF NUTRITION INVESTIGATIONS IN THE UNITED STATES

By C. F. Langworthy, Ph.D.

Office of Experiment Stations, Department of Agriculture

Introduction

For many years, continuous progress has been made in the United States in the study of various questions concerned with human nutrition. In this summary, the attempt has been made to bring together articles on this subject which have appeared in the United States, since the 7th International Congress of Applied Chemistry, thus supplementing a paper of similar scope presented at the 7th Congress.

A survey of the literature under consideration shows that a considerable part of it represents work carried on under governmental or institutional auspices, a considerable part representing the work of the United States Department of Agriculture and other branches of the general Government and the agricultural experiment stations. University laboratories and the laboratories of endowed institutions are also large contributors as are also state boards of health.

In general, it may be said that judging by the amount of work which is published annually, interest in the experimental study of human nutrition is growing very rapidly. The fact is recognized that the record of work here presented is by no means complete but it is believed that it is sufficiently extended to show the character and scope of the work which is being done.

For convenience the material has been arranged under the following heads: Studies of Food and Food Products; Special Studies of Ash, Protein, and Other Food Constituents; Cooking in Its Relation to Nutritive Value; Canning, Preserving, Handling and Storage; Dietary Studies and Dietetics; Digestion; Metabolism; Respiration Calorimeters, Bomb Calorimeters, and Experiments with Them; Foods and Their Relation to Problem of Hygiene; and Cost of Living and Other Statistical Data.

STUDIES OF FOOD AND FOOD PRODUCTS.

As is usually the case, considerable attention has been given to the proximate composition of food products and to the effect of various processes of manufacture or handling upon nutritive value and quality. Many hundreds of proximate analyses, more or less complete in character, have accumulated during the periods under consideration, in connection with inspection work under national and state pure food laws and as a part of other work undertaken for some special purpose aside from analysis.

Many milling and baking tests with different varieties of wheat have been reported, this question being one which is of great interest particularly in wheat-growing regions. E. F. Ladd and Emily E. May (North Dakota Sta. Spec. Buls. 19, pp. 105–114; 24, pp. 179–194, fig. 1) have carried on extensive work of this sort with durum wheat flour. Their studies showed that more power was required to grind durum than Fife or Bluestem but the yield was as large and the bread made equal to that produced from other flours, though not quite so white in color. It was found to hold moisture better than that from commercial flours. A study of milling and baking of durum wheat flour was reported by L. M. Thomas of the North Dakota Experiment Station.

The effect of climatic conditions on the composition of durum wheat has been discussed on the basis of a number of analytical and other studies carried on by the Department of Agriculture, by J. A. LeClerc (U. S. Dept. Agr. Year Book, 1906, pp. 199–212, pls. 2.) Several hundred analyses of spring and winter wheat of different varieties grown in different States showed an average protein content of 12.2 per cent as compared with 14.7 per cent for over 100 samples of durum wheat analyzed by the author.

E. F. Ladd and C. H. Bailey (North Dakota Sta. Buls. 89, pp. 14–80; 93, pp. 204–253, dgms. 5) have reported an extended study of the milling quality of wheats of different varieties and crops.

Similar tests of wheat of different kinds and crops grown in different localities have also been made by R. W. Thatcher (Washington Sta. Bul. 84, pp. 48, figs. 3) and L. R. Waldron (North Dakota Sta., Rpt. Dickinson Substa. 1910, pp. 43, 44),

and with California wheats by G. W. Shaw and A. J. Gaumnitz (California Sta. Bul. 212, pp. 315–394, figs. 18, dgms. 3), and with a variety of wheats by F. D. Gardner (Roller Mill, 28 (1909), No. 5, pp. 201–204).

It is interesting to note the discussion of the future wheat supply of the United States by M. A. Carleton (U. S. Dept. Agr. Yearbook 1909, pp. 259–272, figs. 2), which is based on a digest of statistical data.

With respect to the effect of soaking and germination of wheat on the distribution and yield of milling products, the quality of flour, and bread-making properties, G. A. Olson (Amer. Food Jour., 6 (1911), No. 4, pp. 36–39, figs. 4) found that water-soaked wheat is not necessarily spoiled and can be used for milling purposes, providing it has been thoroughly cleaned and dried. Using small quantities of germinated wheat flour with other flour increased the volume of the loaf, according to the author, without impairing its texture. Each particular flour required a different amount of germinated flour to produce the best results. Too large an amount of diastatic flour is less beneficial than none.

Analyses of a number of sorts of gluten flour manufactured in the United States and of foreign diabetic products were reported in comparison with wheat flour by D. W. Fetterolf (Univ. Penn. Med. Bul., 22 (1909), No. 7, pp. 217–222).

From an experimental study of the starch grain, H. Kraemer (Amer. Jour. Pharm., 79 (1907), pp. 217–229, pl. 1, figs. 3) concludes that "the starch grain consists of colloidal and crystalloidal substances, these being arranged for the most part in distinct and separate lamellæ, that is, at the point of origin of growth and in the alternate lamellæ the colloidal substance preponderates, associated with the crystalloid cellulose; whereas in the other layers the crystalloidal substance, consisting for the most part of granulose, occurs in greater proportion."

Several studies of cane sugar and maple sugar have been carried on.

C. A. Browne, Jr., and R. E. Blouin (Louisiana Stas. Bul. 91, pp. 103) have summarized a large amount of data collected during recent years by the Louisiana Sugar Experiment Station, which have to do with the composition of the stalk, seed, root,

and leaves of sugar cane and of the plant ash. The work as a whole is an exhaustive study of the chemical composition of sugar cane, with reference to its use for sugar making, and of the physiology of the growth and ripening of the cane. Experimental work on sugar making is also reported.

From a study of the question of the influence of micro-organisms upon the quality of maple sirup, H. A. Edson (Abs. in Science, n. ser., 31 (1910), No. 791, p. 308) was able to show by isolation and inoculation experiments that to certain groups of micro-organisms is ascribable the abnormal type of sap of the late runs characterized by green, red, milky, and stringy appearance.

A. H. Bryan (U. S. Dept. Agr., Bur. Chem. Bul. 134, pp. 110, pl. 1, figs. 4, map 1), in connection with a study of maple sap sirup, reports analyses of 481 samples of sirup of known purity collected in maple-producing States in the United States and in Canada, the data being gathered as a basis for comparing and grading maple sirups.

Considering the 395 samples from the United States, the average moisture content was 34.19, sucrose 62.64, invert sugar 1.49, ash 0.66, and undetermined material 1.02 per cent. The polarization values were: Direct, at 20°C.,+60.93; and invert, at 20°C.,−22.16. The average values for the 86 Canadian samples were: Moisture content 34.34, sucrose 62.24, invert sugar 1.41, ash 0.62, and undetermined material 1.59 per cent. The polarization values were: Direct, at 20°C.,+59.33; and invert, at 20°C.,−23.17.

The results of a special study of the constituents of maple-sirup ash are also reported. The average results for 100 samples from different States showed that the ash contained 38.07 per cent potash, 21.88 per cent lime, 5.39 per cent phosphoric acid, and 1.59 per cent sulphates.

Considering the samples from both the United States and Canada, the average basic lead value of 2.70, calculated to dry substance, and the average neutral lead number was 0.79. The average malic acid value determined by the modified calcium chloride method was 0.84, and by the calcium acetate method, 1.01.

Factors which influence the character of the sap and the sirup and related questions are discussed.

Experimental work carried on in an attempt to isolate flavoring substances present in maple sap is described by A. P. Sy (Jour. Franklin Inst., 166 (1908), pp. 249–280); Abs. in Chem. Abs., 2 (1908), No. 24, p. 3376), in a publication dealing with history, manufacture, and analysis of maple products, and work reported on the analysis of maple products.

Housekeepers and manufacturers of food products often express the opinion that there is a difference in the culinary quality of cane sugar and beet sugar. The matter was studied by G. W. Shaw (California Sta. Circ. 33, p. 4), of the California Experiment Station. The sugar is being used for sirup making, for canning fruit, and for jelly making. The beet sugar produced more froth in making sirup, but investigation led to the conclusion that this was due to the finer granulation of the beet sugar, which caused more air to become entangled during the starting than was the case with cane sugar. No differences were observed in the keeping quality of canned goods or the jelly made with the two sorts of sugar from his experimental data and other evidence the author concludes that under commercial and household conditions, beet sugar and cane sugar give equally satisfactory results for these uses.

An exhaustive study was made of the composition of American honeys by C. A. Browne (U. S. Dept. Agr., Bur. Chem. Bul. 110, pp. 1–69, 89–93, pl. 1, fig. 1), of the Bureau of Chemistry, and a microscopical study of honey pollen by W. J. Young (U. S. Dept. Agr., Bur. Chem. Bul. 110, pp. 70–88, pls. 5).

A number of studies of meat, eggs, cheese, and other animal foods have appeared.

The glycogen content of beef flesh and the factors which influence it were studied, in animals recently slaughtered, by P. F. Trowbridge and C. K. Francis (Jour. Indus. and Engin. Chem., 2 (1910), No. 1, pp. 21–24). The length of time which elapses after feeding before the animal is slaughtered, the authors consider an important factor in determining the amount of glycogen which remains stored in the organs and muscles. Their results indicate that there is a rapid enzymatic hydrolysis of glycogen

in flesh under many conditions, but that at 10°C. or lower, it did not take place appreciably.

In connection with an extended study of market classes and grades of meat, L. D. Hall (Illinois Sta. Bul. 147, pp. 147–290, figs. 75; Abstract, pp. 15, figs. 4) has described and illustrated by diagrams or figures the standard grades of beef, veal, mutton, and pork as they are found in the Chicago wholesale trade. Technical terms are defined. The bulletin as a whole furnishes a large amount of data on the subject which is of importance in discussing meat in relation to dietetics as well as for other purposes.

Some data of a similar character have been published by P. F. Trowbridge (Missouri Bd. Agr. Mo. Bul., 9 (1911), No. 2, pp. 69–78).

W. D. Richardson (Jour. Amer. Chem. Soc. 29 (1907), No. 12, pp. 1757–1767) reports the results of the examination of a large number of samples of animal and vegetable foods with a view to securing data regarding the occurrence of nitrates in vegetable foods, cured meats, and elsewhere. He concludes that nitrates are quite generally distributed.

F. C. Cook (U. S. Dept. Agr., Bur. Chem. Circ. 62, pp. 7), of the Bureau of Chemistry of the Department of Agriculture, has reported a large number of analyses of beef extracts and yeast extracts of known origin. According to the author, "the yeast extracts contain approximately 1 per cent ether-soluble material and the beef extracts larger amounts. Cholesterol was not found in the ether extracts, and sarcolactic acid only in the yeast extracts.

"The phosphorus of beef is largely water-soluble, consequently a considerable percentage of the ash of beef extracts is composed of this constituent. Approximately one half of the sulphur of beef is water-soluble. Yeast extracts derived from yeast rich in phosphorus also contain a large amount in the ash. The total amount present is larger than the ash content, showing that some phosphoric acid is volatilized on ashing. The organic phosphorus determined by the Siegfried-Singewald method gives approximately the 1:10 ratio compared with the total as suggested by those authors.

"The total nitrogen of the beef extracts on the water-free and fat-free basis averages 11.82 per cent, that of the yeast extracts averages 7.44 per cent. The amino nitrogen figures for the beef preparations are nearly double those of the yeast extracts.

"Although the water-soluble nitrogen of beef, which constitutes 25 per cent of the total nitrogen, consists of approximately two thirds and one third amino nitrogen, the samples of beef extracts analyzed average 72 per cent of amino nitrogen and 28 per cent of protein nitrogen.

"The general appearance and odor of the two varieties of extracts are very similar. As a food both are extremely limited in value. The beef extracts contain more nitrogenous extractives than the yeast preparations, otherwise their general composition is much the same."

A large number of analyses of samples of meat extract, meat juices, yeast extracts, and similar goods are reported and discussed by W. D. Bigelow and F. C. Cook (U.S. Dept. Agr., Bur. Chem. Bul. 114, pp. 7–56), the methods followed being described.

Meat extracts, yeast extracts, and similar goods were also tudised by J. P. Street, et. al. (Connecticut State Sta. Rpt. 1907–8, pt. 9, pp. 573–716).

On the basis of numerous tests, I. A. Field (U.S. Dept. Com. and Labor, Bur. Fisheries Bul., 28 (1908), pt. 1, pp. 243–257; Doc. 655, 1910, pp. 243–257) reaches the conclusion that the common sea mussel (*Mytilus edulis*) is nutritious, palatable, and easily digested. From tests of culinary qualities, made under a variety of conditions, of the smooth and horned dogfish, he concludes further that the flesh of these fishes is cheap, palatable, nutritious, and easily preserved, and he believes further that it is as digestible as that of other fishes.

In a paper on unutilized fishes and their relation to the fishing industries, I. A. Field (U. S. Dept. Com. and Labor, Bur. Fisheries Doc. 622, pp. 50, pl. 1) discusses methods of profitably using dogfish of different sorts, sand shark, toad-fish, etc., summarizes data regarding the use of fresh, canned and dried dogfish, and gives some results of tests of its culinary quality, which he believes indicate that such dogfish flesh is both palatable and wholesome.

The uniformity with which copper was found in oysters examined by J. T. Willard (Jour. Amer. Chem. Soc., 30 (1908), No. 5, pp. 902–904) led him to conclude that it is to be regarded as a normal constituent.

J. T. Willard and R. H. Shaw (Kansas Sta. Bul. 159, pp. 143–177) analysed all the eggs laid in 6 weeks by 4 lots of pure-bred chickens. On an average the thickness of the shells was 0.0139 in. In addition to usual determinations, they report data regarding the percentage of phosphoric acid, the ash in the yolk, and the ratio of phosphoric acid to ash. The average amount of ash was 1.57 per cent and of phosphoric acid 1.43 per cent, the ratio of phosphoric acid to ash being 1:1.09.

"It is evident that the ash consists almost entirely of phosphoric acid. This is doubtless produced almost entirely, if not altogether, from the lecithin of the egg yolk."

Mary E. Pennington (Jour. Biol. Chem., 7 (1910), No. 2, pp. 109–132) has reported the results of an extended chemical and bacteriological study of fresh eggs, which was reported at the London Congress of Applied Chemistry, in June, 1909, and later published in full.

L. L. Van Slyke and A. W. Bosworth (New York State Sta. Tech. Bul. 4, pp. 1–16, 17–22) at the New York State Station have studied some of the early chemical changes which take place in the proteids and in the calcium and phosphoric acid compounds of Cheddar cheese, and also the acidity of the water extract of Cheddar cheese.

A. W. Bosworth (New York State Sta. Tech. Bul. 5, pp. 23–39) has also reported the results of chemical studies of Camembert cheese.

The manufacture of a food product called buttermilk is described by J. L. Sammis (Wisconsin Sta. Bul. 211, pp. 3-17, figs. 7), in a bulletin of the Wisconsin Experiment Station and some data given regarding its fat content, keeping qualities, etc.

An experimental study of the production of a dairy product called "whey butter" has been reported by C. F. Doane (U. S. Dept. Agr., Bur. Anim. Indus. Circ. 161, pp. 7).

G. A. Olsen (Jour. Biol. Chem., 5 (1908), No. 2-3, pp. 261–281) reports data regarding a proteid found in milk, cream, and but-

ter which he considers new. The chemical and physical character of this proteid are described.

The majority of investigations with fruits and nuts carried on in the United States have had to do with the methods of cultivation, transportation, and shipment rather than with composition, food value, and use in the home.

Cactus fruits, particularly tuna or the fruit of the prickly pear, which is used in southwestern United States and to a greater extent in Mexico as a foodstuff, were studied with respect to its composition and nutritive value, by R. F. Hare and D. Griffiths (New Mexico Sta. Bul. 64, pp. 88, pls. 7, figs. 2).

In the study of the tuna as food for man, by D. Griffiths and R. F. Hare (U. S. Dept. Agr., Bur. Plant Indus. Bul. 116, pp. 73, pls. 6), information is given regarding the use of the fruit for jelly making and preserves as well as for other purposes.

Italian lemons and their by-products and methods of producing lemon oil and citric acid commercially are discussed in a summary of data by E. M. Chace (U. S. Dept. Agr., Bur. Plant Indus. Bul. 160, pp. 35–50, pls. 3, figs. 2).

In connection with a summary of data on the dietetic value of fruit, W. R. Lazenby (Trans. Mass. Hort. Soc., 1910, pt. 1, pp. 89–97) reports data regarding the water content of well-developed and undeveloped specimens. Less than 80 per cent water was found in undeveloped strawberries, peaches, and apples, as compared with 90 per cent in fine but not overgrown specimens. It is further stated that 92 per cent of water was found in fine large peaches, in comparison with 84 per cent in small peaches of the same variety.

Data were also recorded regarding the percentage of shell or waste, and edible portion in nuts, and similar factors. According to the author, there is a loss of nearly 2 per cent of the total weight of kernels in milling or cracking some of the larger sorts of nuts.

Various topics concerned with the composition, nutritive value, and use of fruit as food have been discussed in a summary prepared by C. F. Langworthy (U. S. Dept. Agr., Farmers' Bul. 293, pp. 38, fig. 1).

The occurrence of sucrose in grapes was studied by W. B. Alwood and his associates (Jour. Indus. and Engin. Chem., 2

(1910), No. 11, pp. 481, 482) with a number of varieties. The quantities found in the juice of 3 well-known varieties ranged from 4.49 and 5.66 gm. per 100 cc. of juice. In the juice of a new seedling it was considerably larger.

In a later report, W. B. Alwood (U. S. Dept. Agr., Bur. Chem Bul. 140, pp. 24) states that he and his co-workers have examined practically all the wine and table grapes grown in eastern United States, and with the exception of the varieties mentioned (Hayes Pocklington, and Worden and a seedling), they did not find sucrose in appreciable quantities. Extended studies were also made of varieties grown in other regions and the variations in sugar and acid content studied during ripening. In Catawba grapes the sugar more than doubled after the berries began to color, while the acid was only about half as great. Similar data are reported for many other varieties.

W. P. Kelly (Jour. Indus. and Engin. Chem., 3 (1911), No. 6 pp. 403–405) has studied the composition of Hawaiian pineapples and found them to vary considerably, the sugar content ranging from 9.15 to 15.23 per cent, and the acidity from 0.22 to 1.16 per cent, and increasing generally as the sugar increased. On the whole, Hawaiian pineapples show much the same average composition as those grown elsewhere.

"Green pineapples contain less acidity that the ripe fruit and also a small percentage of fiber, reducing sugar, and sucrose. Dextrin and starch do not occur in important quantities in pineapples at any stage. The reducing sugars and sucrose stand in inverse ratio to that of the ripe fruit. In the ripening of pineapples gathered green, the most important chemical change that takes place is the conversion of reducing sugars into sucrose, but the total sugar content appears not to be increased. . . .

"During the normal ripening of the pineapple, a rapid accumulation of sugars and a slight increase in acidity take place. When the fruit becomes approximately half ripe, it contains at least three-fourths of its maximum sugars."

Bread, milk, vegetables, bananas, and rhubarb were included by H. Ackroyd, (Bio-Chem. Jour., 5 (1911), No. 8-9, pp. 400-406) in a study of the presence of allantoin in certain foods.

His general conclusions are that "the whole quantity of allan-

toin excreted by man on a milk and vegetable diet may be derived directly from the food. Milk, white bread, French beans, green peas, all contain small quantities of allantoin, while none could be isolated from eggs, bananas, or rhubarb."

The food value of nuts and the various ways in which they may be used in the diet have been discussed by M. E. Jaffa (U. S. Dept. Agr. Yearbook 1906, pp. 295–312, pl. 1, fig. 1; Farmers' Bul. 332, pp. 28, fig. 1), in a bulletin published in connection with the nutrition investigations of the Office of Experiment Stations.

In connection with a study of pecan culture, the marketing of pecans, and related questions by W. N. Hutt (Bul. N. C. Dept. Agr., 30 (1909), No. 9, pp. 50, figs. 25), the use of pecans as food is considered, and instructions given for cracking these nuts particularly for commercial purposes.

The care and marketing of vegetables have been more often studied than their composition and nutritive value.

Canned peas and beans of different grades were analyzed by W. L. Dubois (U. S. Dept. Agr., Bur. Chem. Circ. 54, pp. 9), in connection with commercial canning, and particularly with reference to the use of soaked peas and beans in place of the fresh vegetables. In general, the soaked peas had a higher water and starch content and a somewhat higher specific gravity than the fresh canned peas. The author is of the opinion that such determinations may prove useful in connection with physical examinations in judging of the character of such canned goods.

The crude fiber and the crude starch content of the soaked were higher than in the case of the fresh canned beans, though the differences were less pronounced when the results were reduced to a dry matter basis.

The recorded data furnished some information regarding the changes which take place during the growth and ripening of peas.

"As the pea matures the ash decreases, the starch increases, and the crude fiber decreases as a rule, while the conclusions to be drawn from the determinations of nitrogen and ether extract are less decisive. In the peas from one locality the amount of nitrogen decreases as the pea matured, whereas in the same vari-

ety from another locality this variation was not so apparent. Similar changes in composition appear in the canned vegetables. The analyses seem to indicate that during the process of canning the peas take up from 2 to 10 per cent of water. It is difficult from these results to draw any conclusions as to the changes taking place during processing. The principal value of the work . . . is to afford data for the comparison of commercial grades."

Marine algæ are important articles of diet of native Hawaiians. In connection with the work of the Hawaii Experiment Stations, Minnie Reed (Hawaii Sta. Rpt. 1906, pp. 61–88, pls. 4) studied the economic importance and food value of a large number of these marine algæ, reporting cooking tests in addition to analytical work and studies of the value of seaweed mucilage, gelatin, etc.

A digest of data on insoluble carbohydrates, particularly those of marine algæ, and a summary of digestion experiments carried on in the author's laboratory with such foods in comparison with raw Italian chestnuts, have been briefly reported by L. B. Mendel (Zentbl. Gesam. Physiol. u. Path. Stoffwechsels, n. ser., 3 (1908), No. 17, 641–654).

The character and nutritive value of carbohydrates of lichens, algæ, and related substances, particularly marine algæ, as studied by Mary D. Swartz (Proc. Amer. Soc. Biol. Chem., 1 (1910), No. 5, pp. 257, 258; Trans. Conn. Acad. Arts and Sci., 16 (1911), pp. 247–382), the hemicelluloses from 10 species of algæ were found to contain pentosans and galactans. The pentosans, with one exception, were largely found insoluble in cold water, while the galactans were soluble and characterized by their gelatinous nature. Small quantities of soluble pentosans were associated with them in every case.

The resistance to bacterial action was studied, and digestibility was studied in vitro, and in other ways.

They were found to be very resistant to the action of animal and vegetable enzyms. Experiments showed that galactans were not affected by the ordinary aerobic bacteria of the alimentary tract, or by mixtures of soil and fecal aerobes, of soil and fecal anaerobes, or of powerful putrefactive organ-

isms such as *Bacillus anthracis symptomatici and B. maligni aedematis*. Pentosans, mannans, and levulans were found to be gradually decomposed by soil and fecal bacteria and by putrefactive anaerobes, sometimes with the formation of reducing substances.

"When introduced parenterally, either subcutaneously or intravenously, they are not retained or altered by the organisms, but are gradually excreted in the urine. Feeding experiments on dogs and human subjects show that those hemicelluloses most readily attached by bacteria disappear most completely from the alimentary tract. Galactans, which are unaffected to any appreciable extent, are excreted in amounts averaging 75 per cent; pentosans and mannans, hydrolyzed by bacteria, disappear almost entirely during the processes of digestion.

"The experiments give little justification for considering these carbohydrates as typical nutrients for man."

A popular digest of data regarding the composition, food value, digestibility, and place in the diet of potatoes and other root crops used as food is prepared by C. F. Langworthy (U. S. Dept. Agr., Farmers' Bul. 295, pp. 45, figs. 4).

Proprietary foods are made and marketed in large variety. Their composition and food value seem to have been studied much less frequently than many other commercial food products notwithstanding the fact that a knowledge of their real value would seem to be particularly important as they are chiefly recommended by the makers for use in infant feeding and in invalid dietetics.

The composition and true nutritive value of a number of proprietary foods and food products are discussed in a paper by Graham Lusk (Jour. Amer. Med. Assoc., 49 (1907), No. 3, pp. 201, 202, 270), dealing with the general subject of the nutritive value of such foods.

D. L. Edsall (Jour. Amer. Med. Assoc., 54 (1910), No. 3, pp. 193–196) also discusses this general question, as has J. Howland (Jour. Amer. Med. Assoc., 54 (1910), No. 3, pp. 196–201), who pays particular attention to predigested foods.

A published paper gives data regarding predigested foods and similar goods (Jour. Amer. Med. Assoc., 48 (1907), pp. 1612–

1614, 1694; 49 (1908), pp. 1294, 1295; abs. in Chem. Abs., 2 (1908), No. 12, pp. 1740, 1741).

G. F. Richmond and W. E. Musgrave (Philippine Jour. Sci., 3 (1909), No. 2, pp. 87–90) report a study of the composition of malted milk, particularly its fat content, which was found to be 8.18 per cent.

Experiment station investigators have given much attention to the breeding of cereal crops, the influence of fertilizers on composition, and other related questions. This work is perhaps more appropriately considered in connection with agricultural chemistry than with nutrition, though some of it, notably that with wheat and with corn, has an obvious relation to questions of human nutrition.

It is interesting to note that comparatively wide variations are observed in the composition of light and heavy kernels of wheat of the same variety, in the grain from well developed and imperfectly developed ears of corn, and in the kernels in different parts of the ear.

In a study of the improvement of corn, by A. M. Soule and P. O. Vanatter (Virginia Sta. Bul. 165, pp. 91–185, figs. 48), it was observed that many of the best yielding ears did not have as high a protein content as the undesirable ones.

C. L. Penny (Delaware Sta. Rpt. 1904–1906, pp. 13–33) found a wide range in protein content, the minimum being 6.25 in one crop and the maximum 12.69. The smaller kernels at the end of the ear were found to contain on an average 0.3 per cent less protein than the large and well formed kernels.

These matters have been extensively studied at the Illinois Experiment Station. In a report of investigations regarding ten generations of corn breeding, L. H. Smith (Illinois Sta. Bul. 128, pp. 457–575, figs. 2) summarizes data covering the range in protein and fat content. The results obtained show that starting with a single variety it was possible in ten generations to increase the protein content "from 10.92 per cent to 14.26 per cent, a gain of 3.34 per cent, while by breeding in the opposite direction it has been possible to reduce the protein content from 10.92 to 8.64 per cent, a reduction of 2.28 per cent, making a total difference between the two strains of 5.62 per cent. It is

further shown that the high-oil corn has increased from 4.70 per cent to 7.30 per cent of oil, while a low-oil corn has decreased from 4.70 per cent to 2.66 per cent, the difference between the two strains in 1906 being 4.71 per cent.

"High protein and low protein seed were planted together on one plat and high-oil and low-oil seed on another. These plats were continued for 3 years, and the results secured did not indicate that the soil influences the protein or the oil content.

"A study of the secondary effects produced by selection to change the composition of the grain indicated that the change in the composition of the grain has produced no very marked effect upon the composition of other parts of the corn plant."

The composition of corn and corn products, including green corn, their nutritive value and place in the diet, and similar questions have been discussed in a popular summary by C. D. Woods (U. S. Dept. Agr., Farmers' Bul. 298, pp. 40, figs. 2), published in connection with the nutrition investigations of the Office of Experiment Stations, which contains some individual work regarding the composition and digestibility of hulled corn and corn bread and some work regarding the composition of popcorn popped and unpopped.

Alice R. Thompson (Hawaii Sta. Rpt. 1908, pp. 51–58), of the Hawaii Experiment Station, has reported the results of studies of Japanese rice and Hawaiian-grown rice, both polished and unpolished, and rice paddy and straw from imported and Hawaiian rice and from rice grown under different conditions, the nitrogenous constituents being determined in every case, and proximate and ash analyses in the case of rice grain and rice straw and paddy.

Little variation was noted in the chemical composition of the different varieties of rice, and the author is of the opinion that the claim for superiority of Japanese imported over Hawaiian-grown rice is not substantiated so far as nutritive value is concerned.

Comparisons of the analyses of polished and unpolished grain showed that the unpolished rice contained about four times as much fat as the polished, as well as more protein, crude fiber, and ash. Practically all the nitrogen of the rice grain was found to be proteid nitrogen.

The question of the wholesomeness of polished and unpolished rice and the more specific question of the possible relation of polished rice to beri-beri are matters which have been given much experimental study in recent years as a part of the general question of the possible connection between the presence or absence of particular mineral constituents, protein radicals, or other constituents and the occurrence of the disease.

The matter of the possible relation of rice to beri-beri is of particular importance in the regions of the Orient where rice is the principal carbohydrate foodstuff, so naturally the question has been studied as a part of the scientific work undertaken by the Philippine Department of Science. H. Aron and F. Hocson (Biochem. Ztschr., 32 (1911), No. 3–4, pp. 189–203), in an investigation on rice as a foodstuff, have reported experimental studies in which the balance of income and outgo of nitrogen was determined on a rice diet supplemented by other foods chiefly of vegetable origin, including such material as bananas, rice polish, and phytin.

Analyses of a large number of samples showed that relatively more phosphorus than nitrogen was lost by polishing rice. The unpolished rice contained on an average from 0.7 to 0.8 per cent P^2O^5, undermilled rice from 0.4 to 0.6 per cent, and overmilled rice from 0.15 to 0.4 per cent.

The authors conclude that an exclusive rice diet will not supply protein enough to meet man's demands, and that therefore it must be supplemented by vegetable, or better, animal foods rich in protein. Such a mixed diet is satisfactory from an hygienic standpoint, provided the rice has not lost too much phosphorus by overmilling or polishing. From their experimental studies they conclude further that, for a man weighing 50 kg., a diet made up of rice supplemented by vegetable foods must contain at least 75 gm. protein in order to meet hygienic requirements, and that a diet of rice supplemented by fish or meat must contain at least 65 gm., of which at least $\frac{1}{3}$ is supplied by animal foods.

Information gained from practical experience with beri-beri and unpolished rice in the Philippines was summarized by V. G. Heiser (Philippine Jour. Sci., B. Med. Sci., 6 (1911), No. 3, pp.

229–233), particularly regarding the efforts which have been made to encourage the local use of unpolished rice and the success which has attended it.

For purposes of convenience, "a rice containing less than 0.4 per cent of phosphorus pentoxid is regarded as polished and that which contains a greater percentage of phosphorus pentoxid as unpolished rice."

An attempt to secure legislation regarding the use of unpolished rice in the Philippines is briefly discussed.

The question of cotton seed as human food has been considered by G. S. Fraps (Texas Sta. Bul. 128, pp. 5–15), who reports analyses of cotton-seed flour, cotton-seed flour bread, and other cotton-seed bakery products. The general conclusion is that cotton-seed flour is rich in protein and that it may be used alone or mixed with wheat flour for the preparation of appetizing foods. In the author's opinion, there is no reason to believe that cotton-seed flour will not prove a wholesome product when used in small amounts.

In his discussion, the author draws attention to the fact that cotton seed has more or less proved harmful when used as food for domestic animals, particularly pigs, but he is of the opinion that the quantities likely to be used would not prove harmful to man. Nevertheless, he cautions against using too large amounts.

It is interesting to note that F. Russell (Ann. Rpt. Bur. Amer. Ethnol., 26 (1904–5), pp. 66–92, figs. 7) states that cotton seed was formerly used as foodstuff by the Pima Indians of southern Arizona.

The widespread interest at the present time in the possibility of using cotton-seed meal as a food for man lends a special interest to the investigations which have been undertaken to determine the reason why it proves harmful to domestic animals, particularly pigs, when fed a considerable time in fairly generous quantities. Such studies will probably be referred to in detail elsewhere. It may be noted here that it seems to be the case that the renal disturbances or other pathological conditions observed when it is fed to pigs may be postponed or even in some cases materially lessened by feeding a large proportion of green fodder with the cotton seed.

Interesting investigations on the general question of the poisonous properties which cotton seed sometimes exhibits when fed to farm animals have been carried on in the Bureau of Animal Industry and reported by A. C. Crawford[a] (U. S. Dept. Agr., E. S. R., 22 (1910), No. 6, pp. 501–505). His conclusion is that the poisonous principle is not an alkaloid but probably an inorganic compound, namely, a salt of pyrophosphoric acid. Phosphoric acid has long been known to be present in cotton-seed meal in considerable quantity, and has been suggested as having a possible relation to its toxicity, but the methods of study followed have not been such as to bring out this relationship or lend support to the hypothesis.

The conclusions advanced are supported by a large amount of data from a systematic series of laboratory studies and physiological tests and have been further confirmed by feeding experiments with dogs carried on by the Bureau of Animal Industry which were not reported in the preliminary account of the work.

Not all cotton seeds exhibit poisonous properties, particularly being influenced in this respect by variety and by method of cultivation.

To quote from Dr. Crawford's conclusions, "the chief poisonous principle in certain cotton-seed meals is a salt of pyrophosphoric acid. In some, this salt seems to be a simple one, presumably inorganic, while in others, it is more complex, perhaps an organic one. Probably this difference in the combinations of pyrophosphoric acid may aid in explaining the variation in toxicity of different meals. In certain cotton-seed meals one would expect to find salts of metaphosphoric acid entering into this action. To be harmful, the pyrophosphates must be in such a form that they can be absorbed, or the phosphoric acid ionized in the gastro-intestinal tract. The harmlessness of certain cotton seeds and meal is mainly due to the fact that in them the phosphoric acid exists largely, if not entirely, as a compound of ortho, and not as one of the other phosphoric acids. Small amounts of pyrophosphates can apparently be borne without injury. The amount of the salt which may be permitted in cotton-seed meal should be determined."

[a] Jour. Pharmacol. and Expt. Ther., 1 (1910), No. 5, pp. 519–548).

Many summaries of data regarding the composition of foods have appeared, such work not infrequently forming a part of treatises on food and nutrition.

A set of fifteen colored food charts, prepared by C. F. Langworthy (U. S. Dept. Agr., Office Expt. Stas. Food and Diet Chart 15), has been issued in connection with the nutrition investigations of the Office of Experiment Stations, which are designed to show graphically the composition and energy value of the common food materials and to summarize some general data regarding the functions and uses of food.

Special Studies of Ash, Protein, and Other Food Constituents

No new products of particular importance have appeared during the period under consideration in this summary, though many of more or less general importance have been studied, including dairy products, fruits, meats, cereal grains and other materials. Methods of analysis as usual have received a great deal of attention.

Much work has been reported in connection with inspection of food under government and state pure food laws. No attempt can be made here to summarize this. As taken in connection with other pure food work, it constitutes a subject in itself.

In addition to studies of the composition of food already cited, a number of investigations have been reported which have to do with some detailed study of food constituents. For instance, the solubility relations of milk sugar, the vapor pressures of saturated solutions of hydrated milk sugar, the influence of concentration on the equilibrium between the forms of milk sugar, and other similar questions were studied by C. S. Hudson (Jour. Amer. Chem. Soc., 30 (1908), No. 11, pp. 1767-1783, figs. 2).

A bulletin by E. B. Forbes (Ohio Sta. Bul. 207, pp. 23–52), of the Ohio Station, on the balance between inorganic acids and bases in animal nutrition, endeavors to show the bearing on practical animal nutrition of the relationship between those mineral elements of our foodstuffs and of living animal tissues, which in the body give rise to inorganic acids, and the various

means at the disposal of the animal for accomplishing protection from these acids through effecting their neutralization. The relation of ash constituents to human nutrition in general is also considered. The investigations are reviewed in detail and a number of general deductions are drawn.

The available alkali in the ash of human and cow's milk in its relation to infant nutrition was studied by J. H. Kastle (Amer. Jour. Physiol., 22 (1908), No. 2, pp. 284–308). The salient points of difference between the ash of the two kinds of milk, the author points out, are: "Human milk contains relatively more of its mineral matter in utilizable form than cow's milk; it can supply the organism of the child with relatively larger amounts of available alkali in proportion to the proteid than cow's milk; it contains much less proteid; and it contains a more readily absorbable variety of fat."

The nature of the chemical combinations of potassium in the tissues was investigated by W. Koch and C. C. Todd (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, pp. XV, XVI; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 9, 10). The results thus far obtained indicate that "sodium and potassium phosphatid compounds exist in all the tissues of the body and are probably of much more importance than the hitherto assumed ion-protein combination."

H. S. Grindley and E. L. Ross (Jour. Biol. Chem., 8 (1910), No. 6, pp. 483–493) have studied the determination of organic and inorganic phosphorus in meats. Judging from the data which they recorded, it appears that the coagulation of the protein of the aqueous extracts of flesh by heat does not change organic phosphorus to the inorganic form to any appreciable extent.

The subject has also been studied by P. F. Trowbridge and Louise M. Stanley (Jour. Indus. and Engin. Chem., 2 (1910), No. 5, pp. 212–215; abs. in Analyst, 35 (1910), No. 412, p. 311). The proportion of soluble organic phosphorus in total soluble phosphorus in meat was found to vary considerably in different animals and in different parts of the carcass of the same animal. The lowest recorded value (26 per cent) was observed with an emaciated steer, and the highest (91 per cent) with a fat show

steer. "During cooking, a progressive splitting up of the organic phosphorus compounds takes place, and in well-cooked meats practically the whole of the phosphorus is present in inorganic combination."

Data are presented by C. K. Francis and P. F. Trowbridge (Jour. Biol. Chem., 7 (1910), No. 6, pp. 481–501; 8 (1910), No. 1, pp. 81–93) regarding investigations of the kind and amount of phosphorus present in beef cattle in different conditions of fatness. The results were not uniform enough to warrant general deductions. No relation was evident between phosphorus and total ash.

The question was also studied with reference to the kind of phosphorus present in different cuts.

"The round cut of beef contains more phosphorus, in forms which are soluble in cold water than any of the other cuts. Phosphorus is found chiefly in the muscular or connective tissue; the fats contain but little. The flesh of a thin animal contains more soluble phosphorus than that of a fat animal. The quantity decreases with increasing fatness even when it is expressed on a moisture and fat-free basis."

The nature of the phosphorus compounds of the brain, both normal and diseased, was studied by W. Koch (Jour. Amer. Med. Assoc., 52 (1909), No. 18, pp. 1381–1383), the work in considerable part dealing with the phosphorus supply in the diet. The phosphorus required for the growth of the brain the author concludes is amply supplied by the phosphorus of our daily diet. "If desired, the addition of phosphorus-rich foods, such as eggs, sweetbreads (pancreas), liver, and some meats, can be made to meet further requirements, and will far exceed in amount the phosphorus obtained in less natural form from the prescribed doses of any of the various drugs in commercial use. The use of such foods is, however, limited by their richness and their tendency, on account of their rich fat content to interfere with gastric digestion.

"As far as the nervous system is concerned, the addition to the diet of commercial phosphorus compounds, such as hypophosphites, glycerophosphate, phytin, lecithin, etc., is to be discouraged because, in the first place, there is no conclusive

evidence that they have any effect on the growth of the brain, and, second, the amount usually recommended means only a very insignificant addition to the amount of phosphorus (even in its special forms such as lecithin) taken with the daily food."

The relation of brain phosphatids to tissue metabolites was studied by W. Koch and W. W. Williams (Jour. Pharmacol. and Expt. Ther., 2 (1910), No. 3, pp. 253–264). Some of the conclusions follow, which were drawn from experiments with substances which may be regarded as of food value to the tissues, including amino acids, glycocoll and glucose, and with substances having a characteristic physiological action, including among others adrenalin, caffein, and theobromin:

"The changes in state of aggregation of lecithin produced by sodium chlorid are the result of the independent action of the sodium and chlorin ions, whose effects are in opposite directions. Below the concentration of a physiological salt solution (0.12 molecular) the action of the chlorin ion, which decreases the state of aggregation of the lecithin, predominates. Above the concentration of a physiological salt solution, the action of the sodium ion, which tends to increase the state of aggregation of lecithin, comes more and more into prominence.

"It has been suggested that, when the phenomenon of chlorid retention occurs, some change has taken place in the state of aggregation of the cell lipoids which allows this action of the chlorin ion to predominate to a still greater extent.

"Ammonia and bile salts possess the power of altering the physical state of aggregation of lecithin to such an extent as to permit of the conclusion that they can be of functional significance in altering the permeability of cell membranes. . . .

"The ability of the tissue metabolites to combine with lecithin, as measured by the changes in the physical state of aggregation produced by their presence, is in some cases considerable, in other cases entirely lacking. Thus hypoxanthin, creatin, creatinin, adrenalin, and ammonia salts show evidence of combination. Inosit is doubtful and urea is negative.

"The amino acids show varying powers of combination. The dicarboxy-acids, like acids in general, tend to increase the state of aggregation of lecithin."

In connection with the nutrition investigations of the Department of Agriculture, H. C. Sherman (U. S. Dept. Agr., Office Expt. Stas. Bul. 185, pp. 80) studied iron in food and its functions in nutrition, and reported the results of three metabolism experiments in which the balance of income and outgo of nitrogen and iron and other mineral constituents was determined, as well as the results of two dietary studies undertaken with special reference to the iron content of the food consumed. Estimates were also made of the amounts of iron taken per man per day in 20 dietary studies made in connection with earlier nutrition investigations of the Office of Experiment Stations.

"Increase of iron," it is pointed out, "in the diet without a corresponding increase of protein is readily accomplished by the use of vegetable, fruits, and the coarser mill products of the cereal grains. In the experimental dietary here reported, the free use of such foods with milk but without meat or eggs resulted in an increase of 30 per cent in the iron content of the diet, while the protein, the fuel value, and the cost remained practically the same as in the ordinary mixed diet obtained under the same market conditions."

In continuation of the work on mineral constituents, H. C. Sherman, A. J. Mettler, and J. E. Sinclair (U. S. Dept. Agr., Office Expt. Stas. Bul. 227, pp. 70) have studied calcium, magnesium, and phosphorus in food and nutrition, reporting the results of 6 experiments on the metabolism of these constituents and a study of the amounts present in typical American dietaries. In general, the investigations show the importance in the diet of calcium, magnesium, and phosphorus and the possibility of securing them by the use in proper proportion of ordinary food materials.

To quote, "it is entirely feasible to increase largely the calcium and phosphorus intake by making a more liberal use of milk in the dietary. The same may, of course, be said of the various milk products in which the calcium and phosphorus compounds are largely or wholly retained, such, for example, as cheese, junket, kumiss, buttermilk, or cream. This is probably the simplest and more effective means of improving the dietary as regards calcium and phosphorus compounds, without decreasing

its acceptability or materially increasing its cost and with distinct advantages in other directions."

The balance of acid-forming and base-forming elements in foods, was studied by H. C. Sherman and J. E. Sinclair (Jour. Biol. Chem., 3 (1907), No. 4, pp. 307–309). Peas, milk, and prunes are the foods studied containing an excess of base-forming over acid-forming elements. With beef, oatmeal, and entire wheat grain the reverse was the case. It is obvious, the authors note, that "by the free use of meats and breadstuffs on the one hand or of fruits, vegetables, and milk on the other, the net excess of acid or base introduced into the body through the food may be varied at will within wide limits."

The very important work of T. B. Osborne and his associates on the cleavage products of protein has been continued, the studies reported having to do with the hydrolysis of excelsin (Amer. Jour. Physiol., 19 (1907), No. 1, pp. 53–60, pl. 1); hordein (Amer. Jour. Physiol,. 19 (1907), No. 1, pp. 117–124); legumin from the pea (Jour. Biol. Chem. 3 (1907), No. 3, pp. 219–225); glycinin from the soy bean (Amer. Jour. Physiol., 19 (1907), No. 4, pp. 468–474); the crystalline globulin of the squash seed (*Cucurbita maxima*) (Amer. Jour. Physiol., 19 (1907), No. 4, pp. 475–481); amandin from the almond (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 470–476); the proteins of maize (*Zea mays*) (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 477–493); gliadin from rye (Amer. Jour. Physiol., 20 (1908), No. 4, pp. 494–499); vicilin from the pea (Jour. Biol. Chem., 5 (1908), No. 2–3, pp. 187–195); legumelin from the pea (Jour. Biol. Chem., 5 (1908), No. 2–3, pp. 197–205); fish muscle (Amer. Jour. Physiol., 23 (1908) No. 2, pp. 81–89); vitellin from the hen's egg (Amer. Jour. Physiol, 24 (1909), No. 1, pp. 153–160); muscle of scallop (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 161–169); crystalized albumen from hen's egg (Amer. Jour. Physiol., 24 (1909), No. 2, pp. 252–262); ox muscle (Amer. Jour. Physiol., 24 (1909) No. 5 pp. 437–446); casein (Jour. Biol. Chem., 9 (1911), No. 3–4, pp. 333–353); and wheat gliadin (Jour. Biol. Chem., 9 (1911), No. 5, pp. 425–438).

These very important investigations are too extended for summary here.

Supplementing his work on the cleavage products of protein T. B. Osborne and H. G. Wells (Jour. Infect. Diseases, 8 (1911), No. 1, pp. 66-124) have studied the biological reactions of the vegetable proteins, using the globulin from castor bean, flax seed, and squash seed, edestin from the hemp seed, excelsin from the Brazil nut, proteins from the cocoanut, legumin from the vetch, legumin and vicilin from the pea, vignin from the cowpea, glycinin from the soy bean, gliadin from wheat and rye flour, hordein from barley, and zein from maize. All of these were found to produce typical anaphylaxis in sensitized animals, the condition possessing all of the characteristics which are present when anaphylaxis is produced with serum or other animal substances containing soluble proteins.

It was found that considerable differences in toxicity were produced by the various proteins. "The most toxic proteins, as measured by the frequency of severe and fatal reactions, were the globulin of the squash seed, vignin, excelsin, and castor-bean globulin, which usually caused death when given in 0.1 gm. doses to properly sensitized animals. Edestin caused the least severe reactions of any of the proteins, while hordein and glycinin seldom caused fatal reactions; nevertheless, the minimum sensitizing and intoxicating doses of edestin and squashseed globulin are essentially the same."

The experiments showed, furthermore, that where continuous feeding was done with the proteins, the guinea pigs became immune to the proteins and could not be sensitized to them. There was a marked specificity shown within certain limits by the proteins, and a close similarity, if not identity, of the legumins of the pea and vetch and the close relation to the vicilin of the pea was shown by the interaction of these proteins. The probable identity of the gliadin from wheat and rye, or at least their near reaction, was also established. "In some instances doubtful results were obtained, for example, with some guinea pigs castor-bean globulin and flax-seed globulin interacted strongly, while with others similarly treated, no reactions were obtained."

The structure of proteids, enzyms and their relation to biological problems, and related questions are discussed in a paper by R. H. Chittenden (Science, n. ser., 27 (1908), No. 685, pp. 241–254).

D. D. Van Slyke and P. A. Levene (Proc. Soc. Expt. Biol. and Med., 6 (1908), No. 1, pp. 11-13) have reported studies of the cleavage products of plastein, the "protein-like substance or substances precipitated from concentrated albumose solutions by the action of enzyms." Their results "indicate that the plastein is related to the higher albumoses, and apparently, from the resistance of alkali, to the antialbumoses rather than to the native proteins."

T. B. Robertson (Jour. Phys. Chem., 13 (1909), No. 6, pp. 469–489) reported data which showed that the concentration of casein solutions can be very accurately studied by determining their refractive indices. His investigations are discussed at length. He later reported the results of studies of the refractive indices of solutions of certain proteins including the para-nucleins (Jour. Biol. Chem., 8 (1910), No. 4, pp. 287–295), serum globulin (Jour. Biol. Chem., 8 (1910), No. 6. pp. 441–448), casen in alcohol-water mixtures (Jour. Biol. Chem., 8 (1910), No. 6 pp. 507–511), and gliadin (Jour. Biol. Chem., 9 (1911), No. 3-4, pp. 181–184).

Cooking in its Relation to Nutritive Value.

The chemical changes involved in cooking processes have been investigated with a considerable number of materials.

Some data regarding army rations, field ranges, ovens, fireless cookers, the kitchen touring car, portable gas cooker for army use, etc., are included in a report of the U. S. Commissary General, H. G. Sharpe (Rpt. Commis. Gen. [U. S. Army], 1909, pp. 11–15).

Suggestions for a diet kitchen equipment, particularly with reference to naval hospitals, are presented in a paper by W. Wierzbicki (U. S. Naval Med. Bul., 4 (1910), No. 2, pp. 161–163, dgms. 2).

Studies of housekeeping efficiency as a private enterprise, by C. Barnard (Housekeeping Expt. Sta. [Conn.] Bul. 11, pp. 20, pls. 3) form the basis of a discussion of the increased efficiency through correct house planning, the use of conveniences and labor saving devices, the elimination of needless work, and similar questions. Some data are recorded regarding the labor involved in performing a definite household task by different methods.

Studies of the supposed connection between protein coagulation and the heat shortening of muscles were reported by E. B., Meigs (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 178–186 dgms. 6), and are interesting not only from the standpoint of physiological chemistry but also because of their possible bearing on the changes which take place in animal foods during cooking processes. The facts reported, as the author points out, do not preclude "the possibility that the precipitation of protein from its solutions and the shrinkage of animal tissues under the influence of heat may be fundamentally more or less similar processes. They do show, however, that the shortening of striated muscle at temperatures above 50° is independent of the coagulation of myogen, and they make it seem probable that the heat shortening of most animal tissues is dependent, not on the aggregation of the particles of coagulable protein, but on some other process."

Elizabeth C. Sprague and H. S. Grindley (Univ. Ill., Univ. Studies, 2 (1907) No. 4, pp. 37, pls. 4, dgms. 10) studied the cooking of beef with a view to formulating methods which would give uniform results. In connection with this work, temperatures were recorded of the interior of the beef during cookery. If the temperature ranges from 55 to 65°C., the beef will be underdone or rare and red in color. At a temperature of 65 to 70°, it sill be medium underdone, and at a temperature of 70 to 80°, it will be well done.

In connection with his work showing the palatability and wholesomeness of spleens, and studies of their preparation for the table, E. T. Williams (Amer. Med., n. ser., 2 (1907), No. 9, pp. 522, 523) points out that although the raw spleens do not keep well, the cooked material, particularly boiled, has excellent keeping qualities. Attention is directed to the high iron and phosphoric acid content of spleens.

The economical use of meat in the home and many questions which have to do with the nutritive value of meat and the preparation of meat for the table, have been discussed in a popular summary by C. F. Langworthy and Caroline L. Hunt (U. S. Dept. Agr., Farmer's Bul. 391, pp. 43 II).

The question of cooking the cheaper cuts of meat is considered on the basis of the author's experimental study of the problem,

by C. Barnard (Housekeeping Expt. Sta. [Conn.] Bul. 6, pp. 17, dgm. 1).

The results of an extended series of artificial digestion experiments on starch of different sorts as affected by cooking were reported by Edna D. Day (U. S. Dept. Agr., Office Expt. Stas. Bul. 202, pp. 42, figs. 6) in a bulletin of the Office of Experiment Stations. Different sorts of ferments were used with potato, wheat, corn, and other starches. The conclusion was reached that potato, arrowroot, and probably tapioca and sago starches, are made not more easily digestible by long continued cooking, while the reverse is true with cereal starches, though the changes occur very slowly. In general, the experimental data reported are discussed with reference to household problems.

The effect of cooking on cellulose was studied by Edna D. Day (Jour. Home Econ., 1 (1909), No. 2, p. 177), who did not find that cell walls of potatoes when boiled or baked are ruptured, as is generally stated to be the case. When cells from cooked potatoes were examined, it was found that the middle lamella which holds the cells together had dissolved and that the cells had separated from each other, but the cell walls were not ruptured. "If, however, saliva is added to these unbroken cells, the starch filling them is very quickly digested, as shown by the fact that they no longer give the blue color with iodin, proving that the breaking of the cell wall is not at all essential for ease of digestion."

Studies of the etiology of pellagra reported by W. H. Buhlig (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 417–435, figs. 2), particularly with reference to the possible connection of Indian corn with this disease, did not lead to definite results. In connection with culture tests with moldy corn, some cooking tests were made, as certain molds are known to be resistant to heat, and the idea has been advanced that such enzyms may survive cooking. Corn meal mush and hominy made in the usual way, by boiling about 2 hours, was found to be sterile.

In other experiments carried on in the same public institution as Buhlig's work, the question was further studied by J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8), who repeatedly found in corn meal and

hominy a spore-bearing bacterium which survived steaming for 2 hours.

A brief note on the effects of adding sugar to acid fruit at the beginning and end of the cooking period was published by Edna D. Day (Jour. Home Econ., 2 (1910), No. 1, p. 94), as the result of tests with cranberries, grapes, and apples, and also of the comparative sweetness of solutions of the same strength of cane sugar and a mixture of levulose and dextrose.

The conclusion is reached that "in cooking such fruits as apples, cranberries, and grapes, while the product is slightly less sweet if the sugar is added at the beginning than it is if it is added at the end, still the difference is too small to be of practical importance."

The problems of cookery at high altitudes (diminished air pressure) are discussed on the basis of experiments, by Mrs. A. Anderson (Boston Cooking-School Mag., 14 (1910), No. 8, pp. 372, 373, XVI, XVIII, XX). Data on this subject have been summarized in a recent paper (Jour. Home Econ., 3 (1911), No, 2, pp. 176–178).

According to experiments briefly reported by Olive G. Patterson and Clara C. Benson (Jour. Home Econ., 2 (1910), No. 6, pp. 656, 657) on the setting of gelatin, this material may be freed from its mineral matter and tyrosin-holding impurities without affecting the gelatinizing power of its solutions. Boiling for 1 hour did not prevent gelatinization though long-continued boiling diminished it. With respect to the effect of citric and acetic acids, it was found that 4 per cent gelatin solutions containing citric acid to a concentration of 1 per cent would gelatinize in the cold after 15 minutes' boiling, but that after 10 minutes' boiling of a 3 per cent solution with 0.5 per cent citric acid the gelatinizing power had considerably decreased.

The question of bread has received less attention from investigators than it did a few years ago.

The relation of yeast to flavor in bread has been studied experimentally by Ruth A. Wardall (Jour. Home Econ., 2 (1910), No. 1, pp. 75–91), who concludes that the flavor of bread can not be determined by yeast and possibly is not even affected by it.

Since for obvious reasons the time allotted for fermenting bread is short, she regards it as quite possible that an insufficient opportunity to develop flavor is given.

Some experiments were also made upon the effects of adding malt extract to bread dough.

The leavening agent in salt-rising bread was studied experimentally by Winona Woodward (Jour. Home Econ., 3 (1911), No. 1, pp. 100, 101), who isolated an organism which was not a yeast.

H. A. Kohman (Nat. Assoc. Master Bakers [Proc.], 13 (1910), pp. 29–37, fig. 1) studied salt-rising bread making, reaching the conclusion that the fermentation is due to a bacterium and not a yeast. The bacterium was isolated and studied in pure culture.

Among general discussions of bread may be mentioned a paper by M. E. Jaffa (Nat. Baker, 14 (1909), No. 166, pp. 52, 54), which devotes considerable attention to the use of raisins in bread making.

A large amount of data regarding the character and nutritive value of bread of different sorts and similar topics is included in a popular summary, entitled "Bread and Bread Making," by Helen W. Atwater (U. S. Dept. Agr., Farmer's Bul. 389, pp. 47, figs. 7).

A large amount of data has been reported regarding the principles and practice of ice cream making, by R. M. Washburn Vermont Sta. Bul. 155, pp. 92, dgm. 1). The work is based on an exhaustive study of the subject, particularly from a commercial standpoint.

The question of cooking naturally involves the relative value of different kinds of equipment and other similar topics.

Labor and money-saving appliances are discussed in a publication of the American School of Home Economics (Bul. Amer. School Home Econ., Ser. 1, 1908, No. 11, pp. 47, figs. 54), and numerous other discussions have appeared in journals and reports which have to do with such topics.

The matter of fireless cookers has been studied by a number of investigators.

Fireless cookers of special construction have been tested in connection with experiments on the preparation of food made

by the subsistence department of the U. S. Army. This work and data regarding foods supplied in the Philippines are reported by H. G. Sharpe (Rpt. Commis. Gen. [U. S. Army], 1907, pp. 10–14).

The value of different materials for the construction of fireless cookers and the effects of amounts and density of material upon the conservation of heat were studied experimentally by Ellen A. Huntington (Bul. Univ. Wis., No. 217, pp. 38, figs. 10), the article as a whole being an interesting contribution to the subject.

The construction and use of the fireless cooker from a practical standpoint have been studied by Caroline B. Lovewell, Frances D. Whittemore, and Hannah W. Lyon (Topeka, 1908, pp. 211, figs. 11).

A similar summary is also included in the volume dealing with fireless cookers prepared by Margaret J. Mitchell (New York, 1909, pp. XII + 315, figs. 18).

Canning, Preserving, Handling and Storage

The question of canning and preserving food, the changes brought about by cold storage, and related matters have been studied by a number of investigators.

An exhaustive summary of data regarding the question of storage of food products in the District of Columbia is contained in the U. S. House of Representatives report (Report of hearings on H. R. 16925, to regulate the storage of food products in the District of Columbia—Washington: U. S. House of Representatives Committee on District of Columbia, 1910, pts. 1–14, pp. 1–279).

The gases contained in swollen canned goods were studied experimentally by F. O. Tonney and J. B. Gooken (Amer. Food Jour., 3 (1908), No. 6, pp. 20–23, figs. 3). In general, the authors note that the presence of nitrogen indicates putrefaction and carbon dioxid, fermentation, the two processes being often found to be distinct from each other.

G. W. Shaw (California Sta. Circ. 33, pp. 4–8, figs. 3) compared cane sugar and beet sugar with a view to determining whether or not there is ground for the belief that beet sugar is inferior to

cane sugar for jelly making and preserving purposes. His conclusion was that such is not the case and that beet sugar is entirely satisfactory for such purposes.

Information regarding packing house methods, shipping, keeping quality, and similar subjects is included in a summary of the results of field investigations in pomology by G. H. Powell (U. S. Dept. Agr., Bur. Plant Indus. [Circ.], June 7, 1907, pp. 4).

In a volume by G. T. Hamel, entitled "Modern Practice of Canning Meats" (St. Louis, 1911, pp. 100, figs. 19, dgm. 1), the theories of canning are discussed, equipment described, and recipes and formulas given. The subject is considered from the standpoint of the small plant as well as from that of the large establishment.

The principles of canning are discussed and directions for canning a large number of fruits and vegetables and for pickling and preserving meats and fish are included in a bulletin by G. McCarthy (N. C. Dept. Agr., Biol. Div., 1907, pp. 37), designed for the use of housekeepers.

An extended summary of statistics regarding canning and preserving fruits and vegetables, fish, and oysters was presented by E. K. Ellsworth (Bur. of the Census [U. S.] Bul. 61, pp. 9–48), in a publication of the U. S. Census Bureau.

The culinary qualities of dehydrated eggs, fruits, vegetables, and milk were reported upon by H. A. Dent (Navy Dept., Bur. Supplies and Accts., Mem. Inform. Off. Pay Corps) [etc.], No. 85, pp. 626, 627), of the U. S. Navy Department.

The general question of canning vegetables in the home is discussed in a popular summary prepared by J. F. Breazeale (U. S. Dept. Agr., Farmers' Bul. 359, pp. 16, figs. 9), of the Bureau of Chemistry.

A. W. Bitting (U. S. Dept. Agr., Bur. Chem. Bul. 119, pp. 37, pls. 2, figs. 5) discusses the problem of catsup making in connection with an experimental study of the spoilage of tomato catsup.

The cause of cloudy liquor on peas was investigated by E. W. Duckwell (Canner and Dried Fruit Packer, 29 (1909), No. 1, pp. 34, 36), who reached the conclusion that it was caused by starch from the peas and overheating in canning.

A popular summary of data on canning peaches has been published by H. P. Gould and W. F. Fletcher (U. S. Dept. Agr., Farmers' Bul. 426, pp. 26, figs. 14), of the Bureau of Chemistry.

A study of the preparation of sugared and dried pineapple has been reported by H. C. Gore (U. S. Dept. Agr., Bur. Chem. Circ. 57, pp. 8, fig. 1).

An experimental study of packing prunes in cans, with satisfactory results, was also reported by G. W. Shaw (California Sta. Circ. 33, pp. 1–3).

Studies of jelly and jelly making have been reported by Nellie E. Goldthwaite (Jour. Indus. and Engin. Chem., 2 (1910), No. 11, pp. 457–462, fig. 1). A number of small fruits were used as well as orange juice, skins, and whole fruit.

According to the author's summary, "in what is usually a waste product (the white inner skins of oranges and lemons) we have an abundant source of pectin from which excellent jelly can be made if properly acidified. . . .

"It was noteworthy that the purest pectin yet prepared in this research was obtained from oranges and lemons. It was isolated . . . and was reprecipitated three times. By long manipulation of the precipitated pectin (supported on a very fine cloth suspended from the corners) the liquid was so completely worked out of the substance that a powdery white body, somewhat starch-like in appearance, was obtained. This was dried in a current of dry hydrogen over sulphuric acid.

"Ash determinations of orange pectin so obtained showed less than 0.5 per cent of ash—of lemon pectin about 3.5 per cent. . . . No melting point of this pectin could be obtained, but the substance, when out of contact with air, chars strongly at 170°C. It is hoped to continue this work on the isolation and examination of pure pectin."

A chemical-physical study of jelly making was carried on at the Florida Experiment Station, by J. Belling (Florida Sta. Rpt. 1908, pp. CV–CIX), the investigations having particularly to do with the influence of preliminary heating, final temperature, and the percentage of water, sugar, and acid upon appearance and quality of guava jelly.

"In boiling of guava jelly, some acid (the natural acid of the ripe fruit) is absolutely necessary to change much of the sucrose into invert sugar, and if this does not take place then the sucrose crystallizes out. Too much acid (and probably too prolonged boiling) seems to make the jelly sticky from the excess of invert sugar, and also to alter the pectin so that it will not gelatinize. . . .

"The depth of color seems to be increased by additional amount of acid, prolonged boiling, and higher temperature at which the boiling is stopped."

Experiments of great value with reference to the handling and marketing of fruits have been carried on at the Department of Agriculture and elsewhere.

Many questions which have to do with the preparation for shipment and marketing in fresh condition of fruits of different sorts have been studied in a paper by A. V. Stubenrauch (U. S. Dept. Agr. Yearbook 1909, pp. 365–374, pls. 3), of the Bureau of Plant Industry.

The method of pre-cooling fruit for shipment is discussed by G. D. Kellogg (Cal. Fruit Grower, 40 (1909), No. 1120, p. 1) in comparison with results obtained by ordinary methods of shipment.

The problem of time and temperature in cold storage, particularly with reference to the Kieffer pear, was studied by G. H. McKay (Proc. N. J. Hort. Soc., 32 (1907), pp. 127–135).

Experiments on the processing of persimmons to render them nonastringent, carried on by H. C. Gore (U. S. Dept. Agr., Bur. Chem. Bul. 141, pp. 31, pls. 3, figs. 5), though regarding as preliminary, tend to show that carbonic acid gas may be substituted for saké fumes for this purpose with Japanese persimmons, and that, combined with the use of dry starch to prevent cracking of the fruit during the processing, should lead to the perfection and use of this method for the production of nonastringent persimmons which may be pared and eaten like an apple.

A. E. Vinson (Plant World, 10 (1907), No. 11, pp. 259–262) has studied exhaustively the composition of dates with special reference to stages of ripening, the possibility of stimulating ripening, and related questions. As a part of his work, a special study of the endo- and ektoininvertase of the date is reported by A. E.

Vinson (Jour. Amer. Chem. Soc., 30 (1908), No. 6, pp. 1005–1020). The invertase of the date, he notes, remains "insoluble in all ordinary solvents throughout its green stages, but becomes readily soluble on ripening. The change in the behavior of the invertase towards solvents coincides very closely in point of time with the passage of the tannin into the insoluble form."

The influence of chemicals in stimulating the ripening of fruits has been studied further by Vinson (Science, n. ser., 30 (1909), No. 774, pp. 604, 605), who found that dates could be successfully ripened by exposing them to a vapor of acetic acid for 12 or 15 hours. "At the end of this time they have become transparent nearly to the seed and will then ripen naturally without further treatment. The process can be accelerated by exposing them to sunshine, or more rapidly by heating for some hours to 45°C. The process, it is anticipated, will permit the shipping of dates green and ripening them at their destination as bananas are now handled. . . .

"After moderate treatment with acetic acid, the tannin of the date has not yet become entirely insoluble but all astringency disappears in the next few hours. The intracellular invertase, however, passes into solution to quite an appreciable extent immediately after the treatment, and probably other intracellular or insoluble catalytic agents are released simultaneously."

The fresh ripe date is very soft and will not bear shipment. The author believes that by treatment with acetic acid vapor, it may be successfully ripened after shipment, a deduction of much commercial importance.

Continuing his study of the stimulation of premature ripening by chemical means, the author has studied the effect of many other substances beside acetic acid vapor on the ripening of dates and finds that a comparatively large proportion, including among other proprionic acids, ethyl chlorid, chloroform, gasoline, ether, acetone, and volatile oils. A. E. Vinson (Jour. Amer. Chem. Soc., 32 (1910), No. 2, pp. 208–212).

Studies of the artificial ripening of dates by the aid of chemical substances were later reported in greater detail by A. E. Vinson (Arizona Sta. Bul. 66, pp. 403–435), and of ripening by incubation by G. F. Freeman (Arizona Sta. Bul. 66, pp. 437–456).

In a study of methods of canning meat with reference to proper disposal of defective cans, C. N. McBryde (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1907, pp. 279–296, fig. 1) draws a number of general conclusions regarding putrefactive and fermentative changes in the contents of cans, and similar matters.

Packing oysters with and without ice and similar questions were studied by the Indiana State Board of Health, (Mo. Bul. Ind. Bd. Health, 10 (1908), No. 11, pp. 134–136, fig. 1) with reference to the pecuniary loss to the consumer and the melting of ice in the oysters, analytical data being reported.

The possibility of preserving eggs with a number of substances, including sodium silicate of different grades, was studied by R. Berger (Jour. Indus. and Engin. Chem., 3 (1911), No. 7, pp. 493–495; Reprint, pp. 4; Pure Products, 7 (1911), No. 8, pp. 423–425), who reports data regarding the permeability of the shells of preserved eggs and their loss in weight when kept in the open air after preservation in comparison with unpreserved eggs.

Bacteriological studies which have to do with the infection and preservation of eggs were carried on by G. H. Lamson, Jr. (Connecticut Storrs Sta. Bul. 55, pp. 203–214, figs. 7) at the Connecticut Storrs Station, with reference to the cause of decomposition and sources of infection of eggs, the part played by temperature, and the precautions to be observed in preserving eggs.

The diastatic enzym of ripening meat was studied by A. W. Peters and H. A. Mattill (Jour. Biol. Chem., 6 (1909), No. 2, pp. XXIX, XXX). When muscle is autolyzed, the sugar becomes greater, they conclude, provided the meat is fresh and edible; otherwise, the amount diminishes.

The nature of so-called "black spots" on chilled beef was studied by E. Klein (Meat Trades' Jour., 30 (1909), Nos. 1113, p. 234; 1114, pp. 260, 261, figs. 2), who attributes much to the growth of a fungus (*Oïdium carnis*), which is described.

R. Hoagland (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1908, pp. 301–314), of the Bureau of Animal Industry, has studied the action of saltpeter upon the color of salted meat. The red color of uncooked salted meat, to which saltpeter has been added

as a preservative agent, is due, he concludes, to the presence of NO hemoglobin which is formed by the action on hemoglobin of nitric acid due to the reduction of nitrites within the meat. The reduction of saltpeter to nitrites takes place in the meat equally well in either an acid, or an alkaline medium. Neither saltpeter nor nitrites exercise a color-preserving action in meat. The brown color observed when meat is cured with an excessive amount of saltpeter is due to the action of nitrites upon hemoglobin.

In connection with a study of deterioration and commercial preservation of flesh foods, W. D. Richardson and E. Scherubel (Jour. Amer. Chem. Soc., 30 (1908), No. 10, pp. 1515–1564) report the results of a study of experiments on frozen beef, from which the conclusion is drawn that no decomposition is shown in frozen beef stored for a long period, judging by the values obtained for ammonia nitrogen, and hence that no bacterial decomposition occurred in the stored meat. They conclude further that the stored meat did not differ in flavor from the fresh meat and that cold storage below –9°F. is an adequate and satisfactory method for the preservation of beef for long periods.

W. D. Richardson and E. F. Scherubel (Jour. Indus. and Engin. Chem., 1 (1909), No. 2, pp. 95–102) also reported an extended series of analyses of meat kept at room temperature with and without preservatives and of meat stored at 2 to 4°C., and in some cases afterward frozen and held at –9 to –12°C.

"While there are some contradictory figures in the analyses of the samples which were frozen after being stored at 2 to 4°C., from the results the conclusion may fairly be drawn that freezing of meats at –9 to –12°C. arrests bacterial decomposition, but can not in any degree restore the product to its original condition."

The results of an extended study of the effects of cold storage upon beef and poultry are reported by A. D. Emmett and H. S. Grindley (Jour. Indus. and Engin. Chem., 1 (1909), Nos. 7, pp. 413–436; 8, pp. 580–597), as part of an experimental study of the chemistry of flesh. The samples included uncooked refrigerated beef stored for 22 days and for 43 days and frozen drawn and undrawn fowl. Tests were also made of the relative losses in the

cooking of refrigerated beef held in cold storage for varying lengths of time, and the chemical changes resulting therefrom. The cooked meats from the sample stored 43 days were higher in their moisture content than the sample stored 6 days and were therefore juicier, higher in soluble and insoluble dry substance, in nitrogenous, nonnitrogenous and total organic extractives, in fat, in total ash, and in soluble inorganic, total soluble, and total phosphorus. "Further, the percentages of total nitrogen, insoluble and total protein were practically the same as were those for the samples from the 6-day storage meat. Therefore, the cooked meats from the 43-day samples, judging from the chemical composition, were at least as nutritous as were those from the samples stored for the shorter period of time."

The dietetic value of refrigerated foods as a whole is discussed by S. Rideal (Cold Storage and Ice Trade Jour., 36 (1908), No. 4, pp. 32, 33) on the basis of experimental data. The action of diastase, he concludes, is not entirely prevented by cold, but is very much retarded. The tenderness and maturing of refrigeraced meat he attributes not only to the action of sarcolactic acid but also to the gradual and limited work of natural enzyms (pepsin and trypsin) which cause a certain amount of predigestion.

The changes which take place in chickens in cold storage have been exhaustively studied by Mary E. Pennington (U. S. Dept. Agr. Yearbook 1907, pp. 197–206, pls. 7), of the Bureau of Chemistry, which led to the general conclusion that both microscopic study and the taste of the cooked fowl confirmed the fact that microscopically visible degeneration does take place during long-continued storage.

The effects of different methods of handling and storing poultry, particularly with reference to the subject of drawing poultry, and the storage of poultry and eggs have been discussed by Mary E. Pennington (Ice and Refrig., 40 (1911), No. 2 pp. 59–62, charts 6; Nat. Provisioner, 42 (1910), Nos. 4, pp. 16, 23, 24; 5, pp. 23, 24; U. S. Dept. Agr., Bur. Chem. Circ. 64, pp. 42, figs. 9).

A chemical study of drawn and undrawn poultry kept in cold storage was also reported by W. F. Boos (Ann. Rpt. Bd. Health

Mass., 39 (1907), pp. 263-283), and a bacteriological examination of such poultry by H. R. Brown (Ann. Rpt. Bd. Health Mass., 39 (1907), pp. 285–336). According to Brown's conclusions, "decomposition depends largely upon the presence of moisture in the tissues, for moisture is absolutely essential to bacterial growth. In freshly killed birds, ordinarily or properly drawn, the surfaces quickly become dry. In cold storage birds, no matter how they are drawn, the tissues will be moist, because of the melting of the crystals of ice. If properly drawn, there would be but few bacteria present capable of causing decomposition."

Much attention has been devoted to the question of the relative wholesomeness of drawn versus undrawn poultry, by E. W. Burke (Amer. Food Jour., 3 (1908), No. 9, pp. 7–10).

A study of the effects of cold storage on eggs, quail, and chicken was reported by H. W. Wiley, et al. (U. S. Dept. Agr., Bur. Chem. Bul. 115, pp. 117, pls. 13), the general results being unfavorable to this process when long continued.

The effect of low temperatures on ground chicken meat was studied by H. W. Houghton (Jour. Indus. and Engin. Chem., 3 (1911), No. 7, pp. 497–505), in comparison with the original chicken meat. The chemical changes which apparently take place "are (1) slight variations in the case of moisture and other extract; (2) a small increase of ammonia, especially in the case of the light chicken meat; (3) a decided increase of water-soluble nitrogen, total solids, and organic extractives in the light chicken meat, with a slight decrease of the same constituents in the dark meat; (4) a decrease of coaguable nitrogen in both varieties of chicken meat during the first 30 days, followed by a rise which did not reach that of the fresh sample; (5) an increase of amino acids in both kinds of chicken meat, with an increase and decrease of the proteoses and peptones respectively in the light and dark chicken meat."

Chicken fat has been studied extensively at the Bureau of Chemistry. Mary E. Pennington and J. S. Hepburn (U. S. Dept. Agr., Bur. Chem. Circ. 75, pp. 11) report the occurrence of lipase in the crude fat of chickens, and find that it can resist freezing for as long a period as 89 months. (pp. 1–7).

The oxidation of chicken fat by means of hydrogen peroxid was studied by J. S. Hepburn (pp. 8–11), particularly with reference to the effect of prolonged freezing.

"The changes in the fat of chickens during prolonged freezing are similar to the changes called forth by oxidation of the fat with hydrogen peroxid. The Hehner number and the saponification number increase simultaneously, and aldehydes are formed. The increase in saponification number may, therefore, be ascribed to the formation of slightly lower homologues of the fatty acids of fresh chicken fat, while the increase in Hehner number is doubtless due to the formation of aldyhdes and ketones of high carbon content. These changes in the chicken fat in situ are probably produced by the action of enzyms."

The preparation of cod and other salt fish for the market was studied by A. W. Bitting (U. S. Dept. Agr., Bur. Chem. Bul. 133, pp. 63, pls. 6, figs. 4), of the Bureau of Chemistry, who also reports the results of a study of the cause of reddening in fish. This change was found to be due to a micro-organism, a remedy being extreme cleanliness.

The bulletin gives information regarding the composition of salt used in curing, losses in weight during curing, the amount of salt taken up by fish marketed in different forms, and variations in moisture and salt content due to season, style of packing, and other conditions.

L. W. Thomas (North Dakota Sta. Spec. Bul. 24, pp. 179-194, fig. 1) has reported a study of wrapped and unwrapped loaves, with reference particularly to moisture content and keeping quality. The general conclusions drawn are as a whole plainly in favor of wrapping bread, though, as the author points out, wrapping did not prevent loaves from becoming stale after 36 or 48 hours.

H. L. White continued this work by studying the moisture and acidity of samples of wrapped and unwrapped bread. According to his summary, bread made under cleanly conditions from good quality materials did not grow acid, when wrapped or unwrapped, even after 108 hours. Bread wrapped while warm and while hot showed a slight increase in acidity in the inside of the loaf.

C. A. A. Utt (Bul. Kans. Bd. Health, 7 (1911), No. 3, pp. 52–60) has also reported tests of the effect of wrapping bread upon its quality. In general, the unwrapped bread, when kept for 4 or 5 days, lost about twice as much moisture as the wrapped loaf, while the acidity remained practically the same. The wrapped bread was in edible condition for twice the ordinary period.

As pointed out by H. G. Bell (Amer. Miller, 37 (1909), No. 4, pp. 280, 281, fig. 1), in a paper on stored flour, fungi and bacteria are the chief destructive agencies. Flour of different grades was studied with reference to the presence of bacteria and as protection against the growth of these low forms of life, the author suggests storage in well-lighted rooms.

The changes in the weight of stored flour and butter were studied in detail by J. T. Willard (Bul. Kans. Bd. Health, 7 (1911) No. 1, pp. 9–14). The greatest loss in flours stored for a year was 0.79 lb. per sack of about 48 lbs. Loss of weight in butter was determined by the method of packing. Prints wrapped in parchment paper and placed in paraffin cartons, packed in cases, remained practically constant in weight. The loss in weight is chiefly due to loss of moisture by evaporation or in other ways.

Changes which take place in the composition of unground cereals during storage were studied by S. Leavitt and J. A. Le Clerc (Jour. Indus. and Engin. Chem., 1 (1909), No. 5, pp. 209–302). The investigations extended over 2 years. The results demonstrated that "there is more or less change in all cereals under the influence of aging. These changes seem to take place whether the cereal is stored in the whole grain or is ground to a fine powder before storage. In the latter case, however, the changes take place more rapidly. We notice that the principal products which seem most susceptible to change are first the sugars and then the 70 per cent alcohol-soluble proteins, the 5 per cent K_2SO_4-soluble protein and the water-soluble proteins coagulated by so-called Stutzer's reagent.

"Corn, barley, and oats are most subject to loss of sugar during aging. On the other hand, many samples of wheat show a slight loss the first year and then quite a rapid gain in the sugar content, in some cases a gain 24 per cent of the total sugar present being noted at the end of two years."

Dietary Studies and Dietetics

General dietary problems have been considered by many writers with a view to making the work of the laboratory available and useful to the housewife.

Methods of calculating the results of dietary studies and similar topics are discussed in a publication of the American School of Home Economics (Bul. Amer. School Home Econ., Ser. 1, 1909, No. 13, pl. 1, figs. 13), especially with reference to the use of the so-called 100-calorie-portion-method of calculating.

Emma S. Jacobs (Jour. Home Econ., 3 (1911), No. 2, pp. 162–168) in a discussion of family dietetics gives menus for what she believes accurately planned dietaries for families; and a table of weight and cost of protein and energy in different food materials designed to facilitate the computation of the nutritive value of such dietaries.

Food customs and diet in American homes have been discussed in a popular summary of data by C. F. Langworthy (U. S. Dept. Agr., Office Expt. Stas. Circ. 110, pp. 32), which proposes dietary standards for mineral constituents as well as for protein and energy, and discusses dietary standards as distinguished from physiological requirements.

Nellie M. Dickinson (Ill. Agr., 12 (1908), No. 5, pp. 142–145) gives data regarding the preparation of a day's ration designed to conform to dietary standards.

Information regarding dietary habits, food supply, and living conditions of native tribes often appears in descriptive articles, books of travel, reports of ethnological investigations, and other publications not directly concerned with nutrition, which is of importance in discussions of nutrition problems as well as of general interest. As illustrations of such work the following may be cited:

The food of natives of the upper Yukon has been described by F. Schmitter (Smithsn. Misc. Collect., 56, No. 4, pp. 6, 7). The diet of these natives consists of fish, game, and berries, supplemented at the present time by vegetables bought at local stores, though until recently they lived on animal food.

The Mackenzie River natives, it is pointed out, live almost exclusively on meat, and the author states that they are robust and healthy.

A study of the food supply of Pima Indians has been reported by F. Russell (Ann. Rpt. Bur. Amer. Ethnol., 26 (1904–5), pp. 66–92, figs. 7). These Indians subsist on a mixed diet in which vegetable food predominates, but it would seem probable that the proportion of meat used was greater in the past than at present.

Much information regarding the kind and amount of food used by native Indian tribes in the southwestern United States and northern Mexico is included in a physiological and medical study of the Indians by A. Hrdlicka (Smithsn. Inst., Bur. Amer. Ethnol. Bul. 34, pp. IX. + 460, pls. 28, figs. 2). Meat, corn, some vegetables, and other foods make up a simple mixed diet. Cooking processes are described as well as dietary habits and customs.

In a volume on "Mexico" (New York and London, 1909, pp. 213–218, pl. 1) C. R. Enock reports data regarding the Mexican peons, or country people. Corn meal, the native beans, fat, and meat when it can be obtained, are the principle articles of diet.

Of popular summaries which contain data of interest from the standpoint of diet may be mentioned a paper by C. W. Furlong (Harpers' Mo. Mag., 120 (1910), No. 716, pp. 217-229, pl. 1, figs. 7, map 1), which gives considerable information regarding the character of the diet of the natives of Tierra del Fuego, which consists almost entirely of the meat of wild animals, birds, the blubber from stranded whale, fish, and mussels.

In a later paper by Furlong (Harpers' Mo. Mag., 122 (1911), No. 732, pp. 813–827, pl. 1, figs. 9, maps 2) additional information is given on the subject, particularly regarding the food customs and living conditions of the Tehuelches of the Patagonian pampas. Apparently, these natives live very largely upon the meat of mares and game.

Less work pertaining to the food consumption of families and groups has been reported than in some other branches of dietetics.

From data regarding the food of a poor family in Buffalo and one in Boston published by Emma O. Lundberg (Survey 23, (1910), No. 20, pp. 728–730) the protein and energy in the daily food have been calculated.

As part of its nutrition investigations the Office of Experiment Stations has reported the results of 4 dietary studies of farmers' families in Vermont (J. L. Hills), 70 in mountain regions in Tennessee (C. E. Wait), and 14 in Georgia (H. C. White). The cost of nutrients and energy, peculiarities of the diet, adequacy of the food supply, and similar questions are discussed, the results being compared with earlier work of a similar nature. As a whole the bulletin supplies a large amount of statistical and other data regarding living conditions in rural regions, particularly those remote from large centers of population where conditions are very different from those which prevail in towns, cities, and farms which are otherwise situated. (U. S. Dept. Agr., Office Expt. Stas. Bul. 221, pp. 142, pls. 4).

The report of E. T. Wilson (Ann. Rpt. Isthmian Canal Com., 1910, pp. 323–325), the subsistence officer in charge of the subsistence department, Isthmian Canal Commission, contains data regarding the kind and amount of food served to laborers in the Panama Canal Zone. Using the average values for the composition of foods as purchased, it has been calculated that the European laborers' messes would supply 201 gm. protein and 5,428 calories energy per person per day, and the common laborers' kitchens 148 gm. protein and 4,680 calories energy. The amounts actually eaten were not calculated, as no data regarding the waste and refuse were available.

The scientific work organized under government auspices in the Philippines has provided an opportunity for important studies of the native dietary. E. D. Merrill (Philippine Jour. Sci., B. Med. Sci., 4 (1909), No. 4, pp. 219–223) has studied the principal foods used by the natives of Taytay, and H. Aron (Philippine Jour. Sci., B. Med. Sci., 4 (1909), No. 4, pp. 225–231) has studied their food from a physiological standpoint. Rice is the staple nonnitrogenous food and is supplemented by fish, fruits, and some similar foods. The composition of a number of sorts of food was determined.

The matter has also been studied by V. G. Heiser (Ann. Rpt. Bur. Health Philippine Islands, 1909, pp. 25–29), his paper being entitled "Diet and Nutrition of the Filipino People."

That the diet in public institutions may now be passed upon by an expert in a manner profitable to the institution as well as of interest to the investigator is one of the important results of the nutrition work of the last 25 years or more. This has been obtained very largely as a result of the numerous investigations of dietaries in general and public institutions dietaries in particular, carried on as a part of the nutrition investigations of the Office of Experiment Stations and related enterprises.

A report of work of this character issued recently by the Department of Agriculture contains the results of studies in a home for old ladies and an orphan asylum in Philadelphia (Emma Smedley and R. D. Milner) and in orphan asylums, homes for the aged, and a public home whose inmates are chiefly middle-aged or aged people, in Baltimore (H. L. Knight, H. A. Pratt, and C. F. Langworthy). On the basis of the data reported the dietaries are critically considered and some changes suggested. The general problem of the dietary of children and the dietary of aged persons is discussed at length particularly with reference to public institutions. (U. S. Dept. Agr., Office Expt. Stas. Bul. 223, pp. 98).

Considerable information is given regarding the character of the diet in a state hospital for the insane in Illinois in connection with a study of the occurrence of pellagra at the institution, carried on by J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8). On an average the simple mixed diet supplied approximately 30 gm. of protein and 2,000 to 2,500 calories per day.

In connection with an exhaustive and important investigation of the methods of fiscal control of state institutions carried on for the Sage Foundation, H. C. Wright (State Charities Aid Assoc. [N. Y.] Pub. 122, 1911, pp. 353) reports the calculated food supplied per man per year and per man per day in institutions in New York, Indiana, and Iowa, including hospitals for the insane, soldiers' homes, industrial schools, reformatories,

prisons, and institutions for the feeble-minded and for epileptics. The results are summarized as follows:

AVERAGE FOOD PER MAN PER YEAR AND NUTRITIVE VALUE OF DAILY RATION IN PUBLIC INSTITUTIONS

Location of institutions	Food per man per year		Food per man per day	
	Total amount	Cost	Protein	Energy
	Pounds		*Grams*	*Calories*
New York	1,227	$45.05	104.62	3,313
Indiana	1,176	43.03	98.78	3,429
Iowa	1,423	55.48	106.47	3,691

Numerous investigations of the food and diet of children have been made during the period under consideration. Though all that pertains to food from infancy to maturity is obviously of interest and value, special studies of infant feeding have not been included in this summary, since they are commonly regarded as pertaining to the subject of medicine rather than to dietetics.

The bearing of food during early life upon the normal development of the body is a question which has received attention from a number of investigators, the problem having been studied with young animals as well as with children.

In an investigation of this sort experimental studies with dogs were reported by H. Aron, together with the results of general observations on nursing children (Philippine Jour. Sci., B. Med. Sci., 6 (1911), No. 1, pp. 1-52, pls. 4, dgms. 5).

He concludes that "a growing animal which receives only sufficient food to keep its body weight constant, or to allow slight increase, is in a condition of severe scarvation. If by a restriction of food the increase in weight is inhibited, the skeleton grows at the expense of other parts of the body, expecially of the flesh. Most of the organs retain their weight and size, while the brain grows to reach its normal weight. The composition of the body—when at a constant weight—undergoes remarkable

changes. Fat is consumed more or less entirely, the quantity of protein, especially of the muscles but not of the organs, is diminished and a great proportion of the body tissues is replaced by water; thus, this water and the increase of the skeleton together replace the body materials lost. The caloric value of 1 gm. body weight of an animal which has undergone such a process to its extreme limit may amount to only one-third of the normal value.

"It is possible by supplying suitable amounts of food to maintain a dog in an emaciated condition, apparently in good health, and at the weight of a puppy, for nearly 1 year, while its weight at the end of the year should be 3 times as great. If such an animal is thereupon fed amply, it fattens and rounds out, but does not reach the size of a control animal which from the beginning has been normally fed. It is unable to make good the growth suspended by the long restriction of food.

"The 'growth' principally depends on the tendency to grow possessed by the skeleton. The skeleton loses its capability of growing in more advanced age regardless of the size which the animal has reached."

A paper of importance in considering the question of physiological requirement and dietary standard has been published by H. J. Waters (Proc. Soc. Prom. Agr. Sci., 30 (1909), pp. 70–98; Separate, pp. 29, figs. 6), who discusses the influence of nutrition upon the animal form, as the result of a large number of experiments made with farm animals (beef cattle). In general, the results clearly show that a decreased supply of nourishment in the young animals hinders body development. The matter is also taken up in a later paper (Quart. Rpt. Kans. Bd. Agr., 29 (1910), No. 113, pp. 59–86, figs. 7).

The relationship of food to physical development is discussed by D. McCay (Philippine Jour. Sci., B. Med. Sci., 5 (1910), No. 2, pp. 163–170), on the basis of his investigations, his conclusion being that there is a close relationship between the nutritive value of the diet, and particularly its protein content, and physical development. This he believes is clearly brought out in a comparison of the degree of nitrogenous interchanges of a number of native races, arranged according to the amount of nitro-

gen per kilogram of body weight. At the head of the list as regards physical development are the Nepalese Bhutias, with 0.42 gm., and the Tibetan and Bhotan Bhutias, with 0.35 gm., respectively, of nitrogen per kilogram of body weight and with a large amount of animal food in the diet, and at the bottom of the list are the Bengalis and Ooriyas, with 0.116 gm. of nitrogen per kilogram of body weight.

Few dietary studies with children have been reported in the United States during the period under consideration.

The general question of the feeding of young children has been discussed in a popular way by Mary Swartz Rose (Teachers Col. [N. Y.] Bul., 2. ser., 1911, No. 10, pp. 10) and by others.

The dietary studies made in orphan asylums by the Department of Agriculture (U. S. Dept. Agr., Office Expt. Stas. Bul. 223) have been referred to elsewhere. The report of this work contains a discussion of children's dietaries and proposed dietary standards.

An extended summary of data regarding the daily meals of school children was prepared by Caroline L. Hunt (Bur. of Ed. [U. S.] Bul. 3, 1909, pp. 62, pls. 3, dgm. 1) for the U. S. Bureau of Education. The paper is an important contribution to the subject, not only from the material it brings together, but also because of the suggestions it makes regarding the rational feeding of school children.

School luncheons have also been studied under the auspices of the Women's Educational and Industrial Union (Ann. Rpt. Women's Ed. and Indus. Union, 29 (1908), pp. 34, 35).

Lillian D. Wahl (Charities and Commons, 20 (1908), No. 11, pp. 371–374) has reported data on this subject, particularly regarding attempts made to supply food to children in some of the New York public schools.

Contributions to the school luncheon problem are also made by Marion Bell (Boston Cooking-School Mag., 12 (1908), No. 6, pp. 292, 293) in an article describing a luncheon cooked and served in the Honolulu Normal School.

An important contribution to the general question is the study of malnutrition of children in New York public schools made by E. M. Sill (Jour. Amer. Med. Assoc., 52 (1909), No. 25, pp. 1981–

1985). He found that 83 per cent of the 210 cases observed depended practically for their diet on bread with tea or coffee. The importance of a highly nutritious dietary, with a large amount of protein, is recognized, and suggestions given regarding the preparation of such a diet.

Continuing his work regarding the diet of undernourished school children in New York City (Jour. Amer. Med. Assoc., 55 (1910), No. 22, pp. 1886–1891), Sill studied the dietary of 28 families with malnourished children in the thickly congested districts of New York City and of 6 families in more comfortable circumstances but where the children were also undernourished.

In the first group the food cost 19 cents per man per day, and supplied 95 gm. protein, 68 gm. fat, and 407 gm. carbohydrates, the fuel value being 2,614 calories. The families were engaged in active or moderately active muscular work.

In the fairly well-to-do families the diet cost on an average 35 cts. per person per day, and supplied 149 gm. protein, 115 gm. fat, and 569 gm. carbohydrates, the fuel value being 3,884 calories. The families were engaged in moderately active work.

Where the dietaries were up to or above the standard the malnutrition was attributed to such factors as close quarters, over crowding, eating candy between meals, tuberculous infection, enlarged tonsils, or other similar cause. The author states that in his experience such children contract disease much more easily and have less resistance than well-nourished children.

Information regarding undernourished children in New York City was collected by Frances Perkins (Survey, 25 (1910), No. 1, pp. 68–72). According to her summary, "physical disabilities of one kind or another are closely associated with malnutrition, and make it doubly dangerous."

W. C. Hollopeter (Jour. Amer. Med. Assoc., 53 (1909), No. 21, pp. 1727–1730), who has studied the character of breakfasts of over 2,000 school children, has also contributed important information concerning existing conditions with reference to children's diet.

Numerous plans for providing proper food for school children have been proposed, particularly with reference to needy or undernourished children.

A luncheon project which has proved successful in Philadelphia is described by H. H. Bonnell (Starr Center Assoc. [Rpt.] 1909, pp. 18–20, fig. 1), the different articles being sold for a penny.

The subject of penny luncheons for school children in the thickly congested districts of Philadelphia is discussed further by Alice C. Boughton (Psych. Clin., 3 (1910), No. 8, pp. 228–231, fig. 1).

Data regarding the serving of penny lunches to school children in Boston are reported by Ellen H. Richards (Jour. Home Econ., 2 (1910), No. 6, pp. 648–653).

A school luncheon costing one cent per person, which furnishes, in round numbers, 9 gm. of protein, is described by A. L. Benedict (Dietet. and Hyg. Gaz., 23 (1907), No. 7, p. 404).

Some work has been done with older students and pupils.

Daily menus for the school year are presented, together with the results of a dietary study for 1 month, in a report issued by the Institute for Colored Youth (Teachers' Training School) (Cheyney, Pa., 1909, pp. 48). The work was done as a part of a project to prepare at reasonable cost a rational diet with protein and energy in accordance with commonly accepted dietary standards.

Agnes Hunt (Ill. Agr., 12 (1908), No. 5, pp. 146–148) reports data regarding the nutritive value and cost of food served in a students' boarding-house.

From data reported by P. R. Kellar (Cooking Club Mag., 12 (1910), No. 11, pp. 10, 11), regarding the diet in a students' boarding house in the University of Minnesota, the daily food which cost 22 cts. per man per day was calculated to supply 105 gm. protein and 3,715 calories of energy.

Of special investigations of dietary problems the following may be cited.

The possible relation of diet to fatigue, particularly a diet containing the usual average amount of protein, is discussed by I. Fisher (Bul. Com. One Hundred Nat. Health [Washington], 1909, No. 30, pp. VIII+138) in a report on national vitality, its wastes, and conservation. The author is of the opinion, from experiments which he has made, that there is a

relationship between protein consumption and the occurrence of fatigue.

Studies carried on by P. A. Shaffer (Amer. Jour. Physiol., 22 (1908), No. 4, pp. 445–455) with healthy men support the belief that with sufficient food either an increase or a decrease of muscular activity within physiological limits has per se no effect upon protein metabolism, as indicated by the nitrogen and sulphur partitions in the urine. The investigation as a whole was undertaken to secure data regarding diminished muscular activity and protein requirement.

The effect of an ash-free diet was studied experimentally by H. W. Goodall and E. P. Joslin (Trans. Assoc. Amer. Physicians, 23 (1908), pp. 92–106) with healthy individuals, for experimental periods of 13 and 9 days, respectively. The results obtained do not indicate any marked changes in metabolism ascribable to the ash-free diet.

A contest entered into for a wager, in which 48 men endeavored to carry on the back a weight of 100 lbs., for approximately 10 miles, furnished results which were discussed with reference to the strength and endurance of the men by C. F. Langworthy (Science, n. ser., 33 (1911), No. 853, pp. 708–711). Of the number who entered the contest 6 completed the task, while the others dropped out at various stages. The information collected from a number of the men showed that they lived on a simple mixed diet. The energy expended in moving the body and carrying the load over the course was calculated to be 1,137 calories on an average for the 6 successful contestants, of which amount 707 calories would represent the energy expenditure for motion of forward progression and 430 calories for energy expended in moving the load.

Similar calculations for individuals and for groups are reported.

It seems fair to conclude that the men who engaged in the contest were, as regards their food, their occupation, and their general living conditions, representative of a very large group of our population who are living comfortably and meeting their daily obligations in a creditable manner, who are, in fact, living the average life of the average man, with its varied activities and interests.

In so far as the recorded data throw light on the subject, they indicate that the average man living the average life is capable of meeting body demands of considerable severity—a conclusion which perhaps few would question, but which it is interesting to consider in the light of numerical data.

DIGESTION

Studies of thoroughness of digestion have been reported as has work on various details of the general question. The tendency seems to be toward the investigation of special topics rather than the digestion as a whole. Experiments have also been made with reference to ease of digestion, which involve the use of the respiration calorimeter. (See p. 73).

Colloid-chemical aspects of digestion are considered by J. Alexander (Jour. Amer. Chem. Soc., 32 (1910), No. 5, pp. 680–687), who reports ultramicroscopic observations. The author's general conclusion is that chemical analysis alone is not sufficient to express the digestibility and availability of food and that our consideration of such problems must be broadened.

The absorption of fat was studied by L. B. Mendel (Amer. Jour. Physiol., 24 (1909), No. 5, pp. 493–496) who used samples stained with Sudan III. He concludes that "when fat stained with water-soluble dyes, like Sudan III, is fed, the pigments readily pass into the lymphatic vessels and thereby reach the blood stream. Since these compounds are soluble in free fatty acids as well as in neutral fats, their presence in the lymph cannot be taken as evidence either for or against the possibility of the digestion of fats prior to their absorption."

The effect of the presence of carbohydrates upon the artificial digestion of casein was studied by Nellie E. Goldthwaite (Jour. Biol. Chem., 7 (1910), No. 2, pp. 69–81), the recorded data showing, according to the author, that each of the carbohydrates tested (glucose, maltose, dextrose; dextrin, and galactose) retarded the digestion of casein, the retardation being proportional to the amount of added carbohydrate.

The results of experiments by C. H. Neilson and D. H. Lewis (Jour. Biol. Chem., 4 (1908), No. 6, pp. 501–506, fig. 1) on the

effect of diet on the amylolytic power of saliva led them to conclude that "there is a change either in the amount of ptyalin or in its activity, or in the concentration of the saliva, which enables more or less starch to be digested with a given quantity of saliva according to the diet. . . . Whether this change in the amylolytic power of the saliva due to diet should really be called an adaptation to diet is immaterial."

G. Lusk (Amer. Jour. Physiol., 27 (1911), No. 5, pp. 467, 468) has summarized data accumulated in connection with some of his earlier experiments, which have to do with the questions as to whether dextrose arises from cellulose in digestion, the conclusion being that such is not the case.

According to the conclusions reached by J. H. Pratt and L. H. Spooner (Trans. Assoc. Amer. Physicians, 25 (1910), pp. 614–635) in a study of the internal functions of the pancreas in carbohydrate metabolism, there is a rapid decrease in the power to assimilate glucose after the onset of atrophy of the pancreas.

Using dogs with Pawlow fistules, N. B. Foster and A. V. S. Lambert (Proc. Soc. Expt. Biol. and Med., 4 (1906), No. 1, p. 13) studied the influence of water on gastric secretion and the chemical affinity of mucus for hydrochloric acid in the stomach.

"It was observed that with definite amounts of cracker meal as food, the amount and rate of gastric secretion depend to some extent on the amount of water given the dog with his meal, i.e., when small amounts of water are given, the secretion is slow and scanty. If larger quantities of water are mixed in the food the secretion is more abundant.

"The degree of acidity of gastric juice depends upon the amount of secretion. When this is considerable it is much more acid than when the secretion is scanty. . . . The proportion of free acid depends upon the amount of mucus secreted, since mucus protein like other proteins combines with HCl. Mucus in the presence of pepsin combines with HCl to a considerable extent and undergoes digestion, with formation of proteoses."

The results of a large number of experiments carried on by the Office of Experiment Stations coöperating with the Bureau of Animal Industry on the digestibility of cheese of different

sorts have been reported in a summary prepared by C. F. Doane (U. S. Dept. Agr., Bur. Anim. Indus. Circ. 166, pp. 22). In the first series there were 184 tests with 65 young men serving as subjects, and in the second series about 50 experiments. American cheese made by the regular Cheddar process, with varying amounts of rennet and cured for different lengths of time and ripened under controlled conditions, was used in the tests, as well as a number of other sorts of cheese, with a view to determining whether thoroughness of digestion was influenced by the kind of cheese, by the degree of ripeness, and by similar factors.

In general, cheese of all sorts was found to be very thoroughly digested and little or no difference in the comparative digestibility of cheese at different stages of ripeness was observed. It was also found that different kinds of cheese closely resembled cheese made by the Cheddar process in thoroughness of digestion, and, in general, "that all kinds of cheese, even the very high-flavored and so-called condimental cheeses, have a high food value."

Brief statements are also made regarding the experiments on the ease of digestibility of cheese in which the respiration calorimeter was used.

The digestibility of white of egg as influenced by the temperature at which it is coagulated was studied by P. Frank (Jour. Biol. Chem., 9 (1911), No. 6, pp. 463–470, dgms.2). The progress of the hydrochloric acid action and the total digestion is most rapid in the albumin not heated beyond 75°C.

The results are reported of 66 natural and 99 artificial digestion experiments with meat undertaken by H. S. Grindley, T. Mojonnier, and H. C. Porter (U. S. Dept. Agr., Office Expt. Stas. Bul. 193, pp. 100), to determine the ease and thoroughness of digestion of different kinds and cuts of meat cooked in a variety of ways. As regards thoroughness of digestion, the results do not indicate that very appreciable differences exist, and meats of all kinds and cuts can be classed with the very digestible foods, about 98 per cent of the protein and fat being retained in the body on an average.

The erepsin of cabbage has been studied by Alice F. Blood (Jour. Biol. Chem., 8 (1910), No. 3, pp. 215–225). It splits

tryptophan from Witte's peptone and casein, and tyrosin from peptone "Roche." It clots milk and liquefies gelatin. It does not digest fibrin, coagulated egg white, or edestin in neutral, acid, or alkaline solution, or in the presence of HCN. Other characteristics are given.

An extended study of the digestibility and nutritive value of legumes was carried on by C. E. Wait (U. S. Dept. Agr., Office Expt. Stas. Bul. 187, pp. 55), of the University of Tennessee, in connection with the nutrition investigations of the Office of Experiment Stations. In general, kidney beans, white beans, and cowpeas of different sorts were found to supply from 70 to 83 per cent digestible protein and from 87 to 96 per cent digestible carbohydrates. When the digestibility of a diet containing a considerable quantity of beans was considered, rather than that of the beans alone, the values were higher. The experiments as a whole demonstrate the great nutritive value of cowpeas.

A number of foods, particularly tropical fruits and vegetables, were analyzed by L. H. Merrill (Maine Sta. Bul. 158, pp. 219–238), of the Maine Experiment Station, and digestion experiments with hulled corn reported. These show in general that the digestibility of protein and the availability of energy are low in comparison with results obtained with white bread. Analysis of corn before and after popping showed that except for loss of water little change in composition was produced by this process.

Studies of digestibility of carbohydrates of marine algæ by Mary D. Swartz have been referred to elsewhere.

Metabolism; Respiration Calorimeters, Bomb Calorimeters, and Experiments with Them

A fairly large proportion of the work reported during the period under consideration has had to do with the metabolism of nitrogen and other constituents, including numerous studies of the metabolism of the income and outgo of energy made with the respiration calorimeter, and with special studies of body excretions, the influence of different food constituents

upon protein consumption, the effects of water drinking, and similar topics.

The average daily excretion of uric acid of 10 men in normal condition, ranging from 19 to 29 years, and fed a normal diet, was determined by P. J. Hanzlik and P. B. Hawk (Proc. Soc. Expt. Biol. and Med. 6 (1908), No. 1, pp. 18, 19, and found to be 0.597 gm., a value somewhat lower than the generally accepted average of 0.7 gm.

"The average daily protein ingestion from these same subjects, when permitted to select their diet, was 91.2 gm. or 1.33 gm. for such a period."

The metabolism of some purin compounds in the rabbit, dog, pig, and man was studied by L. B. Mendel and J. F. Lyman (Jour. Biol. Chem., 8 (1910), No. 2, pp. 115–143).

In the experiments with man hypoxanthin nitrate, xanthin, guanin, and adenin were added on different days to a purin-free diet. According to the authors, the examination of the urine showed that all four purins produced a marked rise in urinary acid and a small, yet noticeable increase in the elimination of purin bases. The smaller and lighter of the two subjects excreted, in every case, a larger percentage of uric acid and purin bases than the other subject, and, according to the authors, "may possess a more limited power for uric acid destruction."

The effect of meat purins (largely free hypoxanthin), on the elimination of purin compounds is illustrated by data cited from a series of experiments by Hilditch, also made at Yale University, in which meat was substituted for the milk and eggs of a purin-free diet. The resulting increase in the excretion of uric acid nitrogen, it is stated, is quite comparable with the figures obtained in the experiment with pure hypoxanthin.

In discussing their work in comparison with that of earlier investigators, the authors point out that the data which they report "emphasize the fact that all of the familiar purins may lead to an increase in exogenous uric acid in the urine of man, with (quantitatively) little influence on the elimination of the purin bases. They may be interpreted to support the most prevalent view that uric acid is a stage in the metabolism of

exogenous purins in the human body, a view rendered especially plausible by the growing statistics on tissue enzyms."

The composition of dilute renal excretions was studied by A. B. Macallum and C. C. Benson (Jour. Biol. Chem., 6 (1909), No. 2 pp. 87–104), the general conclusion being that the elimination of water, potassium salts, and chloride from the body is not due to filtration, but in the case of water, to the physiological activity of the renal membranes involved, and in the case of the salts, to forces which may be termed "secretory."

T. B. Barringer, Jr., and B. S. Barringer (Amer. Jour. Physiol., 27 (1910), No. 1, pp. 119–121) have compared the total nitrogen excretion of either kidney in normal individuals during varying periods of time. In one case the excretions from the 2 kidneys were found to be equal in quantity. Six times they varied by less than 10 per cent and 4 times from 10 to 20 per cent.

"As regards the total nitrogen, in one case the quanitites were equal. In 7 cases they varied by less than 1 gm. per liter. In 2 cases they varied by between 1 and 2 gm. per liter. The nitrogen-urea plus ammonia-urea showed in 3 cases a variation less than 1 gm. per liter and in 6 cases a variation of between 1 and 2 gm."

L. W. Riggs (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, p. XIX; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, p. 13) studied the chemical composition of human sweat, using 45 samples obtained from persons in normal health and from nephritics. The total nitrogen, nitrogen as urea plus ammonia, inorganic acids, potassium, and chlorin were determined in the majority of the samples.

Factors regulating the creatinin output in man were studied by P. A. Levene and L. Kristeller (Amer. Jour. Physiol., 24 (1909), No. 1, pp. 45–65), the experimental data apparently indicating that the formation of creatin and creatinin represents two phases in the catabolism of a single substance. The constant activity of the creatinin output in normal men, the authors believe, is conditioned by the high velocity of creatin combustion in health.

The process of acid excretion was studied critically by L. J. Henderson (Jour. Biol. Chem., 9 (1911), No. 5, pp. 403-424, dgms.

3), who concludes that, as is the case with temperature and osmotic pressure, normal neutrality or alkalinity is adjusted by a mechanism within the body, but is maintained permanently by exchanges with the environment.

In a study of the nutritive value of gelatin, J. R. Murlin (Amer. Jour. Physiol., 19 (1907), No. 3, pp. 285–313) found that under certain conditions, namely, supplying a large proportion of the energy of the ration in the form of carbohydrates being especially favorable, it was possible in experiments with man and dogs to replace part of the proteid nitrogen with gelatin nitrogen for maintaining nitrogen equilibrium at a fasting level.

Gelatin was one of the materials included in a study of the elimination of total nitrogen, urea, and ammonia following the administration of some amino acids, glycylglycin and glycylglycin anhydrid by P. A. Levene and G. M. Meyer (Amer. Jour. Physiol., 25(1909), No. 4, pp. 214–230). The experiments were made with dogs. According to the authors, all of the excessive nitrogen added as gelatin to a standard diet "is eliminated in the form of urea. Thus, this experiment leads to the conclusion that either diketopiperazins do not enter into the composition of the protein molecule, or that the anhydrids of peptids within the protein molecule offer less resistance than when in a free state."

The significance of glycocol and carbohydrate in sparing the body's proteid was also studied by J. R. Murlin (Amer. Jour. Physiol., 20 (1907), No. 1, pp. 234-258). A specific relationship was shown to exist between carbohydrates ingested and the elimination of nitrogen, carbohydrate not needed for combustion being far more efficient for reducing nitrogen output than carbohydrate coming within the requirement for potential energy. This fact, according to the author, indicates the importance of abundant carbohydrates for convalescence and growth, and may explain the almost universal craving for sweets especially in the young.

From experiments with animals on mucic acid and carbohydrate metabolism L. B. Mendel and W. C. Rose (Abs. in Jour. Biol., Chem., 9(1911), No. 2, p. XII; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, p. 6) conclude that this acid "is presumably not an intermediary oxidative product in the metabolism of galactose

or galactose-yielding carbohydrates. The urinary oxalic acid is only very slightly increased after the ingestion of large amounts of mucic acid. This increase is by no means as large as would be expected if mucic acid were a precursor of oxalic acid."

Some experiments on the influence of caffein on protein metabolism of dogs have been reported by W. Salant and I. K. Phelps (Jour. Pharmacol. and Expt. Ther., 2 (1911), No. 4, pp. 401, 402), who also discuss demethylation in the body. The resistance to caffein, the authors state, "was found to vary with the amounts of the urinary purins eliminated."

W. Salant and J. B. Rieger (Jour. Pharmacol. and Expt. Ther., 2 (1911), No. 4, pp. 400, 401) studied the elimination of creatin and creatinin after the administration of caffein, with rabbits. The results indicate that urinary creatin is increased after the administration of caffein, the size of the dose being an important factor. Neither the increased diuresis nor the diminished appetite observed could, in the authors' opinion, be regarded as a factor in accounting for the increased output of creatin.

On theoretical grounds, G. Lusk (Zentbl. Physiol., 21 (1907), No. 26, pp. 861, 862) discusses the specific dynamic effect of portein.

According to H. McGuigan's (Jour. Biol. Chem., 3 (1907), No. 3, Proc., pp. XXXVII, XXXVIII) studies of sugar metabolism in vitro, the clinical assertion is maintained that levulose, is more easily oxidized than glucose and that it may be used in the body when glucose can not. The order of ease of oxidation of a number of sugars is as follows: Levulose, galactose, glucose, maltose, and saccharose, levulose being the most easily oxidized.

H. McGuigan (Amer. Jour. Physiol., 21 (1908), No. 3, pp. 334–350) found that the living muscles of an animal, when perfused with dextrose, levulose, or galactose, caused a rapid oxidation of these sugars. With maltose direct oxidation was not noted. Other questions were also considered in this experimental inquiry, which is a contribution to the question of the way in which the animal body utilizes a carbohydrate food supply.

The income and outgo of nitrogen of a simple mixed diet are determined and briefly reported by Clara C. Benson et al. (Jour. Home Econ., 2 (1910), No. 6, p. 658).

An extended series of studies with fasting subjects has been reported by P. B. Hawk and his associates, in which the following matters have been taken up: Nitrogen partition and physiological resistance as influenced by repeated fasting (Jour. Amer. Chem. Soc., 33 (1911), No. 2, pp. 215–254, dgm. 1); the catalase content of tissues and organs after prolonged fasting (Jour. Amer. Chem. Soc., 33 (1911), No. 3, pp. 425–434); the nitrogen partition of two men through seven-day fasts following the prolonged ingestion of a low protein diet, supplemented by comparative data from the subsequent feeding period (Jour. Amer. Chem. Soc., 33 (1911), No. 4, pp. 568–598); the allantoin and purin excretion of fasting dogs (Jour. Amer. Chem. Soc., 33 (1911), No. 10, pp. 1601–1622); the influence of an excessive water ingestion on a dog after a prolonged fast (Jour. Biol. Chem., 10 (1911), No. 5, pp. 417–432); distribution of nitrogen during a fast of one hundred and seventeen days (Jour. Biol. Chem., 11 (1912), No. 2, pp. 103–127, dgm. 1); the putrefaction processes in the intestine of a man during fasting and during subsequent periods of low and high protein ingestion (Jour. Biol. Chem., 11 (1912), No. 3, pp. 169–177); hydrogen ion concentration of feces (Jour. Biol. Chem., 10 (1912), No. 2, pp. 129–140); on the differential leucocyte count during prolonged fasting (Amer. Jour. Physiol., 30 (1912), No. 2, pp. 174–181); and glycogen-free liver (Jour. Amer. Chem. Soc., 34 (1912), No. 6, pp. 826–828).

Hawk and his associates have also reported an extended series of experiments on the relative effects of copious and moderate water drinking with meals, some of the experiments being carried on as a part of the series of fasting tests referred to above. The following list of subjects studied show the character and extent of the work: The influence of copious water drinking (Univ. Penn. Med. Bul., 18 (1905), No. 1, pp. 7–25); the stimulation of gastric secretion under the influence of water drinking with meals (Jour. Biol. Chem., 9 (1911), No. 2, pp. XXIX, XXX; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 23, 24); the metabolic influence of copious water drinking with meals (Jour. Expt. Med., 12 (1910), No. 3, pp. 388–410); the uric acid elimination following copious water drinking between meals (Jour. Amer. Chem. Soc., 32 (1910), No. 12, pp. 1686–1691); the excretion of

chlorids following copious water drinking between meals (Reprinted from Arch. Int. Med., 7 (1911), pp. 536-550); intestinal putrefaction during copious and moderate water drinking with meals (Arch. Int. Med., 7 (1911), No. 5, pp. 610–623); the activity of the pancreatic function under the influence of copious water drinking with meals (Arch. Int. Med., 8 (1911), pp. 382–394); the allantoin and purin excretion of fasting dogs (Jour. Amer. Chem. Soc., 33 (1911), No. 10, pp. 1601–1622); the utilization of ingested fat under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp. 1978–1998); the distribution of bacterial and other forms of fecal nitrogen and the utilization of ingested protein under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp 1999–2019), a fecal output and its carbohydrate content under the influence of copious and moderate water drinking with meals (Jour. Amer. Chem. Soc., 33 (1911), No. 12, pp. 2019–2032); the influence of an excessive water ingestion on a dog after a prolonged fast (Jour. Biol. Chem., 10 (1911), No. 5, pp. 417–432); the allantoin output of man as influenced by water ingestion (Jour. Amer. Chem. Soc., 34 (1912), No. 4, pp. 546–550); and the hydrogen ion concentration of feces (Jour. Biol. Chem., 11 (1912), No. 2, pp. 129–140).

As a whole the investigations, which are too extended to be quoted in detail, were favorable to the use of water with meals.

The metabolism of inorganic and organic phosphorus was studied with laboratory animals (rabbits) by F. C. Cook (U. S. Dept. Agr., Bur. Chem. Bul. 123, pp. 63, pls. 3), of the Bureau of Chemistry. The rabbits fed organic phosphorus eliminated a smaller proportion of ingested phosphoric acid in the urine than those fed inorganic phosphorus, and the average amount of calcium absorbed from the intestinal tract was higher in the case of the rabbits fed organic phosphorus, the results agreeing with the theory that calcium and phosphorus fed in inorganic form unite to form insoluble calcium phosphate which is eliminated by the bowels in ingested form. The amount of metabolized magnesium that was retained indicates that the rabbits fed inorganic phosphorus, while metabolizing a smaller amount of magnesium

than did those fed organic phosphorus, retained a larger percentage of the amount actually metabolized.

The sulphur balance in metabolism was studied by A. E. Taylor (Abs. in Jour. Biol. Chem., 9 (1911), No. 2, pp. IX, X; Proc. Amer. Soc. Biol. Chem., 2 (1910), No. 1, pp. 3, 4) with 6 normal men, for periods of nearly 3 months. A condition of equilibrium was not observed, the output being regularly and notably higher than the intake. The author does not consider that the results obtained are trustworthy, the presumption being that errors were involved in the determinations of the sulphur index.

According to data reported by A. O. Shaklee and S. J. Meltzer (Amer. Jour. Physiol., 25 (1909), No. 3, pp. 81–112), shaking may completely destroy the three proteolytic ferments—pepsin, rennin, and trypsin. "They are destroyed more rapidly at higher than lower at temperatures; . . . trypsin is more easily destroyed than pepsin; . . . shaking produced by the respiratory movements is capable of causing some destruction of the ferments. Recent experiments by other investigators show also that other ferments may be inactivated by shaking . . .

"The assumption is here made that the nature of the destruction of ferments is similar to that which takes place in the destruction of living cells, and that shaking affects a certain structure which is common to living cells as well as to red blood corpuscles and to ferments."

In experiments on the effects of respiratory movements, ferments in rubber or glass containers of suitable construction were introduced into the stomach and peritoneal cavity, a dog and rabbits serving as subjects.

E. W Rockwood (Proc. Iowa Acad. Sci., 15 (1908, pp. 99–103) has studied the nature of the uric acid ferments which it is believed are concerned in the formation of uric acid from nucleins in the liver.

The problem of nuclein syntheses in the animal body was studied experimentally by E. V. McCollum (Wisconsin Sta. Research Bul. 8, pp. 75–93; Amer. Jour. Phisiol., 25 (1909), No. 3, pp. 120–141) with young and old rats, with normal and special rations, such materials as edestin, zein, glucose, purified butter

fat, and cane sugar being used. These purin-free diets and diets containing purin bases were also compared.

Some of the author's conclusions were as follows:

"The palatability of the ration is a most important factor in animal nutrition. Without palatability the ration may possess all the necessary food ingredients and yet fail to nourish an animal properly. . . .

"Very young animals adapt themselves to a ration possessing a low degree of palatability much better than do adults.

"Other things being satisfactory, all the phosphorus needed by an animal for skeleton, nuclein or phosphatid formation, can be drawn from inorganic phosphates.

"The animal has the power to synthesize the purin bases necessary for its nuclein formation from some complexes contained in the protein molecule, and does not necessarily use purin bases of exogenous origin for this purpose."

A useful summary and digest of data on the elementary composition of nuccleic acids, their constituents, and related questions has been published by P. A. Levene (Jour. Amer. Chem. Soc., 32 (1910), No. 2, pp. 231–240), and a similar compilation on oxidases by J. H. Kastle (Pub. Health and Mar. Hosp. Serv. U. S., Hyg. Lab. Bul. 59, pp. 164).

Methods and standards in bomb calorimetry have been discussed on the basis of experience by J. A. Fries (U. S. Dept. Agr. Bur. Anim. Indus. Bul. 124, pp. 32; Pennsylvania Sta. Rpt. 1909, pp. 321–345).

An adiabatic calorimeter for use with the calorimetric bomb has been described by F. G. Benedict and H. L. Higgins (Jour. Amer. Chem. Soc., 32 (1910), No. 4, pp. 461–467, fig. 1).

The metabolism as a statistical problem has been considered by H. L. Rietz and H. H. Mitchell (Jour. Biol. Chem., 8 (1910), No. 4, pp. 297–326). Such questions as the application of the laws of probability, together with the various mathematical methods of reducing statistical data, the importance of such procedure in metabolism experiments, and related questions are considered.

Comparative physiology of purin metabolism, by H. G. Wells (Trans. Chicago Path. Soc., 7 (1909), No. 8, pp. 244–248; Jour. Amer. Med. Assoc., 53 (1909), No. 21, p. 1741). According to

his summary, "the invertebrates are able to convert adenin into hypoxanthin and guanin into xanthin, showing the presence of the enzyms, adenase, and guanase, but the metabolism proceeds no further. Passing upward in the scale of animal life to the birds and reptiles we find that nitrogen is excreted chiefly in the form of uric acid. Mammals form uric acid only from the purins and have the power of destroying some of the uric acid formed. The enzyms that destroy uric acid seem to be the last formed in development and are possessed by various mammals in varying degrees and in the same animal often show an uneven distribution in the various organs of the body. This uricolytic power is relatively weak in man." The paper is followed by a discussion.

The output of organic phosphorus in urine was studied by G. C. Mathison (Bio-Chem. Jour., 4 (1909), No. 5–7, pp. 274–279) under conditions of work and rest. In young adults, on an ordinary diet, the organic phosphorus was usually found to be more than 0.1 gm. per day. Occasionally it fell below this, while in one case it reached 0.3 gm.

"The percentage of the total P_2O_5 present in organic combination varies considerably from day to day. In the cases examined it averaged 6 per cent of the total.

"The addition of a large quantity of organic phosphorus in the form of glycero-phosphoric acid to the diet had no distinct effect on the output of organic P_2O_5, while it increased the total P_2O_5 output. Glycerophosphoric acid was not broken down by gastric or pancreatic digestion in vitro, so it was probably absorbed unchanged.

"In the observations made, vigorous exercise was not followed by increased output in organic P_2O_5.

"The N:P_2O_5 ratio was fairly constant in any one individual on a fairly regular diet. It differed greatly in different individuals, and also in the same individual when the diet was irregular."

The construction of improved forms of respiration calorimeters and progress reports of investigations of the Carnegie Institution of Washington are contained in the reports of the Nutrition Laboratory at Boston, which is under the direction of F. G. Bene-

dict (Carnegie Inst. Washington Year Book, 6 (1907), pp. 130-133; Carnegie Inst. Washington Year Book, 7 (1908), pp. 158–162, pl. 1. dgms. 4).

The respiration calorimeters in use at the Nutrition Research Laboratory of the Carnegie Institution located at Boston, Mass., are described in detail by F. G. Benedict and T. M. Carpenter (Carnegie Inst. Washington Pub. 123, pp. VII+102, pls. 5, figs. 25). A general plan is given of the calorimeter laboratory, the principles involved in the construction of the calorimeter are considered, descriptions of different parts of the apparatus presented, and the calculation of results explained. The descriptions are illustrated with diagrams and reproductions of photographs. An account is given of the routine followed in an experiment with a man as subject. It is hardly possible to give an adequate account of this work in abstract.

Control tests of a respiration calorimeter, by F. G. Benedict, J. A. Riche, and L. E. Emmes (Amer. Jour. Physiol., 26 (1910), No. 1, pp. 1–14), in which alcohol was burned in the respiration chamber and the amounts of heat eliminated, water vaporized, carbon dioxid produced, and oxygen consumed were compared with theoretical values. The results showed that the agreement between measured and theoretical amounts was very satisfactory and that the apparatus has proved as accurate as the usual analytical methods employed in the laboratory, with which small amounts of material are studied chemically or calorimetrically.

The direct determination of oxygen in experiments with the respiration calorimeter has been compared with calculated values by F. G. Benedict (Amer. Jour. Physiol., 26 (1910), No. 1, pp. 15–25), who concludes that the direct determination is accurate, "and that experiments on man can be made in which the direct determination of oxygen is fully substantiated by the indirect determination. Personal experience would indicate that the errors involved in the indirect determination of oxygen are such as to preclude its use under conditions that ordinarily obtain in even the most perfect forms of respiration apparatus, and that accurate determinations of the oxygen consumption of man are practicable only by means of the direct method."

The influence of muscular and mental work on metabolism and the efficiency of the human body as a machine were studied by F. G. Benedict and T. M. Carpenter (U. S. Dept. Agr., Office Expt. Stas. Bul. 208, pp. 100, figs. 3) in connection with the nutrition investigations of the Department of Agriculture, the work being done before the respiration calorimeter was taken from Middletown, Conn., to Washington. The first of the two papers included in the bulletin reports data of 19 experiments on the effects of muscular work on metabolism and the efficiency of the body as a machine, respiratory products and oxygen consumption being measured in the usual way with the respiration calorimeter, and the bicycle ergometer employed for measurements of muscular work. The efficiency of the body was found to be 20 per cent; that is, for every calorie of muscular work produced by the body a total of 5 calories is expended. A series of 44 experiments was made to compare mental work (writing the answers to examination papers which were regarded as difficult by the subjects) and mental idleness, perhaps more properly mental occupation which involved no special mental effort, namely, transcribing an amount of very simple material, which gave them the same amount of arm motion required in writing the examination papers. No constant differences in the heat output in the different periods were noted, so it appears that the experiments do not warrant the conclusion that mental work such as was performed had a positive influence on metabolic activity measurable by the very delicate methods employed.

Metabolism in man with greatly diminished lung area was studied by T. M. Carpenter and F. G. Benedict (Amer. Jour. Physiol., 23 (1909), No. 6, pp. 412–419) at the Carnegie Institution Nutrition Laboratory, a respiration calorimeter being used. The only deduction which can be drawn from the experimental data, according to the authors, is that the reduction of the area for oxygen absorption and carbonic acid elimination in the lungs by about one-half did not materially alter the total metabolism.

During the experiments with the respiration calorimeter made at Middletown, Conn., by T. M. Carpenter and F. G. Benedict (Amer. Jour. Physiol., 24 (1909), No. 2, pp. 187–202) several

cases of illness were observed which, after careful investigation, were attributed to poisoning caused by mercury vapor, due to the use of mercury valves in the ventilating air current.

Metabolism during fever was also studied by the same authors, with the respiration calorimeter (Amer. Jour. Physiol., 24 (1909), No. 2, pp 203–233).

"In general the carbon dioxid excretion was apparently greater during fever than during control periods.

"The oxygen consumption during fever is in practically all cases noticeably greater than during control. . . .

"While the data show a slight tendency for the respiratory quotient to increase during fever, the complications attending the ingestion of food, variations in muscular activity, and errors in oxygen determination do not warrant any sweeping deductions from these data."

The recorded data indicate that in general "there was an increase in the water of vaporization during fever over that during the control period. Since, however, the control experiments showed marked variations when compared with the fever experiments during periods when there was no appreciable fever, it is obvious that here again we can not draw any sweeping deductions regarding this point."

As regards heat elimination, the authors state that "in view of the necessarily tentative nature of all deductions made from these experiments, it has not been deemed advisable to attempt to discuss the influence of fever on the various paths of heat elimination."

The metabolism of man during the work of typewriting was studied by T. M. Carpenter and F. G. Benedict (Jour. Biol. Chem., 6 (1909), No. 3, pp. 271–288), who conclude that "it seems reasonable to assume that the work of writing some 1,500 to 1,600 words per hour on the typewriter results in an increase over the resting metabolism of some 10 to 14 gm. of carbon dioxid, 10 to 13 gm. of oxygen, and 20 to 30 calories of heat per hour. Of these factors of metabolism, it is highly probable that the truest factor is presented by the total energy exchange as directly measured, and hence taking into consideration all the data furnished by these two experiments, we can tentatively say that

the writing of 1,600 words per hour on the typewriter results in a heat transformation over and above the resting metabolism of not far from 25 calories. At present too little is known regarding the energy transformation of various everyday activities to make any striking comparison, but [by other investigation] . . . it has been computed that there is an hourly energy expenditure of about 160 calories over and above the resting maintenance requirement of a man of 70 kg. walking along a level road at a rate of 2.7 miles per hour. It is seen, therefore, that the work of typewriting calls for very much less transformation of energy than does that of ordinary walking."

An apparatus for studying respiratory exchange has been described by F. G. Benedict (Amer. Jour. Physiol., 24 (1909), No. 3, pp. 345–374, figs. 6), which is similar in principle to that portion of a respiration calorimeter which has to do with gaseous exchange and respiratory quotient measurements.

An important digest of data accumulated in experiments with the respiration calorimeter, extending over 10 years, is included in a bulletin on the metabolism and energy transformations of healthy man during rest, by F. G. Benedict and T. M. Carpenter (Carnegie Inst. Washington Pub. 126, pp. VIII + 225). Tentative tables are given for computing the metabolism of normal individuals with varying degrees of muscular activity.

The table on page 217 gives the carbon dioxid eliminated, the oxygen absorbed, and the heat produced per hour during various activities, the data as to standing and very severe muscular exercise being calculated, using as a standard the results obtained with 55 men awake and sitting up.

"The results presented in this report are to be considered simply as indicating the normal metabolism of healthy young men at rest and under several conditions of muscular activity. The variations from the normal exhibited by the individual can be seen by an examination of the tables. The attempt is made to point out the cause of the variations in so far as possible, but with so complex a process as the energy transformation and catabolism in the body, it is clearly futile to attempt to predict with great accuracy either the catabolism or the energy transformations of a given individual. Approximate values that may prove

CARBON DIOXID ELIMINATION, OXYGEN ABSORPTION, AND HEAT PRODUCTION PER HOUR HOUR DURING VARIOUS ACTIVITIES

Degree of Muscular Activity	Number of subjects	Average body weight	Carbon dioxid eliminated	Oxygen absorbed	Heat produced
		Kg.	*Grams*	*Grams*	*Calories*
Man at rest, sleeping.....	17	66.6	23	21	71
Man at rest, awake, sitting up.....	55	64.5	33	27	97
Man at rest, standing, calculated.....	.....	64.5	37	31	114
Man at very severe muscular exercise, calculated ..	.....	64.5	248	213	653

of practical use can be obtained by means of some of the factors outlined in this report. With more accurate and improved calorimeters, there should be in the course of a few years the addition of many factors, at present entirely unknown."

The influence of the preceding diet on the respiratory quotient after active digestion has ceased has been studied by F. G. Benedict, L. E. Emmes, and J. A. Riche (Amer. Jour. Physiol., 27 (1911), No. 4, pp. 383–405).

In general, the conclusion was drawn that the respiratory quotient determined 12 hours after a meal rich in carbohydrates was higher than when the last meal contained only a small amount of carbohydrates. The possibility of this high respiratory quotient being due to the delayed absorption and combustion of carbohydrates in the alimentary tract is discussed, but the authors believe that the evidence is rather against the theory.

"Obviously the previous body condition play a very important role. The extent to which the body storage of glycogen has been drawn upon, the muscular activity of the day previous to the experiment, possibly the temperature of the surrounding air, the general diet of the individual for several days before—in fact, anything which contributes to a disturbance of the storage of glycogen in the body—may alter the influence of the in-

gestion of a carbohydrate-rich meal. If the glycogen storage in the body is at a low point, the ingestion of a carbohydrate-rich meal does not result in an increased respiratory quotient in accordance with the amount ingested, as a not inconsiderable proportion of the carbohydrate may be stored immediately as glycogen. Until this glycogen storage has been replenished the combustion of carbohydrate in the food may be delayed. On the other hand, with individuals subsisting without food and remaining quiet in a respiration chamber, the store of glycogen may last for some time. From these data we may infer, then, that muscular activity may play an important role in effecting the storage of glycogen."

Other questions which have to do with the general subject are discussed.

The respiratory exchange as affected by body position was studied at the Carnegie Institution of Washington by L. E. Emmes and J. A. Riche (Amer. Jour. Physiol., 27 (1911), No. 4, pp. 406–413). In general, the authors found that the pulse rate lying down was on an average 63, the carbon dioxid excretion 209 cc., and the oxygen consumption 236 cc. per minute. With a subject in a sitting position the pulse rate was 71, the carbon dioxid excretion 218 cc., and the oxygen consumption 254 cc. per minute.

In their discussion of the data reported the authors point out that for experimental purposes, when metabolism at a given condition of body rest is to be determined, it is of value to know, "as a result of experiments with the respiration apparatus, that the metabolism of a subject when sitting absolutely quiet in a chair, without extraneous muscular activity, represents a metabolism 8 per cent greater than that of a subject lying on a couch, with similar muscular rest. The difference in metabolism is then due, primarily, to the difference in the internal muscular activity necessitated by the sustaining of body parts. This is in conformity with the well-known fact that the pulse rate of an individual when sitting is always noticeably higher than when he is lying down. From these tests we could infer that if it were possible to so support the body of the subject in a sitting position that the pulse rate would be no greater than when the subject

was lying down, the metabolism would be essentially the same in both positions."

Using a small apparatus for studying respiratory exchange, G. Lusk (Amer. Jour. Physiol., 27 (1911), No. 5, pp. 427–437) investigated the influence of cold baths on the glycogen content of man.

"Immersion of normal men in cold baths at a temperature of 10° when the intestine is free from carbohydrates induces shivering, which causes a rapid utilization of body glycogen, as determined by a fall in the respiratory quotient to the fasting level. In one very muscular individual this result could not be obtained.

"In one individual in whom the shivering had been severe, a quotient of 0.67 and another of 0.62 were found during subsequent periods of rest, which correspond to those observed during rest after a period of exhaustive exercise (glycogen formation from protein).

"The greatest increase in heat production which was brought about by the cold baths was 181 per cent above the normal. The urine remained free from albumin and from sugar."

A respiration apparatus for the determination of the carbon dioxid produced by small animals has been constructed at the Boston Nutrition Laboratory of the Carnegie Institution of Washington and described by F. G. Benedict and J. Homans (Amer. Jour. Physiol., 28 (1911), No. 1, pp. 29–48, dgms. 2).

The effects on men at rest of breathing oxygen-rich gas mixtures were studied by F. G. Benedict and H. L. Higgins (Amer. Jour. Physiol., 28 (1911), No. 1, pp. 1-28, fig. 1), with normal individuals. The air mixtures contained 40, 60, and 90 per cent oxygen, respectively, and the tests were made in a condition of complete muscular rest 12 hours after the last meal was taken. It was found "that there is no apparent difference between the metabolism as indicated by the gaseous exchange (i.e., the carbon dioxid output, oxygen consumption, and respiratory quotient) and the metabolism when breathing ordinary air; that there is no change in the respiration, either as to character, depth, or frequency, as compared with the same factors when breathing ordinary air; [and] that the pulse rate is lower with oxygen-rich mixtures than when breathing ordinary air; furthermore, that

the higher the percentage of oxygen breathed (up to 90 per cent), the lower the pulse."

Data regarding the relative ease of digestion of cheese as compared with beef have been reported by C. F. Langworthy and R. D. Milner (2. Cong. Internat. Hyg. Aliment. Bruxelles [Proc.], 2 (1910), Sects. 4–7, pp. 249–253). No constant differences in the heat elimination per hour were noted when comparable amounts of cheese and meat (beef) were added to a uniform basal ration. So far as the reported data and results of later experiments not yet reported show, there were no marked differences with respect to ease of digestion of these two staple foods.

The success which attended preliminary experiments with the respiration calorimeter on the possibilities of studying physiological processes by means of the gaseous exchange and heat output led to the construction of an instrument of suitable size for this special work and other related problems. C. F. Langworthy and R. D. Milner (U. S. Dept. Agr., Office Expt. Stas. Circ. 116, pp. 3) have described briefly the new calorimeter of small size and also the large apparatus used for experiments with man, as well as a micro-calorimeter for use in the experimental study of very small quantities. The calorimeters as now installed are equipped with recording and controlling devices of special construction. The devices of this character used in connection with the small respiration calorimeter, designed for the study of vegetable problems, involve much that is new and original, so that the calorimeter is very largely automatic in operation. Important modifications have also been introduced into the construction of the calorimeter itself which make for ease of operation.

The work which has been done thus far with ripening fruit has demonstrated that the respiration calorimeter is fully as well suited to the study of certain fundamental problems of plant life as to the study of similar problems of animal life.

The apparatus can also be used, it seems safe to conclude, in studying such problems as the changes which take place when meat or cheese or other similar products are cured or ripened, and factors which influence these changes; that is, problems which are

of commercial interest as well as of agricultural, domestic, and scientific importance.

The construction of the small respiration calorimeter for use in the study of problems of vegetable physiology is given by C. F. Langworthy and R. D. Milner in a later paper (U. S. Dept. Agr. Yearbook 1911, pp. 491–504).

The respiration of apples and its relation to their keeping quality were studied by F. W. Morse (New Hampshire Sta. Bul. 135, pp. 85–92, figs. 2). The results of experiments in one season, calculated on the basis of 1 kg. of fruit for 1 hour, showed that the average exhalation of carbon dioxid was 18 mg. at summer temperature, 8.1 mg. at cellar temperature, and 2.7 mg. at cold storage temperature (32 degrees). The apples obtained in a second series of experiments were 13.2 mg. at cellar temperature, 21.9 mg. at summer temperature, and 5.2 mg. at cold storage temperature.

The expired breath was studied with reference to the presence of organic matter, by M. J. Rosenau and H. L. Amoss (Jour. Med. Research, 25 (1911), No. 1, pp. 35–84, figs. 5). Using the reaction of anaphylaxis, the authors conclude from experiments in which the liquid obtained by condensing the moisture from the expired breath of man was injected into guinea pigs, that the presence of organic matter in expired breath has been demonstrated.

"The logical conclusion from our results is that protein substances under certain circumstances may be volatile. It seems unlikely that such a complex molecule should possess the power of passing into the air in a gaseous form. The volatility, however, now in question may resemble that solubility which deals with particles in suspension in a physico-chemical state (colloidal suspension). The protein may simply be carried over in 'solution' in the water vapor.

"Our experiments are too few to state that albuminous substances such as egg white, milk, or blood serum in vitro is 'volatile.' However, they are sufficiently suggestive to stimulate further work along this line."

FOODS AND THEIR RELATION TO PROBLEMS OF HYGIENE

The need for clean food is much more generally recognized than was formerly the case. This matter and many others pertaining to food in its relation to hygiene have been studied during the period under consideration in the summary.

Studies of the influence of various dietary conditions on physiological resistance have been reported by N. B. Foster (Jour. Biol. Chem., 7 (1910), No. 5, pp. 379–419). The results perhaps pertain more to pharmacology than to dietetics.

The influence of dietary alternations on the types of intestinal flora was studied experimentally by C. A. Herter and A. I. Kendall (Jour. Biol. Chem., 7 (1910), No. 3, pp. 203–236, pls. 3), who observed a marked bacterial degeneration following pronounced physiological alternations in the flora of the intestines as a result of changes in the diet. Experiments were made with laboratory animals. The authors consider it probable that analogous conditions would be found to exist in man.

The question has been further discussed by C. A. Herter (Internat. Beitr. Path. u. Ther. Ernährungsstör. Stoffw. u. Verdauungskrank., 1 (1910), No. 3, pp. 275–281) in a paper.

The results of an extended study of fecal bacteria of healthy men have been reported by W. J. MacNeal, L. L. Latzer, and J. E. Kerr (Jour. Infect. Diseases, 6 (1909), No. 2, pp. 123–169, fig. 1). In general, they conclude that "the direct quantitative determinations of the fecal bacteria furnish evidence of the extent and nature of the bacterial growth in the intestines. This seems to be a delicate index of intestinal conditions."

Numerous studies on the effect of lactic acid ferments on intestinal putrefaction have been reported, including a paper by Helen Baldwin (Jour. Biol. Chem., 7 (1909), No. 1, pp. 37–48).

Coagulated milk and a large number of other preparations were used by P. G. Heinemann (Jour. Amer. Med. Assoc., 52 (1909), No. 5, pp. 372–376) in a study of lactic acid as an agent to induce intestinal putrefaction.

The results obtained led the author to conclude "that so far as the therapeutic effect is concerned, there is yet no convincing evidence that sour milk prepared with commercial cultures is

preferable to naturally soured milk. Yeasts were present in all but one of the commercial preparations."

The bacterial condition of protected and unprotected foods in restaurants, meat markets, grocery stores, bakeshops, and fruit stores was studied by H. E. Barnard (Ann. Rpt. Bd. Health Ind., 27 (1908), pp. 517–523, pls. 4), who found that foods kept in glass cases were in every case practically free from dust and accompanying bacteria, while food on exposed tables and racks was surrounded with air heavily laden with dirt and bacterial life. It was also found that cleanliness of floors and utensils lessened to a certain extent the number of bacteria present, and that on the contrary counters and stands near sidewalks are always surrounded with atmospheric dust and dirt.

The author's studies were concerned chiefly with the relative number of bacteria found on the culture plates inoculated under different conditions and the types of bacteria were not thoroughly differentiated. He believes that pathogenic bacteria were present.

G. W. Stiles (U. S. Dept. Agr., Bur. Chem. Bul. 136, pp. 53, figs. 15) studied shellfish contamination from sewage-polluted waters and from other sources, the observations made in many localities being supplemented by bacteriological work.

According to the author, "there is undisputed evidence to show that shellfish become contaminated when placed in sewage-polluted water, and that *Bacilluscoli* and *B. typhosus* will survive for variable lengths of time in the liquor and the body contents of such shellfish after their removal from infected water. . . .

"Oyster beds should be protected from every possible source of contamination, and they should be located in water proven to be pure by repeated examinations. . . .

"The practice of floating oysters in water of questionable purity should be absolutely prohibited because of the probability of sewage contamination. . . .

"Like other perishable food products, oysters may become unfit for use if stored or kept under insanitary conditions. This spoilage, however, may take place wholly from the length of time out of water. . . .

"The liquor in the shell surrounding the oysters contains more bacteria than does an equal volume of meat from the same oyster. This liquor, together with any sand in the gills of the oyster, can be removed and the meat chilled at the same time by the use of pure ice and water. This washing process can be done efficiently within 3 to 10 minutes, depending upon the method employed. Oysters should not be allowed to soak in fresh water, as they increase in volume, change in appearance and flavor, and decompose more rapidly than those not soaked.

"[As shown by cooking tests], steaming contaminated oysters and clams in the shell, or cooking them after shucking for 15 minutes at boiling temperature, practically destroys all organisms of a questionable character, but since in practice shellfish are never cooked for this length of time, cooking can not be depended upon to remove this danger. . . .

"The investigations show that vast areas of valuable shellfish grounds in this country are now reasonably free from sewage pollution, but this territory will gradually diminish in size if sewage is not properly cared for in the future. Comparatively speaking, only a small acreage is now subject to serious pollution."

The absorption of aluminum from aluminized food was studied by M. Steel (Amer. Jour. Physiol., 28 (1911), No. 2, pp. 94–102). When alum was administered in aluminum-free foods to dogs or when they were given biscuits baked with alum baking powder, "aluminum in comparatively large amounts promptly passed into the blood.

"Absorbed aluminum circulated freely, but as it did not show any pronounced tendency to accumulate in the blood, its full effects must have registered outside of the circulation."

When aluminum chlorid was administered intravenously, from 5.55 to 11.11 per cent of the aluminum passed from the blood into the feces during the 3 days immediately following the injection. "Whether the aluminum passed directly through the walls of the intestine or was excreted by the liver, or whether both channels (or others) were followed, has not yet been ascertained."

The use of metallic containers for edible fats and oils was studied by J. A. Emery (U. S. Dept. Agr., Bur. Anim. Indus. Rpt. 1909, pp. 265–282), of the Bureau of Animal Industry, with

vessels and sheets of tin plate, galvanized iron, copper, tin, lead, zinc, aluminum, and iron, for the purpose of determining the action of fats and oils upon metals, with particular reference to the utility of these metals as containers.

The work shows that where an increase in the acid content of the fat or oil was noted there was an increase in the solvent action of the oil for metals, particularly where other favorable conditions, such as heat, moisture, and exposure to the atmosphere, were present. With cotton-seed oil, however, an exception was noted, as this oil, when prepared with a corn oil of lesser or approximately the same acidity, showed little or no effect upon metals.

"It [was] demonstrated that zinc, copper, and lead are somewhat readily acted upon, while aluminum, iron, and tin, in the order in which they are named, have offered evidences of higher resisting power and are the metals which would more satisfactorily meet the requirements of both manufacturer and consumer."

Much attention has been given in the United States to the discussion and study of pellagra, on account of its supposed relation to Indian corn. The agricultural aspects of the pellagra problem in the United States were studied by C. L. Alsberg (N.Y. Med. Jour. and Phila. Med. Jour., 90 (1909), No. 2, pp. 50–54). Of important discussions may be mentioned the report of C. H. Lavinder (Pub. Health and Mar. Hosp. Serv. U. S., Pub. Health Rpts., 24 (1909), No. 37, pp. 1315–1321), and the paper on the etiology of pellagra by the same author (N. Y. Med. Jour. and Phil. Med. Jour., 90 (1909), No. 2, pp. 54–58), who has also reported considerable data regarding pellagra and its possible relation to maize according to some recent views (Pub. Health and Mar. Hosp. Serv. U. S. Pub. Health Rpts., 26 (1911), No. 8, pp. 199–208). Particular interest attaches to his discussion of Raubitschek's photodynamic theory that the disease is ascribable to the joint action of a substance present in corn meal fat and sunlight.

W. H. Buhlig (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 417–435, figs. 2) and J. F. Siler and H. J. Nichols (Ill. Bd. Health Mo. Bul., 5 (1909), No. 7, pp. 437–478, figs. 8) have made exten-

sive studies of the possible relation between corn in the diet and the occurrence of pellagra. Final conclusions were not drawn. In connection with this work some data were reported on experiments in cookery as well as regarding institution dietetics.

Cost of Living and Other Statistical Data

The collection of statistical data has continued as an important part of the general activity in nutrition. Much of the work in the United States has been done under government or state auspices.

A select list of references on the cost of living and prices, by H. H. B. Meyer (Washington: Library of Congress, 1910, pp. V+107), has been published by the Library of Congress.

A large amount of statistical data on wages and prices of commodities has been reported in various Senate documents (Washington: U. S. Senate Select Committee, 1910, vols. 1, pp. 658; 2, pp. III+659-875—Hearings held before the Select Committee of the Senate relative to wages and prices of commodities). Topical digest of evidence submitted in hearings held before the Select Committee of the Senate relative to wages and prices of commodities (Washington: U. S. Senate Select Committee, 1910, pp. XCV).

In connection with the work of the U. S. Census many studies have been reported on the production and value of food products; for instance, those on rice cleaning and polishing, by H. McK. Fulgham (Bur. of the Census [U. S.] Bul. 61, pp. 49-58, dgm. 1), and beet sugar, by Z. C. Elkin (Bur. of the Census [U. S.] Bul. 61, pp. 59-69); slaughtering and meat packing (Bur. of the Census [U. S.] Bul. 83, pp. 7-41; and starch, by R. H. Merriam (Bur. of the Census [U. S.] Bul. 64).

The Bureau of Labor has published a summary of retail prices of food from 1890 to 1906 and discussed the data with reference to the cost of living in the United States (Bur. of Labor [U. S.] Bul. 71, pp. 175-328).

A large amount of data regarding the prices of foodstuffs in different New Jersey cities and towns has been summarized in an article on the cost of living in New Jersey (Ann. Rpt. Bur. Statis. Labor and Indus. N. J., 30 (1907), pp. 141-157).

Data regarding the prices of meat in the United States have been summarized in the Yearbook of the Department of Agriculture, by the Secretary, James Wilson (U. S. Dept. Agr. Rpts. 1909, pp. 15–31; Rpt. 91, pp. 10–24; Yearbook 1909, pp. 15–31).

Much statistical data regarding Hawaiian honey are included in a paper on Hawaiian bee keeping, by E. F. Phillips –U. S. Dept. Agr., Bur. Ent. Bul. 75, pt. 5, pp. 43–58, pls. 6).

Information regarding the cost of living of wage-earners and other similar material, including a paper on the preparation of a rational diet at a reasonable cost, are incorporated in the report of the Committee on Social Betterment, by G. M. Kober (Reports of the President's Homes Commission. Washington, D.C., 1908, [pt. 5], pp. 281, pls. 4; Reprint, pp. 281, pls. 4; see also Alimentation and foods (U. S. Senate, 60th Cong., 2. Session Doc. 644, pp. 121–157).

R. C. Chapin (New York, 1909, pp. 372, dgms. 16) has reported an extended investigation of the standard of living among workingmen's families in New York City, which presents and discusses a large amount of statistical data.

The cost of living in American towns has been exhaustively studied by the British Government (London: Govt., 1911, pp. XCII+533, map 1; U. S. Senate, 62 Cong., 1. Sess., Doc. 22, pp. XCII+533), the results being presented in a report by H. L. Smith which was reprinted by the U. S. Senate, and summarized in publications of the U. S. Senate (U. S. Senate, 62 Cong., 1. Sess., Doc. 38, pp. 74) and of the Department of Commerce and Labor (U. S. Dept. Com. and Labor, Bur. Labor Bul. 93, pp. 500–570).

Included in the study were 28 American towns on or east of the Mississippi, with an aggregate population in 1910 of 15,500,000, in round numbers.

The range of price levels for rents was found to vary greatly, being the highest in New York City. The prices of the principal foodstuffs, such as bread, flour, meat, potatoes, and sugar, did not show great range in the different towns, as was evident from the fact that when each article is considered in its relative importance the lowest level is 91 and the highest 109, with New York midway counting as 100. "If the towns are grouped geo-

graphically the New England and southern groups show the highest food price levels, the Midle West towns the lowest, the position of the New England towns in regard both to wages and rents being here reversed."

As regards retail prices of foods, the conclusion is that the ratio between the United States and England and Wales is 138 to 100.

"One peculiarity shown by the budgets is the comparatively small consumption of baker's bread in the average American working-class family, the consumption being 8¼ lbs. weekly per family as against 22 lbs. in the United Kingdom, the place of bread being taken in the United States to some extent by rolls, cakes, biscuits, etc., on which the expenditure is about three times as great as that shown in the average British budget. On the other hand, the consumption of meat is much larger in the United States, and the consumption of vegetables is also larger. The budgets indicate in general that the dietary of American working-class families is more liberal and more varied than that of corresponding families in the United Kingdom."

In addition to general discussions the report contains the details of the family budgets and other statistical data collected.

Conclusion

In the foregoing summary of work in human nutrition which has been carried on in the United States since the Seventh International Congress of Applied Chemistry, the attempt has been made to give some idea of the general condition of nutrition investigations and to cite examples of investigations along the proper lines of work into which the subject naturally divides itself.

That the list of investigations is by no means complete is recognized but it is believed that enough has been brought together to show that progress has been continuous and to make it clear that important contributions have been made not only to the fund of available data of interest to the students of nutrition and to practical workers, but also to methods of investigations as well as to the more important matter of fundamental theories of nutrition.

AN IMPROVED FORM OF RESPIRATION CALORIMETER FOR THE STUDY OF PROBLEMS OF VEGETABLE PHYSIOLOGY

By C. F. Langworthy and R. D. Milner
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Theoretical considerations regarding ripening fruit led to the attempt to study such questions of plant life by methods which have given good results in investigations of topics pertaining to the nutrition and energy expenditure of man. It was found that ripening fruit (bananas) could be studied in this way, since when they were kept during the active ripening period in the chamber of the large respiration calorimeter described in a publication of the Department of Agriculture,[1] carbon dioxid and water vapor were given off and oxygen was absorbed and heat liberated, all in measurable quantities. In other words, conditions were present which could be studied with great exactness with the aid of this apparatus.

It was furthermore apparent from the results obtained in this preliminary work that many other problems of plant life could be studied by such methods and that results of both practical and scientific value could be secured, since a knowledge of the factors determined is of great importance in the consideration of questions pertaining to vegetable metabolism and to the handling and storage of fruit in the home and under commercial conditions.

The chamber of the respiration calorimeter used for experiments with man is of such a size that it will accommodate seven or more large bunches of bananas; that is, its capacity is too great to make it useful for experiments with vegetable products, except those which can be obtained in uniform condition and in fairly large quantities. Furthermore, the entrance to the respiration calorimeter and all its internal arrangements are designed with

[1] U. S. Dept. Agr. Yearbook 1910, p. 307.

respect to experiments with man and are not particularly well suited to experiments with fruits or similar products. It was obvious, therefore, that a smaller respiration calorimeter with special equipment suited to experiments with plant products would be a great convenience and in view of the fact that it would be useful in the study of problems of interest to the Department of Agriculture, such an instrument was constructed. In plan and principle it corresponds to the large respiration calorimeter used for experiments with man although some improvements in grouping of accessory apparatus have been introduced and some new automatic regulating devices which greatly lessen the labor of conducting the experiments have been added, which make for greater accuracy as well as ease of operation.

A respiration calorimeter is thus designated because such an instrument combines in the same device a respiration apparatus for the determination of gaseous exchange of the subject in the respiration chamber during a given period, and a calorimeter for measuring heat liberated in the respiration chamber. Though there are conditions under which it is convenient to use the apparatus either as a calorimeter only, or as a respiration apparatus only, the two operations are usually carried on simultaneously. The two functions of the apparatus, however, are perhaps best considered separately.

The Apparatus as Used for Studying Respiratory Exchange

The chamber of the apparatus is 18 by 18 by 36 inches inside measurements, the walls being copper attached to a wooden framework, and double for purposes which are explained later. The top of the chamber has a cover that may be lifted off so that material may be put inside. The edges of the cover are formed so as to fit into grooves in the upper edges of the sides, and by means of a special wax (mixture of beeswax and Venice turpentine) pressed into the grooves the cover is sealed tight. In the upper part of each of two opposite sides is a window 6 inches by 8 inches sealed into its frame, which forms part of the copper wall. These afford opportunity to observe the fruit during ripening, or to remove a sample if desired. In the third wall is an "outlet," likewise sealed air-tight, through which pass two $\frac{3}{8}$ inch pipes for

the passage of air into and out of the chamber. On the inside the pipe carrying the ingoing air opens near the top of the chamber, while that carrying outgoing air is extended to the bottom, so that air passsing from one opening to the other must traverse the chamber.

The chamber is of sufficient size to accommodate a large bunch of bananas suspended from a framework supported on brackets in the corners near the top; and other brackets have been attached at different levels so that trays, shelves, or other supports may be used. Thus it is possible to place under investigation, at different times, not only a bunch of bananas, but also larger or smaller quantities of apples, or potatoes, or other materials. This material may be packed in the calorimeter in a manner approximating commercial conditions, or, if desired, in some other manner.

On the outside the pipe for outgoing air is connected with a rotary compressor, operated by a small electric motor which withdraws the air from the chamber and forces it any desired rate through a purifying system of special gas washing bottles arranged in series. The first two bottles of the series contain sulphuric acid, which removes from the air all water vapor brought out by it from the chamber. The next two bottles contain granular soda lime (a mixture of caustic soda and quick lime) which removes from the air all the carbon dioxid carried out of the chamber. Following these is another bottle of acid which catches moisture imparted to the dry air by the moist soda lime. The final bottle in the series contains granular sodium carbonate, which catches any sulphuric acid vapor or spray that might be carried from the preceding bottle. The air then returns to the chamber through the pipe for ingoing air. Just before entering the chamber oxygen may be admitted to the ingoing air to replace that used by the material in the chamber.

A small copper pipe, from the side of the chamber, is connected outside with a rubber bag or a spirometer or similar device, the purpose of which is to indicate the volume at any given time, and to maintain atmospheric pressure within the chamber.

The different bottles of the purifying system are weighed on a large sensitive balance to an accuracy of 0.05 of a gram. The

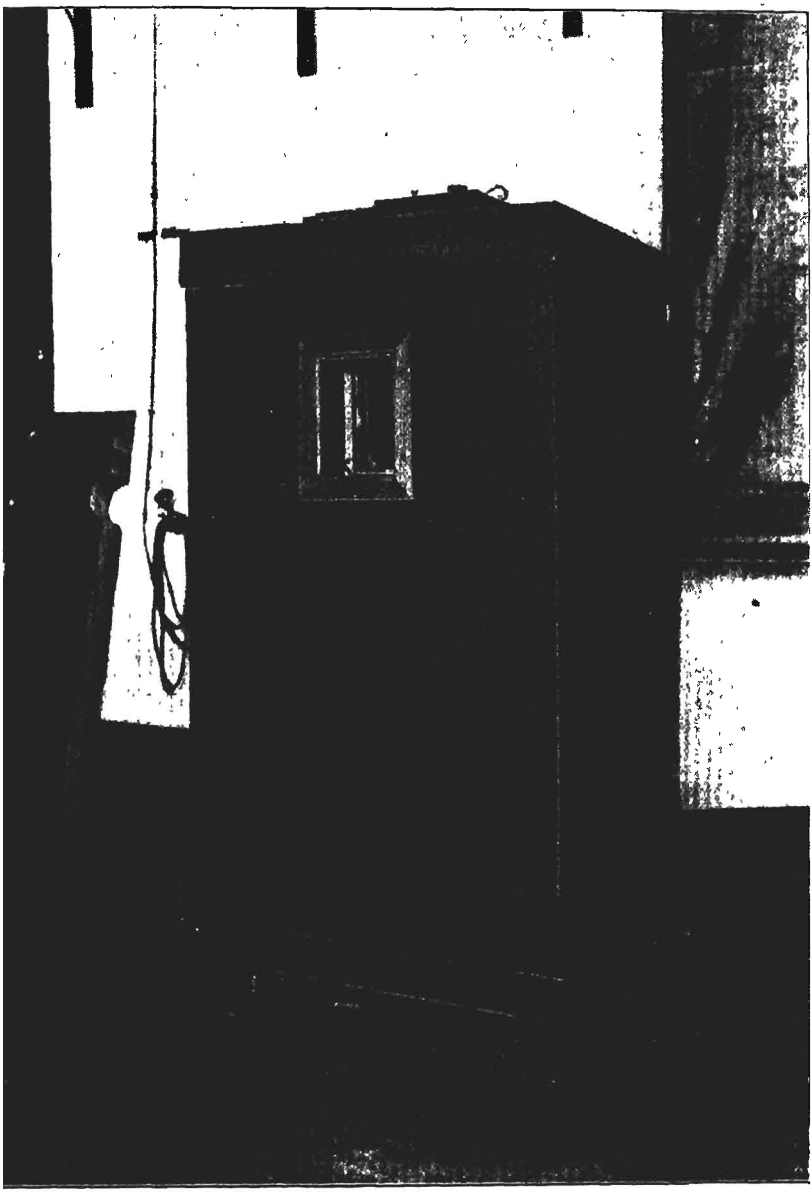
increase in weight of the first two, which contain sulphuric acid, shows how much water was removed from the chamber in the air current during a given experimental period, and that of the two soda lime bottles and of the acid bottle following them shows the amount of carbon dioxid brought out. Under ordinary conditions, in an experiment with fruit, these would represent the amounts actually produced during the period, though whenever necessary samples of the air may be analyzed to determine the amounts residual in the air of the chamber, and allowance may be made for any difference in these amounts at the beginning and the end of the period.

With a ventilating system such as described above, as rapidly as any gaseous substance is removed other gas must be introduced to maintain atmospheric pressure in the chamber. Ordinarily, oxygen is admitted, since oxygen is utilized by the ripening fruit or other material from the air of the chamber. In case the fruit were ripened in some inert gas, such as carbon dioxid or nitrogen, this gas would be admitted instead of oxygen. The gas admitted to the chamber is drawn from a supply under pressure in a steel cylinder that is suspended on a sensitive balance, and the amount admitted is determined very accurately by weighing. In the case of oxygen, for example, from the change in the weight of the cylinder and in the amount residual in the air at the beginning and the end of the period, the amount used by the fruit during the period may be ascertained.

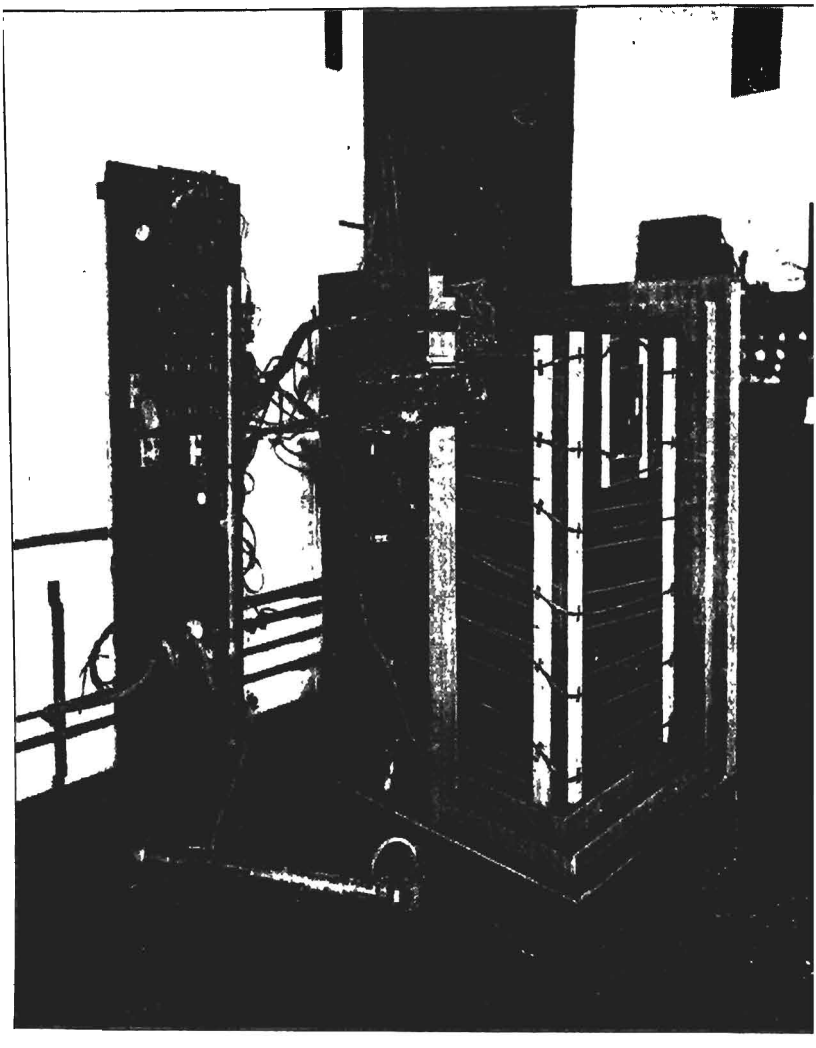
Experimental periods may be of any duration. Since the ripening process continues for several days in the case of bananas, it has been sufficient in these experiments to weigh the different bottles in the purifying system once a day.

The Apparatus as a Calorimeter

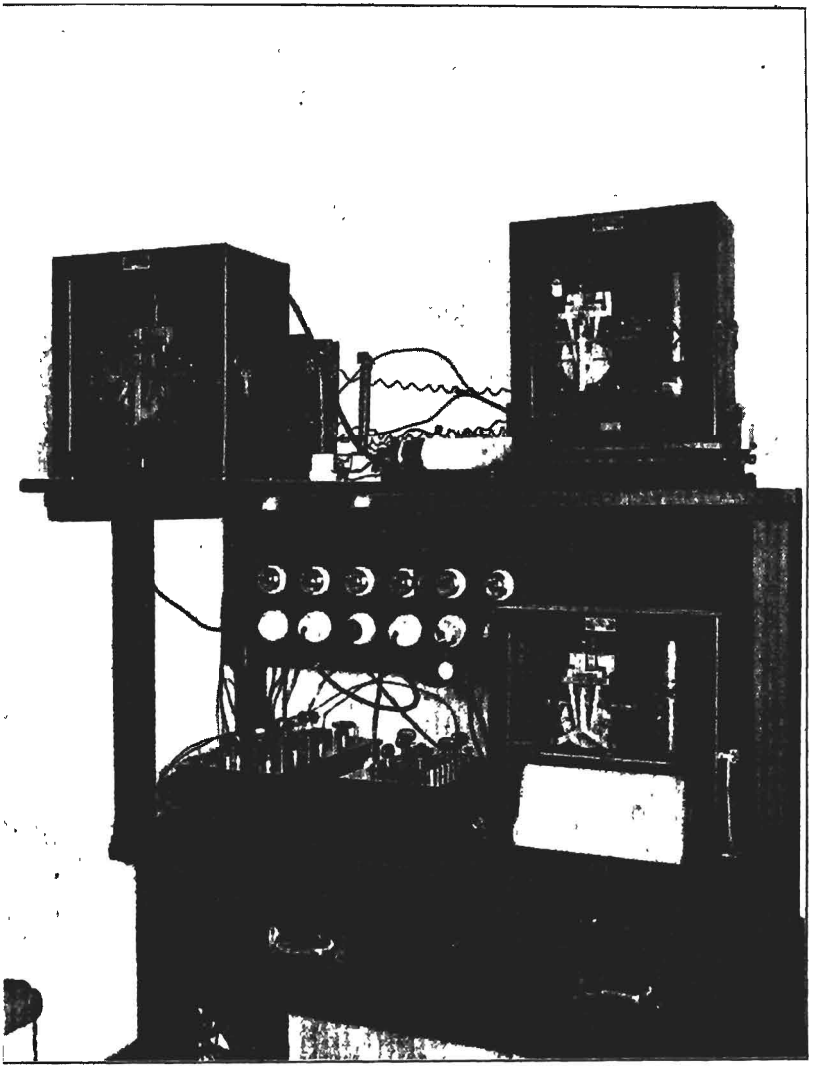
The heat generated by the ripening fruit or other material in the respiration chamber is carried out by a current of cold water flowing through a coil of copper pipe. From the weight of the water flowing through the coil during a given period, and the mean temperature difference between the incoming and outgoing water during the period, the quantity of heat carried out is determined. This quantity and that carried out as latent heat



Photograph No. 1.—This is a view of the exterior of the respiration calorimeter for the study of vegetable problems. The protective covering is in place and the window in one side is shown.



Photograph No. 2.—This is a view of the respiration calorimeter showing how portion of the protective covering may be removed. The cold-water pipes for cooling the ai in the space between the protective covering and the walls of the respiration chambe are also shown. The cover of the respiration chamber is raised. On the wall pane are shown switches for various electric currents, the preheater for warming, and th bridge for determining the temperature of the water entering the heat absorbing sys tem, and other devices.



Photograph No. 3.—In the lower right-hand corner of this photograph is shown the automatic temperature recording device with its pen which draws the line representing temperature differences. Next to this on the left is shown the bridge pertaining to this device, by means of which it is possible to vary the range of the records and to test the accuracy of the recording device. Immediately above the recorder is shown a device for the automatic control of the temperature of the water which enters the heat absorbing system in the respiration chamber. At the left on the same shelf is a device which automatically controls the heating of the air adjacent to the top, sides, and bottom of the exterior wall of the respiration chamber and of the ingoing air in the circulating air system. The bridge for this device is shown on the shelf immediately below it.

of water vapor in the out going air represent, with small corrections for changes in temperature of the chamber and of material inside, the amount of heat generated by the material in the chamber.

The quantity of water that flows through the coil in the chamber is ascertained by weighing. Water is kept flowing through the coil within the chamber at as constant a rate as possible. For this purpose the water is drawn from a constant level tank on a shelf above the calorimeter. Since distilled water is used in the circulating system, after it has passed from the calorimeter, it is collected in a tank from which, by means of a small gear pump, it is raised again to the constant level tank. This small pump is driven by the motor which operates the rotary compressor for the circulating air. The purpose of using distilled water in this cooling system is to avoid difficulties due to a presence of air in the water, which tends to collect in bubbles in the pipes and thus form temporary obstructions, causing an irregular flow of water from the heat absorbing system.

The temperature of the air in the calorimeter may be regulated by controlling either the temperature of the water that enters the calorimeter, or its rate of flow, or both. The most convenient practice is to maintain a constant rate of flow and to regulate only the temperature. To this end the water leaving the pressure tank is cooled by passing it through a coil immersed in cold water or brine and then is raised to the desired temperature by means of an electric heating device introduced in the water circuit just before it enters the calorimeter. The ingoing water is kept at such temperature that the heat will be taken up just as fast as it is liberated, so that the temperature of the air in the chamber is kept constant.

This heating device consists of a pre-heater and of a final heater. The pre-heater, which is operated manually, has a total capacity of $5\frac{3}{4}^{\circ}$ C., with a rate of 500 cubic centimeters of water per minute, and heat may be added as needed in small increments. To secure uniformity of temperature, the final heater is separated from the pre-heater by a mixing bottle. The final heater, which is automatic, has a much smaller range than the pre-heater, but it can be adjusted within very narrow limits. The sensitive

portion of the final heater consists of a very delicate electrical resistance thermometer, of a type designed by the National Bureau of Standards, which comprises one arm of a Wheatstone bridge, the slide wire of which is designed to cover by tenths of a degree a range of temperature from 0° to 35°C., shown on the dial of the bridge. This thermometer is placed in the water pipe immediately above a small heating coil of electric resistance wire, so that the water flows from the coil directly over the thermometer. If the temperature of the water flowing over the thermometer differs from the desired temperature, at which the pointer of the bridge is set, the needle of the galvanometer with which the bridge is connected is deflected accordingly; and the automatic controller, of which the galvanometer forms a part, alters the position of a sliding contact on a variable resistance which is in series with the heater just below the thermometer. This results in a change in the amount of electric current through the heater, its heating effect is varied accordingly, and the temperature of the water flowing past the thermometer is regulated until it reaches the desired constant temperature.

The difference between the temperature of the water entering the calorimeter and that leaving is determined by means of a pair of electric resistance thermometers placed in the water line, one just as it enters and the other just as it leaves the chamber, and connected with a Leeds and Northrup temperature recorder. This temperature recorder consists of a self-balancing Wheatstone bridge, two arms of which are formed by the resistance thermometers. The amount of change necessary in the balancing point of contact on the slide wire to balance the bridge at any given time is indicated by a pen that is drawn back and forth on a record sheet that moves forward at a rate of 2½ inches per hour. Since the balance is determined by the mechanism every five seconds, a virtually continuous record of the temperature differences is drawn by the pen. The scale on the paper is 10 inches wide and represents a total range of 2°. The scale is ruled with 100 lines, each representing 0.02°, but the distance between the lines is so wide that 0.01° is very easily read. The bridge part of the apparatus is so constructed that the slide wire may be made

to represent a temperature difference of from 0 to 2°, 1 to 3°, 2 to 4°, or 3 to 5°.

In order that the quantity of heat generated within the calorimeter chamber may be accurately determined, it is necessary to prevent either gain or loss of heat through the walls of the chamber. To this end the respiration chamber has double metal walls and the outer wall is kept at exactly the same temperature as the inner wall, in which case there will be no transference of heat between them. In order to accomplish this it is necessary to provide means for determining any difference of temperature between the two walls and for heating or cooling one wall until it has the same temperature as the other. In this calorimeter the outer metal wall is surrounded by a covering of heat insulating material, between which and the metal wall is an air space about 1 inch across. In this space and surrounding the outer wall of the chamber are a coil of copper pipe to carry cold water for cooling it, and a coil of resistance wire to carry an electric current for heating it. If the outer wall of the calorimeter is too warm it may be cooled by passing cold water through the copper pipe, or if it is too cold it may be heated by passing an electric current through the resistance wire; but in practice it is found most convenient to allow water to flow continually through the coil of pipe and to vary only the heating. This is done automatically.

On the inner and outer copper walls of the calorimeter chamber are electric resistance thermometers which comprise the two arms of a Wheatstone bridge, which have exactly the same resistance when the walls are at the same temperature. As the temperature of one wall varies from that of the other the resistance of the arms of the bridge varies and this causes a corresponding change in a mechanism which controls a variable resistance in series with the heating system surrounding the outer wall, and thus regulates the heating of the outer wall. By this means the temperature of the exterior wall is maintained automatically in balance with that of the interior wall. The controlling mechanism regulates the temperature of the top, sides, and bottom of the chamber independently. The temperature of the air entering the calorimeter is likewise maintained by it

exactly the same as that leaving the calorimeter, so that no heat will be carried in or out in the moving air current.

Possible Uses of the New Respiration Calorimeter

The control experiments and the experimental studies of ripening fruit (bananas) already undertaken have demonstrated the great accuracy of this respiration calorimeter as an instrument of precision and have given interesting results regarding gaseous exchange and heat production which will appear in Department of Agriculture publications.

Although the new calorimeter is arranged with special reference to experiments with fruits and other vegetable products, it is so constructed that the respiration chamber can be removed and another substituted for it of the same size but with different interior arrangements, or of smaller size, should this be desirable. In other words, it would be possible, with little additional labor, since no change in the recording and controlling devices and other accessory apparatus would be involved, to adapt the apparatus to the study of additional problems, such, for instance, as the incubation of eggs and the changes which take place in curing and storing meat products and cheese, or by making suitable provision for the collection of excretory products and for the comfort of the subjects, it would be possible to adapt the calorimeter to experiments with laboratory animals, should the work of the Department make this necessary.

SUR LE ROLE ANTISEPTIQUE DU SEL MARIN ET DU SUCRE

PAR M. L. LINDET
Paris, France

Il est facile de concevoir comment certains corps qui sont pour nous des poisons, comme les composés de l'arsenic et du mercure, peuvent arrêter le développement des microbes; mais l'action du sel marin et du sucre, dont nous faisons un usage journalier, me semble ne pas avoir été suffisamment envisagée.

Elle s'explique cependant par la facilité avec laquelle les microbes se plasmolysent; ils cèdent à une solution concentrée de sel ou de sucre une partie de leurs éléments constitutifs, s'affaiblissent, et ne présentent plus la même capacité de reproduction.

J'ai voulu rechercher dans quelle mesure la composition des microbes est capable de se modifier sous l'influence de solutions sucrées ou salines, de concentration variable, et j'ai choisi celui des microbes qu'il est le plus facile de se procurer en masse, la levure de distillerie; celle-ci provenait de la distillerie Springer à Maisons-Alfort (Seine). Dans le but de mesurer la sensibilité du phénomène, je n'ai laissé la levure en contact de la solution que pendant 24 heures, et j'ai dosé l'azote, l'acide phosphorique et la potasse dans les liquides filtrés. J'ai rapporté les chiffres obtenus à la quantité de matières que la levure contenait primitivement:

	Azote	% des éléments contenus dans la levure:	
		Acide phosphorique	Potasse (KO^2)
Témoin Eau pure	1.89	0.57	73.3
Solution de sel à 2%	1.99	1.32	75.4
Solution de sel à 4%	2.19	1.60	77.8
Solution de sel à 8%	2.65	1.77	82.1
Témoin Eau pure	1.89	1.78	73.3
Solution de sucre à 20%	"	5.33	92.6
Solution de sucre à 40%	"	"	93.8
Solution de sucre à 80%	11.13	11.38	96.8

Evidemment les quantités d'azote et d'acide phosphorique dont la cellule s'est appauvrie ne sont pas très considérables, surtout en présence de la solution de sel; mais il faut songer qu'elles représentent les matières les plus solubles de la cellule, celles que la cellule mettra en jeu dès les premiers moments de son évolution. La solubilité des composés potassiques au contraire leur confère un coefficient de diffusion considérable.

L'étude au microscope des levures ainsi soumises à l'action des solutions salines ou sucrées révéle avec netteté leur amaigrissement.

En présence de ces faits, il était intéressant de rechercher comment se reproduisent, sur bouillon de touraillons, gélatiné et sucré des globules de levure qui ont séjourné 48 heures au contact des mêmes solutions. J'ai appliqué, pour la numération des levures la technique que j'ai exposé dans un précédent travail (Comptes-rendus de l'Académie des Sciences, 1910, T. 150, p. 802), et j'ai rapporté le nombre des colonies comptées au mmg. de levure.

Colonies par mmg. de levure:

Témoin	4.514.000
Solution de sel à 5%	4.370.000
Solution de sel à 10%	1.733.000
Solution de sel à 20%	600.000
Solution de sucre à 20%	1.525.000

Il convient en outre de faire remarquer que les colonies de ces différentes levures ont apparu sur la gélatine avec un retard d'autant plus grand qu'elles avaient séjourné au contact de solutions plus concentrées. Une fois apparues, elles n'ont pas augmenté sensiblement en nombre du jour au lendemain; mais celles qui ont été formées au début ont grossi régulièrement, à fur et à mesure qu'elles retrouvaient dans le bouillon gélatine les éléments qu'elle avaient perdus.

J'ai commencé des expériences analogues avec le ferment lactique et avec les champignons; mais ces expériences sont plus difficiles à réaliser, et je demande crédit pour quelque temps.

SALMON CANNING INDUSTRY OF NORTH AMERICA

H. M. Loomis

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The salmon of the North Pacific Ocean has now become one of the most important marine food products on this continent and its popularity is fast increasing in Europe and other countries. The catching and packing of salmon in the Northwest has developed into such a large industry that it ranks second only to the lumber business. As the growth has been so rapid some apprehension has been felt that the fish might be gradually exterminated but the Federal government and the governments of the various states and of Canada are striving to overcome any such danger by regulating the industry and by establishing hatcheries at various favorable localities.

There are five principal varieties of salmon packed along the Pacific Coast, each one of which is known by several names, depending on the locality where it is caught. The fish with the reddest flesh and most oil are held in the highest esteem by consumers, and in the following list they are given in the commonly accepted order of quality.

1. Red Salmon, Sockeye, or Blueback.
2. Chinook, King or Spring Salmon.
3. Medium Red Salmon, Cohoe or Silverside.
4. Humpback or Pink Salmon.
5. Chum or Dog Salmon.

The 1911 pack of salmon amounted to 290,000,000 cans or six million cases. Of this entire output Alaska produces nearly one half, Puget Sound district about one quarter, British Columbia one sixth, Columbia one twelfth and the balance is caught in various rivers and bays along the coast of California, Oregon and Washington.

For 14 years the United Kingdom has taken an average of over 900,000 cases a year while Australia, the East Indies and South America make continually increasing demands on the product.

The habitat and history of the Sockeye and other salmon is unknown from the time they first reach the sea as young fish until they return to spawn and die—a period of about four years. These fish are not caught by hook and line but in seines and traps of various forms. The traps are located along the shores of the mainland or islands at points which the large schools of fish pass on their journey from the ocean to the rivers, and each trap consists of a row of piling, running out from the shore a distance of several hundred feet. To this, nets are hung vertically to a considerable depth below the water, which depth is regulated by law. These serve to divert the course of the fish into the trap proper, which are rectangular enclosures, formed of nets supported vertically by piles and into which the fish are directed by V-shaped openings. To empty the trap a sort of apron net is raised horizontally until the fish are near the surface and then a bail net is used to transfer the fish to a scow or steamer. As many as 50,000 fish are sometimes taken in a trap at once.

The Sockeye Salmon is comparatively small, weighing from 5 to 10 lbs., while other varieties of salmon are larger—the largest variety, the Chinook, averaging 30 lbs.

Until very recently the older type of soldered can was used for packing salmon but the solderless, so-called sanitary can, is rapidly growing in favor and in 1911 about 1,700,000 cases of fish were packed in the latter form of cans.

To manufacturing chemists it may be of interest to know that in this industry alone 840,000 lbs. of hydrochloric acid, 180,000 lbs. of caustic soda, 6,000,000 lbs. of solder, 137,000,000 sq. ft. of tin plate, and 375,000 gallons of lacquer are used.

Of the 325,000,000 lbs. of salmon caught last year about 225,000,000 were canned and the rest were cured in light brine, frozen, salted or smoked.

I wish to make acknowledgment to "The Pacific Fisherman" for most of the statistics given above.

At the cannery the fish are unloaded and carried by conveyors to:

1. The "iron chink"—a machine which removes the heads, tails, fins, and entrails.

The further steps in the canning process may be briefly enumerated:

2. Cleaning.

3. Washing.

4. Slicing by a machine which cuts the fish transversely into pieces the right size to fit the cans.

5. Adding of salt to cans.

6. Packing of fish in cans. This is usually done by white women in the United States and by Indians in Alaska.

7. Covers put on, crimped and soldered by machine.

8. Cans cooled and vent hole soldered by hand.

9. Cans tested for leaks by immersing in a hot water bath.

10. Placed in steam retort for 1-2 hour.

11. While still hot, covers are punctured, allowing most of the air and some of the liquor to escape. The sound made by striking the can also serves to detect leaks.

12. Cans resealed.

13. Cans heated in steam retorts about one hour at 240° to cook and sterilize contents.

14. Cans are scoured with caustic soda solution and washed.

15. Cans tested for leaks from the sound emitted in tapping the cover.

16. Cans lacquered and labeled.

After the process of canning and before shipment every can is usually tested several times for leaks.

In the United States canneries the labor is almost entirely done by Chinese or Japanese men—the transferring of the fish to the cans employing white women. In Alaska, owing to the scarcity of white labor, most of the canneries employ Orientals and Indians exclusively.

It might be well to mention that the low form of soldered cans, or "flats," are filled by hand, while the tall cans, or "talls," are

filled by machinery and the latter contain a product which is inferior in appearance and price to the former.

In packing the so-called sanitary cans, the number of steps in the process is considerably less and, while the cans are more expensive in the first place, the saving in solder, labor, and other items amounts to about 25c a case. With this form of can there are no vents and the filled cans are run on conveyors through a steam chest, (to heat the contents and expel air), covers are crimped immediately and the cans placed in the retort for final cooking and sterilization.

The methods in use for preparing canned salmon in the United States are generally adapted to the production of a fresh, clean and high grade product.

Since the passage of the National Food and Drugs Act and promulgation of Food Inspection Decision No. 105, regarding the labeling of canned salmon, misbranding is rarely resorted to and the cans are generally labeled to show the variety of salmon contained in them. The public is further safeguarded in the labeling of salmon by the provisions of the Alaska Fisheries Law of June 26, 1906, requiring that in the labeling of canned salmon no false or misleading statement or designation shall be made. The enforcement of this law is in the hands of the Bureau of Fisheries.

A proper study of the composition of canned salmon requires the analysis of many samples of known history, for the different varieties of salmon vary in composition in different parts of the body and at different seasons, the earlier runs of fish being much fatter and finer than the later.

Only a few analyses are reported herewith, but they represent the beginning of a more extended investigation of the composition and quality of fresh and canned salmon at the Seattle Laboratory of the Bureau of Chemistry. The fresh salmon were analyzed within 24 hours after being taken from the traps and were kept on ice as much as possible during that time.

The following notes on the analytical methods seem necessary:

Ammoniacal nitrogen:—For this determination two similar methods were employed. The first method is substantially the alcoholic distillation method of Richardson and Scherubel,

(J. A. C. S., Vol. 30, page 1515), with certain modifications proposed by W. B. Smith. 450 cc. of 95% alcohol by volume were used instead of 60% alcohol, with 25 grams of material and 5 grams of freshly ignited magnesium oxide and the fish was added directly to the distilling flask without previous extraction. 750 cc. Kjeldahl flasks were used and three 150 cc. portions were distilled into N–10 acid, making up the volume in the distilling flask to 450 cc. with 95% alcohol between each portion. The excess of acid is titrated with N–10 caustic soda and cochineal indicator.

The second method was to place 25 grams of fish in a 500 cc. Florence flask, add 5 grams magnesium oxide and 100 cc. of 95% alcohol by volume and distill in a current of boiling 95% alcohol vapor, using an apparatus like that shown on page 37 of Gattermann's "Practical Methods of Organic Chemistry." 400 cc. of distillate were collected in N–10 acid and the excess of acid titrated as above.

In both methods blank determinations were made with the reagents and the necessary correction applied.

CANNED SALMON

1911 pack

	WATER	ETHYL ETHER EXTRACT	PROTEIN (N x 6.25)	TOTAL ASH	NaCl.	AMMONIACAL NITROGEN	
						Richardson method	Alcohol vapor method
No. 1. Puget Sound Sockeye Salmon.....	62.44	15.17	20.25	2.50	0.79	0.0403	0.0348
No. 2. Puget Sound Sockeye Salmon.....	61.84	13.74	21.77	2.73	1.10	0.0437	0.0410
No. 3. Alaska Medium Red Salmon....	69.97	7.81	20.40	2.58	1.09	0.04965	....
No. 4. Alaska Chum Salmon....	73.48	2.88	21.33	2.57	0.83	0.0563	0.0557
No. 5. Alaska Pink or Humpback Salmon	74.12	4.75	19.75	1.98	0.50	.0404	....
No. 6. Alaska Red Salmon.....	70.88	5.26	21.79	2.35	0.64	.0455	....

Each sample was average of two or more cans.
All samples, except No. 2, were old form 1 lb. tall cans.
No. 2 was ½ lb. flat cans.

ANALYSES OF FRESH SALMON, EDIBLE PORTIONS

	WATER	ETHYL ETHER EXTRACT	PROTEIN (N x 6.25)	TOTAL ASH	NaCl	AMMONIACAL NITROGEN	
						Richardson method	Alcohol vapor method
Puget Sound Sockeye Salmon, caught May 7, 1912.	67.48	8.86	22.24	1.36	..	0.0121	0.0205
Puget Sound Steelhead or Salmon Trout, caught May 7, 1912.....	67.89	9.39	21.80	1.35	..	0.0135	0.0218

From a comparison of the fresh and canned Puget Sound salmon there is evidently considerable reduction in water content during the canning process. As all samples of canned salmon were in good condition and gave no indication of deterioration as far as the senses could detect it, the results on "ammoniacal nitrogen" are also of interest, being two or more times greater in the case of the canned product than in the fresh fish.

PROPOSED METHOD FOR THE ESTIMATION OF TIN IN CANNED FOODS

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Immediately before and subsequent to the issue of Food Inspection Decision 126, which limits the amount of tin in canned foods to less than 300 milligrams per kilogram, it was necessary in the course of routine work in the New York Laboratory, for a large number of analyses for tin to be made.

The first method used was the Munson Combustion Method as given in Bulletin 107, U. S. Dept. Agriculture. This method had to be discarded because of its length, and its doubtful accuracy in the case of canned foods containing salt, as then there was a loss of volatile tin salts during the combustion. It thus became necessary to find a method that would fulfill two conditions:

I. Accuracy: II. Rapidity:

The first method tried was practically that described in the report to the Local Government Board of England, by Drs. Buchanan and Schryver relative to the presence of tin in certain canned foods, published at London, 1908. In this method the organic material is destroyed as in a nitrogen determination by means of potassium sulphate and concentrated sulphuric acid. While this proved accurate enough, it was discarded because of its tediousness and the constant breaking of flasks. I attempted a modification of this method by using potassium permanganate in conjunction with the sulphuric acid. This was not entirely successful because of the large amounts of permanganate necessary and the constant attention it required. Finally a method was tried using nitric and sulphuric acids to destroy the organic matter. This proved successful from the start, not only being rapid, but also yielding practically 100% recovery, in the case of known amounts of tin. The method was developed not only for

canned materials such as fish, vegetables, fruits, etc., but also for foods high in sugars, such as maple syrup, molasses, jam, etc.,

Below are directions for each class:

Directions for Material such as Fish

Place 25 to 100 grams of the well mixed and finely ground-sample (the quantity employed depending upon the amount of fat or oil present) into a kjeldal flask (800–1000 c.c.), and add 25 to 50 c.c. of concentrated sulphuric acid, the amount depending upon the weight of the charge. Place the flask on a hot plate or on wire gauze over free flame; add about 30 c.c. of concentrated nitric acid, raise the temperature to boil and heat till white fumes are generated, then without cooling add 10 c.c. of nitric acid and continue heating as before. Repeat the nitric acid addition until the solution remains clear (usually straw color), after boiling off the nitric acid fumes. The digestion can be easily accomplished in about one hour with three or four additions of nitric acid. Let the solution cool, and dilute to about 400 c.c. with water. Neutralize with concentrated ammonia, transfer the solution to a beaker, rinse out flask with a little concentrated ammonia, add to main solution, make slightly acid with sulphur ric, and saturate with H_2S gas. Let the precipitate settle on steam bath, filter, wash with a little hot water saturated with H_2S, and then dissolve the precipitate in hot yellow ammonium sulphide. Reprecipitate with acetic acid or hydrochloric acid, filter on ashless paper, ignite, moisten with nitric acid, ignite and weigh as stannic oxide. SnO_2.

Directions for Material such as Syrup

Weigh 50 to 100 grams in kjeldal flask (800–1000 c.c.) and add about 100 c.c. of water and 150 c.c. concentrated nitric acid. Boil until all the fumes are driven off, then add a few c.c. more of nitric acid, and boil to see if there is any further action. Repeat addition of nitric acid and boiling until there is no further action. Then add concentrated sulphuric acid, a few c.c. at a time, heating until all the nitrous acid fumes are driven off. When 20 to 25 c.c. of sulphuric acid have thus been added, boil until

sulphuric acid fumes are driven off. Now add concentrated nitric acid, five c.c. at a time, until the solution is clear. Then proceed as in case of canned goods given above.

NOTE: Fifty c.c. of concentrated ammonia will nearly neutralize 25 c.c. concentrated sulphuric acid. Make usual tests for complete precipitation in the filtrate from the first tin sulphide precipitate. In the case of canned vegetables, as high as 100 grams may be taken without using more than 50 c.c. of sulphuric acid. With fish it is best to take as many c.c. of sulphuric acid grams as grams of fish. The rapidity of the digestion depends on the temperature maintained—the higher the temperature, the faster the material is oxidized.

ON THE PREPARATION OF "NATTO"

S. MURAMATSU

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There are several kinds of *natto* prepared in Japan, but here I mean common *natto* which is a kind of vegetable cheese made by fermenting boiled soya beans wrapped in rice straw and set in a warm cellar for one or two days. Thus the product becomes white and mucilageous by the development of bacteria. *Natto* is consumed as an accessory after having been mixed with table salt and several stimulants, amongst others the powdered mustard is preferred. It is chiefly consumed in Tokyo and the north-eastern districts of Japan and for the production of it Aizu is the noted place. It is chiefly consumed in Tokyo in the summer time, but in the north-east during the winter time, as these are rather poor in vegetables at that season.

There exist several studies on *natto* so far as to its constituents and the micro-organisms forming it, but no exact investigation is known of about its preparation. So, its manufacturers suffer under many difficulties of preparing *natto* of good quality; for this reason, I was obliged to make a study of the method of preparing it and several other points. Besides, I think it is very useful to prepare *natto* of good quality and increase its consumption by the people, as it is a very good and economical food stuff, being cheap and containing much protein, especially in our country where rice is the principal food.

I. SOYA BEANS

Soya beans are the principal raw material of *natto*. There are numerous varieties of soya beans cultivated in Japan, which, for instance, we can distinguish by their color as yellowish white, green, black, spotted, etc. I prepared *natto* with these different kinds and could not find a more suitable kind than the small yellowish white bean.

The beans which serve for the preparation of *natto* are first sorted and all that are broken or imperfectly developed are picked out; besides, it is better to sift them through sieves with proper meshes to separate too small or too large ones. They are then washed and allowed to steep in clean water for several hours, after that they are boiled in a large iron kettle with sufficient water for ca. 5 hours. Thus the beans become moderately soft and their color darker.

Their constitution was as follows:

	In 100 pts. air-dry beans:—	
Moisture	7.14	
Dry matter	92.86	
	In 100 pts. dry matter:—	
Crude protein	50.156	
Crude fat	22.453	
Crude fibre	6.420	
N-free extract	11.871	Soluble in water 4.329 Insoluble in water 7.542
Ash	3.600	
Total-N	8.025	
Albuminoid-N	7.953	Soluble in water—trace Insoluble in water 7.953
Non-albuminoid-N	0.072	

II. Rice Straw

Rice straw is used for the wrapper of the boiled soya beans. Fresh straw is preferable to old, as its smell is better than that of the latter. The straw is cleaned by taking the muddy leaf away from the under part of the stem and washed with clean water; afterwards it is well tied at its two ends, leaving several inches apart and bundled after filling the bag with the beans. As to the reason of using straw for the preparation of *natto*, it was considered that the straw supplies the proper bacteria to the beans but I do not think this the sole reason, for we can prepare it another way, as, for instance, by setting it in a sterilized Petri-dish or in a basket. When it is made in a basket, which after filling it with beans is put in a warm cellar covered with a straw

mat, it is called basket-*natto*. From this and other facts it is reasonable to consider the principal objects of using straw for the preparation of *natto* to be:—

1. To supply the good aroma of straw to *natto*.
2. To take away ammonia from *natto*.
3. To offer good ventilation of air to the loosely packed beans.

The bacteria which produce *natto* from soya beans are always present on the surface of the beans and their spores being very strong against high temperature, they are not easily killed by boiling, as we can see from the following experiment: The grains which were boiled for several hours are taken in sterilized Petri-dishes after each hour and placed in the incubator at 42°C. By this means, I found that the beans which were boiled for 8 hours become *natto* rich in mucilage and with good aroma.

The fact that the basket-*natto*, which does not come in touch with straw, does not sell as well as common *natto*, for, when we prepare it in the straw bundle its flavor is always superior to one which is made in Petri-dish, as it contains an aroma somewhat like that of straw. So I think that the straw which is used as a bag for the beans gives its good aroma to *natto*.

When the bacteria grow on the beans they produce so much ammonia that we can perceive it by its peculiar smell. As the straw absorbs ammonia, the smell of it is more feeble when we use straw bundle than in the case of glass dish. We can understand this fact when we see that the straw which has been used as a bag always contains much more ammonia than the same fresh material, and *natto*, made in the dish is richer in it than that from bundle.

	Amount of ammonia
In the fresh straw	0.035%
In the straw used as wrapper	0.065%
In *natto* made in a glass dish	0.235%
In *natto* made in straw bundle	0.188%

For these reasons, *natto* prepared in straw bundle must have better flavor than any other, by taking its flavor from straw and giving off the disagreeable smell of ammonia to straw.

The bacteria producing *natto* want much oxygen for their proper growth, as it is an obligate aerobe. So, when we prepare it by heaping up many bundles the interior ones become inferior in quality and also the interior beans of a large bundle become not so viscous as the outer parts. For this reason, it is recommendable to use small bundles for the preparation of superior *natto*.

III. Cellar

The cellar for the preparation of *natto* is made with bricks or with pillars surrounding them with thick layers of straw and plastering the walls with mud; the entrance is furnished with a thick door preventing the entering of air. Along the inside of the wall a long shelf two feet wide is set up at the height of ca. two feet and one or two large hearths are made on the floor for the purpose of warming the room.

IV. The Preparation of Natto

For the preparation of *natto* the soya beans are sorted at first and all beans that are broken or imperfectly developed are picked out. After washing with clean water, they are soaked for several hours and boiled in an iron kettle until they become moderately soft (ca. 5 hours). The boiled beans are put into the straw bundle while they are still hot and the bundles are placed, standing obliquely, on the shelf in the cellar, which is previously warmed by charcoal to about 40°C. The cellar is then shut up carefully, avoiding the ventilation of air; thus, the beans become *natto* after one or two days and are ready for consumption.

V. The Microbes of Natto

As to the micro-organisms of *natto* several authors have made investigations. Dr. Yabe isolated three species of micrococci which formed yellow, orange, and white colonies respectively, and a bacillus which is not motile, liquefying gelatine and producing a greenish fluorescence. He attributed the production of the characteristic aroma of *natto* to the development of the micrococcus which produces yellow colonies; but no explanation was given about the formation of the viscous substance.

Dr. Sawamura isolated various kinds of bacilli and micrococci from *natto* and regarded the following two bacilli as the chief microbes for the production of *natto*.

Bacillus No. 1. is a motile and facultative aerobe. *Natto* produced by this bacillus had a good taste and aroma, but its viscosity was not so great as that produced by the other. The author gave the name of *Bacillus natto* to this bacillus, considering it as the chief microbe in the fermentation.

Bacillus No. 2 is a rarely motile and facultative aerobe. *Natto* produced by this bacillus showed a stronger viscosity but a less nice taste and aroma than that produced by the B. *natto;* he recognised it as a variety of *Bac. mes. vulgatus*. Thus, he concluded that for the formation of good *natto* both bacilli must be present.

Mr. Monzen isolated several kinds of bacteria, among them one bacillus to which Dr. Ōmori gave the name of *Bacillus viscosus natto* and which he said, is the principal microbe that produces strong viscosity. The two kinds of bacilli which he named *Bacillus odorans natto* I and *Bacillus odorans natto II*, produce good aroma in *natto;* and another one which he named *pseudomonas odorans natto*, produces also good aroma. The latter three did not produce good *natto*, unless the material is inoculated also with *B. viscosus natto*. Thus the author concluded that there are necessary for the preparation of *natto* at least two kinds of bacteria, one producing the peculiar aroma and the other strong viscosity.

Mr. Muto isolated several bacteria and concludes that only one bacillus belonging to B. subtilis group is necessary for the production of natto.

I isolated also several bacteria from *natto*, prepared in Tokyo, Aizu, and Morioka, and found that these all contain the same micro-organisms, amongst which the following three bacilli are the principal ones; several other bacilli are not suitable for the preparation of *natto*, as they produce bad color or smell and make the *natto* unfit for eating. Two micrococci were found, one of which was analogous to Mic. flavus, and the other producing a translucent colony on agar plate-culture; but, both the micrococci having no relation to the preparation of *natto*, I gave up their further investigation.

Bacillus No. 1

This bacillus develops most energetically at high temperature (40—50° C.) and produces the best quality of *natto*, providing much mucilage and good aroma.

Form:

The cells grown in bouillon at 40° C. are 1 μ thick and 5-8 μ long.

It moves energetically, providing long cilia around its body.

Spore:

An oval spore is formed principally in one end of the cell, which is 0.8 μ thick and 1.6 μ long; the formation of spore requires 4 hours at 42° C. and germination of it begins equatorial after $2\frac{1}{2}$ hours at the same temperature.

Oxygen:

Obligate aerobe.

Coloring:

It is colored readily with aniline coloring matters and also after Gram's method.

Bouillon culture:

Bouillon remains almost clear after its development, and a strong folded film, colored slightly grayish brown, is formed after 10 hours at 38° C.

Sugar bouillon becomes slightly turbid changing its reaction to acidic at the beginning, which turns alkaline gradually; gas is not formed.

Peptone-water culture:

It produces a grayish white film on its surface and the liquid becomes slightly turbid.

Gelatine plate-culture:

Small white colonies are formed which liquefy it quickly.

Gelatine stab-culture:

It develops vigorously at the surface and liquefies gelatine in the shape of a funnel; the liquefied part remains transparent and a film is formed.

Agar plate-culture:

White and mealy-looking colony, that has a rough wristle at its centre but delicate at its edge, spreading very rapidly at 40° C.

Agar slope-culture:

Colony develops along the line and spreads rapidly all over the surface with mealy appearance; the condensed water remains transparent with a film on its surface, but no sediment.

Potato culture:

Elevated colony is formed in the beginning, which spreads soon over the whole surface of the medium; the colour of the colony is yellowish brown and it is folded with mealy appearance, the medium becoming brown.

Milk culture:

It is coagulated at first and is dissolved again.

H_2S:

Is formed.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon but does not develop in the glucose nitrate medium.

Ammonia:

Is formed in the culture of bouillon and Soya beans.

Enzym:

Diastase and proteolytic enzym of tryptic nature are recognised.

Behaviour to temperature:

It develops very vigorously at 50° C., but not at 60° C. It is killed at 60° C. after two hours, and after one hour at 80° C.

The resistance of the spores against heat is very strong, for it wants one hour to be killed in Koch's steam-steriliser.

Behaviour to several compounds:

Table salt:

In bouillon containing 15% NaCl it develops slowly, but not in 20% solution.

Alcohol:

It develops in bouillon containing 4%, but not in 5% alcohol.

The spore is not killed readily with alcohol, as it is yet alive after ten days and more, when put either in 50% or absolute alcohol at 20° C.

HCl:

It develops in bouillon containing 0.025% HCl, but not in 0.05% The spore which is put in 3% HCl is alive after one day, but not

after two days. In 4% HCl it is alive after one hour, but not after two hours.

Acetic acid:

It develops in bouillon with same concentration as hydrochloric acid.

The spore is not killed by glacial acetic acid after 10 days and more.

NaOH:

It develops in bouillon containing 0.2% NaOH, but not in 0.3%.

The spore is killed when it is put in 35% solution after one day.

Phenol:

It develops in bouillon containing 0.1% Phenol, but not in 0.2%.

The spore is not killed after ten days when it is put in 5% solution.

Corrosive sublimate:

It develops in bouillon containing 0.0025% $HgCl_2$, but not in 0.005%.

The spore is killed after 50 minutes when it is put in 0.1% solution, but it was alive after 40 minutes in the same solution.

This bacillus may be the same as those which Dr. Sawamura represented as *Bacillus No.* 2 and *Bacillus viscosus Ōmori*, and also that which Mr. Muto thought was the only bacterium which produces *natto*, though there are several differences in its behaviour investigated by these authors.

Bacillus No. 2

This bacillus develops most energetically at high temperature and produces *natto* of the best quality, forming much mucilage and rather higher aroma than Bacillus No. 1.

Form:

The cells grown in bouillon at 40° C. are 0.8-1 μ thick and 4-10 μ long.

Motility:

It moves vigorously providing long cilia around its body.

Spore:

An oval spore is formed in one end of the cell, and it is 0.8 μ thick and 2 μ long.

The spore wants 4 hours at 42° C. for its formation and it germinates equatorial after $2\frac{1}{2}$ hours at the same temperature.

Oxygen:

Obligate aerobe.

Colouring:

The cell is coloured easily by aniline colouring matters and also after Gram's method.

Bouillon culture:

Bouillon remains almost clear after its development and a strongly folded film of slightly grayish brown is formed after twelve hours at 38° C. Sugar bouillon becomes slightly turbid, changing acidic at the beginning which turns alkaline gradually; gas is not formed.

Peptone-water culture:

It produces a grayish white film on its surface and the liquid becomes turbid slightly.

Gelatine stab-culture:

It develops on the surface quickly and liquefies gelatine in the shape of a funnel; the liquefied part remains transparent and a film is formed.

Agar plate-culture:

There is formed a white and mealy-looking colony with rough wristle at its centre but delicate at its edge, spreads very quickly at 40° C.

Agar streak-culture:

Colony develops along the line and spreads rapidly all over the surface with mealy appearance. The condensed water remains transparent with a folded film on its surface but no sediment.

Potato culture:

Elevated colony is formed in the beginning, which soon spreads over the whole surface of the medium; the colony is folded and has brownish yellow colour and mealy appearance; the medium becomes brownish gray.

Milk culture:

It is coagulated at the beginning and is dissolved again.

H_2S:

Is not formed.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon and produces ammonia by reduction of nitric acid in the glucose nitrate medium.

Ammonia:

It is formed in the culture of bouillon and soya beans.

Enzym:

Diastase and proteolytic enzym of the tryptic nature are recognised.

Concerning the behaviour against heat and several compounds as formerly mentioned, there is not much difference with *Bacillus No. 1.*

This bacillus may be the same as that which Dr. Sawamura named *Bacillus natto,* though there are several differences in its behaviour investigated by us. As this bacillus does not produce any mucilage at low temperature (say, 35° C.) he thought it, perhaps, to be one which produces aroma peculiar to *natto;* but, as I mentioned already, this bacillus produces much mucilage at higher temperature and makes good *natto* with high aroma.

Bacillus No. 3

This bacillus develops most energetically at 40° C., and when it is developed on boiled soya beans at this temperature, it produces good *natto* with strong viscosity and good aroma; but its mucilage is somewhat less than *Bacillus No. 1* and *Bacillus No. 2.*

Form:

The cells grown in bouillon at 40° C. are 1.2 μ thick and 6-10 μ long.

Motility:

It moves providing long cilia around its body.

Spore:

An oval spore is formed in one end of the cell, which is 1 μ thick and 1.5 μ long.

The spore is formed after 4 hours at 42° C. and germinates equatorial after 2½ hours at the same temperature.

Oxygen:

Obligate aerobe.

Colouring:

It is coloured readily with aniline colouring matters and also after Gram's method.

Bouillon culture:

Bouillon becomes slightly turbid and a brittle film of slightly grayish brown colour is formed after ten hours at 38° C. and produces a small amount of sediment. The film is broken easily by shaking and sinks to the bottom. Sugar bouillon changes to slightly acidic at the beginning and turns slightly alkaline afterwards.

Peptone-water culture:

It becomes slightly turbid and forms a yellowish white film on its surface. Gas is not formed.

Gelatine plate-culture:

Small white colonies are formed which liquefy it quickly.

Gelatine stab-culture:

It develops on the surface at the beginning and liquefies gelatine in the shape of a funnel, afterwards thoroughly.

Agar plate-culture:

White colony with rough wristle at its centre but delicate at its edge, spreads very rapidly at 40° C.

Agar slope-culture:

The colony develops along the line and spreads rapidly in the shape of a feather; the condensed water is transparent with a film on its surface, but no sediment.

Potato culture:

Yellowish gray colony is formed, somewhat elevated in the beginning; it spreads soon over the whole surface of the medium. The colony has strong viscosity and it is folded shallower than *Bacillus No. 1. and Bacillus No. 2.*, the medium becoming gray.

Milk culture:

It is coagulated and dissolved again.

H_2S:

Is produced.

Indol reaction:

Is not obtained from old bouillon culture.

Reducing property:

It reduces methylene blue in bouillon, and ammonia is formed by the reduction of nitric acid in the glucose nitrate medium.

Ammonia:

Is formed in the culture of bouillon and soya beans.

Enzym:

Diastase and proteolytic enzym of tryptic nature are recognized.

The behavior to heat and several compounds is almost the same as with Bacillus No. 1, although there are some differences.

This may be the same bacillus as *Bacillus grossus*, but as there is no detailed description of it, I cannot make a precise comparison.

VI. The Application of Cultured Bacteria for the Preparation of Natto

As mentioned already, when we prepare *natto* in a glass dish at ca.30°C. inoculated with *Bacillus No. 1* it has some viscosity, while others have not, but the aroma was inferior to that made in straw bundles, for it does not touch with straw. At 45°C. all bacilli produce *natto* of fine quality providing strong viscosity and good aroma; the aroma produced by *Bacillus No. 1* was the best, while *Bacillus No. 2* produces a rather strong smell of ammonia, and *Bacillus No. 3* being the worst; moreover, I prepared *natto* according to the common way differing only on the point of inoculating these bacilli separately and also mixing them with one another. The result was that *natto* which was produced by the inoculation of *Bacillus No. 1* was the best, as it has much mucilage and fine aroma, while *Bacillus No. 2* produced an inferior and *Bacillus No. 3* the worst quality. *Natto* produced by the inoculation of mixed baccilli was not so good as that produced by each bacillus; so, there is no necessity that two or more bacilli present for the formation of good *natto*. By the inoculation of cultured bacteria we can entirely avoid failures and can prepare good *natto* by selecting the bacteria. Otherwise, it is sufficient to put it in the cellar for only one day, after which the *natto* will be ready for consumption. So, I recommend to use the pure culture of proper bacteria according to the following way:

The bacteria developed on the slope culture medium of agar are mixed with juice produced by the boiling of beans. This is poured over the surface of boiled beans while they are still in the kettle, the further process being the same as usual. There is no necessity of mixing several bacilli.

VII. Natto as a Food Accessory

As *natto* is prepared from soya beans which are rich in protein and carbohydrates, it contains much protein and carbohydrates; the nutritive value of it is greater than that of boiled soya beans, for it is rich in soluble matters produced by the micro-organisms.

The composition of *natto* differs exceedingly with age, but its mean composition is as follows: (Compare with the composition of boiled soya beans.)

	In 100 pts. of fresh *natto*.		
Moisture	53.480		
Dry matter	46.520		
	In 100 pts. of dry matter:		
Crude protein	46.088		
Crude fat	20.216		
Crude fibre	6.140		
N-free extract	3.348	Soluble in water	2.495
		Insoluble in water	0.853
Ash	5.010		
Total-N	7.374		
Albuminoid-N	5.458	Soluble in water	1.141
		Insoluble in water	4.317
Non-albuminoid-N	1.916		

The micro-organisms which grow on the soya beans secrete trypsin and diastase; so, when we take it together with several foods rich in protein or starch, they may be digested more rapidly than when they are taken alone.

I express many thanks to Dr. Satō, Director of our College, who helped me in determining the quality of *natto* that I prepared, and also to Mr. N. Nitta and Mr. Y. Tanaka who assisted me in these investigations.

CONTRIBUTION TO THE CHEMISTRY OF THE RIPENING OF "SHIOKARA"

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Although the isolation and identification of some nitrogenous compounds in "Shiokara" has been undertaken about two years ago by Prof. U. Suzuki, Yoneyama and Ōtake in this laboratory, no chemical investigation about the ripening process of this interesting food material has yet been reported. So I have tried to contribute something on this line. I have observed that the autolysis and the action of microbes are two indispensable factors for the preparation of "Shiokara."[1] Some trials have also been made to isolate the enzymes which play an important rôle in this process, and finally, I have carried out some quantitative determinations to see the chemical changes at different stages of ripening.

I. Autolysis and the Action of Microbes

1). To see whether the autolysis is going on during the ripening process of "Shiokara," very fresh organs[2] of a bonito fish were minced with a meat-chopping machine, and rubbed with some quartz sand in a mortar. 40g of the paste thus prepared was divided into two equal parts, and put in the flasks A and B. After adding 100 c.c. of water to each flask, A was boiled for a few minutes to destroy the enzymatic action. Both flasks were then shaken with enough toluol and a little chloroform, and kept for 4 days at ordinary temperature. No bacterial growth was observed during that time. The flask B was

[1] "Shiokara" made from the organs of bonito was used.
[2] The stomach, the intestines, and the pyloric coecum.

now boiled, and the contents of both flasks were then filtered and analysed with the following results:—

	A (boiled)	B (not boiled)
Total soluble-N	1.697%	1.895%
Soluble Alb.-N	0.184 "	0.141 "
Non-alb.-N	1.513 "	1.754 "

2). The fresh "Shiokara" two days after preparation was chopped and crushed in a mortar, 200g of the paste were divided into two equal parts and put in two flasks of 1 litre capacity, and stoppered with cotton plugs. After adding 500 c.c. water, one flask was boiled. To each flask was now added enough toluol and chloroform and after keeping for 4 days at room temperature, the contents of both flasks were filtered and analysed.

	A (Boiled)	B (not boiled)
Total soluble-N	1.848%	2.052%
Soluble Alb.-N	0.056 "	0.038 "
Non-alb.-N	1.792 "	2.014 "
Amino-N (after formol method)	0.604 "	1.023 "

We see from the above two experiments that autolysis is going on in the fresh organs of bonito fish, and also in the freshly prepared "Shiokara."

3). The microbes, predominating in "Shiokara" seem to be quite different at different stages of its ripening. In three preparations, made in April and two months old, we found immense numbers of yeasts, bacilli and cocci, but only few moulds, while in a sample prepared early in October and about one and a half months old, were found numerous yeasts, the other microbes being relatively very few.

The isolation and identification of these microbes will be reported afterwards.

4). 120g of the "Shiokara", which was two months old, were well crushed and equally divided into three Erlenmeyer

flasks containing each 100 c.c. of saturated natrium chloride solution and treated in the following way:—

A. Control:—Not boiled, no antiseptics added.

B. Not boiled, toluol and chloroform added to prevent the bacterial growth, but not the enzymatic action.

C. Boiled and antiseptics added to prevent both bacterial and enzymatic action.

After keeping for ten days at 34—38°, they were boiled and filtered, and the filtrates were analysed with the following results:—

	A (Control)	B (Not boiled, but antiseptic added)	C (Boiled and antiseptic added)
Total soluble-N.....	2.404%	2.305%	2.305%
Soluble alb.-N.....	0.049 "	0.090 "	0.184 "
Non-alb.-N.....	2.355 "	2.215 "	2.121 "

The above experiment shows that both autolysis and the action of microbes are going on very slowly in the old preparations compared to fresh ones. The investigation of Wehmer[1] on salted herring has shown that the action of microbes upon proteins is somewhat retarded in 5 per cent. common salt solution, but it does not entirely stop even in 30% solution. As the concentration of the salt in "Shiokara" usually is 15%, there is no doubt that the microbes can still play an important rôle on the ripening process, especially at the early stage of its preparation.

II. Enzymes in 'Shiokara"

Trypsin, diastase and lipase were identified in the fresh organs of a bonito fish and also in the fresh preparations of "Shiokara." In the old preparation, however, their action seems to be much retarded. This observation agrees well with the experiments mentioned above.

[1] Wehmer: Abhandlungen des deutschen Seefischerei—Vereins, III, 1898.

1). Fresh organs. The stomach, intestines and pyloric coecum of a fresh bonito were freed from their contents and rubbed with some quartz sand in a mortar, and filtered through the cloth filter. The faintly acid extract thus obtained has shown its peptonifying power upon milk and fibrin, either in the faintly acid reaction or after addition of 0.2% sodium carbonate. But no action was observed in presence of 0.2% hydrochloric acid in the medium, thus the absence of a pepsin is most profitable.

The existence of diastase was shown by the saccharification of starch paste and glycogen in the neutral reaction.

For the detection of lipase, the minced and ground organ was extracted with a mixture of 90 parts of pure glycerine and 10 parts of 1% sodium carbonate, 10 c.c. of the mixture being used for 1 g of the sample. The liquid was filtered through a piece of cloth and exactly neutralized. By the addition of some milk or olive oil to this extract, the increase of acidity due to the formation of fatty acids by the action of lipase upon neutral fats was observed. Of course some toluol and chloroform being added to prevent the bacterial growth.

2). "Shiokara" at different stages of ripening. The following observation was made with the samples collected at different stages of ripening:—

(a). "Shiokara," two days old.

Trypsin.	Present, active.
Diastase.	Do.
Lipase.	Present, but the action was very weak.

(b). "Shiokara." 40–50 days old.

Trypsin.	Present, but very weak.
Diastase.	No reaction.
Pepsin.	Do.

(c). "Shiokara," 50–60 days old.

Trypsin.	Very weak.
Lipase.	Do.
Diastase.	No reaction.

3). Isolation of enzymes. For this purpose, about 200g of the fresh sample, 3 days old, were finely minced and ground with some quartz sand in a mortar and macerated with a little

distilled water. The liquid was strained through linen cloth, and after dialysing for about two hours to get rid of the greater part of the common salt, it was poured in a mixture of absolute alcohol and ether, the grayish white voluminous precipitate thus produced was then collected on a filter, washed with absolute alcohol and ether, and dried over sulphuric acid. The crude enzyme preparation obtained in this way, when dissolved in a little water has shown strong diastatic and tryptic action while that of lipase was very weak. When the solution of this crude enzymes was added to a solution of various amino-acid, no liberation of ammonia was observed, showing the absence of amidase.

The proteolytic enzyme which acts in weak alkaline as well as in neutral or in faintly acid reaction, but not in a 0.2% hydrochloric acid solution, was also found by Blanchard[1] in several fishes and by Roaf[2] in two crustaceae.

[1] Blanchard, Jahresbericht für Their-Chemie, 13, 1883—Orig. Compt. rend. 96, 1241.

[2] Roaf, Jahresber. f. Tier Chem. 36, 1906—Orig. Biochem. Journal, 1. 390–97.

III. Chemical Changes During the Ripening Process

1). The sample[1] used for this determination was prepared on the 17th of June, 1911, and after 3, 6, 12, 25, and 40 days respectively a portion was taken for analysis. Thus the following results were obtained:—

Date of analysis	3	6	12	25	40	Days after preparation
In 100 parts of fresh samples						
Water	64.95	64.78	64.58	64.25	63.99	
Dry matter	35.05	35.22	35.42	35.75	36.01	
Total-N	1.98	2.04	2.05	.....	2.07	
Alb.-N	0.35	0.35	0.28	0.26	0.14	
Ether-extract	1.83	1.81	1.81	1.71	1.74	
Soluble matter	27.25	28.45	29.24	31.10	31.14	
Non-alb.-N	1.63	1.69	1.83	1.98	2.01	
Ammonium-N	0.15	0.15	0.25	0.18	0.13	
Organic base-N	0.84	0.72	0.69	0.63	0.64	
Other-N	0.74	0.80	1.09	1.17	1.43	
Total acid (as lactic)	1.43	1.42	0.97	0.96	0.98	
NaCl (calculated from total chlorine)	17.31	.....	17.34	.....	17.71	
In 100 parts of dry matter						
Total-N	5.64	5.79	5.79	.....	5.76	
Alb.-N	1.00	1.00	0.80	0.71	0.38	
Ether-extract	5.21	5.13	5.10	4.77	4.84	
Soluble matter	77.74	80.76	82.53	86.99	86.49	
Non-alb.-N	4.64	4.79	5.18	5.54	5.57	
Ammonium-N	0.43	0.43	0.70	0.51	0.36	
Organic base-N	2.39	2.04	1.97	1.77	1.79	
Other-N	2.12	2.26	3.09	3.26	3.98	
Total acid	4.07	4.02	2.73	2.67	2.72	
Common salt	49.37	.....	48.95	.....	49.17	

[1] This sample contained the stomach, intestines, pyloric coecum and very little liver.

2). The second sample[1] was prepared on the 3rd of Oct. 1911, and after 1, 14, and 53 days respectively, a portion was taken for analysis:—

	In 100 parts of fresh sample			In 100 parts of dry matter			Days after preparation
Date of analysis	1	14	53	1	14	53	
Water.....	64.39	63.00	60.33	0.0	0.0	0.0	
Dry matter	35.61	36.99	39.67	100.0	100.0	100.0	
Total-N.....	2.29	.....	.....	6.42	.....	.....	
Total acid.....	0.95	1.05	1.14	2.65	2.85	2.88	
Ether-extract.....	6.84	6.89	.....	19.19	18.61	.....	
Soluble matter.....	25.61	27.14	28.90	71.92	73.27	72.86	
Non-alb.-N.....	1.59	1.85	2.07	4.45	4.99	5.21	
Ammonium-N.....	0.10	0.12	0.14	0.28	0.32	0.35	
Organic base-N.....	0.81	0.73	0.71	2.27	1.96	1.79	
Creatinine-N.....	0.01	Trace	Trace	0.02	Trace	Trace	
Creatine-N.....	0.02	Trace	Trace	0.04	Trace	Trace	
Xanthine base-N ...	.....	0.06	0.03	.....	0.16	0.08	
Other-N.....	0.66	1.00	1.21	1.86	2.71	3.05	
Natrium chloride....	13.51	13.94	.....	37.94	37.62	.....	

The results of the above two analyses may be summarized as follows:—

	(1)	(2)
Soluble organic matter	Gradually increased	Do.
Alb.-N	" decreased	.....
Non-alb.-N	" increased	Do.
Ammonium-N	Increased at first and decreased hence-forward.	Gradually increased
Monoamino-N	Gradually increased	Do.
Organic base-N	" decreased	Do.
Creatine-N	.....	Gradually decreased
Creatinine-N	.....	" "
Xanthinbase-N	.....	" "
Ether-extract	Somewhat decreased	Do.
Total acid	Decreased	Somewhat increased

[1] This sample contained more liver than the former one.

Thus the results of two analyses resemble each other in general respects, only the contradictory results were observed with ammonia and with total acid. This may be due to the differences of materials and also the temperature during the experiments.

3). I will add here some qualitative tests made about the distillates obtained by the steam distillation of two shiokara-preparations, in neutral as well as in acid reaction.

In the distillate	10 days after preparation	61 days after preparation
Alcohol	(+)[1] (very little)	(−)[1]
Aldehyde	(−)	(−)
Acetone	(−)	(−)
Indol	(−)	(−)
Phenol	(−)	(−)
Formic acid	(+) (trace)	(+) (distinct)
In the residue		
Lactic acid	(+) (distinct)	
Succinic acid	(−)	
In the water extract of the natural sample		
Tryptophan	(+)	(+)

Summary of the Results

1). Various samples, examined at different stages of ripening, gave all acid reaction chiefly due to lactic acid.

2). Autolysis is going on in the freshly prepared "Shiokara," and decreases gradually as the ripening process proceeds.

3). The enzymes found in "Shiokara" are diastase, lipase, and trypsin. The last one acts not only in weak alkaline solution but also even in neutral or in faintly acid reaction.

4). Micro-organisms play also some important rôle during the ripening process.

[1] (+), indicates presence; (−) absence.

5). Temperature has also great influence upon the action of enzymes and microbes.

6). During the ripening process, the increase of soluble matter non-albuminoid nitrogen, especially monoamino-nitrogen, and the decrease of protein, organic bases, creatine, creatinine, and purin bases were observed.

In conclusion I express my thanks to Profs. U. Suzuki and S. Machida for their kind advices given during the work.

QUANTITATIVE DETERMINATION OF CREATINE, CREATININE AND MONOAMINO-ACIDS IN SOME FISHES, MOLLUSCA AND CRUSTACEA

By Y. Okuda

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I. Creatine and Creatinine

For the determination of creatine and creatinine, the flesh freed from bones, heads, fins, scales, and internal organs[1] was chopped and extracted with water at 50–55° for one hour. The residue was treated two times more in the same way. The whole extract was now boiled for a short time to remove most of the proteins by coagulation and filtered. The filtrate was evaporated under diminished pressure to a small volume and was divided into two portions. One portion of it served directly for the determination of creatinine after Folin's colorimetric method, while the other portion was previously boiled with nearly 4 per cent. sulphuric acid for two hours, to convert the creatine present into creatinine, and after removing the sulphuric acid by means of barium hydroxide, it was subjected to the determination after Folin. From the difference of these two determinations we can calculate the quantity of creatine originally present in the flesh, 1 mg creatinine being equivalent to 1.16 mg creatine. The results obtained were as follows:

[1] The case of clam was exception, as its whole body was used.

Name	In 100 parts of fresh substance			In 100 parts of dry matter	
	Water	Creatine	Creatinine	Creatine	Creatinine
	g	g	g	g	g
Bonito (Gymnonsarda affinis Cantor)	72.165	0.649	0.134	2.011	0.481
Tunnyfish (Thunnus schlegeli Steined)	72.402	0.497	0.064	1.800	0.232
"Katsuobushi" (Steamed and dried bonito)	14.808	0.453	0.660	0.531	0.775
Salmon (Oncorhynchus tshawytscha Walbaum)	63.300	0.560	0.067	1.525	0.182
Snapper (Pagrus major)	77.340	0.754	0.070	3.327	0.308
Carp (Cyprinus carpio L)	79.160	0.421	0.077	2.020	0.369
Shark	79.800	0.655	0.134	3.242	0.663
Lobster (Palinurus japonicus Gray)	79.920	Trace?	Trace?		
Crab (Neptunus pelagicus M-Edw)	84.500	Trace?	Trace?		
Cuttle-fish (Sepia esculenta Hoyle)	81.699	Trace	Trace		
"Kakisurume" (Chopped and dried cuttle-fish)	27.570	Trace	Trace		
Clam (Cytherea meretrix L)	90.490	Trace	Trace		

The materials used for the determination were very fresh, except the salted flesh of salmon.

We see from the above result that all of the examined fishes contained comparatively much creatine and creatinine,[1] on the contrary in mollusca and crustacea, the existence of these two compounds was doubtful, at least, they must be present only in traces. In fresh fish we found generally more creatine than creatinine, while in dried bonito the reverse was observed. It is therefore possible that a part of creatine is transformed into creatinine during the preparation of the food.

[1] Van Hoogenhuyze and H. Herploegh found per kilogramm flesh of ox, sheep, pig and horse 4.4, 4.1, 4.5 and 3.8 g creatine respectively. (Zeitschr. f. physiol. Chem., 1905, 46, 432.)

It may be mentioned here that the water extract of clam gives only slight yellowish red coloration instantly after addition of picric acid and soda after Folin, thus showing that only a trace of creatinine is present in it, but after standing for many hours at room temperature, it takes a dark red color. After some tests we found that the glycogen, originally present in the extract, is gradually acted upon by diastatic ferments of clam itself, and the sugar thus resulted may impart this red coloration. The presence of diastatic ferment in the clam is easily shown in the usual way.

II. Monoamino-acids

For the determination of monoamino-acids Sörensen's formol titration method was adopted. Of course, this method does not hold good for every monoamino-acid, but in the case of fish flesh, the quantity of the amino-acids being very little, the method of Van Slyke is not conveniently applied.

I have made some preliminary tests also, and found that the presence of organic bases, like arginine, lysine, histidine, etc., more or less interferes with the result of the formol method, so it is better to remove these bases previously. But the presence of creatine has apparently no effect upon this determination.

150 g minced fresh flesh, free from bones, heads, fins, scales and internal organs was extracted in the similar way as mentioned above and the aqueous extract was boiled and slightly acidified with acetic acid to remove coagulable proteins, filtered, neutralized and evaporated by a low pressure to a small volume, acidified with sulphuric acid and precipitated with phosphotungstic acid in the usual way. The filtrate of the phosphotungstic precipitate, after the removal of the phosphotungstic and sulphuric acid by means of barium hydroxide, was evaporated, in neutral reaction, again to a small volume and titrated according to the usual formol method. Thus the following result was obtained:

Substance	Water	N of Monoamino-acids in g.		Remarks
		In 100g fresh flesh	In 100g dry flesh	
Carp I.....	76.609	0.022	0.094	Tunny, bonito, porgy and cuttle fish applied to the above determination were fresh. Spiny lobster, Crussian carp and Carp I were still living when they were analyzed. Carp II was analyzed standing 50 hours after death at room temperature (12°C). The increase of monoamino-acids after that time was very insignificant.
Carp II.....	76.789	0.024	0.103	
Tunny.....	73.516	0.011	0.041	
Bonito.....	69.371	0.022	0.072	
Porgy.....	76.787	0.016	0.069	
Crussian carp..	81.998	0.035	0.194	
Spiny lobster...	75.975	0.146	0.608	
Cuttle fish.....	81.671	0.089	0.485	

We see from the above results that the contents of monoamino-acids are generally very little in fish, while mollusca and crustacea contain a little more.

III. On Different Forms of Proteins in the Flesh of Fish

For this purpose, the flesh was extracted[1] with water, alcohol, NaCl, and KOH, respectively, and the quantity of total and albuminoid nitrogen in each extract was determined according to Kjeldahl's method.

[1] 10 g fresh flesh was extracted with 100 c.c. of solvent for 24 hours at 10°C.

	Flesh	Solvent	In 100 g fresh flesh		In 100 g dried flesh		Sum of each N as 100	
			Total N	Prot. N	Total N	Prot. N	Total N	Prot. N
1.	Crussian carp (Carrassius auratus L)	H_2O	0.746	0.476	4.144	2.644	17.171	13.583
		0.2%KOH	2.003	1.793	11.127	9.960	46.077	51.125
		70%Alcohol	0.386	0.174	2.144	0.966	8.‘99	4.961
		10% NaCl	1.212	1.064	6.732	5.910	27.881	30.339
2.	Carp (Cyprinus carpio L)	H_2O	0.479	0.326	2.182	1.485	11.129	10.326
		0.2% KOH	1.715	1.341	7.812	6.108	39.809	43.477
		70% Alcohol	0.492	0.240	2.241	1.093	11.420	7.602
		10% NaCl	1.622	1.250	7.388	5.694	37.649	39.605
3.	Spiny lobster (Palinurus japonicus Gray)	H_2O	1.600	0.736	6.659	3.063	23.808	22.816
		0.2% KOH	2.138	1.212	8.898	5.044	31.813	37.572
		70% Alcohol	0.934	0.119	3.887	0.495	13.898	3.689
		10 % NaCl	2.048	1.158	8.544	4.819	30.474	35.898
4.	Cuttle fish (Sepia esculenta Hoyle)	H_2O	0.932	0.351	5.085	1.915	19.984	
		0.2% KOH	1.775	1.223	9.864	6.673	38.059	
		70% Alcohol	0.602	—	3.285	—	12.908	
		10% NaCl	1.354	—	7.387	—	29.032	

The amount of proteins extracted by alkali was generally much greater than that extracted by other solvents. The proteins soluble in 10% NaCl, as globulins, were also much, water soluble proteins as proteose and albumine not much and the proteins as prolamins very little.

IV. Form of Nitrogen in Some Marine Animals

The analytical results are shown in the following table:

Summaries

1. All of the examined kinds of fish contained comparatively much creatine and creatinine, but the flesh of mollusca only trace, in the flesh of crustacea the existence of these compounds

was doubtful. The quantity of creatine was generally much more than that of creatinine, in all fresh fishes.

2. In all marine animals examined the quantity of organic base nitrogen is much more than that of monoamino-acid nitrogen, and the amount of the latter is generally very little in fish, but somewhat much in lobster and cuttle fish.

3. Most of proteins are soluble in dilute alkali solution, the proteins soluble in 10 per cent NaCl were also much, this fact must be cared on the preservation of fish.

The experiments have been made by the writer under the direction of Professor Dr. U. Suzuki, and it is my pleasant duty to thank him for his kind advices given during the progress of the work.

	Carp I. Cyprinus carpo		Carp II. Cyprinus carpo		Bonito Gymnonsarda affinis		Porgy Pagrus major		Crussian carp Carassius auratus		Tunny Thunnus schlegeli		Spiny lobster Palinurus japonicus		Cuttle-fish Sepia esculenta	
	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.	In 100g of fresh flesh. g.	In 100g of dry flesh. g.
Water[1]	76.609	—	76.789	—	69.371	— 6	76.787	—	81.998	—	73.516	—	75.974	—	81.671	—
Dry matter	23.391	100.000	23.211	100.000	30.629	100 000	23.213	100.000	18.002	100.000	26.484	100.000	24.025	100.000	18.329	100 000
Total-N	2.608	11.149	2.590	11.057	4.479	14 621	3.121	13.445	2.655	14.746	3.549	13 397	3.558	14.808	2.490	13.619
Alb.-N	2.107	9.007	1.972	8.495	3.799	12.401	2.685	11.584	2.285	12.691	—	—	2.637	10.975	2.209	12.051
Non-alb-N	0.501	2.142	0.618	2.562	0.680	2.220	0.432	1.861	0.370	2.055	—	—	0.921	3 833	0.287	1.568
Warm water soluble-N.	0.884	3.779	—	—	1.628	5.314	1.025	4.419	1.039	5.771	1.530	5.776	1.586	6.601	0.919	5.013
Under that: Alb.-N	0.431	1.843	—	—	0.948	3 094	0.503	2.555	0.456	2.533	0.812	3.065	0.621	2.585	0.479	2 613
Organic base-N	0.226	0.966	0.262	1.129	0.189	0.617	0.088	1.379	0.177	0.983	0.285	1.076	0 610	2.539	0.201	1.096
Monoamino-N	0.022	0.094	0.024	0.103	0.022	0.072	0.016	0.069	0.035	0.194	0.011	0.041	0.146	0.608	0.089	0.485
Ammonium-N	Trace		Trace		Trace		Trace		Trace		Trace		Trace		Trace	
Reaction of flesh	Amphich romatic		Very faintly acid		Acid		Faintly acid		Amphich romatic		Acid		Amphich romatic		Amphich romatic	
Water[2]	79.160	—			72.165	—	77.340	—			72.402	—	79 920	—	81.699	—
Creatine	0.421	2.020			0.649	2.011	0.754	3.327			0.467	1.800	Trace?	—	Trace	—
Creatinine	0.077	0.369			0.134	0.481	0.070	0.308			0 064	0.232	Trace?	—	Trace	—
Creatine-N	0.135	0.648			0.208	0.645	0.242	0.767			0.159	0.577				
Creatinine-N	0.029	0.137			0.050	0.179	0.026	0.115			0.024	0.086				

[1] Determined September, 1910.
[2] Determined February, 1911.

THE EFFECT OF MODIFYING THE GLUTEN SURROUNDING OF FLOUR

GEO. A. OLSON

Pullman, Wash.

The results included in this article are a part of our cereal investigation work. This particular article deals with the modifying of the gluten surroundings in flour with a view of searching for the causes which affect the quality of the flour for baking purposes.

The oldest idea is that the gluten in flour holds the gas in panary fermentation. The capabilities to distend depends upon the physical qualities of gluten. Wood believes the quantity of the gluten is modified according to the amount of salts and acid present, which in turn influences the shape of the loaf, and treats this subject fully in his article on "The Chemistry of the Strength of Wheat Flour." *

The writer in the following experiments studied the effect of adding directly definite amounts of acid, alkali, or salt to original dialyzed, decanted, dough, gliadin-free and nitrogen-free with gliadin added flours. The nitrogen components of treated and untreated flour have also been included. The quantity of dry gluten and the nitrogen in the gluten of these respective flours was determined in each case.

In order to remove the salts from a flour by either dialysis or decantation, it is obvious that the flour must be soaked in distilled water and then dialyzed or allowed to stand and the supernatant liquid removed after the flour has settled to the bottom of the jars. This method at once brought up the question as to whether or not a flour could be water soaked and remilled into flour again. The first trial with dialyzed flour, however, determined that such a procedure was possible.

Perhaps the greatest difficulty experienced in this undertaking was the drying of the water-soaked flour. Aside from avoiding

* Journ. Agr. Sc. Vol. 2, No. 2, Apr. 1907, p. 139, ibid. Vol. 2, No. 3, Dec. 1907, p. 267.

too high temperatures, in the drying process, the possibilities for fermentation were most favorable and in consequence the flour while drying required the strictest attention of both an assistant and myself. The tendencies for fermentation were particularly noticeable in the flour which had been dialyzed, in spite of the fact that they had been treated frequently with small quantities of chloroform.

In addition to recovering the water-soaked flour, the dialysate and water decantations were saved and either reduced to small volume or evaporated to dryness.

The method of preparing the dialyzed, decanted, and dough flours are described in the following paragraphs:

Dialyzed Flour. Approximately kilo lots of flour were thoroly worked into paste with water and then dialyzed in a cool room, frequently changing the dialysate for a fresh supply of distilled water. Sufficient chloroform was used to check fermentation. After a course of three days the dialysis was considered complete. The colloidal material was then thoroly stirred and poured thinly over glass window panes and allowed to dry (frequently stirring) at a low temperature. When it solidified into lumpy masses all danger of fermentation was apparently removed. The lumpy masses were then worked out into thin layers and allowed to dry over night without any attention. The following morning these thin layers of flour crumbled readily and were then ground into small pieces about the size of clover seed, when it was dried further. When satisfactorily dry the work was considered complete after milling and finally bolting thru a 10xx bolting cloth.

Decanted Flour. Kilo lots of flour were worked into paste and then diluted with four liters of distilled water for every kilo of flour; after settling, the supernatant liquid was removed, and the operation frequently repeated for a period of three days, after which the flour was finally dried in the same manner as described for the dialyzed flour. Sufficient chloroform was used throughout the experiments to allay fermentation.

Dough Flour. Not knowing what influence the water would have upon the physical properties of the flour, it was necessary, in order to obtain checks, to treat the flour with enough water to make a dough, allowing it to stand for three to four hours,

then rolling this dough out into thin sheets, crumbling and milling as described for the dialyzed flour.

Plan of Investigation. After the treated flours were prepared as described above, the next step consisted in planning a method whereby the physical properties of the glutens could be studied. After some consideration and thought it was decided that in place of taking bits of gluten prepared from these flours and subjecting them to acid, alkali or salt as Wood did, that the flour be treated with these agents directly and the changes occuring, if any, be noted, and the amount of dry gluten and the nitrogen in the gluten be determined. It can readily be seen that such a plan takes into account other things besides salts and acids, since it would also determine the effect of adding these reagents to the flour directly. Later baking tests of flour treated similarly could be made and the influence of the reagents upon the shape of the loaf be noted.

Gluten Determinations. Ten gram lots of the treated and original flour were mixed with six cubic centimeters of either distilled water or N–10 normal hydrochloric acid, sulfuric acid, phosphoric acid, sodium hydroxide, potassium hydroxide, dipotassium acid phosphate, disodium acid phosphate, dicalcium acid phosphate, sodium chloride, sodium sulfate, aluminum sulfate and magnesium sulfate, and worked up into small wads. These wads of flour were then allowed to stand for one hour, after which they were finally washed over silk with running water. In some cases the particles of gluten cohered and were easy to gather, while in other instances the gluten particles scattered and fell upon the silk. All scattering glutens which fell upon the silk, together with those that cohered, were gathered together, washed, dried and weighed according to the usual method. The length of time required for drying was 20 hours at maximum temperature of the water oven. Nitrogen determinations of the dry glutens were finally made by the Kjeldahl method. The results of the percent of gluten, weight and percent of nitrogen in the gluten, percent of total nitrogen and percent of total nitrogen calculated from the nitrogen in the gluten from the original flour as 100, are given in the following table, which is subdivided into four separate parts according to the treatment of the flour.

TABLE I (a, b, c and d)

EFFECT OF MIXING ACID, SALT OR ALKALI WITH DIFFERENTLY PREPARED FLOURS UPON THE YIELD OF AND NITROGEN IN GLUTEN

		H_2O	H_2SO_4 N/10	H_3PO_4 N/10	HCl N/10	NaOH N/10	KOH N/10	Na_2HPO_4 N/10	K_2HPO_4 N/10	$CaHPO_4$ N/10	NaCl N/10	Na_2SO_4 N/10	$MgSO_4$ N/10	$Al_2(SO_4)_3$ N/10
(a) *Original*	Per Cent Gluten	11.94	7.73	6.43	3.56	9.30	8.84	12.07	10.85	10.89	12.82	11.59	11.70	10.39
	Weight of Nitrogen	0.1521	0.101	0.079	0.043	0.121	0.120	0.149	0 142	0.141	0.149	0.155	0.146	0.139
	Per Cent Nitrogen	12.75	13.08	12.22	12.09	13.02	13.58	12.34	13.10	12.97	11.60	13.41	12.46	13.42
	Per Cent of total Nitrogen	93.30	61.97	48.15	26.35	74.25	73.56	91 33	87.12	86.52	91.07	95.34	89.50	85.55
	Per Cent Gluten Nitrogen in Original Flour	100.00	66.42	51.61	28.34	79.58	78.84	97.89	93.38	92.73	97.61	102.18	95.93	91.69
(b) *Dough*	Per Cent Gluten	11.51	5.84	9.20	1.48	9.47	9.18	11.75	10.92	10.80	11.21			
	Weight of Nitrogen	0.152	0.074	0.125	0.020	0.116	0.126	0.151	0.150	0.151	0.151			
	Per Cent Nitrogen	13 19	12.33	13.54	13.54	12.20	13.25	12.88	13.66	13.95	13.49			
	Per Cent of total Nitrogen	93.05	45.32	76.37	12.27	70.94	77.34	92.79	91.42	92.36	92.70			
	Per Cent Gluten Nitrogen in Original Flour	99.72	48.58	81.85	13.16	76.03	83.54	99.45	97.98	98.99	99.36			
(c) *Dialyzed*	Per Cent Gluten	10.47	0.951	3.02	0.381	9.48	9.21	11.70	10.41	9.91	10.69	11.04	11.03	7.13
	Weight of Nitrogen	0.147	0.013	0.040	0.005	0.133	0.124	0.156	0.144	0.136	0.152	0.152	0.152	0.094
	Per Cent Nitrogen	14.00	13.70	13.38	13 23	14.02	13.48	13.36	13.84	13.72	14.18	13.97	13.81	13.11
	Per Cent of total Nitrogen	86.53	7 79	23.80	2.97	78.51	73.31	92.23	85.04	80.25	89.50	90.00	90.13	55.34
	Per Cent Gluten Nitrogen in Original Flour	96.32	8.67	26.49	3.31	87.39	81.60	102.67	94 66	89.33	99.63	99.90	100.09	61.45
												Ca $(OH)_2$	C_3H_5 $(OH)_3$	C_2H_5 OH
(d) *Decanted*	Per Cent Gluten	0.0041	0.004	0.000	0.000	6.88	6.98	0.000	0.000	0 000	0.008	5.46	0.59	0.000
	Weight of Nitrogen					0.0998	0.0948					0.0713	0.008	
	Per Cent Nitrogen					14.55	13.58					13.05	12.80	
	Per Cent of total Nitrogen					60.12	57.10					43.92	4.55	
	Per Cent Gluten Nitrogen in Original Flour					65.58	62.28					46.82	4.93	

Comparing the data obtained for dry gluten in the original dough, dialyzed, and decanted flours as modified by either water, acid, alkali, or salt, it will be noted that with the exception of sodium sulfate none of the N/10 solutions of salt, acid, or alkali gave as high results as was obtained with the water in the original flour. In Table 1 (a) the prejudicial* influence upon the gluten increased in the order named, disodium acid phosphate, sodium chloride, magnesium sulphate, dipotassium acid phosphate, dicalcium acid phosphate, aluminum sulfate, sodium hydroxide, potassium hydroxide, sulfuric acid, phosphoric acid, and hydrochloric acid.

It will be further noted from the results given in table 1 (b) that the dough flour agrees fairly well with the original flour, the widest variations occurring where the flour had been treated with sulfuric acid, phosphoric acid, and hydrochloric acid. In the same way the weight of nitrogen varied and the percent of total nitrogen calculated on the basis of 100 for the original flour perhaps illustrates more clearly the effect of the acid upon the dough flour when compared with the original.

In regard to the dialyzed flour, when compared with the original, water has affected the yield of gluten and the weight of nitrogen to a slight extent. Sulfuric acid, phosphoric acid and hydrochloric acid have been prejudicial even to a much greater extent than was the case in the dough flour. Sodium hydroxide and potassium hydrocide were apparently beneficial,** the former more so than the latter. Comparing salts of phosphoric acid, the sodium salt increased the gluten and nitrogen content to a greater extent than the potassium salt, while the calcium salt was slightly prejudicial in this case, being an exception to that observed in case of the dough flour. Sodium chloride was beneficial when weight of the nitrogen contained in the gluten is considered. On the basis of weight of nitrogen, aluminum sulfate was prejudicial, sodium sulfate was without effect and magnesium sulfate was beneficial. Comparing the influence of

* Prejudicial refers to decreased amounts of dry gluten or nitrogen in the gluten.

** Beneficial refers to an increased amount of dry gluten or nitrogen in the gluten.

the reagents on the gluten content and weight of nitrogen with water, it will be noted that disodium acid phosphate, magnesium sulfate, sodium sulfate and sodium chloride were beneficial in the order named, while dipotassium acid phosphate, dicalcium acid phosphate, sodium hydroxide, potassium hydroxide, aluminum sulfate, phosphoric acid, sulfuric acid and hydrochloric acid were prejudicial in the order named.

The dalysate from dialyzed flour (based upon the weight of flour used) contained 0.655 percent total solids, of which 0.57 per cent was combustible and 0.084 percent was ash.

The flour obtained after decanting the soluble extract gave the most remarkable results of any in the series. Neither water, salts, nor acid yielded gluten, while sodium and potassium hydroxides were beneficial to gluten formation. In addition, calcium hydroxide, glycerol, alcohol and flour extract were tried and only the glycerol and calcium hydroxide were found to be beneficial.

Collectively, the results given in Table I (a, b, c and d) clearly show that acid is more prejudicial than alkali of same normal strength and the salts with one exception (aluminum sulfate in case of the dialyzed flour) has practically a very slight effect upon the physical properties of the gluten. On this basis, sodium was least active and calcium most active on gluten disintegration. There is no doubt but that the substances contained in the water extract play an important part in modifying the physical properties of the gluten. Whether the substances contained in this extract, which play so important a role, are inorganic or not must be determined. It appears that the (OH) radical tends to produce coherence. This view is supported from the results obtained in the dialyzed flour when compared with the original, and in the decanted flour where other substances failed to bring on coherence. Just what causes the disintegration of the gluten complex has not as yet been satisfactorily established.

Physical Condition of the Gluten. The gluten in the flour studied from physical appearances was fair. None of the salts used showed any marked variation in the physical appearances of the gluten other than was observed in the case of water. N/10 acids tended to produce scattering glutens, which when

TABLE II

EFFECT OF MIXING ACID, SALT OR ALKALI WITH PATENT FLOURS FROM DIFFERENT LOCALITIES UPON THE YIELD AND NITROGEN IN GLUTEN

	HCl N/100	HCl N/10	HCl N/1	H_2SO_4 N/100	H_2SO_4 N/10	H_2SO_4 N/1	$C_3H_6O_3$ N/100	$C_3H_6O_3$ N/10	$C_3H_6O_3$ N/1	NaOH N/100	NaOH N/10	Na_2HPO_4 N/10	NaCl N/10	Na_2SO_4 N/10	H_2O
Pullman Flour.															
Per Cent dry Gluten	7.85	0.94	trace	7.45	1.90	3.11	7.43	3.17	none	7.77	4.96	8.33	8.06	8.04	8.01
" Nitrogen	12.43	10.85	.....	13.04	13.03	10.52	12.90	.....	.....	11.90	12.15	12 40	12.68	12.78	12.32
" of total Nitrogen	82.70	8.66	.....	82.00	21.03	27.77	81.15	.....	.....	78.30	51.02	87.56	86.61	87.09	83.64
Bridgeport Flour.															
Per Cent dry Gluten	12.56	2.55	0.07	12.40	4.44	4 53	11.74	0.98	0.11	14.38	7.96	12.51	11.85	12.13	12.09
" Nitrogen	12.00	12.86	.....	12.09	13.77	11.93	12.10	13.47	.....	10.84	14.30	12.73	12.70	13.02	12.62
" of total Nitrogen	84.26	18.30	.....	83.69	34.10	30.19	79 39	73.58	.....	87.10	63.59	88.93	84.00	88.22	85.25
Vancouver Flour.															
Per Cent dry Gluten	10.90	5.36	0.08	9.53	6.68	6 45	.....	.....	.....	12.43	9.30	12.07	12.82	11.59	11.94
" Nitrogen	12.54	12.04	.....	13.89	13.83	10.77	.....	.....	.....	11.50	13.02	12 34	11.60	13.41	12.75
" of total Nitrogen	83.83	39.56	.....	81.25	56.69	42 58	.....	.....	.....	89.57	74.30	91.39	91.13	95.33	93.36
Anatone Flour.															
Per Cent dry Gluten	8.52	2.48	0 000	9.06	4.22	0.00	8.33	0.000	0.000	.....	4.11	8.53	8.45	8.47	8.18
" Nitrogen	13 03	13.88	.....	13.06	12.75	.....	13 28	.....	.....	.....	13 90	13.00	14.17	13.93	14.28
" of total Nitrogen	83 00	24.57	.....	84 50	41.07	.....	79.07	.....	.....	.....	40.79	79.21	85.50	84.28	83.50

It is difficult to obtain gluten from flour treated with N/1 sodium hydroxide.

gathered were dry and seemingly free from water, while alkalies of the same strength affected the gluten in such a way that in the process of washing out the starch the gluten rolled or washed down with the water on to the silk in little bits, which when collected together, (the gluten) had a very dry touch, indicating that glutens treated in this way contain very little water. The amount of water contained in N/10 acid and alkali prepared glutens was not determined.

Influence of Acids, Alkalies and Salts on Gluten from Patent Flours. Four patent flours were next studied with the object of determining whether or not the addition of acid, alkali, or salt to the flour modified the physical properties, the yield and the nitrogen content of the gluten in these flours. The flour selected for this series of investigations represent four different localities with different climates, soil and types of wheat. The glutens from these flours varied considerably in their physical qualities.

As in the case of the previous experiments, ten gram samples were mixed with six cubic centimeters of either N/10 acid, alkali or salt, and in addition to these like qualities of N/100 and N/1 acid and alkali were tried. These different lots were worked up into wads and the gluten collected by washing over silk. The weight and the per cent of the nitrogen in the gluten are recorded in Table II. The grams of nitrogen in the gluten and the ratio of total nitrogen in the flour to nitrogen in the gluten are recorded in Table III. In place of phosphoric acid, lactic acid was used in these experiments. Potassium hydroxide was not tried.

It will be noted from the data given in Table II that the acids, alkali and salt affected the weight of gluten similarly as found in Table I (a). While the strength of N/100 acid was slightly prejudicial, increasing the strength to N/10 and N/1 had marked effects upon the yield of gluten when compared to those treated with distilled water and run as controls. The increased or equal weight of gluten in the flour treated with N/1 sulfuric acid compared to N/10 sulfuric acid was due to the fact that the former was poorer in nitrogen. Disodium acid phosphate was beneficial in all cases. Sodium chloride and sodium sulfate were beneficial in three out of four instances. It was impossible

TABLE III

GRAMS OF NITROGEN RECOVERED IN THE GLUTEN AND THE RATIO OF TOTAL NITROGEN TO GLUTEN NITROGEN FROM PATENT FLOURS TREATED WITH ACID, SALT, OR ALKALI

	HCl N/100	HCl N/10	HCl N/1	H_2SO_4 N/100	H_2SO_4 N/10	H_2SO_4 N/1	$C_3H_6O_3$ N/100	$C_3H_6O_3$ N/10	$C_3H_6O_3$ N/1	NaOH N/100	NaOH N/10	$NaHPO_4$ N/10	NaCl N/10	Na_2SO_4 N/10	H_2O
Pullman Flour.															
Grams N. in Gluten...	0.0976	0.0102	None	0.0967	0.0248	0.0328	0.0958	None	None	0.0924	0.0602	0.1033	0.1022	0.1028	0.0987
Ratio of total to Gluten Nitrogen	1:0.827	1:0.087	.....	1:0.820	1:0.210	1:0.278	1:0.811	.....	.....	1:0.783	1:0.510	1:0.876	1:0.866	1:0.871	1:837
Bridgeport Flour.															
Grams N. in Gluten...	0.1508	0.0328	None	0.1498	0.0610	0.0540	0.1421	0.0132	None	0.1558	0.1138	0.1592	0.1504	0.1579	0.1526
Ratio of total to Gluten Nitrogen.....	1:0.843	1:0.183	.....	1:0.837	1:0.341	1:0.302	1:0.794	1:0.736	.....	1:0.871	1:0.636	1:0.889	1:0.840	1:0.882	1:0.853
Vancouver Flour.															
Grams N. in Gluten ...	0.1366	0.0645	None	0.1324	0.0924	0.0694	.....	.....	.....	0.1460	0.1211	0.1490	0.1485	0.1554	0.1522
Ratio of total to Gluten Nitrogen.....	1:0.838	1:0.396	.....	1:0.813	1:0.567	1:0.426	.....	.....	.....	1:0.896	1:0.743	1:0.914	1:0.911	1:0.953	1:0.934
Anatone Flour.															
Grams N. in Gluten ...	0.1162	0.0344	None	0.1183	0.0575	None	0.1107	None	None	.....	0.0571	0.1109	0.1197	0.1180	0.1169
Ratio of total to Gluten Nitrogen.....	1:0.830	1:02.46	.....	1:0.845	1:0.411	.....	1:0.791	.....	.....	.....	1:0.408	1:0.792	1:0.855	1:0.843	1:0.835

to obtain gluten from flour treated with N/1 sodium hydroxide. N/100 sodium hydroxide was less prejudicial than N/10. The most peculiar modification of gluten that took place was with N/100 sodium hydroxide in case of the Bridgeport bluestem where the weight of the gluten increased. The per cents of nitrogen in the glutens varied irregularly.

The data given in Table III show the actual amounts of nitrogen entering into the gluten make-up. The ratio of total nitrogen in the flour to that found in the gluten shows how reagents of identical normal strengths modify the nitrogen complexes in the gluten. Particular attention should be called to the effect of N/100 acid. The ratio constant was remarkably uniform for each regardless of source or nature of the flour. Slightly higher ratios were obtained with hydrochloric acid and sulphuric acid than with lactic acid. With N/10 solution, sulphuric acid was the least prejudicial. Hydrochloric acid disintegrated the gluten rapidly at this strength, while with lactic acid practically no gluten was obtained. No results could be obtained with either N/1 hydrochloric acid or N/1 lactic acid. The accompanying cut illustrated the effect of N/100, N/10 and N/1 sulphuric and hydrochloric acids upon the yield of gluten.

(CUT) (1) (See legend on back of photo.)

The physical condition of the glutens resulting from washing the flour after it had been treated with hydrochloric acid, lactic acid, and sodium hydroxide of N/100, N/10 and N/1 strengths are tabulated in Table IV.

From the physical appearances of the gluten as affected by either acid or alkali, it may be said that alkali behaves similarly to acid of the same normal strength.

Flour with Gliadin Removed. Since it is more difficult to remove glutenin than gliadin from flour it was decided to study the influence of acid, alkali, and salts on flour with the gliadin removed. Flour was repeatedly extracted with cold 70 per cent alcohol and finally dried, milled and bolted thru a 10xx bolting cloth. As in previous experiments, 10 grams of flour and 6 cubic centimeters of water or specified reagent were used. The amount of gluten obtained with water 0.14%, N/10 sodium

TABLE IV

Physical Properties of Gluten as Affected by Acid and Sodium Hydroxide

	HCl	H_2SO_4	$C_2H_4O_2$	Na (OH)
N/1	disintegrated	soft, massive, lack cohesiveness.	disintegrated	tough dough, soapy and difficult to remove gluten
N/10	separated in particles, firm and seemingly dry	same as for N/10 HCl	disintegrated	separates into particles, firm and dry
N/100	excellent cohesion, elastic	same as for N/100 HCl	same as for N/100 HCl	soft, voluminous gluten, easy to wash, elastic

hydroxide 0.712%, N/10 sodium chloride 0.100% and N/10 hydrochloric acid none, was insignificant when compared with flours treated by dialysis or decantation and as a result it was considered futile to try other reagents on this gliadin-free flour. These results, however, appear to indicate that gliadin does not act as an acid, alkali, or salt, for if such was the case, it is reasonable to believe that all of the glutenin could have been recovered by the addition of an acid, alkali, or salt.

Nitrogen-free Flour with Gliadin Added. In the previous paragraph, the effect of acid, alkali, and salt upon gliadin-free flour was discussed. In a similar way the same original flour was treated with weak potassium hydroxide N/50 several times. The supernatant liquid resulting after the flour stood for four hour intervals was replaced with fresh lots of potassium hydroxide and finally the treated flour was washed with frequent changes of distilled water, then rolled into thin layers, dried and milled. The amount of nitrogen components present after the above treatment was determined and found to be a trace of globulin and albumin nitrogen, amid nitrogen 0.119%, alcohol soluble (gliadin) nitrogen 0.133%, total nitrogen 0.301%. The total nitrogen in the original flour was 1.63%.

It appears from these results that water is more prejudicial than either N/100 hydrochloric acid or sodium hydroxide and N/10 hydrochloric acid or sodium chloride. It is reasonable to believe, however, that such was not the case, since the gliadin in the course of preparation and purifying has been altered and when dried and ground is made up of minute hard particles of gliadin. Just as dried gluten takes up water only after long periods of soaking, so it is with these hard particles of gliadin which, when mixed with water, require long periods of time to even swell. Thus when flour is incorporated with an addition of these hard particles of gliadin and then mixed into wads with water and gluten determinations made, the finest particles of gliadin pass thru the fine silk and are lost. The only gliadin that can be recovered are the partly swelled particles which are too large to pass thru the silk. The presence of N/100 hydrochloric acid or sodium hydroxide and N/10 hydrochloric acid and sodium chloride, undoubtedly, has increased the tendencies of the hard particles of gliadin to absorb more water than was the case with distilled water and as a result increased the number of particles too large to pass thru the silk hence a larger yield. With larger quantities of water than was used in these experiments more gliadin would undoubtedly have resulted since gliadin takes up water in time and with an increased amount accelerates water absorption thereby increasing the number of larger particles.

Nitrogen Components of Treated and Untreated Flour. Aside from studying the effect of acid, alkali and salts upon the yield of gluten and the weight of nitrogen in the same, a systematic study of the nitrogenous components in the flours mentioned above was made. The first flours investigated were the dough, dialyzed, and decanted compared with the original. All nitrogen determinations were made according to the straight Kjeldahl method. The total nitrogen, the percent of nitrogen of flour entering into the gluten makeup, the percent of alcohol soluble nitrogen (using 70% alcohol by volume), the percent of nitrogen compounds precipitated by phosphotungstic acid from a one percent sodium chloride soluble of flour and the percent of nitrogen in the salt soluble extract not precipitated by phosphotungstic acid were determined.

TABLE V

Nitrogen Components in Differently Treated Flours

	Nitrogen in Flour Per Cent.	Nitrogen in Gluten Per Cent.	Gliadin Nitrogen Per Cent.	Edestin and Leucosin Nitrogen Per Cent.	Amide Nitrogen Per Cent.	Glutenin Nitrogen based on Flour Per Cent.	Glutenin Nitrogen based on Gluten Per Cent.
Original	1.63	1.5218	0.602	0.378	0.056	0.594	0.4858
Dough	1.63	1.5176	0.609	.....	.....	.....	.....
Dialyzed	1.69	1.4658	0.623	0.308	0.070	0.689	0.4648
Decanted	1.66	1.4255*	0.756	0.273	0.077	0.554	0.3195

The results given in Table V show a higher nitrogen content for the dialyzed and decanted flours, than was found in case of dough and original flour. These differences in total nitrogen in the dialyzed and decanted flours may be due to the removal of nitrogen-free or nitrogen-poor material. The fact that the decanted flour contains less nitrogen than the dialyzed flour would tend to indicate that this was not the case; on the other hand, it must be considered that small quantities of the more highly complex nitrogen bodies are carried away with water and with the large quantities of water used repeatedly for decantation some of the higher nitrogen bodies have been affected and their losses have resulted in diminishing the nitrogen content. The amount of nitrogen in the glutens (expressed in percent of the nitrogen of the flour in gluten), also supports this thought, being higher in amounts for the original and dough than in either the dialyzed or decanted. Although, it was impossible to obtain any gluten with water (see Table 1, d) in the decanted flour, the result 1.4295% nitrogen cannot be far off from the actual amount present if a gluten could have been made. This result has been calculated from the data given in table 1, a & d, and confirmed by those given in Table V. The increased amount of alcohol soluble nitrogen in the decanted flour cannot be entirely accounted for by the removal of the nitrogen-free and nitrogen-poor material. In this same flour there is less precipitated nitrogen and more nitrogen not precipitated by phosphotungstic acid. The same is true to a less

* Calculated.

extent in case of the dialyzed flour. The glutenin was obtained by difference between the nitrogen obtained in gluten and the sum of alcohol and the salt soluble. Another column for glutenin has been inserted for the benefit of those who subtract the alcohol and salt soluble from the total nitrogen.

In Table VI the results given in Table V are calculated as proteins using the factor 6.25.

TABLE VI

Nitrogen Components Calculated as Proteins

	Protein in Flour Per Cent.	Gliadin Per Cent.	Edestin and Leucosin Per Cent.	Amides Per Cent.	Glutenin based on Flour Per Cent.	Glutenin based on Gluten Per Cent.	Gluten Per Cent.
Original	10.19	3.76	2.36	0.36	3.71	3.04	9.51
Dough	10.19	3.81	....	....	....	....	9.49
Dialyzed	10.56	3.89	1.93	0.43	4.31	2.91	9.16
Decanted	10.38	4.73	1.70	0.49	3.46	2.00	8.91

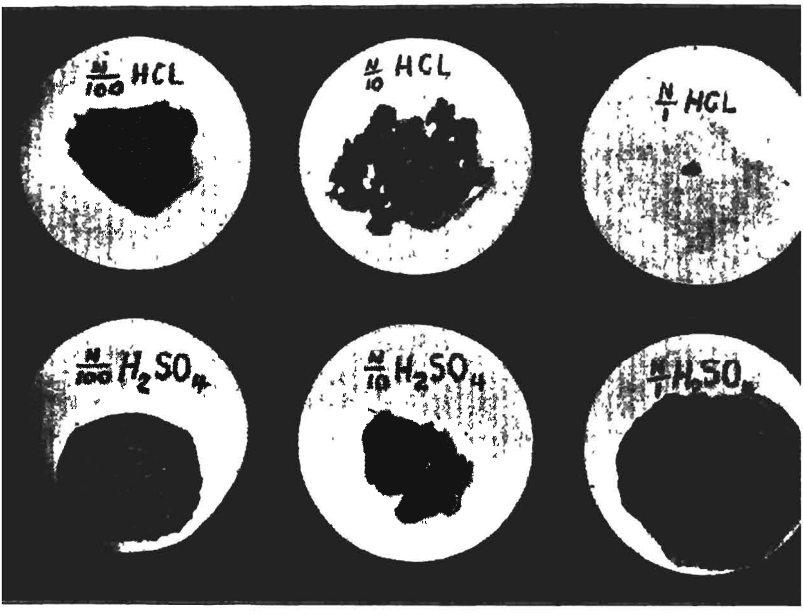
Owing to the increased amount of alcohol-soluble nitrogen obtained in the decanted flour over that found in the original, two other flours were treated by the decantation method. The nitrogen components of these flours in conjunction with the same flours untreated were also made. The data for the original and decanted flour given in Table V are included in Table VII for comparison.

TABLE VII

Nitrogen Components in Patent Flour Compared with the Same Flour after Decantation.

	Total Nitrogen Per Cent.	Gluten Nitrogen Per Cent.	Gliadin Nitrogen Per Cent.	Edestin and Leucosin Nitrogen Per Cent.	Amide Nitrogen Per Cent.	Glutenin Nitrogen based on Flour Per Cent.	Glutenin Nitrogen based on Gluten Per Cent.
Pullman, Original	1.18	0.987	0.651	0.329	0.028	0.172	None
Decanted	1.20	.....	0.686	0.217	0.021	0.256	.....
Bridgeport, Original	1.79	1.526	0.910	0.350	0.049	0.481	0.217
Decanted	1.74	.....	0.950	0.245	0.035	0.510	.....
Vancouver, Original	1.63	1.5218	0.602	0.378	0.056	0.594	0.486
Decanted	1.66	1.4255*	0.756	0.273	0.077	0.554	.....

* Calculated.



Increasing the strength of acid alters the physical quality of gluten.

It will be seen from the results given in Table VII that the amount of alcohol-soluble nitrogen in the decanted flours were again found to be higher than that found in the original flours. The amount of amid nitrogen found was, however, the reverse of that found in Table V. When the nitrogen in the gluten makeup is assumed to be composed of the gliadin and glutenin it will be noted that the Pullman flour is devoid of glutenin. On the other hand, if the total nitrogen is considered instead of the nitrogen entering into the gluten makeup there is found 0.172% of gluten.

Extract from Decanted Flour. In addition to having determined the nitrogen components of both the control and the decanted flours, the extract of the decanted flour was also studied in regard to the total nitrogen, nitrogen precipitated and not precipitated by phosphotungstic acid. The total solids and total ash were also made. These results are recorded in Table VIII.

TABLE VIII

Nitrogen Components in Decanted Liquid from Patent Flour

	Total Nitrogen Percent.	Edestin and Leucosin Nitrogen Percent.	Amide Nitrogen Percent.	Total Ash Percent.	Total Solids Percent.
Pullman.....	0.094	0.079	0.015	0.36	3.00
Bridgeport.....	0.081	0.074	0.007	0.38	2.74

The results given in Table VIII show that the total solids and nitrogen components for the Bridgeport flour are slightly lower than was the case in the Pullman flour. It may be of interest to know what these extracted substances are, since all or some one of them may be important in determining the baking qualities of a flour. Although some work has been done by the writer along this line of research, nothing definite has been determined thus far.

In Table IX an attempt has been made to correlate some of the data given in preceding tables. The ratio of total nitrogen to gluten nitrogen, as affected by water, sodium hydroxide, disodium

acid phosphate, and sodium sulphate on the one hand, with the ratio of gliadin to glutenin nitrogen on the other, are recorded.

TABLE IX

Ratio of Total Nitrogen to Gluten Nitrogen as Affected by Alkali, Water and Salt and Ratio of Gliadin to Glutenin Nitrogen

	Ratio of gliadin to-gluten nitrogen	Total nitrogen to gluten nitrogen ratio			
		N/10 Na (OH)	Water	N/10 $Na_4H_2(PO_4)_2$	N/10 $Na_2(SO_4)$
Pullman	1:0.264	1:0.57	1:0.837	1:0.87	1:0.87
Bridgeport	1:0.528	1:0.636	1:0.852	1:0.88	1:0.88
Vancouver	1:0.986	1:0.743	1:0.934	1:0.91	1:0.95

When flour was treated with either water, alkali or salt the ratio of total nitrogen to gluten nitrogen decreased as the ratio of gliadin nitrogen to glutenin nitrogen decreased; this fact is clearly brought out in table IX. In other words, the more glutenin nitrogen a flour contains the higher will the gluten nitrogen be. On the other hand, this view is contradicted, since the flour which had the gliadin removed practically yielded no gluten when treated with acid, alkali, or salt. In the same way decanted flour having nearly all of its glutenin and gliadin yielded only a part of its gluten when treated with sodium hydroxide, potassium hydroxide, calcium hydroxide, and glycerol. From these observations there appears to be some as yet unknown substance or physical change which is more important in causing a transformation of the physical properties of gluten than either acid, alkali, or salt. Whether this be in the form of some organic salt, acid, or alkali, or not is a problem for the future.

Conclusions

1. Flours were either made into dough with water, dialyzed or decanted, then dried, remilled and bolted into flour again. Gluten determinations were made, using different reagents in order to note the differences in yield caused by modifying the surroundings.

2. Mixing untreated flour with N/10 solutions of different salts, acids and alkali was prejudicial to the yield of gluten. The prejudicial influence increased in the following order: Sodium phosphate, sodium chloride, magnesium sulphate, potassium phosphate, calcium phosphate, aluminum sulphate, sodium hydroxide, potassium hydroxide, sulphuric acid, phosphoric acid and hydrochloric acid.

3. Flour treated with sufficient water to form a dough then dried and remilled into flour again, has resulted in slightly modifying the gluten of that flour when compared with the original. N/10 solutions of sulphuric acid, phosphoric acid and hydrochloric acid were more prejudicial to gluten formation in dough flour than in the original one.

4. The gluten from flour which has been dialyzed has been affected to a greater extent than was the case with the dough flour.

5. No gluten could be obtained from the residual flour by decantation when mixed with either water, salts or acids. Mixed with N/10 solutions of sodium, potassium, and calcium hydroxides and glycerol, varying amounts of gluten were obtained. The amount of gluten obtained decreased with the hydroxides used in the order mentioned above in No. 2 of conclusions.

6. N/10 solutions of salts appear to have no effect upon the physical appearance of the resulting gluten when compared with similar ones which were mixed with water. N/10 acid and alkali tend to produce scattering glutens which when gathered appear to be rather free from water. The amount of water held by such glutens was not determined.

7. Using patent flours from various sources it was found that the glutens prepared from these behaved similarly to those previously obtained and treated in like manner.

8. Patent flours were treated with N/100, N/10, and N/1 sulphuric acid, hydrochloric acid, lactic acid and sodium hydroxide. N/100 strength solutions had the least effect upon the yield of gluten. N/10 strength solutions, the hydrochloric acid yielded some and the lactic acid practically no gluten. Using N/1 strength solutions non-cohering gluten resulted with sulphuric acid and no yield with hydrochloric acid and lactic acid.

9. In determining the nitrogen present in the glutens resulting from treating flour with N/100 strength, solutions of sulphuric acid, hydrochloric acid or lactic acid, gave fairly concordant results when compared with one another and the ratio of total nitrogen to gluten nitrogen was practically constant, regardless of the kind of acid used.

10. Flour with the gliadin removed does not form gluten either in the presence of water, acid, alkali or salt. This fact indicates that gliadin does not behave either as an acid, alkali or salt.

11. Nitrogen free flour with gliadin added to it does not form gluten either in the presence of acid, alkali or salt. The failure to recover the admixed, previously dried, gliadin is undoubtedly due to the limited time the gliadin was exposed to water; in addition to the small quantity of water used.

12. In studying the nitrogen components of the original dough, dialyzed, and decanted flours, it was found that the dialyzed and decanted flours showed slightly higher total nitrogen and alcohol soluble nitrogen contents than was found in either the original or dough flour.

13. An attempt was made to correlate the ratio of gliadin nitrogen to glutenin nitrogen with the ratio of total nitrogen to gluten nitrogen as affected by either water, sodium hydroxide, sodium phosphate and sodium sulphate. According to the data obtained it appears that the ratio of total nitrogen to gluten nitrogen decreased as the ratio of gliadin nitrogen to glutenin nitrogen decreased. On the other hand, it must be considered that gliadin-free flour yielded no gluten and decanted flour yielded only a part of its gluten. Accordingly there appears to be as yet some unknown substance which is important in causing a transformation of the physical properties of the gluten.

A METHOD FOR THE DETECTION OF COLOR IN TEA

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The following method was devised for the purpose of detecting color on tea, the United States Treasury Department having issued regulations prohibiting the entry into this country of colored teas.

For the demonstration of color and facing on tea, the chemical methods, as suggested by Allen (1), Leach (2), Villiers et Collin (3), and The International Committee (4), have usually been employed. The difficulty with such methods is in the small amount of color used and the masking of the color reactions by the solution of natural color in the tea.

The method suggested in this paper has the advantage in that it can detect much smaller amounts than can be found by chemical methods, but at the same time overlooks traces of color which would be found by a compound microscope.

Hilger and Mayrhofer (5) suggest a method of rubbing wet tea leaves on white paper, to detect artificial color.

The method suggested in this paper has an advantage over the use of wet tea leaves in that a larger sample can be used in a single examination, and it is more easily and quickly handled. In cases where there is a blending of colored and uncolored tea, many of the wet leaves might be used without detecting the color. These authors also suggest the sifting of the tea, but no

(1) Commercial Organic Analysis, 1911; V, 658.

(2) Food Inspection and Analysis, 1911; 375.

(3) Traité des Altérations et Falsifications des Substances Alimentaires, 1900; 258.

(4) Report on the Unification of Analytical Methods for Food-products; 1912; 148.

(5) Vereinbarungen zur Einheitlichen Untersuchung und Beurtheilung von Nahrungs-und Genussmitteln fur das Deutsche Reich, 1–3, 1897–1902; 54.

mention is made in the article of crushing the particles on paper to demonstrate the presence of color, and thus not only affording a method surer and safer for the chemist but also one which can be used by men untrained in Science; therefore, making it available for tea examiners at the ports and for the tea tasters employed by the importers and dealers in tea. It also allows the accurate handling of large numbers of samples within a short time, thus preventing the detention of tea at the ports for any considerable time and consequent financial loss to the importer.

The articles needed for testing the tea are sieves, 16 to 24 meshes to the centimeter, a spatula or case knife and a piece of unglazed, white paper.

A small amount of tea, about 25 to 50 grams, is placed in a sieve and shaken over a piece of white paper. If the tea is tightly rolled, it should be slightly crushed, either before putting into the sieve or by rubbing it against the sieve. The dust on the paper is then crushed by dragging over it a spatula or case knife, pressure being applied by the finger to the end of the spatula. This crushes not only the tea dust, but any particles of color which are present. The process of dragging the knife across the paper, streaks the color, making it more easily seen. A lens with a magnification of 8 to 12 diameters is useful in detecting the smaller streaks. Sunlight is desirable; bright light is essential for this work.

This method will detect any coloring as blue, turmeric or carbon. An application of the method has been made by Mr. G. F. Mitchell, Supervising Tea Examiner, Treasury Department, to the detection of facing on tea. Black, unglazed paper is used in place of the white paper. The facing leaves a white streak on the black paper.

Microchemical Tests for Color:

A black streak would suggest carbon; the blue may be Prussian blue, indigo or ultramarine; and a yellow streak suggests turmeric. These may be identified as follows: The carbon, by its glossy appearance; the blue and turmeric can be tested directly on the paper or by mounting on a microscopic slide. To the blue streak on the paper or to the particle on the microscopic slide, add a drop of 40 % sodium hydroxide. Prussian blue will turn

yellowish-brown; indigo or ultramarine will remain unchanged in color. Ultramarine is discolored by acid; indigo remains unchanged when treated with either acid or alkali. Turmeric turns bright red when a drop of a mixture of equal parts of boracic acid and concentrated hydrochloric are added to the yellow streak. Concentrated sulphuric also turns turmeric bright red.

RECHERCHE DE PETITES QUANTITES DE GRAISSE DE COCO DANS LE BEURRE DE VACHE

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La méthode d'analyse du beurre que j'expose dans un premier mémoire, est assez sensible pour déceler 10% de coco. En y apportant quelques variantes, on pourrait en augmenter encore la sensibilité, ainsi que j'ai pu le vérifier déjà par un certain nombre d'expériences que je tiens à renouveler encore, et dont les résultats me semblent assez intéressants pour que je les fasse connaître.

Le beurre de coco renfermant beaucoup d'acides caprique, caproïque et laurique dont la majeure partie se trouve dans le groupe des acides gras insolubles dans l'eau, et ces acides ayant un indice de saponification élevé, j'avais pensé qu'en isolant assez d'acides gras de ce groupe pour en déterminer cet indice, je trouverais peut-être là un renseignement précieux.

Un certain nombre de tentatives de ce genre m'ont bien fait voir que l'indice de saponification des acides de ce groupe obtenus avec le coco, était relativement élevé, et le plus souvent supérieur à celui que donnent les mêmes acides retirés du beurre pur, mais que pourtant on ne pouvait espérer pouvoir s'en tenir exclusivement à cette détermination pour affirmer la présence de 5% de coco.

Mais il n'en est plus de même, si l'on compare les rapports établis comme je vais l'indiquer:

Appelons I S l'indice de saponification du beurre
i s celui de ses acides gras insolubles dans l'eau
I E les acides gras insolubles dans l'eau dosés suivant ma méthode (1er mémoire).
S E les acides solubles dans l'eau dosés aussi suivant ma méthode.

Si l'on établit les rapports suivants:

$$\frac{I\ S}{S\ E} = R^1 \text{ et } \frac{i\ s}{I\ E} = R^2$$

on constate que les beurres purs donnent

R^1 toujours inférieur à R^2

tandis que les beurres cocotés à 5% seulement donnent le contraire: R^1 supérieur à R^2
si l'on ajoute 10% d'um mélange à parties égales de coco et de margarine, R^1 reste encore le plus souvent supérieur à R^2 malgré la margarine ou les deux rapports deviennent au plus égaux.

Mode opérative

Je vais indiquer rapidement comment j'isole une quantité suffisante d'acides gras insolubles dans l'eau pour déterminer en suite leur indice de saponification.

Il consiste à suivre la technique exposée dans le premier mémoire, avec cette différence, que je prélève 10 grammes de beurre fondu et filtré au lieu de 5 grammes; prends un ballon jaugé à 300 au lieu de 150, je saponifie avec 50 cent. cubes de liqueur de saponification au lieu de 25, et j'ajoute 34 cent. cubes d'eau au lieu de 17, pour amener le titre alcoolique à 56°5.

Après la filtration à la température de 15°, je dose les acides gras solubles dans l'alcool à 56°5 et ceux solubles dans l'eau, sur le filtratum, exactement comme je le fais savoir dans le ler mémoire. Ce qui reste de liquide filtré est introduit dans un bécherglass de 250 et évaporé au bain-marie jusqu'à réduction au volume de 45 cent. cubes. La liqueur concentrée, sur laquelle les acides gras insolubles dans l'eau surnagent, est versée étant chaude, (car la filtration est alors plus rapide) sur un petit filtre sans plis que l'on a préalablement échauffé lui-même, en le remplissant d'eau bien chaude.

Après avoir lavé le bécherglass avec de l'eau chaude, pour entraîner le plus possible d'acides gras qui y adhèrent, ceux-ci sont lavés aussi 4 ou 5 fois sur le filtre avec de l'eau bien chaude.

On laisse alors le filtre s'égoutter 2 ou 3 minutes, puis à l'aide d'un tube de très petit diamètre, et à extrémité légèrement

effilée, on prélève les acides gras retenus sur le filtre, pour les introduire dans un petit ballon taré (1).

Une nouvelle pesée fait connaître le poids de ces acides.

On les dissout par addition de 20 cent. cubes d'alcool neutre à 90° 95° et après agitation on prend l'acidité en présence de 2 gouttes de solution de phtaléine, en se servant de potasse déci normale dont on cesse l'addition, dès que se manifeste le virage au rose très faible.

Supposons avoir opéré sur 0 gramme 453 d'acides gras et que leur saturation a nécessité 20 cent. cubes de potasse décime; l'indice de saponification sera donné par le calcul ordinaire à savoir:

$$\frac{20\times 0.0056}{0.453} = 247 \text{ d'indice de saponification.}$$

Le tableau suivant donne une idée des renseignements que peut fournir l'application des opérations que j'ai citées.

Il est bien certain que si l'on peut ainsi découvrir 5% de coco, on comprend qu'à fortiori, il sera aisé d'en trouver une teneur plus élevée; le rapport R^2 pourrait être utilement cherché; du reste sa détermination ainsi que l'on a pu s'en rendre compte, peut se lier à la pratique de ma méthode rapide d'analyse des beurres (voir ler mémoire).

	R^1	R^2	
B.P.....	38	43	
B.C.....	40	35	abréviation
B.CM.....	41	38	B.P. —beurre pur
B.P.....	34	37	B.C. —beurre÷5% de coco
B.C.....	36	34	BCM. —beurre÷5% de coco÷5%
BCM	38	36	margarine

(1) Il est bien de chauffer un peu ce tube, pour que les acides ne s'y solidifient point.

THE CHEMIST IN THE SERVICE OF THE PACKING HOUSE

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There is perhaps no other industrial line of work embracing so great a variety of subjects as packing house chemistry. This work may vary from the routine analysis of occasional samples of a few fats and fertilizer materials such as blood, bone and tankage, often performed under contract by a commercial chemist, to the great variety of work performed in the laboratories of the larger packing houses by a corps of chemists and trained helpers.

Confining the subject under discussion to the larger laboratories, the work may be classified in a general way into three fairly distinctive lines, namely analytical, research, and consulting work.

The analytical work embraces two principal lines; first, the examination of purchased materials, so far as they can be purchased on specifications involving chemical control, and second, the control of the finished manufactured products, often including the various stages of manufacture as well. Under the head of purchased materials may be mentioned, for example, steel, iron and cement for construction work; paper, tin plate, wood, fiber board, jute, and burlap, for containers or wrapping purposes; and such raw materials as edible and inedible fats and oils, phosphate rock, and potash salts, which enter into the manufacture of finished goods.

Very often unusual specifications have to be made for purchased materials, as for example in the case of special coverings or special containers for meats which have to withstand the deteriorating influence of tropical, humid climates.

In the control of products manufactured in the packing house, one of the principal aims in the case of edible products is, of course, to comply fully with the letter and spirit of the foreign and

domestic laws, including the Meat Inspection Regulations, the Foods and Drugs Act, and the laws of the various individual states. It is also important, however, that the product shall suit the consumer, who often enough seems to know better what he doesn't want, than exactly what he does want. Local customs, likes, dislikes and often prejudices on the part of the consumer and retailer must be met, and as in other selling transactions, this is often possible only by an analysis or examination of a sample of the product which suits the consumer.

In the long list of work on edible packing house products may be included the control of the fresh, cured, smoked, and canned meats, sausages, edible fats, and the processes by which they are produced. To this may also be added the animal products, used chiefly for medicinal purposes, such as pepsin, pancreatin, desiccated glands and their active principles.

Aside from the routine analytical work there are often special analytical problems presented in the control of manufacturing processes. In the manufacture, for instance, of pemmican for polar expeditions it is absolutely necessary to have not only a well-balanced ration of protein, fat and carbohydrates, but the percentage of moisture in the meat, the sweetness of the meat and fat, the amount of salt, spices, sweetening, etc., must be rigidly controlled. Again, in an emergency methods can and must be changed or adapted to meet the situation. Some years ago, for example, it became necessary to determine the absence of boron compounds in not less than 500 samples of cured meat daily. To incinerate and extract such a number of samples daily was impossible with the equipment and help at hand. A number of comparative analyses confirmed the idea that the qualitative test for boric acid could be made on the brine in which the meats had been cured by simple treatment with hydrochloric acid, and direct application of the Goske method, the results being just as satisfactory as if the determinations had been made on the ash of the meat. In this manner it was easily possible for one man to carry on the work at the rate of 500 samples daily.

In the case of soaps, glues, fertilizers, inedible fats and oils, and other inedible products such partial or complete analyses are made as will assure the selling department that the product

will fully meet with the requirements of the purchaser and his specifications, or in the case there are no such requirements or specifications that the products will prove acceptable for the purposes for which they are intended. In a very large part of the work the highest accuracy must be sacrificed to a certain extent to the necessary speed. For instance, it is far more important to avoid per diam penalties or so-called demurrage charges on cars held on the track, to secure cash discounts on materials bought subject to analysis, to avoid costly interruptions in manufacturing processes, the adjustment of which depends on analyses made on the finished product, or to furnish a telegraphic quotation on the day of the inquiry, than to employ unnecessarily refined methods of analysis.

Where so much analytical work must be done, economy of materials and of operation becomes a very important matter. It is necessary, for instance, to arrange the apparatus, for determinations which have to be made in great numbers, in the most compact, simple and convenient form possible, so that the operator will not have to waste time in carrying out the different steps of his work. The expense of reagents can often be materially reduced. For example, the potassium sulphate for nitrogen determinations can be bought from the wholesale druggist in the powdered form in which it is used in medicinal preparations, much more cheaply than the so-called chemically pure article of the chemical supply houses. The only requisite in this case is its freedom from nitrogen, and this must be determined in any event by blank determinations. Again, ammonium nitrate, which is used so largely in phosphoric acid determinations, can be purchased at a relatively low price, if the specifications regarding its purity be limited to absence of phosphates.

It may not be amiss to refer to a mistaken notion which seems to have grown up regarding the value of such analytical work, a notion which has crept even into undergraduate life, namely that analytical work is something to be shunned because of the lack of opportunity for advancement. Nothing could be further from the truth. There is no more valuable training for the industrial chemist and chemical engineer than that which he can obtain in such work as has been described above. If he will

stop to realize what an important bearing even the simplest routine operation may have on manufacturing processes and how often large money values are involved and depend on the faithfulness with which such a simple routine determination is carried out, then his work will take on a new meaning, and actual experience shows that he will make a better man in every way, other things being equal, than the one who lacks such experience and training. Advancement simply depends upon whether or not he is content to remain a routine analyst.

Little can be said regarding the research work which falls to the lot of the packing house chemist. Like all industrial research work it is, from its very nature and purpose, confidential in character, although more frequently than ever before, certain phases of it may be of sufficient scientific interest to bear publication without destroying the value of the general proposition at hand. If this is so, it will doubtless be due to the fact that the methods employed are becoming more and more scientific and less empirical than they have been.

It is out of the question to indicate even in the briefest way the variety of subjects considered in the consulting work required of the packing house chemist, except perhaps, to say that the executive heads, general and department superintendents, engineers, architects, attorneys and many others daily refer questions to the chemist for answer. Many of these subjects are of the greatest interest, sometimes involving careful and extended experimental work, at other times a carefully planned series of analytical determinations or of microscopical or bacteriological work. Occasionally, however, emergencies arise where decisions must be made on the spur of the moment without time for a search of the literature or for actual experiments, otherwise the time for decision would be past.

In conclusion, I cannot emphasize too strongly a fact quite commonly overlooked by the chemist, namely that his work is of no value to the business man unless results, conclusions, judgments, or opinions be reported in simple, clear and concise language, avoiding as far as possible the use of technical, involved, or indefinite expressions, or conditional statements.

AN INVESTIGATION ON THE MANUFACTURE OF TEA

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I. Effect of Steaming on the Activity of the Enzyms of Tea Leaves

In green tea leaves there are present abundant oxydising enzym, wherefore Mann in India holds an opinion that oxydising enzym is one of the factors which determine the quality of tea. In the manufacture of green tea, however, oxydising enzym of tea leaves is killed by steaming, because when it is active the green color of tea leaves can no more be retained. The author[1] found in another investigation that the formation of some aroma of manufactured tea, which takes place usually during the rolling of tea leaves, is due to the action of a certain enzym on a certain compound of tea leaf. Hence if steaming kills all the enzyms of tea leaves the production of aroma may be more or less hindered.

In 1909 I tried to know whether all the enzyms of tea leaves lose activity by steaming in the usual manner. In these trials green leaves were steamed in the usual manner, respectively for 30 seconds, 50 seconds and one minute, and the steamed leaves as well as the unsteamed were crushed and extracted with 40% alcohol. The extracts are precipitated with ether-alcohol and filtered. The precipitates were washed with alcohol and again dissolved in water. The solution gave no reaction with Fe_2Cl_6, proving the absence of tannin. Oxydising enzyms were tested with guayak tincture, and guayacol and H_2O_2, by which the solution obtained from unsteamed leaves showed the characteristic reaction, while the steamed did not. Steaming for 30 seconds killed oxydising enzyms completely. In another trial tea leaves steamed for 20 seconds were tested for the presence of oxydases, and a faint reaction was observed. From these

[1] Bulletin of Agric. Exp. station, No. 1.

facts we know that the oxydising enzyms of tea leaves lose activity when they are steamed only for 30 seconds.

I tried then to see whether the enzyms other than oxydase lose activity by steaming for a short time. Preliminarily I detected diastase in tea leaf by the following manner. Green tea leaves were crushed in a mortar and extracted with 40% alcohol. To the extract ether-alcohol was added, and the precipitate thereby formed was washed and again dissolved in water. In this solution tannin was removed by hide powder and putrefaction was prevented by the addition of thymol. It was filtered, and the filtrate which gave no reaction with Fe_2Cl_6 and did not reduce Fehling's solution, some boiled starch and thymol were put in. The solution after having been kept at 40° C. for 4 days, reduced Fehling's solution considerably. We confirmed by this trial that diastase of tea leaves can be detected in this manner.

The tea leaves steamed for 30 seconds, in which oxydase was completely killed, reduced also Fehling's solution when treated in the same manner. Hence we know that oxydase is much more sensible than other enzyms such as diastase and it is highly probable that some enzymatic actions take place in the first stage of rolling tea leaves, and the production of some fine aroma is due to them. In practice, therefore, steaming of tea leaves must be so regulated as to kill only oxydising enzyms but not other enzyms.

II. Effect of Rolling on the Solubility of Tea

Whether the object of rolling tea leaves in the manufacture of green tea is to give tea a fine shape or to press out the juice in order to accelerate the desiccation of the leaves, or to break the cells in order to increase solubility is, as far as I know, not yet decided. According to the investigation of Dr. Kozai[1] the solubility of green tea was little increased by the manufacture, but Rombe and Roman's experiment[2] showed on the contrary the decrease of soluble tannin and thein.

To settle this question I made an experiment in 1905, in which

[1] Bulletin of College of Agriculture and Dendrology No. 7.
[2] König. Chemie der Nahrungs und genussmittel B. II.

fresh tea leaves, picked at a sheltered tea garden, were divided into three parts, and one of them was steamed and dried without rolling which served as control; the second part was prepared into green tea (Gyokuro), and the third part into Tencha, which is usually prepared without rolling the leaves. The infusion of these three kinds of tea was found to be as follows:—

	Control	Tencha	Gyokuro
Color	light	deeper	deepest
Flavor	weak	stronger	strongest
Taste	faint	good	best

The reaction of the infusion with Fe_2Cl_6 was not the same in three kinds; that of Gyokuro produced deep black color, while control and Tencha a very faint black color. The solubility of tea was determined as follows:—400 cc. of boiling water were poured on 10 gr. of the powdered sample which had been kept at 100° C. for an hour. It was filtered after leaving it to stand for 5 minutes and washed on filter with 100 cc. of boiling water, and soluble matters were estimated in it.

The composition of the control tea was as follows:—

In 100 pts. of air dry substance	
water	6.215
In 100 pts. of dry substance	
Crude protein	41.984
Albuminoids	28.252
Ethereal extract	9.042
Crude fiber	12.012
Nitrogen free extract	14.101
Thein	3.529
Tannin	15.968
Crude ash	6.883
Total nitrogen	6.717
Albuminous nitrogen	4.520
Thein nitrogen	0.934
Amide nitrogen	1.263

The soluble constituents of the three samples were as follows:—

IN 100 PTS. OF DRY MATTERS

	Control	Tencha	Gyokuro
Dry matter.....	34.057	34.130	33.862
Tannin.....	7.083	6.939	6.477
Thein.....	3.124	2.996	3.088
Ash.....	5.249	5.373	5.197

According to these results Gyokuro, which was prepared by rolling the leaves, showed no greater solubility than the other two. Soluble tannin decreased in Gyokuro probably in consequence of oxydation during the rolling.

In the other experiment I determined solubility of three samples in a different manner. 10 gr. of whole, not powdered sample were put in a beaker, and after keeping it at 100° C. for an hour 200 cc. of boiling water were poured on and filtered through glass wool after leaving it to stand 5 minutes. In the filtrate dry substance, crude protein, tannin, thein and ash were estimated. They were as follows:—

	Control	Tencha	Gyokuro
In 100 part of air dry substance			
Water.....	8.375	7.953	7.638
In 100 pts. of dry matter there were soluble			
Dry matter.....	16.076	21.190	29.233
Nitrogen.....	1.885	2.141	2.313
Tannin.....	0.659	1.312	5.492
Thein.....	1.975	2.243	2.804
Ash.....	3.405	4.411	4.385

	Control	Tencha	Gyokuro
In 100 pts. of each constituent there were soluble			
Dry matter.....	17.545	23.021	31.656
Nitrogen.....	28.068	31.869	34.427
Tannin.....	4.127	8.216	34.374
Thein.....	55.965	63.559	79.453
Ash.....	49.750	64.083	63.708

The increase of solubility compared with the control was found to be as follows:—

	Tencha	Gyokuro
Dry matter.....	5.476	14.111
Nitrogen.....	3.801	6.359
Tannin.....	4.089	30.247
Thein.....	7.594	23.488
Ash.....	14.333	13.958

We see that, when the whole, not powdered samples were used, there were greater increase of solubility in the rolled leaves. Hence we may conclude, that the rolling of tea leaves has the effect of increasing easily soluble matter by crushing the cells and pressing out the juice and making it dry on the surface of the leaves.

Second experiment on the same subject was carried on in 1906 with tea leaves picked in unsheltered tea garden. The leaves were divided into two parts, and one part was dried after steaming and served as control, and the other part prepared into green tea. The infusion of the two samples was found to be as follows:—

	Control	Green Tea
Color.....	For lighter	Common
Flavor.....	Nearly null	Good
Taste.....	Faint	Good

The composition of the original leaves was found to be as follows:—

In 100 pts. of air dry substance	
water	6.008
In 100 parts of dry substance	
Crude protein	33.209
Ethereal extract	25.656
Tannin	18.889
Thein	3.266
Ash	5.719
Soluble matter	44.525
Total nitrogen	5.313
Thein nitrogen	0.864

The solubility which was determined in whole, not powdered samples, was found to be as follows:—

	Control	Green tea
Dry matter	9.879	26.692
Nitrogen	0.969	1.410
Tannin	4.883	12.802
Thein	1.995	2.136
Ash	1.383	3.077

	Control	Green tea	Increase in Green tea
In 100 pts. of each constituent there were soluble			
Dry matter	22.119	59.948	37.829
Nitrogen	18.227	26.531	8.304
Tannin	25.850	67.778	41.928
Thein	61.074	65.403	4.329
Ash	24.186	53.811	29.625

The result of this trial agreed with that of the former one, showing the increase of easy solubility in the rolled leaves. Hence we may conclude, that the chief effect of rolling tea leaves is the increase of easy solubility of the constituents. The desiccation of the leaves will also be accelerated by rolling by pressing out the juice from the interior of the cells. From these facts we are justified in testing tea-infusion to take whole, not powdered sample, and to infuse it only for a few minutes. Total solubility as was determined in the usual method is not of much use for practical purpose.

III. The Effect of Firing on the Chemical Composition of Tea

Green tea as well as black tea are usually refired some days later after the manufacture. By refiring the flavor is much improved, but the infusion becomes usually darker in color. In 1908 and 1909 I made some investigation on the effect of refiring on the quality and composition of tea. I kept respectively green tea and black tea at various temperatures for one hour and then analyzed. Tannin was estimated by Löwenthal's method and thein by Mulder's method. Solubility was determined by infusing 2 gr. of whole tea leaves in 400 cc. of distilled water for 2 hours, and after 100 cc. of water had been added it was filtered. The temperature used for firing, the color and flavor of the infusion and the color of the infused leaves were found to be as follows:—

1908

1. GREEN TEA

No.	Temperature	Color	Flavor	Color of the infused leaves
1	Control (not fired)	Little lighter than No. 3	Weaker than No. 2	Greenish yellow
2	61°C	Nearly same as No. 3	Best	
3	82°C	Best	Little too strong	
4	101°C	Rather red	Bad smell	Little reddish
5	123°C	Reddish than No. 4		Reddish
6	140°C	More reddish No. 5		
7	160°C	Reddish		Blackish brown

2. BLACK TEA

No.	Temperature	Color	Flavor	Color of the infused leaves
1	Control (not fired)	Lighter than No. 2	Weaker than No. 2	Brown
2	62°C	Lighter than No. 5	Weaker than No. 3	
3	81°C	Lighter than No. 1	Best	
4	101°C	Lighter than No. 3	Bad smell	Blackish brown
5	119°C	Most reddish		
6	141°C	Lighter than No. 4		
7	156°C	Lighter than No. 6		

1909

1. GREEN TEA

No.	Temperature	Color of infusion	Aroma of infusion	Taste of infusion	Color of the infused leaves
1	Control (not fired)	Faint	Weak	Weak	Usual
2	60°C	Best	Weak	Astringent	
3	70°C	Lighter than No. 4	Best	Good	
4	80°C	Lighter than No. 2	Good	Best	
5	90°C	Reddish	Bad	Bitter	
6	100°C	Worst		Most bitter	Little burnt

2. BLACK TEA

1	Control (not fired)	Not clear	Faint	Weak	Usual
2	60°C	Light		Good	
3	70°C		Best	Best	
4	80°C	Best	Weaker than No. 3	Weaker than No. 3	
5	90°C	Worse than No. 4	Bad	Bad	
6	100°C	Blackish			Blackish

The chief constituents of the tea were found to be as follows:—

1908

1. GREEN TEA

No.	Temperature	In 100 pts. of air dry subst.	In 100 pts. of dry substance				
		Water	Tannin	Thein	Solubility	Soluble tannin	Soluble thein
1	Control (not fired)	5.228	15.690	3.210	37.458	11.724	2.506
2	61°C	4.633	.....	.....	36.786	11.906	2.600
3	82°C	3.158	.....	.....	35.417	11.785	2.601
4	101°C	1.383	14.602	3.101	35.553	11.688	2.444
5	123°C	2.045	.....	.....	37.364	11.096	2.455
6	140°C	2.453	.....	.....	35.162	10.400	2.287
7	160°C	3.005	13.248	3.098	29.898	6.470	2.317

2. BLACK TEA

1	Control (not fired)	4.445	8.575	3.075	27.393	4.045	2.518
2	62°C	3.985	.....	.....	27.027	4.151	2.676
3	81°C	3.703	.....	.....	27.415	4.390	2.290
4	101°C	2.293	7.247	3.130	27.020	4.450	2.705
5	119°C	4.875	.....	.....	26.281	3.682	2.477
6	141°C	2.230	.....	.....	24.957	2.594	2.443
7	156°C	1.460	5.797	3.135	22.757	1.961	2.500

1909

1. GREEN TEA

No.	Temperature	In 100 pts. of air dry subst.	In 100 pts. of dry substance				
		Water	Tannin	Thein	Solubility	Soluble tannin	Soluble thein
1	Control (not fired)	5.157	15.857	3.077	37.557	11.621	2.191
2	60°C	4.512	15.844	3.134	37.252	11.883	2.425
3	70°C	3.903	.....	.....	37.463	11.936	2.483
4	80°C	3.168	15.418	3.209	36.455	11.711	2.536
5	90°C	2.105	.....	.....	35.569	11.236	2.442
6	100°C	1.844	14.586	3.061	24.666	11.451	2.487

2. BLACK TEA

1	Control (not fired)	6.320	8.484	3.165	27.556	3.917	2.348
2	60°C	4.541	8.632	3.163	27.137	3.844	2.456
3	70°C	3.574	.....	.....	27.378	3.955	2.403
4	80°C	3.218	7.402	3.147	27.098	3.979	2.407
5	90°C	2.271	.....	.....	26.727	3.479	2.221
6	100°C	2.049	7.093	3.046	26.475	3.283	2.200

From these results we may conclude, that green tea is improved in quality by being fired at 70° C. for one hour, and temperature higher than 70° C. spoils both the flavor and color. The optimum temperature for firing black tea lies little higher than for green tea; viz. 80° C , and like green tea higher temperature spoils the flavor and color. By refiring tannin and thein decrease more or less, probably the former being due to oxydation and the latter to volatilization. Solubility increases little when tea is not strongly heated, but when temperature is high total soluble substance and tannin decrease remarkably. Therefore in firing tea temperature must of course be properly applied. If it is too high, the quality of tea is much deteriorated.

WHEAT FLOUR. A MONOGRAPH

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In considering the chemical composition of a flour, its moisture content and the basis upon which the results are reported, are matters of first importance. Flour freshly milled may contain from 12.50 to 13.50 per cent of moisture. When stored under the best conditions its moisture content appreciably decreases. Often the chemist receives for analysis a small, over-dried sample sent in paper envelope. This sample may contain less than 8% moisture. Comparison of such a sample with one freshly milled is inconsistent unless the necessary corrections for differences in moisture content be made. This difference in moisture content, unless corrected, disturbs comparative analytical results and is the occasion of much unfavorable comment as to the value of a chemical analysis of flour.

The extent to which a variation of 6% in moisture may affect results is large. A freshly milled flour with 13.50% moisture and .40% ash, would when dried to 7½% moisture show nearly .43% (.427) ash.

The maximum standard of 13.50 per cent of moisture adopted by the U. S. Department of Agriculture is reasonable when the wide range in moisture content of wheat is considered. As an illustration of this range, 278 cars of wheat tested for moisture by myself during the present year showed 48 cars with less than 13.5 %moisture and 230 cars with more than 13.5% moisture.

The question of the moisture content of flour is a disturbing factor not only in chemical analysis but also in the matter of weight of a package. Any loss of moisture causes a proportional loss of weight. Since the Government has established the maximum moisture content of flour at 13½%, that necessarily establishes the minimum dry matter at 86½% and in turn determines the approximate tolerance allowable for shrinkage in

weight. To illustrate: if a 98-lb. package of flour after storage and handling weighs 97 lbs. and is carefully sampled and its moisture content found to be 11½%, it means that the 97 lbs. on an 11½% moisture basis contains 85.84 lbs of dry matter. If the flour had been packed with 13.50% the 98-lb. package would have contained 84.77 lbs. of dry matter. As it is, it contains about one pound of dry matter or dry flour more than is called for on the minimum dry matter basis and hence cannot be considered as short in weight. On the basis of dry matter it is not short in weight and when packed it did not contain the maximum moisture content allowed by the Government standard. Hence in the consideration of both weight and chemical composition of flour the moisture content is a matter of first importance. All comparisons of composition should be made on a uniform moisture basis. The extent to which a flour sample may dry cannot be anticipated and the moisture removed by drying must in turn be added in bread-making. Thus the consumer is in no way defrauded by the drying of flour provided it is packed full weight and without excessive moisture, that is above 13½%. Indeed more water than that would endanger the keeping qualities of the flour and entail loss on the part of the manufacturer. Flour with either an excessively low or high moisture content is not normal flour.

Over-dryness of flour may affect the analytical results by translocation of soluble ingredients. In the case of a sample of flour freshly milled with .40% ash, about one-fourth is mechanically combined—or chemically united with the gluten, one-fourth with the starch and one-half is capable of being dissolved in distilled water. When a flour dries an uneven distribution of the soluble constituents may occur, depending entirely upon governing conditions. Thus if a sample of flour be drawn from only one part of a large flour package it may show an abnormal ash content.

Example of Translocation of Flour Ash.

	Ash Content of Flour	Moisture Content*	
1. Fresh Flour 140-lb. packages	.37+	12.92	.37+
2. Sample from exterior of package after storage 3 mos. in flour store room.....	.41	10.84	.40+
3. Sample from Center of pkg..	.37	11.58	.36+

Drying of a flour accompanied by translocation, and drawing of the sample from a portion only of the package may affect the ash results to the extent of .06 of a per cent. Not infrequently do the results of two laboratories reporting on the same sample of flour show as large and even a larger difference than .06%.

In the determination of the ash of flour other errors that may occur result from: too high a temperature during combustion, incomplete combustion, fusion and occlusion of particles of carbon and failure to make the necessary distinction between crude ash and pure ash. The temperature during combustion should be appreciably below 675°C. and the combustion should be continued until a light grey granular ash is secured, reasonable constant in weight. The ash should not be fused and should be corrected for carbon and combined carbon dioxide. The ash from a refined flour can be obtained quite free from carbon and combined carbon dioxide, so that there is no appreciable difference between the crude ash and the pure ash. But, if the combustion is not made with care, the difference is large.

The main loss caused by high temperatures for combustion is sulphur. This, however, affects the ash percentage less than .01 per cent. There appears to be no loss of phosphorus during the combustion process at low temperature as there is sufficient alkaline matter to form non-volatile pyro phosphates. In fact I have been uuable to obtain any difference in the phosphorus

*Ash on basis of uniform moisture content.

content of a flour from the analysis of the ash, and from the analysis of the residue of the calorimeter where the flour is burned in such a way as to preclude any possible loss of phosphorus compounds.

No official method has yet been adopted by the Association of Official Agricultural Chemists for the determination of the ash of cereals. In the work on flours and cereals by the U. S. Department of Agriculture a just distinction has been made between crude ash and pure ash along the lines laid down by European Chemists.

Methods have been proposed for the complete analysis of the inorganic constituents of plants in which calcium acetate is used to prevent volatilization of sulphur, and then corrections are made for the lime and carbon dioxide introduced by its use. The application to flour of such a method for obtaining ash is not feasible as corrections for carbon dioxide &c. must be made on each individual sample tested. This in turn calls for the combustion of 100 grams of flour so as to get enough crude ash to determine the quantities of impurities to be deducted. Such a method would be impracticable in flour mill work. No common factor could be assumed for correction as a more or less richly carbonated ash of a variable degree of purity is obtained. The acetate introduces one-fourth as much mineral matter as is naturally present in the flour and its use may occasion the introduction of a larger error than* it is intended to correct: viz. volatilization of sulphur.

When made under uniform conditions and by one person, the ash results indicate the mechanical uniformity of a flour to a high degree and are of value in flour mill control work but alone—the ash results are incapable of determining the bread-making value of a flour.

When the ash results of two chemists working on the same sample of flour are compared wide differences may be observed because of variation in moisture content, occlusion, translocation, incomplete combustion and failure to make the necessary distinction between crude and pure ash. These variations are often so large, and unnecessarily so, as to cast much discredit on

* Through formation of carbonates.

chemical tests and their application to determining the value of flour.

Another source of confusion in the interpretation of a flour analysis is the occasional use of the factor 5.7 for converting total nitrogen into protein, instead of 6.25 and then no mention being made of the factor used. In technical scientific investigation the use of special factors for protein determination is necessary and unquestionable, but to use a special factor for wheat and a general factor (6.25) for all other foods is inconsistent. From a nutritive point of view the 6.25 factor for wheat is more correct than 5.70 because the wheat proteins are concentrated in nitrogen containing 17.50% against the general average of 16.25. It is the nitrogenous part of the molecule which gives the unique food value to the proteins, and in the wheat proteins the consumer gets more of this material. In fact the 5.7 factor assigns too low a nutritive value to wheat. Wheat proteids are too concentrated in distinctive nitrogenous material to be assigned so low a percentage value—when compared with other foods where the proteins are of lower nitrogen content. It is the quality of the protein that determines its nutritive value as well as the amount, and in wheat the proteins are of strong nitrogenous character.

The general method for the determination of crude fiber is not satisfactory for determining the fiber content of flour, as the action of the acid and alkali solutions for the removal of non-fiber materials is not complete. By extracting the flour with 70% alcohol, after extraction with ether, better results are obtained as the gliadin is removed, foaming is prevented and the material is in better mechanical condition for extraction with acid and alkali solutions. This extraction with alcohol is beneficial in determining the fiber of wheat products.

In conclusion, it may be said that the chemist can do most in the way of flour investigations by making a study of bread-making processes and the factors which control them. Flour making is distinctly a mechanical process and the whole tendency of modern flour manufacture is in the direction of producing cleaner flour.

Since wheat flour takes such an important part in the dietary and because it supplies such a large amount of nutrients at com-

paratively low cost, it is consistent that the efforts of the chemist be directed toward encouraging the farmer to raise the best of bread wheats in the most approved ways through scientific agriculture, and that a broader knowledge be secured and generally disseminated concerning the principles of bread-making and the nutritive value of bread and foods in general. In this work the food chemist must necessarily take the leading part. If indifferent, he is not doing his duty.

ON SOME DRIED MILKS AND PATENT FOODS

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Whether dried milks, infants' foods and other milk food preparations are becoming more popular or that mothers find it more convenient to bring up their children on these products, the fact remains that there is a steady increase in the number of these preparations. Although some are of a wholesome nature there are on the market nevertheless a large number whose chief ingredient is starch, contained in such a proportion as to be totally unsuitable for infants. Artificial products cannot effectually replace what nature has supplied. The characters of human milk are such that its imitation seems almost irrealisable. It is true that by diluting cows' milk with water and adding lactose and cream, an article can be produced much resembling human milk, but it has not those properties that render mothers' milk the ideal food for children. Sommerville pointed out the value of dried milk for infant feeding on account of its relative sterility and the absence of a dense clot in the infant's stomach. When one studies the results of the analyses of the infants' foods on the market, the unsuitability of the majority of them is greatly in evidence. The Australian Food Standards Committee 1906, recommended that "Infants' food shall contain no woody fibre, no preservative substance and no mineral substance insoluble in water; and unless described or sold specifically as a food suitable only for infants over the age of seven months shall, when prepared as directed by an accompanying label, contain no starch and shall contain the essential ingredients of and conform approximately in composition to normal mothers' milk." Apart from the high starch percentage of a number of brands, there are not many which form even in 10% solutions anything approaching a homogeneous solution. In several cases this was not possible, the result being a thick

pasty mass caused by the swelling and cohesion of the starch. The small % of fat is an outstanding feature of inferior brands; they are deficient in the chief body fuel namely fat. Abundance of fat should be the main characteristic of the diet of infancy just as abundance of carbohydrates is the chief feature of the adult and laborious life. The frequent connection between rickets and deficiency in fat is an undeniable clinical fact. Again, the desiccating process destroys the enzymes always present in raw milk and to which its anti-scorbutic properties are generally ascribed.

Preparation

Until lately the process of separating the solid and liquid constituents of milk was too costly to render the manufacture of "dried milk" a profitable industry. The machine used in the "Just-Hatmaker" process appears to give the most satisfactory results; it consists of two large metallic drums 28 inches in diameter and 5 feet long, mounted horizontally in a framework with a space of about $\frac{1}{8}$th inch between them. High-pressure steam, admitted to the drums through axial pipes, raises their surfaces to a temperature of 220° F. The milk is allowed to flow in thin streams over the revolving drums, the heat of which quickly evaporates the water. A coating of solid matter gradually forms which is scraped off by a knife and falls into a receptacle. The milk is not boiled, though completely sterilised by the heat. A slight decomposition of the proteins and fat probably takes place.

Classification

1. Dried milks and milk products; they consist of milk evaporated and dried, with or without the addition of lactose and fat. They are free from starch. The results are given in table I.

2. Farinaceous and malted foods; in this are included foods containing either malt or starch or both. In many cases the diastase was not active. The results are given in table II.

3. Miscellaneous products, oats, barley, etc., for growing children. The results will be found in table III.

Methods of Analysis

Water: This was estimated by drying 10 grams of the sample at 100° C. for 4–6 hours.

Ash: The residue was incinerated and weighed in the usual manner.

Total P_2O_2: Where it was desirable to estimate the phosphates, a titration with N–10 uranium nitrate using $K_4Fe(CN)_6$ as indicator was made.

Total proteins: This was estimated by the usual Kjeldahl process. The factor N x 6.38 was used unless otherwise stated. A verification of this factor was effected by D. Richmond (Analyst, 1908, p. 179). When the nitrogen is present as casein or albumen the factor 6.38 should be used but when its origin is uncertain 6.25 is recommended as a general factor.

Soluble albumen: 10 cc. of a 10% solution are digested with 20 cc. of saturated $MgSO_4$ solution and crystals of magnesium sulphate are added until they no longer dissolve. An excess does not matter, provided the $MgSO_4$ is free from Na_2SO_4. The solution is put aside till the next morning, when it is filtered and washed with a little saturated $MgSO_4$ solution. The filtrate is then treated with Almen's reagent (4 gram tannic acid, 190 cc. 50% alcohol, 8 cc. 25% acetic acid) and the precipitate allowed to settle till the next day. It is then filtered, paper and contents transferred to a Kjeldahl flask and the estimation of the proteins carried out in the usual way.

Lactose and Carbohydrates. These were obtained by difference.

Fat: We utilised 3 methods: (1) Leffmann and Beam, centrifugalisation of fat (2) Adams and Soxhlet process, the latter in certain cases where the Adams process could not be applied owing to the product not forming a solution with water (3) Werner-Schmidt. The last named is the only reliable method combining accuracy with rapidity. The Adams and Soxhlet extraction were often found to be unreliable. This is in agreement with McLellan (*Analyst*, 1908, p. 353); he found that the incompleteness of the extraction of the fat is due to the coating of the fat globules in the milk during the process of evaporation with an impermeable substance which prevents the solvent from penetrating. He found that it was possible to completely

extract the fat from dry milk in a soxhlet apparatus if the sample was soaked overnight, then extracted for 8 hours and allowed to soak again overnight and finally extracted for 1 to 2 hours. That the results obtained by the Adams process are too low is further borne out by Siegfeld (*Molkerei Zeitung*, 1909, N. 25), Grünhut (*Zeit f. Anal. Chem.*, 1911, p. 649), and Anton Burr (*Milchwirtschaftl. Zentralbl.*, 7, p. 118). The latter found the Werner-Schmidt and Rose-Gottlieb processes to give good results, the last named requiring special conditions to be complied with according to D. Richmond (*Analyst*, 1908, p. 389).

Physiological Fuel Value: The results are expressed in large calories per 100 grams of the sample. The factors used were fat 9, protein 4, and carbohydrate 4, these being the physiological fuel values of food constituents.

Nutritive Ratio: is the ratio of proteid to carbohydrate and fat, i.e., the ratio of nitrogenous to non-nitrogenous nutrients compared on the basis of fuel values. It is deduced from the formula (Sherman, Chemistry of food and nutrition 1911):—

$$\frac{\text{carbohydrates} + 2\frac{1}{4}\ \text{fat}}{\text{proteins}}$$

No preservatives (formaldehyde, boric acid) were found in any of the samples analysed.

TABLE I

DRIED MILKS AND MILK PRODUCTS EXEMPT FROM STARCH

Mark		Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Lactose	Fuel Value	Nutritive Ratio
Dried human milk	(1)	.....	30.02	2.46	18.18	10.00	49.32	540	1:11
Cow and Gates	(2)	3.36	25.00	6.32	27.5	2.23	37.82	486	1:6
id.	(3)	4.58	10.00	6.98	30.90	2.23	41.54	433	1:4
id.	(4)	4.76	1.86	8.43	33.75	2.74	51.20	356	1:1.8
Trumilk	(5)	1.46	30.00	5.57	27.69	4.91	35.28	521	1:7
id.	(6)	7.3	1.04	7.41	32.85	3.57	51.40	346	1:1.7
Allenbury No. 1	(7)	2.12	18.46	3.80	11.23	1.87	64.39	468	1:14
Allenbury No. 2	(8)	3.70	16.28	3.56	11.55	2.23	64.91	452	1:13
Proteid Huxley A	(9)	17.21	.13	8.30	45.36	.....	29.00	298	1:1
Proteid Huxley B	(10)	14.99	5.62	8.14	50.72	.....	20.53	335	1:1
Neaves Milk Food	(11)	3.64	25.37	4.28	19.65	3.03	47.06	495	1:9
Sanatogen	(12)	8.28	.12	5.46	84.15	.....	.....	337	....
Albulactin		8.70	.....	5.64	85.30 (13)	.....	.....	341	....
Hogg's Protein Nerve Food		11.08	.78	6.30	80.38 (14)	.....	.....	328	....
Robb's Soluble Milk Food 1	(15)	2.52	14.50	3.46	14.67	1.70	64.85	448	1:9
Robb's Soluble Milk Food 2	(16)	4.38	9.83	4.74	18.37	1.70	62.68	404	1:6
Plasmon	(17)	9.71	.52	7.68	75.92	.....	.....	308	....
Majax	(18)	.....	.....	1.03	.....	.....	98.2	392	....
Humanoid	(19)	66.60	11.58	.70	4.78	1.78	16.34	188	1:16

(1) Figures calculated from König: Chemie der Mensch. Nahrungs ü Genussm. (2) Full cream. (3) Half skimmed. (4) Machine skimmed. (5) Full cream. (6) Skimmed. (7) Dried milk with added lactose. (8) Idem. (9) Contains sodium glycerophosphate. (10) Made from milk and eggs with addition of glycerophosphate. (11) Dried milk with added lactose. (12) Milk caseine with glycerophosphate P_2O_5=.71%. (13) Lactalbumin. (14) Milk caseine. (15) Dried milk with added lactose. (16) Idem. (17) Milk caseine P_2O_5=2.94%. (18) Lactose. (19) Concentrated and modified milk.

TABLE I—*Continued*

DRIED MILKS AND MILK PRODUCTS EXEMPT FROM STARCH

Mark		Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Lactose	Fuel Value	Nutritive Ratio
Paget's Modified Milk 1.....	(20)	71.58	7.83	.57	3.12	1.27	16.90	150	1:19
Welford Humanized Milk.....	(21)	88.98	3.36	.24	1.22	.14	6.20	60	1:18
Vitafer.....	(22)	9.85	.48	9.25	71.90	.....	.....	292	....
Cibrola.....	(23)	9.32	.62	10.29	72.35	.....	.....	295	....
Glaxo.....	(24)	1.89	27.0	5.55	24.62	6.06	40.94	505	1:7
Trufood.....	(25)	4.76	13.45	6.88	20.0	3.82	54.91	421	1:6
Norwegian.....	(26)	7.30	.34	7.38	32.15	1.25	52.83	343	1:2
Dutch.....	(27)	4.43	24.0	6.33	24.11	1.60	41.23	477	1:6
id.	(28)	7.75	.86	7.26	35.72	2.86	48.41	344	1:1.5
id.	(29)	5.45	.35	8.17	31.96	8.03	54.07	346	1:1.7
English.....	(30)	5.52	26.87	5.59	23.92	3.21	38.10	490	1:7
id.	(31)	8.0	.80	8.13	34.64	5.18	48.43	339	1:6
American.....	(32)	6.86	1.0	7.88	34.64	4.37	49.62	346	1:1.6
French.....	(33)	4.36	23.55	5.72	25.62	3.75	40.75	477	1:6
id.	(34)	7.36	.73	7.55	34.64	1.35	49.72	344	1:1.6
New Zealand "Defiance"	(35)	3.70	29.70	5.33	26.79	2.76	34.48	512	1:7

(20) idem. An enclosed packet contained .2112 grm. $NaHCO_3$. (21) Supposed to resemble human milk. (22) P_2O_5= 3.97%; milk caseine with glycerophosphate. (23) P_2O_5=3.19%; idem. (25) Modified dried milk. (26) Skimmed. (27) Full cream. (28) Best quality separated. (29) Ordinary qual. separated. (30) Full cream. (31) Separated. (32) idem (33) Full cream. (34) Extra fine qual. separated. (35) Full cream.

TABLE II
MALTED AND FARINACEOUS FOODS

Mark	Water	Fat	Total Ash	Total Proteins	Soluble Albumen	Carbohydrate	Fuel Value	Nutritive Ratio
Horlick's malted milk	3.58	7.65	3.67	14.55	3.04	70.54 (1)	409	1:8
Allenbury malted food No. 3	4.68	.94	1.01	11.80	1.16	81.57 (2)	381	1:7
Milo	3.26	6.40	1.26	12.86	1.61	76.22 (3)	413	1:8
Virogen (4)	7.21	.54	6.15	47.65	.....	38.45	349	1:1
Neaves Food for infants	5.40	.91	.68	13.71	.....	79.30 (5)	380	1:6
Benger's Food	7.47	.82	1.06	12.50	.....	78.15 (6)	370	1:6
John Bull Malt-and-Milk 1	1.42	17.0	4.43	19.14	.....	58.01 (7)	461	1:7
John Bull Maltassa 2	4.20	1.00	1.58	13.93	1.78	79.29 (8)	381	1:6
Miriam's Food	10.41	4.25	1.03	11.87	.....	72.44 (9)	375	1:8
Ridge Patent Food	4.70	1.34	.98	11.25	.....	81.73 (10)	384	1:8
Neaves Health Diet	5.41	15.13	3.10	19.07	.....	57.29 (11)	441	1:1
Chapman malted food	9.93	1.86	1.36	12.25	.....	74.60 (12)	364	1:1
Mellin's Food	2.81	.21	3.18	14.00	.....	79.8 (13)	377	1:6
Mellin's Lactoglycose	4.94	7.10	4.06	18.37	3.21	65.53 (14)	400	1:6
Ovaltine (15)	3.98	5.40	3.63	15.31	2.76	71.68	396	1:6
Savory-and-Moore	5.07	1.43	.77	12.93	.....	79.80 (16)	383	1:7
Carnrick's Soluble Food (17)	1.71	1.76	3.58	19.14	5.1	73.81	387	1:4
Chapman's Food	9.37	1.98	1.64	12.75	.....	74.26 (18)	365	1:6
Frame Food	3.37	.46	1.52	13.31	.....	81.34 (19)	383	1:6

(1) Dextrine, maltose, lactose; contains active diastase. (2) Lactose maltose; wheat flour and malt. (3) Dextrine, maltose, lactose, sucrose; Nestle's desiccated milk with baked wheat starch. (4) Caseine with sodium glycerophosphate. Trace of starch. (5) Wheat flour. (6) Wheat flour with pancreatic extract. (7) Maltose, dextrose, dextrine, lactose; active diastase. (8) Starch, maltose, dextrose, dextrine, lactose. (9) Baked wheat flour N x 6.25. (10) idem. (11) Dried milk and wheat flour. (12) N x 6.25. Flour and malt. (13) No starch; a completely malted product. (14) Mellin's Food with dried milk. (15) No starch; malt extract, dried milk, eggs, a little cocoa. (16) Wheat flour N x 6.25. (17) Dried milk with wheat flour and a little malt. (18) N x 6.25. Wheat flour without malt. (19) N x 6.25. Starch, maltose, dextrine, sucrose. Baked wheat flour.

TABLE III

MISCELLANEOUS PRODUCTS

Mark		Water	Fat	Total Ash	Total (1) Proteins	Carbo-hydrate	Fuel value	Nutri-tive ratio
Maltcon	(2)	25.06	1.28	9.54	14.12	50.00	268	1:4
Quaker Oats	.....	9.95	7.43	1.70	15.56	65.36	370	1:7
Plasmon Oats	(3)	8.98	9.28	2.14	18.87	60.73	402	1:6
Plasmon arrowroot	(4)	13.19	.46	2.15	18.06	66.14	341	1:4
Glidine	(5)	7.56	.45	.53	87.27	4.19	369	....
Virol	(6)	24.10	15.00	1.04	3.37	55.49	374	1:40
Cerola	(7)	10.56	1.00	.52	12.50	75.42	360	1:6
Farola	(8)	10.79	1.39	.44	13.00	74.38	362	1:6
Robb's Nursery Biscuits	.....	5.91	5.36	1.01	10.37	77.35	399	1:10
Manhu prepared barley	.....	10.72	1.96	1.24	9.37	76.71	362	1:9
" flaked "	.....	10.66	1.72	1.73	10.18	75.71	359	1:8
" " rye	.....	10.48	1.49	1.64	10.75	75.64	359	1:7
" " wheat	.....	10.16	1.86	1.64	11.43	74.91	362	1:7

(1) N x 6.25. (2) Starch maltose, dextrose; extract of yeast. (3) Oats with Plasmon. (4) Arrowroot with Plasmon. (5) Pure gluten N x 5.7. (6) No starch; bone marrow, yolk of eggs, malt extract, flavored with lemon juice. (7) Granulated wheat. (8) Idem in fine powder.

The question of starch in infants' foods

A short perusal of the results tabulated will show that nearly 50% of the samples analysed contained starch. Nearly all of these milk food preparations are intended for infants' food, so that this question is of capital importance in the bringing up of children from birth. This subject was dealt with in an interesting paper by Cautley.—(Lancet, Nov. 6, 1909), amongst whose conclusions are:—

(I) A diastasic ferment is secreted by the salivary glands and pancreas of new-born infants; the salivary secretion however is scanty in young infants and rarely appreciable before the age of two months.

(II) Barley water contains about 2% of starch; mixtures containing this percentgae of starch are not injurious but may be beneficial, for the growth of lactic acid bacilli and the formation of lactic acid are thereby encouraged. These organisms are of undoubted advantage in the prevention of the growth of proteolytic bacteria.

(III) The evil effects of starch in early life are due to (a) excess, (b) its admistration in the form of a more or less insoluble emulsion instead of as soluble starch (c) the substitution of starch for the necessary protein, fat and salts.

Under the title "Patent Foods" an interesting pamphlet has been written by R. Hutchison. The defects of artificial foods may be summed up as follows:—

(I) They are often recommended when there is marked loss of appetite; they do not promote it.

(II) It is often contended for these products that they are more easily digested than natural foods and many of them exist because they are pre-digested. The necessity for peptonizing foods is greatly exaggerated. In pathological chemistry pepsin is almost never absent from the gastric juice unless HCL is also absent. If HCL can be found in the stomach pepsin is sure to be there too; there is therefore little necessity for predigested foods.

(III) The claim often put forward for artificial foods that they enable you to enrich the diet in certain constituents is largely fallacious.

(IV) None of them is worth the money asked for it; some of them contain a ricidulously small amount of nourishment at the price. It is vastly more expensive to rear a child upon one of them than upon fresh or even condensed milk.

ÜBER DIE CHEMISCHE ZUSAMMENSETZUNG DES "SALZBREIES" VON BONITO ("SHIOKARA")

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Zur Bereitung des "Salzbreies" wird der Magen und Darm des Bonitos vom inneren Inhalt befreit, gut gewaschen, fein zerhackt und mit viel Kochsalz vermischt, so dass es einen dicken Brei gibt. Die Leber wird auch manchmal dazu gemengt. Man lässt nun den so bereiteten Brei wochenlang bei Zimmertemperatur stehen und rührt öfters um. Es tritt dabei allmählich die Reifung ein; es entwickelt sich ein eigentümlicher Geruch und Geschmack, und von vielen Leuten, besonders von Sakekennern wird der Artikel als Delikatesse mit Vorliebe genossen. Die an der Reifung des Breies teilnehmenden Mikroben sind bis jetzt nicht untersucht, und die chemischen Vorgänge, die während des Reifeprozesses vor sich gehen, sind auch noch nicht näher erforscht. Nur vermutet man, dass sie den bei der "Shōyu" Bereitung auftretenden ziemlich ähnlich sind. Durch Einwirkung von Mikroben und Enzymen werden verschiedene Stoffe, besonders Eiweissstoffe, allmählich gelöst und abgebaut, unter Bildung von Peptonen und Aminosäuren, die zum Teil weiter desamidiert, oxidiert oder reduziert werden. Es entstehen dabei verschiedene Säuren, Alkohole, Amine u.s.w. Die Zusammensetzung des Breies ist deshalb sehr kompliziert. Es kommen bei verschiedenen Reifestadien verschiedene Stoffe zum Vorschein.

Wir beschränken uns vorläufig mit der Untersuchung der stickstoffhaltigen Bestandteile des käuflichen, gereiften Breies. Das von uns untersuchte Material war aus Odawara bezogen. Es war grau-rötlich-braun gefärbt und reagierte ziemlich stark sauer. Die quantitative Bestimmung gab folgendes Resultat:

In 100 Teilen frischen Breies

Wasser	65.13
Trockensubstanz	34.87

In 100 Teilen Trockensubstanz

Organische Stoffe	30.06
Asche	69.94
Chlor	29.80
(Als NaCl berechnet)	49.18

	In 100g frischen Breies	Gesamt-N als 100
Gesamt-N	1.735	100.0
Eiweiss-N	0.472	27.2
Org.-Basen-N	0.447	25.7
Ammoniak-N	0.131	7.6
Anderes N	0.685	39.5

Zur Isolierung der stickstoffhaltigen Stoffe wurden 4 Kilo Brei aus gepresst. Der Rückstand wurde drei mal mit warmem Wasser (40–50°) extrahiert. Die vereinigten Auszüge, die schwach sauer reagierten, betrugen rund 9 liter. Sie wurden mit 20% iger Tannin lösung gefällt. Der Tannin-Niederschlag (B) wurde abgesaugt und mit Wasser gewaschen. Das Filtrat vom Tannin-Niederschlag wurde mit verdünnter Natronlauge verstezt, bis es schwach alkalisch reagierte. Es entstand dabei eine flockige Fällung (C) in reichlicher Menge. Man saugte davon ab, und setzte dem Filtrat viel Baryt zu, um das Tannin zu entfernen, saugte wieder ab und nach dem Entfernen des Baryts mittelst Schwefelsäure dampfte man bei niederem Druck stark ein. Es schieden sich dabei Tyrosin, Leucin und anorganische Salze aus. Aus heissem Wasser umkrystallisiert, erhielt man zuerst 3 g Tyrosin und von der Mutterlauge desselben 2.1 g Leucin. Beide Aminosäuren wurden nochmals für sich umkrystallisiert und analysiert.

Tyrosin:

0.1604 g Subst. gaben 10.7 c.c. N (16° 760 mm)

		N
$C_9H_{11}NO_3$	Ber.	7.70
	Gef.	7.78

Leucin:

0.1719g Subst. gaben 15.7 c.c. N (14° 758 m.m.).

		N
$C_6H_{13}NO_2$	Ber.	10.07
	Gef	10.71

Die Mutterlauge von Leucin und Tyrosin wurde mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt.

A Der Phosphowolframsäure-Niederschlag

Die aus diesem Niederschlag dargestellte alkalische Flüssigkeit, die freie Basen enthielt, lieferte nach starkem Einengen im Vakuum keine Krystalle, so wurde sie mit Kohlensäure gesättigt und mit Quecksilberchlorid gefällt.

a) Der Quecksilberchlorid-Niederschlag wurde mit Schwefelwasserstoff zerlegt, im Vakuum ein gedampft und mit Pikrinsäure erwärmt. Nach dem Erkalten schieden sich 8.5 g fast reines Lysinpikrat aus, welches aus heissem Wasser umkrystallisiert und analysiert wurde.

0.1414 g Subst. gaben 22.0 c.c. N (13° 766 mm)
0.1491 g " " 0.2094 g CO_2 0.0612 g H_2O
0.4149 g " " 0.2533 g Pikrinsäure

		C	H	N	Pikrinsäure
$C_6H_{14}N_2O_2$, $C_6H_3N_3O_7$	Ber.....	38.40	4.53	18.67	61.07
	Gef.....	38.34	4.56	18.53	61.05

Im Kapillarrohr erhitzt, zersetzte sich das Pikrat gegen 247° (unkorr.).

Das Platinchlorid-doppelsalz des Lysins waren hygroskapische goldgelbe lange Prismen. Es schmolz bei 205° (unkorr.).

Für die Analyse wurde es im Vakuum bei 100° getrocknet.

0.3014 g Subst. gaben 0.1055 g Pt.

		Pt.
$C_6H_{14}N_2O_2$, H_2PtCl_6	Ber.....	3.500
	Gef.....	35.00

b) Das Filtrat vom Quecksilberchlorid-Niederschlag wurde nach dem Entfernen des Quecksilbers durch Schwefelwasserstoff und der Salzsäure durch Silbernitrat, mit einem Überschuss von Silbernitrat und Baryt versetzt. Der braune Niederschlag lieferte 1.3 g Lysinpikrat.

Die Analyse des gereinigten Salzes gab folgendes Resultat:

0.1313 g Subst. gaben 21.3 c.c. N (20° 760mm)

		N
$C_6H_{14}N_2O_2$, $C_6H_3N_3O_7$	Ber.....	18.67
	Gef.....	18.56

c) Das Filtrat vom Silbernitrat und Baryt-Niederschlag wurde in bekannter Weise mit Phosphowolframsäure gefällt. Aus diesem Niederschlag erhielt man wieder 6 g Lysin pikrat.

0.1374 g Subst. gaben 22.3 c.c. N (21° 763 mm)
0.1526 g " " 0.2160 g CO_2 0.0672 g H_2O

		C	H	N
$C_6H_{14}N_2O_2$, $C_6H_3N_3O_7$	Ber.	38.40	4.53	18.67
	Gef.	38.60	4.89	18.55

B. Der Tannin-Niederschlag (Trytophan)

Der vom wässerigen Extrakt des Salzbreies durch Zusatz von Tannin erhaltene Niederschlag wurde mit 3% iger Schwefelsäure wiederholt verrieben. Ein Teil ging dabei in Lösung. Man filtrierte nun vom unlöslichen Rückstand ab und setzte dem Filtrate viel Baryt zu, um damit Tannin und Schwefelsäure wegzuschaffen. Das vom dabei entstandenen Niederschlag abgesaugte Filtrat wurde mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt.

a) Durch Zerlegung des phosphowolframsauren Niederschlags wurde eine Flüssigkeit erhalten, welche schöne Tryptophanreaktion gab. Wird diese Flüssigkeit mit Essigsäure angesäuert und mit einigen Tropfen Bromwasser versetzt, so entsteht eine rot violette Färbung. Beim Schütteln mit Amylalkohol geht der Farbstoff in das letztere Reagenz über. Um das Tryptophan zu isolieren, wurde die Flüssigkeit mit so-viel Schwefelsäure versetzt, bis sie 5% der Säure enthielt, und mit Hopkinischem Reagenz gefällt. Es enstand dabei eine weisse flockige Fällung, die absesaugt, mit 5% iger Schwefelsäure gewaschen und mit Schwefelwasserstoff zerlegt wurde. Beim Eindampfen des Filtrats im Vakuum schied sich ein Teil des Tryptophans krystallinisch aus. Die Hauptmasse blieb jedoch amorph, so dass das gereinigte Tryptophan nicht zur Analyse ausreichte.

Aus dem Filtrate vom Quecksilbersulfat-Niederschlag des Tryptophans wurde eine Base als pikrinsaures Salz isoliert. Dies genügte auch zur weiteren Untersuchung nicht.

b) Das Filtrat vom Phosphowolframsäure-Niederschlag lieferte, nach der Estermethode verarbeitet, eine kleine Menge Leucin.

C. Der Tannin=und Natronlauge Niederschlag

Wie oben erwähnt, lieferte das Filtrat vom Tannin Niederschlag (B) durch Zusatz von verdünnter Natronlauge, wieder eine reichliche Fällung, die eine nicht unbeträchtliche Menge Basen enthielt. Um die Basen zu isolieren wurde der Niederschlag mit 5% iger Schwefelsäure verrieben, wobei ein grosser Teil in Lösung ging. Die braune Flüssigkeit wurde nun mit einem Überschuss von Baryt versetzt, vom dabei entstandenen Niederschlag abgesaugt, mit Schwefelsäure angesäuert und mit Phosphowolframsäure gefällt. Nach Zerlegung des phosphowolframsauren Niederschlags in bekannter Weise erhielt man eine alkalische Flüssigkeit, die freie Basen enthielt. Diese Flüssigkeit wurde nun mit Kohlensäure gesättigt und mit Quecksilberchlorid gefällt.

a) Der Quecksilberchlorid-Niederschlag (Histidin)

Aus diesem Niederschlag erhielt man eine alkalische Flüssigkeit, die sowohl starke Paulysche Reaktion, wie auch Biuretreaktion beim Erwärmen gab. Bei Zusatz von Pikrinsäure wurde 0.5 g Histidinpikrat gewonnen, welches aus heissem Wasser umkrystallisiert, im Vakuum bei 100° getrocknet und analysiert wurde.

0.1430 g Subst. gaben 27.8 c.c N (24° 759 mm)
0.1517 g " " 0.2034 g CO_2 0.0457 g H_2O

		C	H	N
$C_6H_9N_3O_2$, $C_6H_7N_3O_7$	Ber.	37.50	3.13	21.91
	Gef.	37.56	3.35	21.77

Die Analyse stimmt also mit dem Histidin pikrat überein. Der Schmelzpunkt war jedoch viel höher als beim gewöhnlichen Histidin pikrat, welches aus den Spaltungsprodukten des Eiweisses dargestellt wird. Im Kapillarrohr erhitzt, wurde es gegen 200° braun und zersetzte sich gegen 210° (unkorr.) unter Schäumen. Es handelt sich wahrscheinlich um eine Isomerie des Histidins. Wegen Mangel an Material konnten wir das optische Verhalten nicht untersuchen.

b) Das Filtrat vom Quecksilberchlorid-Niederschlag des

Histidins wurde mit Silbernitrat und Baryt gefällt. Aus diesem Niederschlag isolierte man eine Base als pikrinsaures Salz, welches 1.5 g betrug. Das Pikrat bestand aus rot braunen blättrigen Krystallen mit dem Schmelzpunkt 225° (unkorr.). Die Analyse gab folgendes Resultat:

0.1171 g Subst. gaben 24.2 c.c. N (20° 760 mm)
0.1372 g " " 0.1715 g CO_2 0.0496 g H_2O
0.2504 g " " 0.1919 g Pikrinsäure.

		C	H	N	Pikrinsäure
$C_6H_{14}N_4O_2$ $(C_6H_2N_3O_7)_2$	Ber.....	34.18	3.16	22.15	72.47
	Gef.....	34.09	4.02	23.64	76.63

Die Analyse stimmt also mit dem Arginindipikrat, nur ist der Gehalt an Stickstoff und Pikrinsäure etwas höher.

c) Das Filtrat vom Silbernitrat und Baryt-Niederschlag (b) wurde wieder mit Phosphowolframsäure gefällt. Der Niederschlag lieferte 2.34 g Lysinpikrat mit dem Zersetzungspunkt 245° (unkorr.).

D. Das Filtrat vom phosphowolframsauren Niederschlag

Das Filtrat vom phosphowolframsauren Niederschlag wurde nach der Estermethode verarbeitet, indem die Phosphowolframsäure und Schwefelsäure durch Baryt entfernt und der Überschuss vom Baryt mittelst Schwefelsäure beseitigt und im Vakuum stark eingedampft wurde. Der zurückgebliebene Syrup wurde nun mit absolutem Alkohol versetzt, mit trockenem Salzsäuregas gesättigt und in bekannter Weise in die freien Estern der Aminosäuren verwandelt. Nach fraktionierter Destillation der Estern, wurden die folgenden drei Fraktionen erhalten:

	Temperatur	Estermenge	Aminosaüren nach der Verseifung
1	bis 75° (20mm)	9.0 g	3.0 g
2	75–100° "	10.5	7.8
3	über 100° "	7.0	6.0

Fraktion 1 bestand aus Alanin. Aus heissem Wasser umkrystallisiert, bildete es farblose Nadeln mit süssen Geschmack und zersetzte sich gegen 270°. Für die Analyse wurde es im Vakuum bei 100° getrocknet.

0.1553 g Subst. gaben 21.3 c.c. N (17° 760 mm)
0.1530 g " " 0.2248 g CO_2 0.1040 g H_2O

		C	H	N
$C_3H_7NO_2$	Ber.	40.45	7.87	15.88
	Gef.	40.07	7.55	16.03

Fraktion II. bestand auch hauptsächlich aus Alanin, nebst einer Kleinen Menge Prolin.

Analyse des Alanins:

0.1476 g Subst. gaben 20.4 c.c. N (21° 751 mm)

		N
$C_3H_7NO_2$	Ber.	15.88
	Gef.	15.62

Kupfersalz des Alanins:

0.2114 g Subst. gaben 0.0703 g CuO.

		Cu
$(C_3H_6NO_2)_2Cu$	Ber.	26.22
	Gef.	26.57

Fraktion III. bestand zum grössten Teil aus Leucin, nebst Alanin und Prolin. Zweimal aus heissem Wasser umkrystallisiert wurde das Leucin in ziemlich reinem Zustande erhalten. Es schmeckte schwach bitter und zersetzte sich gegen 280°.

Analyse des Leucins:

0.1467g Subst. gaben 13.4 c.c. N (19° 762 mm)

	N
$C_6H_{13}NO_2$ Ber.	10.07
Gef.	10.56

Das Prolin wurde isoliert, indem die nach der Verseifung des Esters erhaltenen Aminosäuren mit heissem absolutem Alkohol extrahiert wurden. Der vereinigte alkoholische Extrakt wurde eingedampft und der Rückstand nochmals mit absolutem Alkohol extrahiert. Nach dem Verdampfen des Alkohols wurde das Prolin in bekannter Weise in das charakteristische Kupfersalz verwandelt, welches in heissem absolutem Alkohol löslich war.

Die Ausbeute an Kupfersalz betrug 1.03 g. Für die Analyse wurde das gereinigte Salz im Vakuum bei 100° getrocknet:

0.1613g Subst. gaben 13.8 c.c. N (22° 752 mm)

	N
$(C_5H_8NO_2)_2Cu$ Ber.	9.60
Gef.	9.59

Ferner wurde das Vorhandensein von Glutaninsäure im Filtrat des phosphowolframsauren Niederschlags durch ihren charakteristischen faden Geschmack ausser Zweifel gestellt. Wegen Mangel an Zeit haben wir diese Säure nicht isoliert.

Aus 4 Kilo Salzbrei des Bonitos wurden isoliert:

1.	Lysin pikrat	18.14g
2.	Histidin pikrat	0.50
3.	Tyrosin	3.00
4.	Leucin	4.06
5.	Alanin	10.80
6.	Leucin+Alanin	4.00
7.	Prolin Kupfer	1.03
8.	Tryptophan	Vorhanden
9.	Arginin di pikrat(?)	1.50
10.	Glutaninsäure	Vorhanden

ON THE CHEMICAL COMPOSITION OF "SAKÉ"

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On the chemical composition of Saké many reports have been made: Some authors write on the composition which is contained originally in the fermented mash and others on the constituents which have been derived from the vat or cask which contain saké. In the factories of saké the fermenting vat and the cask which is employed for the transportation of saké, are made from cryptomeria japonica; and therefore certain components of the material of the vessels must be present and dissolved in saké Ch. Kimoto[1] reported on sughi-oil in 1903 and N. Nagai[2] and T. Kimura made some researches on its chemical composition and found protocatechin, a phinon-like substance, vanillin and a kind of terpene and they have proved the presence of the same substances in saké. The terpene, mentioned by the above authors, was by K. Keimatsu[3] proved to be a sex-terpene and he also added as a new component of cryptomeria japonica a phenol-like substance, having reducing property. In the same year Yamamoto and Ishikawa[4] made studies on the same reducing substance as regards its influence in determining the reducing sugar in saké.

On the proper composition of saké K. Keimatsu[5] made a report on the furfurol in saké and one of us[6] made research on the contents of furfurol and found that in young saké, or shortly after the fermented mash is pressed, there was no furfurol or, if

[1]Bulletin of the College of Agriculture Tokyo Imp. Univ. vol. 4. page 403.

[2]In a speech made at the Tokyo Chemical Society (1904).

[3]Yakugakuzasshi (Journal of the Pharmaceutical Society of Japan, 1905, March.)

[4]do: Sept. 1905.

[5]do: Decemb. 1904.

[6]T. Takahashi: The Journal of the Agricult. Society of Japan. May, 1905.

present, it was a trace; while in aged saké, i.e., after the storage during summer, it was always present in it. In the same year H. Nishizaki[7] made a quantitative determination of furfurol, but arrived at somewhat different results from the writer. Recently H. Ito made experiments on this subject[7'] with about 111 samples of saké and arrived at the same conclusion as the writer. One of us[8] has made a report in respect to the quantity of fusel oil and pointed out that the quantity of the substance must be examined when we classify saké as regards quality. Moreover, a small quantity of methyl alcohol was proved to be present in ordinary saké[9], and a somewhat evident quantity of methyl-lactate in certain samples of saké was found by one of us[10].

H. Nishizaki made a report on free acids[11] and sugars[12] of saké and mentioned that the latter consisted chiefly of glucose, while K. Suda[13] already sixteen years ago made experiments on the contents of the sugar of saké and reported that the sugar consists chiefly of maltose. It seems to the writer that both authors may be right; because they used only a limited number of samples and arrived at altogether opposite results. If they had examined a wider range of samples they would probably have found out that their results were one sided. K. Keimatsu[14] and Shimizu reported on the presence of acetaldehyde, fusel oil, succinic acid, lactic acid and acetic acid in saké.

On animo-acids, however, no one has reported yet and we therefore present here the results of our investigations about amino-acids and other components.

The general chemical composition of the samples was as follows:

[7]Journal of the Pharmaceutical Society of Japan. Novemb. 1905.

[7']Journal of Tokyo Chemical Society. Vol. 32, No. 7. He proved directly, chiefly by anillin acetate, but the quantity was too small; he has distilled saké under reduced pressure and below 35°C neutralized saké.

[8]T. Takahashi. Journal of the Agricu. Society of Japan. Apr. 1905.

[9]T. Takahashi. Bulletin of the Agr. Coll. Tokyo Im. Univ. Vol. 6. No. 4.

[10]T. Takahashi. Bulletin of the Agr. Coll. Tokyo Im. Univ. Vol. 7. No. 4.

[11]Journal of the Pharmaceutical Society of Japan. May, 1905.

[12]do: May, 1906.

[13]do: Apr. 1890.

[14]do: Decem. 1905.

1).	Total—N	0.1865 %
2).	Protein—N.	
	a). Stutzer's method	0.0072 %
	b). Rumpler's method[15]	0.00672%
	c). Precipitate by Pb–acetate and Pb-oxide (Bungener u. Fries. Zeit. f. d. ges. B. 1894.69.)	0.00447%
	d). Precipitate by basic lead acetate	0.00435%
3).	Non-albuminoid—N.	
	a). Ammonia—N. (Wurster's method)	0.00629%
	b). Organic base—N	0.0598 %
	c). Other—N. (chiefly amino-acids)	0.1131 %
4).	Esters (as acetic ester)	0.0457 %
5).	Total acid (as succinic acid)	0.2666 %
	a). Non-vol. acid (as succinic acid)	0.2596 %
	b). Volatile acid (by difference) (as acetic acid)	0.00715%
	c). Volatile acid (determined in the distillate) (as acetic acid)	0.0216 %

The protein nitrogen determined by Stutzer's and Rümpler's method is always higher than that of the nitrogen found in the basic-lead-acetate precipitate of lead–acetate and lead oxide; because the former two methods always precipitate in a certain degree a part of albumoses and peptones beside proper proteins. For this reason therefore, we must assume the presence of albumoses and peptones in saké; but we can prove directly the presence of both substances. If we take 100-200 c.c. of saké and after removing the protein by basic lead acetate and from this filtrate after removing lead by H_2S and evaporating to a small volume a sufficient quantity of Zu-sulphate is added to suturaled the solution, acidifying with sulphuric acid, there will be found a precipitate of *albumoses*. From the filtrate of albumoses, after removing zinc by H_2S and condensing in a small

[15]Rümpler: Deutsch. Zeit. Ind. 1898, 1729.

The difference between contents of nitrogen determined by Stutzer's method and that of the precipitate made by lead-acetate is shown from the above table to be 0.0027%, and this must be the least quantity of the nitrogen of albumose and peptones.

volume, this will contain *peptones* in solution, which will be proved easily by Biuret reaction.

(I) Organic Bases

10 litres of the samples were evaporated under reduced pressure and at 60° C. to a small volume, almost equal to $\frac{1}{8}$ of the original volume. After this operation the protein-substance was removed by basic lead-acetate and researches were made about bases according to Kossel's method; but of histidine only a trace was found and the characteristic crystals of the chloride were not obtained, giving Pauly's diazo-reaction (1904). Arginin was not found. Picrate of lysin, about 1.1 grms, was obtained as fine needles and platy crystals, which melted at 230° C. (uncorr.), so that it contained some impurities.

(II) Mono-amino-acids

Ten litres of saké were used for the isolation of mono-amino-acids after E. Fischer's well-known ester method.

a) Under the pressure below 20 m.m.
(Esters prepared from 10 l. saké.)

First fraction.....	60°–92° C.	1.0 grms.
Second fraction.....	92°–150° C.	1.5 grms.
Third fraction.....	150°–235° C.	Trace.

b) Under the pressure below 18 m.m.
(Esters prepared from 10 l. saké.)

First fraction, below.....	41° C.	0.6 grms.
Second fraction.....	41°–60° C.	1.0 grms.
Third fraction	60°–98° C.	3.0 grms.
Fourth fraction.....	98°–150° C.	4.5 grms.

From the first fraction of series (a), 0.2 grms of alanin, 0.2 grms of leucine, and 0.1 grms of prolin were obtained. In the second series 0.2 gr. of alanin from first fraction and 1 g. of leucine, trace prolin from third fraction, were obtained. The Alanin obtained from first series was added to the same from the second series and after purification analysis was carried out. It was very sweet, having a melting point of 243–245° C. (uncorr.)

and decomposed at the point with evolution of gas. The result of analysis was as follows:—

Substance taken:—0.972.
Nitrogen=12 c.c. (at 15° C. 760 m.m.)
Calculated as $C_3H_7NO_2$ N=15.67%
Found.....N=14.51%

Prolin was bitter in taste and its copper salts contained two components, one of which dissolved in absolute alcohol, while others do not, indicating the presence of active and inactive prolin.

Leucin was bitter in slightest degree so that it was washed with absolute alcohol. It gave a melting point of 290–293° C. (uncorr.), changing in brown at 270° C. already. It decomposed at the melting point with emission of gas. The analytical result was as follows:

Substance taken: 0.1226 grms.
Nitrogen.....10.7 c.c. (at 11° C. 758 m.m.)
Substance taken: 0.1102 grms.
Co_2.....0.2165 grms.

Calculated as $C_6H_{13}No_2$	Found.
N=10.85.....	10.46
C=55.80.....	53.57
H=10.07.....	9.73

The above result tells us that the substance was not pure.

Phenylalanin and glutannic acid were not found, but trace of aspartic acid was found.

Leucinimid. ($C_{12}H_2N_2O_2$). After all esters were evaporated, the residue was treated with acetic-ester, which dissolved a part of it. When the dissolved part was evaporated, there remained hexagonal or quadratic plate, which tasted very bitter. The platy crystals were dissolved again in acetic ester and after evaporaton ether and ethyl-alcohol were added, but there were found no crystals. So we could not prove the presence of leucinimid.

Tyrosin. The presence of this substance was easily proved by Millon's reaction by the filtrate from the precipitate of protein—substance of saké. But for the isolation of this substance we followed Brown[16] and Willer's operation, which they employed for the isolation of this substance from malt. Thus 1 grm. of tyrosin was obtained from three litres of saké. While, as in other cases, 10 litres of the sample was evaporated to a small volume and after separation of protein, the filtrate free from lead, was evaporated to almost one-fifth of the original volume of saké and left standing over night in a cool place, there appeared the characteristic silky crystals of tyrosin amounting almost to 6 grams. The isolated tyrosin gave strong red coloration by Millon's reagent, faint reaction of Pauly's[18] diazo-reaction strong by Wurster's reaction[17] and Denige's reaction.[19] The analytical result was:

Substance taken: 0.1048 grms.
Nitrogen.....6.8 c.c. (at 10° C. 762 m.m.)
Calculated as $C_9H_{11}NO_3$..... N=7.777%
Found..... N=7.79%

Cystin. This substance was proved very easily in the filtrate which was obtained in removing the protein-substance of saké, but the quantity was too small to isolate it.

Tryptophane, ($C_{11}H_{12}N_2O_2$). This substance was obtained from 110 c.c. of saké by Hopkins' and Cole's[20] method. The crystals were platy and bright; giving red coloration with bromine-water and precipitated by phosphotungstic acid. A few crystals were mixed with caustic potash and after fusing and subjected to the dry distillation, pyroll reaction was observed in this distillate. However, the presence of tryptophane in saké is limited only to young saké, or not aged saké.

[16] Brown u Willer. Woch. f. Brauerei, 1907. Nr. 11. S. 139.

[17] Beautiful red color by acetic acid and natrium-nitrite (c. f. Ph. 1. 1903 (1888).

[18] Hoppe-Seyler's zeit. f. ph. ch. 42, 517 (1904).

[19] Wine-red by form aldehyd and H_2SO_4 (comptes rendus, 130, 583, 1900).

[20] Journal of Phys. 27, 418 (1902), 29, 451 (1903).

On this fact one of us[21] has reported already. On the question, that why this substance disappears in the aging process of saké, H. Ito[22] has made some observations and arrived at the conclusion that the tryptophane in young saké is assimilated or rather decomposed by so-called aging yeast[23]:—Willia anomala varieties.

The Substances which dissolved in Ether

1.) Succinic acid.

Ten litres of saké were evaporated to about 400 c.c. under reduced pressure below 70° C. The syrup thus obtained was extracted with ether, using Kumazama and Sudo's extraction apparatus and dried. From ether extract, the ether was evaporated for a long time in sulphuric acid containing desiccator, then there appeared long platy or mono-crinic prismatic crystals in a brown colored syrup. The crystals were separated by filtration and after washing very quickly with a small quantity of cold absolute alcohol, there remained rather small quantities of the crystals, which melted at 181°–182.5° C. (uncorr.). The substance behaves very strongly acidic to litmus, tasting characteristic to succinic acid, and gave pyroll reaction when subjected to Neuberg's prove (1900–1901). Such pure substance obtained from ten litres of saké was about 3 grams.[24]

From another 18 litres, 4.7 grams of the substance was obtained as raw product, which was brown in color, so that it was first neutralized with a 5% solution of natrium hydroxide, and after evaporating to syrup, absolute alcohol was poured on there appeared almost spontaneously fine crystals of natrium succinate. The natrium salt thus obtained was washed repeatedly with absolute alcohol. It was silky white and gave a brown precipitate with ferric chloride.

[21] T. Takahashi. Journal of the Tokyo Chemical Society of Japan, Vol. 32, No. 3, 1911. Also, Journal of the College of Agr. Tokyo Imp. Univ. Vol. 000, No. 0.

[22] H. Ito. Journal of the Tokyo Che. Soc. of Japan, Vol. 32. No. 7, 1911. Also, Journal of the College Agr. Tokyo Imp. Univ. Vol. 00, No. 00.

[23] T. Takahashi's report. Journal of the College of Agr. Tokyo Imp. Univ Vol. 1, No. 3.

[24] The inactive form of zinc salt contains 18.17% of water of crystallization.

2). Lactic acid.

The syrup obtained after removal of succinic acid crystals was neutralized with zn-carbonate to attain a thick pasty mass from which, after standing overnight, the crystals of zinc lactate were separated by "nutsche" and washed with absolute alcohol. The salt thus obtained was treated with H_2S to separate zinc, and the colorless solution of free acid thus obtained gave intense reaction of Ueffermann's proof. Zinc salt was reprepared from this pure lactic acid and after drying well in the desiccator, the water of crystallization was determined, drying several hours at 105–110° C. The water[25] of crystallization amounted to 17.0%, almost equal to that of the inactive form of lactic acid.

3). Tyrosol. $OHC_6H_5CH_2CH_2OH$.

For the isolation of tyrosol we followed Fe. Ehrlichi's[26] method. Ten litres of the sample were evaporated to almost 400 c.c. and after basifying with $NaHCO^3$, ether extract was made by Sudo and Kumagawa's extraction apparatus. The yield was 4 grams as raw product. It was dissolved again in absolute alcohol and decolorized with animal black. It behaved very strongly to Millon's reagent and diazobenzol sulfonic acid and faintly to ferric chloride and Denigè's–Moerner's proof. Tyrosol-di-benzoate prepared by the addition of benzoyl chloride and natroulye, melted at 113° C. (uncorr.).

Summaries

The above statements are summarized below:
The yield of the observed substance from 10 litres of Saké:—

1). Glycocoll..... Not found.
2). Alanin..... 0.2 grams.
3). Leucin..... 0.6 grams.
4). Prolin (active and racenic)..... 0.1 grams.
5). Phenylalanin..... Not found.

[25] Ber. I. Deut. ch. G XLIV, Heft I. S. 139–146, 1911, u Bioch. Zeit, 36 Band, 15 Heft. S. 477, 1911.
[26] F. Ehrlich, Ber. d. Deut. ch. Ges. 1911, XLIV, Heft I. S. 143.

6). Glutamic acid..... Not found.
7). Aspartic acid..... Trace (?)
8). Leucin-imid..... Not found.
9). Tyrosin..... 6.0 grams.
10). Cystin..... Trace.
11). Tryptophane (only present in young saké)..... 1.0 grams.
12). Lysin..... 0.25 grams.
13). Ristidin..... Trace.
14). Arginin..... Not found.
15). Tyrosol..... 4 grams (raw products).
16). Succinic acid..... 3 grams (in the least).
17). Lactic acid (inactive)..... 2 grams (in the least).
18). Albumoses and peptones..... present.

The yield of amino-acid is too small in comparison to the nitrogen contents of amino-acids obtained from calculation. The main cause is that the pressure, under which we have made fractions was rather high (18–20 mm.), but beside this saké contains rather high percentage of carbohydrate, which makes it difficult to isolate the esters. In conclusion the writer must offer many thanks to his assistant M. Sato, for his faithful help during this research.

(*Abstract*)

A STUDY OF THE COMPOSITION OF CIDER VINEGAR

MADE BY THE GENERATOR PROCESS

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This paper is the result of factory experiments on a very large scale of the conversion of fermented cider into vinegar in the ordinary commercial type of vinegar generator.

Experiments were carried on for a period of several months, and some 40,000 to 50,000 gallons of hard cider were run through a series of generators, samples being taken from the material going on to the generator and from the finished product, and analyses made in detail to show the changes which took place. Work was undertaken largely because the existing data published, regarding American cider vinegars was based wholly upon the analyses of vinegars made in a small way, and fermented under the slow fermentation process, which takes place in a barrel or cask. It was found at the beginning of this investigation that the results of analyses on this type of vinegar showed such a wide variation that it was practically impossible to detect any forms of adulteration. And it was concluded from our analyses of ciders existing at that time that this wide difference in results was due to the method of manufacture; and as the method of manufacture in this country at the present time is practically confined to the generator process, it was found that it was necessary to have data upon this product.

Results of the investigation showed that vinegars made by the generator process were practically as uniform in composition as the cider from which they were made, showing a very different condition from that found to exist in the existing data on cider vinegar.

THE MICROSCOPICAL EXAMINATION OF VEGETABLE PRODUCTS AS AN ADJUNCT TO THEIR CHEMICAL ANALYSIS

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In solving the problems of man and nature the analytical chemist too often limits himself to chemical or physico-chemical methods. He is an analytical chemist in the strict sense of the word and not an analyst, which implies a man of broader training and experience, utilizing the principles of other sciences as means to his end. He turns his back on the methods of vegetable and animal histology, physiology and bacteriology, asserting with satisfaction that he is a specialist and as such must limit his field of activity.

This attitude of the analytical chemist may be traced to a misapprehension as to the province of a specialist. Such a worker must be limited only in the field of application and not in training or the methods employed. An occulist, for example, limits himself to defects of vision and diseases of the eye and allied organs, but in order to properly carry out the work of his specialty he must have broad medical training and be conversant with the general principles of optics, bacteriology, chemistry and perhaps other sciences. Specialists in other sciences, both pure and applied, must also have good general training if they are to achieve distinction in their limited fields; otherwise they are in much the same position as the mechanic who, instead of mastering his trade, learns to operate one machine, thus becoming a mere automaton.

Botany and chemistry are generally considered incompatibles. The student of chemistry sometimes takes up bacteriology as a minor subject, but comparatively seldom studies advanced botany, even though he intends to specialize in food analysis, textile chemistry, paper technology or some other subject dealing chiefly

with materials of vegetable origin. No physiological chemist would think of pursuing his investigation of animal materials without a working knowledge of animal anatomy, yet agricultural and food analysts and others dealing with vegetable materials too often limit themselves to a knowledge of chemical constituents, ignoring the relation of composition to histological structure.

This is most remarkable, since the methods of vegetable histology, as well as of chemistry, are invaluable in solving problems relating to the nature or constituents of foods, drugs, fibers and other products of vegetable origin. Sometimes one line of investigation alone is useful, sometimes the other, but often each throws some light on the subject, and the corroboratory results obtained by such widely differing means furnish an indisputable chain of evidence.

Let us look more closely into the nature and relation of these two applied analytical sciences.

Chemical analysis deals with chemical constituents; microscopical analysis deals largely with the form of some of these constituents. Chemical analysis determines the amount of fiber, starch, protein, oil, etc.; microscopical analysis determines the shape, size, and other characteristics of the cells and cell contents. Chemical analysis usually stops with the mere determination of the amount of chemical constituents; microscopical analysis goes further and names the particular product from which they were derived. Chemical analysis answers a question only in scientific terms; microscopical analysis, in terms which all can understand.

In many cases, the best idea of a material is gained by following out both lines of investigation. By chemical analysis we learn the percentage of protein, fiber, starch, etc., but not the ingredients from which they were derved; by microscopical analysis we learn the ingredients, but usually gain only an approximate idea of their proportion. Given the results of both analyses, we may often calculate with some exactness the percentage of the different materials present.

If, for example, we find in a sample of wheat bran 11 instead of 16 per cent. of protein, and 15 instead of 8 per cent. of fiber, we know it is not pure bran but we do not know the adulterant;

if we find corn-cob tissues under the microscope, we learn the adulterant but not the amount. Knowing that the material is a mixture of bran and ground corn-cob, and knowing the average percentage of protein and fiber in both, we are in a position to calculate from the results of the chemical analysis the relative amounts of these ingredients.

Again, if we find in ground mace 40 per cent. instead of 20 per cent. of fixed oil, we know it is not pure mace; if we find under the microscope a large amount of tissues of the Bombay mace, a material worthless as a spice containing about 60 per cent. of fixed oil, we learn the adulterant. Knowing all this, and knowing the average percentage of oil in true mace and Bombay mace, we have the data for calculating roughly the percentage of each in the mixture.

Still again, if in a textile fabric we find a certain percentage of organic fiber insoluble in boiling alkali, we know that the fabric is not all wool. If under the microscope we identify this insoluble fiber as cotton, we have found the missing link in the chain of evidence.

In the analysis of complicated mixtures, we must often rely entirely on microscopical examination. For example, chemical analysis of a mixture of wheat, buckwheat and corn flours gives us little information, and it is only after the characteristic starch granules and tissues of each have been found under the microscope that we gain a definite idea of the nature of the constituents.

Again, in the examination of paper, the microscope is our sole dependence in learning the nature and approximate percentages of the fibers employed, chemical analysis serving merely to determine the kind and amount of sizing, coating and other non-fibrous constituents.

Among some condimental cattle foods examined by the writer some time since was one, the chemical analysis of which disclosed but one proximate constituent, viz., common salt; the microscope, however, disclosed linseed meal, corn meal, wheat feed, mustard hulls, cocoa shells, malt sprouts, fenugreek and turmeric. In such a case, dependence must be placed entirely on the microscope, except for mineral ingredients.

Chemical analysis of another sample demonstrated the presence

of ground bone, carbonate of lime, iron oxide and free sulphur; microscopical examination disclosed linseed meal, wheat feed and charcoal. This is a striking example of a material in which half the constituents (all mineral) can only be detected by chemical analysis; the other half (all vegetable) by the microscope.

Many other equally striking examples of the interdependence of these two applied analytical sciences might be cited.

The point now arises as to who is to carry on these two lines of investigation so different in details but so similar in purpose.

One plan is for a chemist to make the chemical analysis and a botanist the microscopical examination. This plan has the advantage that each can confine his attention to one specialty, but it had the disadvantage that the close partnership between the two, which is essential to the best results, outside of large institutions, is both difficult and expensive. Such a division of labor would usually be as impracticable as to divide the work of a chemical laboratory between a chemist and a physicist, the former conducting the precipitations and other chemical processes the latter, polarizations, determinations of specific gravity, refractive index and the like.

The rational plan is for one man to master both lines of research. Such a man need not execute all the details, but he should be thoroughly acquainted with them and should interpret the results. We will call him an analyst, not a chemist or a botanist, and his laboratory an analytical laboratory, not a chemical or botanical laboratory. His equipment should consist of the necessary apparatus for a wide variety of chemical work and a complete microscopical outfit, including micro-reagents and a set of standard specimens of economic seeds, roots, barks, fibers, woods, etc.

But in order to have workers in this field, we must have suitable courses of instruction in our schools of science. The subject has a recognized place in many continental universities, particularly in the schools of medicine, pharmacy and hygiene, but outside of a few institutions, receives little attention in America.

The student who seeks to prepare himself for this field should take both chemical and botanical studies. In chemistry, he

should study the branches taught in a well-regulated chemical course—elementary chemistry, qualitative and quantitative analysis, organic and physical chemistry, and so on. In botany he should take up successively elementary botany, systematic botany (at least of the phanerogams) and vegetable anatomy and physiology. These studies are all on the curriculum of every college and school of technology, although the student of chemistry does not usually take all the botanical studies named. Without a certain amount of botanical training, however, a chemist is no more fitted to take up microscopical analysis than a botanist without chemical training is fitted to work at quantitative analysis.

After his preliminary studies in chemistry and botany, the student is ready to take up a course in the methods for the chemical and microscopical examination of the various raw materials and of the products derived from them. This course should be so arranged that the student will carry along his chemical and histological practice side by side, as he must do afterwards in practical work. For example, in studying the cereal grains, he should devote part of his time to the methods of determining water, ash (including ash analysis), protein, fiber, starch, fat, pantosans, etc., and another part to a systematic study of the starches and the histological elements of the bran coats both in sections and in powdered form. In like manner, he should take up a chemical and histological study of leguminous seeds, oil seeds, spices, tea, coffee, cocoa, drugs, fibers, etc.

His work in the chemical laboratory should teach him not only the strictly chemical methods but also the use of the polariscope, the spectroscope and other physical apparatus, and his microscopical instruction should fit him not only to differentiate organized forms but other characteristic elements, such as fat crystals, mineral crystals, and the like.

After such a course, he should be able not only to undertake investigations in physiological or plant chemistry but also the laboratory work of an official food department or a custom house, a flour mill, a brewery, a sugar refinery, a candy works, a fruit cannery, a drug mill, a textile mill, a paper mill, etc.

It is my firm belief that courses similar to that outlined should

be conducted in all our leading universities and schools of technology, and the student should be taught the use of the microscope in conjunction with the balance in solving the analytical problems which every day become more numerous and intricate.

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THE IODINE CONTENT OF THE SMALL, MEDIUM AND LARGE THYROID GLANDS OF SHEEP,[1] BEEF AND HOGS

By T. B. Aldrich

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

It is conceded by a majority, if not all writers on the subject of thyroid therapy, that the thyroid gland (or its preparations) to be physiologically active, must contain at least *some* iodine, furthermore that this iodine to be of the greatest value therapeutically, must be combined or associated with some protein or organic complex found in the gland. Presumably the iodine is the more important constituent, the two, however, associated or combined seem to give the best therapeutic results and to-day the efficiency of a thyroid preparation is generally measured, or should be by its iodine content. In fact for some time a number of pharmaceutical houses have been putting out thyroid preparations with a guaranteed percentage of iodine. Since then the iodine content of a thyroid preparation is a measure of its therapeutic efficiency, it is desirable to select if possible those glands which contain the most iodine, providing other factors are equal, and from those animals, whose thyroids contain the most of this constituent.

The following work was taken up with the object of determining the iodine content and thereby the therapeutic efficacy of some thyroid glands, especially the small, medium and large thyroid glands of sheep, beef and hogs by means of the method employed by Hunter (Journal of Biolog. Chemistry 1909-1910, VII, p. 321) which is very accurate and detects the presence of very small amounts of iodine, that are incapable of being detected by the older method of Baumann (J. Physiol. Chem. 1896, 21, p. 489; Ibid, 22, p. 1) which has been the method usually employed heretofore. (See Rigg's work J. of American Chem. Soc. 1910) XXXII, p. 692; Ibid, 1909 XXXI, p. 710).

[1] The iodine content of some mixed sheep thyroids was also determined.

The glands received came from the Chicago stock yards, were placed in Mason jars as soon as removed from the animals, subsequently placed in cold storage and shipped packed in ice. They were all received in the best possible condition. Six lots were obtained as follows:

(1) Mixed sheep thyroids (Lots A and B).
(2) Small, medium and large sheep thyroids (Lot C).
(3) " " " " " " (Lot D).
(4) " " " " beef " (Lot E).
(5) " " " " hog " (Lot F).

In lots A, B and C the glands were not counted.

After freeing from superfluous tissue and weighing, the glands were ground very fine, defatted and desiccated in the usual manner and eventually reduced to a very fine powder by passing through a 60 mesh sieve.

The following information obtained by one* of our staff from a packer, relative to thyroids, may be of interest at this point.

(1) Sheep thyroids are subject to great variation in size. The sex factor is not the determining factor for the size of the gland, nor has the condition of nutrition of the sheep any decisive bearing on the size of the gland. They run from the size of an almond to the size of a lemon.

(2) Steers' thyroids are larger than cows', this variation in size being a *constant* factor; size of gland varies again with the condition of the animal. Well-nourished cattle have larger thyroids than poorly fed ones.

(3) In hogs the thyroids vary little in size and present only slight variation in general appearance.

The thyroids of cattle are removed after head has been severed; same is true of hogs. Cattle thyroids are often cut, those of hogs not.

The following method of assaying the iodine, somewhat abbreviated was employed. (For details see Hunter, The Journal of Biological Chemistry 1909-1910, VII, p. 321).

Exactly one gram of the body was taken, placed in a nickel crucible (125 c.c.), 14 grm. of the following oxidation mixture added (106 parts Sodium carbonate, 75 parts Potassium Nitrate,

and 138 parts Potassium carbonate) and the two intimately mixed by means of a nickel spatula. Over this was dusted 4 gm. of the oxidation mixture. The nickel crucible was then heated over a flame until contents of the same was perfectly white. This more or less fused mixture was dissolved in water and brought into an Erlenmeyer flask (800 cc.). After cooling 35 cc. of Sodium Hypochlorite solution was added, and while holding the flask in a slanting position in cold water and agitating at the same time, 65 cc. of 42½% phosphoric acid was added. The solution should, after the addition of the acid, be colored slightly yellow from the slight excess of chlorine liberated. The mixture was then boiled briskly, a funnel with a short stem being placed in neck of flask to avoid any loss. When all the free chlorin was expelled, recognized by holding filter paper moistened with starch solution containing Potassium iodine in the steam (blue color if present), the flask containing now about 70-80 cc. was allowed to cool and brought up to about 200 cc. by the addition of water. To this cold solution 10 cc. of a 1% Potassium Iodide solution was added, which causes the liberation of 6 times the amount of iodine originally in the product to be assayed, according to the following equation:

$$KIO_3 \cdot HIO_3 + 10\ KI + 11\ HCl = 11\ KCl + 6\ H_2O + 12\ I$$

This liberated Iodine is immediately titrated with a standard solution of Sodium thio sulphate solution approximately N/200, a few drops of starch solution being added toward the end of the reaction.

The number of Cc. of Sodium thio sulphate used multiplied by the iodine factor then divided by 6 gives the amount of iodine in the original sample.

A blank test using casein, or some other body free from iodine was made to insure the absence of iodine in the reagents, and the usual precautions employed in analytical methods observed.

The following table gives the number (except in the lots A, B and C), total weight of glands, average weight of glands and iodine content in each lot with average iodine content and percentage in each gland, where the number of glands is known.

SHEEP THYROIDS (LOTS A AND B)

	No. of Glands	Total Wt. of glands gms.	Av. Wt. of glands gms.	Iodine % (moist gland tissue)		Av. iodine in each moist gland mg.	%
(A)		2925 (MX)[1]		.032	.025		
(B)		5120 "		.022			

SHEEP THYROIDS (LOT C)

(1)		6000 (S)		.027	.019		
(2)		5200 (M)		.018			
(3)		5805 (L)		.01			

SHEEP THYROIDS (LOT D)

(1)	397 (S)	540	1.36	.044	0.028	.6	.04
(2)	192 (M)	650	3.38	.028		.9	.027
(3)	48 (L)	675	14.00	.015		2.1	.015

BEEF THYROIDS (LOT E)

(1)	98 (S)	620	6.32	.039	0.036	2.47	.04
(2)	53 (M)	405	7.64	.030		2.40	.031
(3)	34 (L)	425	12.20	.038		4.70	.038

HOG THYROIDS (LOT F)

(1)	108 (S)	725	6.7	.054	0.047	3.6	.05
(2)	70 (M)	765	10.9	.048		5.2	.047
(3)	40 (L)	735	18.37	.041		7.5	.04

[1]The letters (S) (M) (L) and (MX) stand for small, medium, large and mixed glands.

The average iodine content of the mixed sheep thyroid glands, Lots (A), (B), (C), and (D) where over 50 lbs. were employed, is about .025% while in some cases with selected glands 0.044% has been obtained and .027 where relatively large amounts (6000 gms.) were used.

The beef thyroids (Lot E) gave an average of .036% iodine, and in selected cases nearly .04%. The hog .047% average, in selected glands over .05% iodine.

It will be noted that the greatest variation in iodine content in the different sized glands of the same animals, is in that of the sheep where it varies from .01%-.027% in Lot C and from .015-.044 in Lot D. This variation being no doubt due to the greater prevalence of goiter in sheep.

Next to sheep the iodine content of the hogs' thyroids vary the most .041-.054% while in the beef we have the least variation .030-.39%.

The hogs' thyroids[1] contain the highest percentage of iodine, with the small sheep glands in one case higher, in the other lower than the mixed beef glands. Assuming the iodine content to be a measure of the therapeutic activity, the mixed thyroids of the hog are superior to the beef and the latter superior to sheep thyroids, small sheep thyroids being about equal to mixed beef thyroids.

Weight for weight the small glands of all the animals studied, nearly without exception contain the most iodine (excepting beef where the large and the small have nearly the same .038%).

In general the larger glands contain the most iodine and the ratio of the iodine content of the small, medium and large glands is approximately as follows:

In the sheep 2: 3: 7
" " beef 1: 1: 2
" " hog 3: 4: 6

The mixed glands arranged according to their iodine content stand about in the following ratio:

[1]It is interesting to note that Baumann found very little iodine in pigs' and hogs' thyroid glands; *very much* less than in beef and sheep.

Sheep	Beef	Hogs
5	7	9

From whatever standpoint we take we must conclude from the above that the employment of either hogs or beef thyroids for therapeutic purposes would be more rational than the employment of sheep glands, even if small selected sheep glands are employed, thus eliminating the goiterous glands.

*It is a pleasure to thank Dr. Baeslack, of our staff, for looking after the collection of the glands and also for the information relative to the same.

BIOCHEMICAL AND TOXICOLOGICAL STUDIES ON PENICILLIUM STOLONIFERUM — THOM

By C. L. Alsberg and O. F. Black

United States Department of Agriculture, Washington, D. C.

Whether molds or the products of their growth have an injurious effect on animals is a question which has not yet been conclusively settled. The literature contains many records of alleged intoxications due to these fungi. Certain diseases of men and domesticated animals have been attributed to this cause. Though, obviously, the solution of this problem is urgent, few serious attempts have been made to identify chemically the alleged toxic substances. Chemical studies of this kind have been undertaken in the Poisonous Plant Laboratory of the Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, of the Bureau of Plant Industry, U. S. Department of Agriculture. The present paper is the second of this series of studies.

The genus Penicilium was chosen for study because, owing to the investigations of Thom[1] it is now well systematized. The necessity of using pure cultures of identified molds in an investigation of this kind is obvious. Nevertheless, in most previous investigations these factors have been neglected. Many of the studies on molds deal with the action of unidentified mixtures of molds on complex substrata like maize or wheat. In many instances in which pure cultures growing upon simple media were studied, the identity of the species of mold employed can no longer be established. This is due to the fact that these investigations were, ordinarily, not conducted with the help of a trained mycologist. Such help is absolutely essential, for the difficulties of distinguishing between species are ordinarily underestimated

[1]Thom, C. H., "Cultural Studies of the Species of Penicillium," Bulletin 118, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C.

by the clinical bacteriologist. In the present series of investigations the molds were isolated by Dr. Erwin F. Smith and identified by Dr. Chas. Thom. Without such aid these studies could not have been undertaken.

In the first study of this series it was found that *Penicillium puberulum* Bainier, produces a phenolic acid of the empirical formula $C_8H_{10}O_4$, for which the name penicillic acid was suggested.[1] This acid gives a brownish red color with ferric chloride, reduces Fehling's solution and yields a deep red dye when acted upon by ammonium hydroxid. It is also somewhat toxic and antiseptic. The lethal subcutaneous dose is from .2 to .3 grams per kilo of body weight. It was not possible to identify penicillic acid with any known compound. In its general properties it resembles very greatly certain of the lichen acids found in lichens.

In the present paper a similar study upon a closely related organism, *Penicillium stoloniferum*, Thom, is reported. This organism was isolated from a specimen of spoiled Italian maize which was very kindly secured by Dr. C. H. Lavinder, of the Hygienic Laboratory of the Public Health and Marine Hospital Service while studying pellagra in Italy.

The examination of the specimen of Italian spoiled maize was undertaken because as stated in a former publication[2] it seems to differ from American spoiled maize in its behavior toward the ferric chloride reaction of Gosio. In Italy this reaction for phenolic substances is regarded as one of the most reliable tests for the determination of deterioration of maize. According to Schindler it is not highly esteemed in the Tyrol.[3] American spoiled maize when tested by the method recommended by Gosio[4] only occasionally gives the reaction. The color obtained in the few posi-

[1]Alsberg, C. L., and Black, O. F., "Biological and Toxicological Studies upon Penicillium puberulum, Bainier. Proceedings of the Society for Experimental Biology and Medicine, IX, p. 6, (1911)."

[2]Alsberg, C. L. and Black, O. F., "The Determination of the Deterioration of Maize with Incidental Reference to Pellagra. Bulletin 199, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., 1910."

[3]Alsberg, C. L. and Black, O. F. op. cit. page 27.

[4]Gosio, B.: Alterazioni Del Grantureo E Loro Profilassi. Page 35. Rome 1909. Tipografia Nazionale Di G. Bertero E C.

tive cases has always been red or brownish red, never violet or green as described by Italian investigators. Recently the test has been improved in this laboratory so as to render it more delicate. The essential improvement in the procedure as now conducted consists in extracting directly with chloroform. Fifty grams of ground corn or meal are placed in a stoppered flask and covered with chloroform. After two hours the chloroform is filtered off and concentrated to a bulk of 10-15 cubic centimeters. This is transferred to a small separatory funnel or test tube and covered with about 5 cubic centimeters of water containing a trace of ferric chloride. If substances like penicillic acid are present the characteristic color develops in the aqueous layer. Conducting the tests in this way a greater number of samples of obviously deteriorated maize show the reaction than was the case with the old test. Nevertheless a positive result seems to be less frequent in American maize than in Italian maize.

The Italian spoiled maize mentioned above gave an intense ferric chloride reaction of a violet color. Moreover, when grown on Raulin's medium it gave the same characteristic reaction. It is certainly a remarkable fact that the first sample of spoiled Italian corn examined gave the violet color described by Italian authors, whereas no American sample has been found giving a similar tint.

It was, therefore, decided to isolate, if possible, the substance responsible for the ferric chloride reaction. For this purpose the organism from Italian spoiled corn was grown on Czapek's medium and on Raulin's medium. It was found that the organism grew more rapidly upon the latter. Therefore, for the preparation of material Raulin's medium only was used.

The substance responsible for the ferric chloride reaction was isolated by the following procedure. The culture fluid and the mycelium were transferred to an evaporating dish and rendered weakly alkaline with sodium carbonate. The contents of the dish were then heated to boiling and filtered hot. The mycelium remaining on the filter was thoroughly expressed. It was then again extracted with water, rendered weakly alkaline with sodium carbonate. The combined extracts were evaporated to a small bulk over a free flame and filtered hot. To the clear filtrate a

slight excess of hydrochloric acid was added. An abundant precipitate was produced which consisted of a mixture of needle clusters and amorphous material. The precipitate was separated by filtration and washed with cold water. After drying spontaneously it was extracted with hot toluene and the hot extract filtered. Only the crystalline portion of the precipitate dissolved. The amorphous dark brown material which remained on the filter was discarded, for it did not give a color reaction with ferric chloride. The toluene extract, on cooling and spontaneous evaporation, precipitated in the form of needles, the material giving the ferric chloride reaction. These were still slightly colored but were finally obtained white, either by decolorizing with boneblack in hot toluene solution or by dissolving in alcohol and adding alcoholic potassium hydroxide to form the potassium salt which is insoluble in alcohol. This salt was then washed free from color with alcohol. From the potassium salt the free acid was recovered in the form of white needles by dissolving the salt in water and precipitating with hydrochloric acid.

The substance thus obtained consists of white needles with a melting point of 140°, uncorrected. The name mycophenolic acid is provisionally suggested for it. It is almost insoluble in water but freely soluble in alcohol, in ether or in chloroform. It is somewhat less soluble in benzene and only moderately soluble in cold, though very soluble in hot toluene. With ferric chloride it gives a violet color in aqueous solution, though its solubility in water is not sufficient to render the color intense. In alcoholic solution it gives a bright green color with ferric chloride. It does not react with Millon's reagent. It does not give Lieberman's reaction and could not be diazotized. It does not reduce Fehling's solution nor ammoniacal silver nitrate. It is fairly resistant to sodium, ammonium and potassium hydroxide and hydrochloric, sulphuric and acetic acid, not being affected by boiling in 10 per cent. solutions of any of these reagents. It does not contain water of crystallization. Its salts of potassium and sodium are very soluble in water. The former is soluble in dilute, but insoluble in absolute alcohol. The latter is soluble in absolute alcohol but may be precipitated in crystalline form by adding ether. The salt of barium is only very slightly soluble in water

and forms clusters of minute needles. The copper, lead and silver salts are amorphous and insoluble in water. In characterization of the substance the facts collected in Table I were ascertained by analysis of the free acid, by titritation of the alcoholic solution of the free acid with N/10 sodium hydroxide, using phenolphthalein as indicator, and by the determination of the barium content of the salt on ignition in platinum with sulphuric acid.

TABLE I. Analyses of Mycophenolic Acid

Weight of Substances Grams	CO_2 Grams	HO_2 Grams	C Percent	H Percent	$BaSO_4$ Grams	Ba Percent	N/10NaOH Cubic Centimeters
0.2316	0.5419	0.1315	63.81	6.30			
0.2044	0.4770	0.1161	63.64	6.31			
0.2494					0.1256	29.65	
0.1990							11.53
Average			63.725	6.305			

Calculated for $C_{17}H_{20}O_6$ Carbon 63.74 Hydrogen 6.25 %
Found Carbon 63.72 Hydrogen 6.30 %
Calculated for Ba ($C_{17}H_{18}O_6$) Barium 29.15 %
Found Barium 30.09 %

A molecular weight determination by the elevation of the boiling point in chloroform solution gave the results in Table II.

TABLE II. Ebulioscopic Determination of the Molecular Weight of Mycophenolic Acid

Weight of substance	Weight of solvent	Rise of boiling point	Molecular weight
Grams	Grams		
0.1641	30.32	0.065°	308
0.1578	30.32	0.060°	321
Average			314.5

Molecular Weight calculated for $C_{17,}H_{20}O_6$ 320
Molecular weight found from titration 345.4
Molecular weight found from barium content of salt 328
Molecular weight found from boiling point elevation 314.5

The formula $C_{17}H_{20}O_6$ may therefore be assigned to mycophenolic acid. It does not readily decompose carbonates at ordinary temperatures. It is apparently a dibasic acid, or at any rate, combines with two atoms of a monovalent base. Whether the base combines entirely with carboxyl groups or with phenol groups has not been determined. The acid seems to form two series of salts. Presumptive evidence on this point was obtained by the following experiments.

0.2 grams of free acid were suspended in water and one equivalent of potassium hydroxid added. Unfortunately, this was not sufficient to put the substances completely in solution, so that a slightly greater quantity of the alkali had to be used. This solution was then treated with one equivalent of barium chloride. On standing in the dessicator a crystalline barium salt formed. This salt was evidently different from the normal barium salt which is so insoluble that it precipitates at once. It was also of different appearance under the microscope, consisting of a few small needles in clusters, which apparently were the normal salt and more abundant larger single needles, apparently the acid salt. The presence of the normal salt in small quantities under the conditions of the experiment was probably due to the fact that an excess of alkali had to be used in dissolving the substances. The barium content of this preparation was determined, 0.207 grams yielding 0.0692 grams of $BaSO_4$, equivalent to a barium content of 20.2%.

Calculated for $Ba(C_{17}H_{18}O_6$	28.1 per cent.
Calculated for $\frac{5}{2}Ba(C_{17}H_{19}O_6)_2$,	17.7 per cent.
Found	20.2 per cent.

Apparently, as shown by the microscope, the preparation consisted of a mixture of two salts.

It has not been found possible to identify the substances with any known compound. In very many respects it resembles the class of substances found in lichens and classed vaguely as lichen acids. To find a substance of this class in molds is not surprising since lichens are symbiotic forms composed of fungi and algae. Mycophenolic acid also resembles very greatly a substance isolated by Gosio from a species of Penicillium. The formula

$C_9H_{10}O_6$ calculated for the latter by Gosio is based on a single combustion. As far as may be judged from Gosio's records, it is probably not identical with mycophenolic acid, though resembling it greatly. However, Gosio's characterization of the substance was based on a very small quantity of material, so that it cannot be regarded as final. The chief points of difference between the substance described by Gosio and mycophenolic acid are the percentage composition and the bahavior with ferric chloride. Gosio's substance gives an intense blue color with ferric chloride in alcoholic solution. Mycophenolic acid gives a violet color in aqueous solution, while in alcoholic solution with a trace of ferric chloride it gives a violet color which becomes bright green on addition of an excess of the reagent.

In one particular mycophenolic acid resembles Gosio's substance but differs from penicillic acid. It is not toxic. Ten milligrams were dissolved in water, with the aid of a little sodium carbonate and injected subcutaneously into a mouse. No untoward effects whatever were noted. From penicillic acid, furthermore, it differs in being present chiefly in the mycelium in the early stages of growth. In old cultures it is found both in the culture fluid and in the mycelium, perhaps because with the gradual production of basic substances it is dissolved. The question whether toxic phenolic substances are found in the culture fluid or only in the mycelium is one that has been much discussed by students of pellagra. When the substances are insoluble acids with soluble salts like mucophenolic acid, their distribution is probably only a question of the reaction of the medium. When the reaction is acid they will be found in the mycelium as lichen acids incrust the lichen thallus. When the medium contains available bases they will become more or less dissolved in the medium.

With the advancing age of the culture mycophenolic acid gradually increases in quantity until under the conditions employed in these experiments the maximum yield is obtained in about two weeks. After that time the quantity present is apparently constant. When grown in rectangular quart bottles known in the trade as "long Blakes" turned on their sides in order to have a large surface and charged with about 250 cubic

centimeters of culture fluid the yield at the end of about two weeks averages per bottle about 0.07 grams of the crude acid.

Since *P. stononiferum* is found so commonly in the United States it is not easy to understand why it is so rarely, if ever, causes spoiled maize in the United States to give the ferric chloride reaction. The first explanation to present itself was that the American organism might be a different strain or perhaps a "physiological variety."

To solve this question Dr. Thom very kindly furnished a specimen of his type culture. This was grown side by side with the Italian organism. It grew rather more slowly than the latter and there were slight differences in appearance. The cultures gave a good ferric chloride reaction very similar in shade to that given by the Italian organism. When, however, the attempt was made to separate mycophenolic acid from the cultures of the American organism none could be found. In its place was found a quite different substance or mixture of substances. As this material has not yet been obtained in satisfactory crystalline form not much can at present be said of its properties.

The different biochemical behavior of the two strains from the two continents is certainly suggestive. Whether these two strains are really physiologically different can not as yet be decided. The American organism used is an old one, having been propagated by Dr. Thom in the laboratory for a number of years. Possibly this long artificial propagation has altered its behavior. It is proposed to continue the investigation of this problem by comparing the two cultures on hand with a number of new recently isolated strains.

No extended physiological studies were undertaken on *P. stononiferum.* A few observations were made incidentally. The organism always produced alcohol as shown by applying the iodoform test to the distillate. No quantitative determinations were made but the amount of alcohol formed as judged by the iodoform test seemed to be decidedly less than that produced by *P. puberulum. P. stoloniferum* produces a small amount of oxalic acid. To detect it the medium was concentrated to a syrup and mixed with clean sand and plaster of paris. The hardened mass was pulverized and extracted with ether in a Soxhlet apparatus.

The oxalic acid, identified by the melting point and insolubility of the calcium salt, crystallized in the extract. Oxalic acid seems to be present in somewhat larger amounts and at an earlier stage of growth than in cultures of *P. puberulum*. Finally the mycelium of *P. stoloniferum* seems to be very rich in mannitol.

Summary

From cultures of *Penicillium stoloniferum* Thom obtained from a sample of spoiled maize from Italy a new phenolic acid of the formula $C_{17}H_{20}O_6$ was isolated in crystalline form. It resembles the lichen acids, is not toxic and is one of the substances causing the ferric chloride reaction of Gosio in deteriorated maize.

THE EFFECT OF SODIUM CHLORID AND COLD STORAGE UPON THE ACTIVITIES OF PROTEOLYTIC ENZYMES

By William N. Berg

From the Dairy Division Research Laboratories, Bureau of Animal Industry, Washington, D. C.

At low temperatures and in the presence of sodium chlorid the activity of a proteolytic enzyme may be inhibited, if the amount of enzyme is small. If the amount of enzyme is large, proteolysis takes place rapidly and apparently is not interfered with by the low temperatures and sodium chlorid. These observations were made during the course of some investigations on the chemical changes taking place in cold storage butter. A detailed account of these and related investigations is soon to be published by the Dairy Division, Bureau of Animal Industry.

The Inhibiting Effect of Cold Storage and Sodium Chlorid on the Activity of Galactase in Buttermilk

Buttermilk obtained from a churning pasteurized or of unpasteurized cream may contain galactase, a proteolytic enzyme very similar in its general characters to trypsin. When buttermilk is preserved with chloroform and kept at room temperature, the galactase will slowly digest the proteins present. Some quantitative data are given in a previous publication from this laboratory.[1]

In buttermilk containing 18% of sodium chlorid placed in cold storage, (at 0 F or minus 18 C.) for as long as nine months, no proteolytic action was detected.

For the detection of proteolytic action, water soluble nitrogen was determined in the buttermilk before and after storage, as follows:

[1]Rogers, L. A., Berg, W. N., and Davis, B. J., Circular 189, Bureau of Animal Industry, 1912, p. 315.

Transfer 200 cc of the sample to a 500 cc volumetric flask. Dilute with water to about 450 cc. Add 20 cc 10% acetic acid. This will flocculate the casein. A cc more or less of the acid will make no difference when sodium chloride is present. Make up to the mark, filter on a 32 cm folded filter (S & S No. 595 or 588) and determine total water soluble nitrogen in two 200 cc portions of the clear filtrate.

By this method, the results for water soluble nitrogen in buttermilk containing 18% of sodium chloride and placed in cold storage for 9 months, were the same, practically, before and after storage. This indicates that the action of the enzyme was inhibited under those conditions.

The Effect of Cold Storage and Sodium Chlorid on the Activities of Proteolytic Enzymes in Sterilized Skimmilk

Digestive mixtures were prepared as follows: Skimmilk was sterilized by heating for two hours at 94, 99 C. in a steam sterilizer. The skimmilk was quickly cooled to 35 C. and to three 3 liter portions in separate containers, 540 grams of sodium chlorid was added, making the salt concentration approximately 18%. To one of these mixtures there was added 3 grams of pancretin, dry, U. S. P., to the second, 3 grams of pepsin, dry, U. S. P. and to the third 15 grams of a dry proteolytic enzyme preparation obtained from cultures of lactic acid bacteria which also digested protein. The enzyme was precipitated from the cultures (by L. A. Rogers) with alcohol in the manner usually used for such preparations. The enzyme preparation was tested before hand and found to liquefy gelatine.

Controls were likewise prepared, which differed from the before described mixtures only in the fact that the enzyme preparations were added to them while the shimmilk was near the boiling point.

The mixtures were kept in sealed cans having a capacity of 1 liter. Each can contained 600 cc of the sample, and was kept in cold storage for 9 months at 20 F (minus 7 C). Water soluble nitrogen was determined in these mixtures before and after storage by the method used for galactase.

It was found that under these conditions, the pancreat in (trypsin) was very active, pepsin acted a little more slowly while the bacterial enzyme preparation showed very little, if any activity. The controls showed no change. In the trypsin mixture 2-3 of the total nitrogen present was rendered water soluble. In the pepsin mixture 1-3 of the total nitrogen was rendered water soluble. When these mixtures were allowed to stand at room temperature, further digestion took place. In the trypsin mixture practically all the protein became water soluble.

It is to be noticed that both pepsin and trypsin acted vigorously in different portions of the same substrate.

However, the claim is not made that sodium chlorid does not exert an inhibiting influence. Under certain conditions it does. Experiments were made in this laboratory in the spring of 1909, in which the speed of digestion of casein in several pepsin-acid solutions was compared with that in the same solutions to which 20 grams of sodium chlorid had been added to 100 cc of acid solution. The presence of the salt almost completely inhibited the action of the pepsin-acid during the experiment—40 minutes' digestion. It is of course probable that digestion would have taken place had the digestion period been several months. The method of comparing speeds of digestion was that described by Gies.[1]

The results show that whether sodium chlorid does or does not inhibit proteolysis depends upon the amount of enzyme, to a very large extent.

[1]Berg, W. N., and Gies, William J., Journal of Biological Chem. Vol. 2, pp. 489-546, 1907.

FATTY ACID ESTERS OF GLUCOSE

By W. R. Bloor

(From the Laboratories of Biological Chemistry of Washington University, St. Louis, Mo.)

This paper is a preliminary report on the preparation and properties of a new class of compounds—the fatty acid esters of glucose. The interest of compounds of this type is threefold: (1) the relationship which has been shown to exist between carbohydrates and fats in metabolism; (2) the possibility of the natural occurrence of these and similar compounds; (3) the possible usefulness of such compounds in the study of fat metabolism. A relationship between fat and carbohydrates in metabolism has been repeatedly noted. In the absence of a sufficient supply of available carbohydrate, as in starvation or severe diabetes, the fats are incompletely burned and the unburned residues are excreted as B-oxybutyric acid and its derivatives, diacetic acid and acetone. The fact has been crystallized in the statement attributed to various investigators that "fats can burn only in the fire of the carbohydrates." The acidosis may be decreased or made to disappear if carbohydrates can be fed and utilized.

That the condition may be reciprocal, i.e., that the fats, under certain conditions may help in the metabolism of the carbohydrates has, so far as I know, never been suggested but it seems something more than a coincidence that the carbohydrate of oats—the grain which has the highest per cent. of fat of all ordinary grains—should be the best utilized by diabetics.

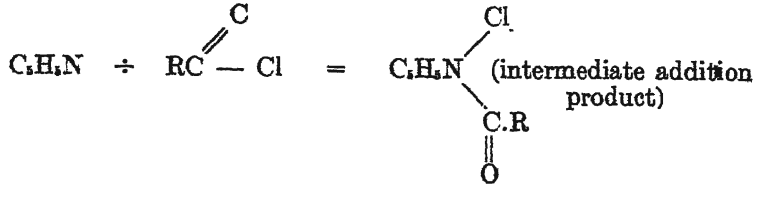
The nature of the relation of carbohydrates to the combustion of fats has been the subject of many theories, the most reasonable of which is that the glucose acts as a catalyser, either by furnishing readily available oxygen, or by the formation of a compound with the fatty acid which is more readily burned than the fatty acid alone.

Glucose esters of the fatty acids have so far not been found in nature. As may be seen from a study of the properties of the compounds already prepared they are so much like the fats and lipoids in their solubilities, etc., that they may well have escaped detection. Compounds of galactose with fatty acids and other substances are well known to occur in the brain substances (cerebrosides).

Compounds of this sort are of interest also because they may be useful in solving the problem of the absorption and transportation of fats. If absorbed unchanged they could be readily recognized in the chyle and if injected into the blood stream could be readily traced. Some work along this line has already been done using analogous compounds—the mannite esters of the fatty acids.[1]

Glucose esters of butyric acid (di-butyrate) and stearic acid (di-stearate) have been prepared by Berthelot[2] by direct combination at high temperatures. The yield was slight and his description of the compounds is limited to their physical appearance and two or three solubilities. Because of the small yield and the instability of glucose at high temperatures this method does not lend itself to the preparation of the compounds in large quantity.

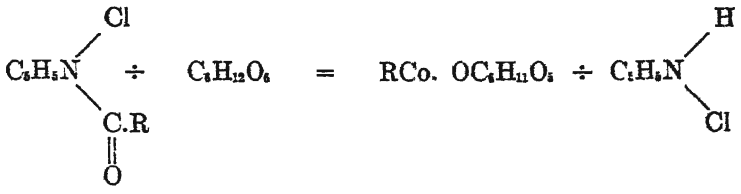
The synthesis adopted depends on the action of the chlorides of the fatty acids on glucose in solution in pyridin, the pyridin acting both as solvent and catalyser somewhat as follows[3]:



[1]Bloor, Journal of Biological Chemistry, XI, p. 429.

[2]Berthelot Annals de Chemie et de Physique, (3) 60. 96. (quoted from Beilstein Handbuch der organischen Chemie, 3rd ed. vol. 1, p. 1049).

[3]Einhorn and Hollandt-Liebig's Annalen, 301. 95 (1898).



Process.

25 grm. of dry glucose is dissolved with the aid of heat in five to ten times its weight of dry pyridin, the solution cooled, and an equimolecular amount of the chloride of the fatty acid added in small portions with cooling. The mixture is allowed to stand over night, then poured into iced dilute sulphuric acid. The esters separate and float on top, and are freed from the liquid (in the case of the higher fatty acids) by filtering on a suction funnel. The mass is then boiled out several times with water, until it is free enough from electrolytes to form a colloidal solution. It is caused to separate from the colloidal condition by the addition of sodium sulphate, let cool and the solid cake removed and dried. The mixture is first fractioned with ether to separate the higher esters and then with alcohol.

The following is a brief description of the compounds which have been separated. Because of the great difficulty in making the separations the data given are regarded as only approximately correct.

General properties of the esters.

The compounds all reduce Fehling's solution and are optically active, the optical activity being less than that of glucose. They form colloidal solutions with water (best made by pouring the hot alcoholic solutions into water). They are readily saponified by acids or by alkalies (watery or alcoholic). They do not ferment with yeast. The presence of glucose was shown by saponification with alcoholic hydrochloric acid and preparation and identification of the osazone. Some of the compounds possess the property, in common with glucose, of forming sodium compounds when treated in alcoholic solution with sodium ethylate.[1] The compounds precipitate out as a gummy mass.

[1]Honig, Rosenfeld: Berichte der Deutsch. Chem. Gesellsch. 10. 1871.

Stearic acid esters.

Yield from 20 gm. glucose and 30 gm. stearyl chloride — 35 gm. of crude esters.

Monostearate m.p. 110°. Specific rotation ÷ 36.25°.

Slightly soluble in cold ether and alcohol; readily soluble in hot alcohol and ether; separates slowly from cold alcohol. The separation is hastened by the addition of ether.

Distearate m.p. 90-95°. Dextro rotatory

Slightly soluble in cold ether and alcohol; readily soluble in them hot; separates quickly from alcohol on cooling.

Tristearate m.p. 60°. Specific rotation ÷ 12.00°.

Soluble in cold ether; soluble in hot alcohol from which it separates on cooling.

Lauric acid esters.

Yield from 25 gm. glucose and 26 gm. lauryl chloride = 38 gm. crude esters.

Monolaurate—well crystallized in shining rhombic plates. m.p. 110°. Specific rotation ÷ 30°.

Slightly soluble in cold alcohol and ether; readily soluble in them hot. The separation from alcohol in the cold is aided by the addition of ether.

Dilaurate α m.p. 55°. Specific rotation ÷ 21.8°.

Somewhat soluble in cold ether. Soluble in hot alcohol from which it separates on cooling.

Dilaurate β m.p. 33°. Specific rotation ÷ 30°.

Readily soluble in cold ether. Soluble in hot alcohol from which it separates on cooling.

Butyric acid esters.

Yield from 25 gm. glucose and 12 gm. normal butyryl chloride ÷ 3 gm. of mixed esters.

This synthesis is evidently not adapted to the preparation of the butyric esters. The ester mixture agrees with Berthelot's[1] description of the dibutyrate. "Very bitter liquid, somewhat soluble in water, very easily in alcohol and ether." Strongly dextro rotatory (÷ 31°).

[1]Berthelot: loc. cit.

Animal experiments.

The material for the animal experiments was prepared from the fatty acids of cocoa-nut oil after separation of oleic, palmitic and stearic acids.[1] The fatty acid mixture used had a mean molecular weight of from 200-210 and a melting point of 30°-36° C. depending on the sample. Acid chlorides were prepared from this product by the method of Krafft and Burger.[2] The glucose esters were prepared from the acid chlorides in the way described above. The ester mixture so prepared and which was used for the animal experiments had a m.p. of 41° but remained soft and sticky at 30°. It was readily and completely soluble in cold ether.

Specific rotation ÷ 21°.

Feeding experiments.

The animal used for the feeding experiments was a cat, weight 2 k.

5 gm. of the ester mixture together with 25-30 gm. of lean meat, 5 gm. cotton seed oil and 2-3 gm. bone ash was fed every third day. In the intermediate days and on the first two days of the experiment the animal received the diet without the ester and containing 1-2 gm. of wood charcoal in place of the bone ash. It was hoped in this way to get sharply divided feces corresponding to the feeding periods. As may be seen from the results it was not possible in this experiment. Charcoal feces very often had a core of bone ash feces and the two were otherwise so mingled that only an approximate separation was possible. The feces from each period were extracted with ether in a Soxhlet extractor for 3-4 hours and the extract examined polarimetrically for unabsorbed esters.

Preliminary period. Two days.

50 c.c. ether extract, reading in 1 dcm. tube = ÷ 0.03° corresponding to a weight of ester of 0.10 gm.

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[1]For a description of the method of separation see Bloor: Jour. Biol. Chem. XI, p. 421 (1912).

[2]Krafft and Burger: Berichte der Dutsch. Chem. Gesellsch. 17, 1378 (1884).

This figure was used as correction in the other periods.
First two ester periods (bone ash) extracted together.
65 c.c. of ether extract
Polariscope reading in 1 dcm. tube + 1.05°
Corresponding to a weight of ester of 3.40 gm.
Correction for blank 0.10 gm.
Corrected weight 3.30 gm.
First two control periods (charcoal)
28 c.c. of ether extract
Polariscope reading in 1 dcm. tube + 0.20°
Corresponding to a weight of ester of 0.28 gm.
Correction for blank (4 days) 0.20 gm.

Corrected weight 0.08

Summary of first two periods	Ester fed	10 gm.
	Ester recovered	3.40 gm.
	Absorbed	6.60 gm.

Per cent. absorption 66%.

Third period (some diarrhoea)
Ester feces 98 c.c. of ether extract
Polariscope reading 0.50°
Corresponding to a weight of ester of 2.45 gm.
Correction for blank 0.10 gm.

Corrected weight 2.35
Control feces 65 c.c. ether extract
Polariscope reading 0.03°

For third period	Ester fed	5 gm.
	Ester recovered	2.35 gm.
	Absorbed	2.65 gm.

Per cent. absorption 53.0

Fourth period
Ester feces 120 c.c. of ether extract
Polariscope reading in 1 dcm. tube 0.12°
Corresponding to a weight of ester of 0.72 gm.
Correction 0.12 gm.

Corrected weight 0.60 gm.

Control feces 40 c.c. ether extract
Reading in 1 dcm. tube 0.20°
Corresponding to a weight of ester of 0.40 gm.
Correction for blank 0.10 gm.

Corrected weight 0.30 gm.

For fourth period	Ester fed	5 gm.
	Ester recovered	0.90 gm.
	Absorbed	4.10 gm.

Per cent absorption 82%

Summary of experiments:

First two periods	Absorbed	66%
Third period	"	53% (diarrhoea)
Fourth period	"	82%
Average absorption		67%

Injection experiments.

The material used for the injections was a colloidal solution of the esters in water made by pouring a hot alcoholic solution into water, filtering hot and boiling until the alcohol had evaporated and the solution had reached a concentration of about 10%. The milky suspension so obtained could be flocked out by the addition of acids or of strong salt solutions, but could be diluted with several volumes of normal salt solutions without separation taking place for several hours. The ester mixture used in preparing the solution was prepared for use by washing the ether solution with dilute alkali until free from fatty acids, then several times with distilled water.

Intraperitoneal injections.

The animal used was a young rabbit (Belgian hare) weighing 1.5 K. Two injections were made on succeeding days of (1) 5 c.c. of suspension containing 0.5 gm. of ester and (2) 10 c.c. of solution containing 0.9 gm. of ester. The animal showed no bad effects. Postmortem[1] examination two weeks later showed that part of one injection had lodged between the muscular coat and the peritoneum. Microscopic examination of the cheesy mass

[1] I am indebted to Doctor W. S. Thomas of the Department of Pathology of this School for the postmortem examination.

showed it to consist practically entirely of leucocytes. Extraction of the substance with ether showed that the ester had disappeared. Below the mass, the peritoneum was united to the intestines by many adhesions. Scattered through the intestines and also in the diaphragm and one edge of the liver were many encapsulated masses of the same nature. Except for the above all organs were normal.

Intravenous injections.

Made on rabbits.

Experiment I. Large (3 k.) rabbit with light brown spots. Ten c.c. of solution containing 1 gm. of ester was injected into the lateral ear vein during about 30 minutes. After the injection the animal appeared normal and was put back into the cage. Ten minutes later (about 40 minutes after beginning injection) it was down and kicking convulsively and one minute later respiration had ceased, although the heart continued to beat for a short time longer. Autopsy showed a marked injection of the vessels on the left side of the pons. Otherwise no abnormality.

Experiment II. Young rabbit weight 1.5 k. (the same one as was used for the intraperitoneal injections above). First injection—10 c.c. of solution containing 0.8 gm. of ester injected into the lateral ear vein, the injection lasting fifteen minutes. After the injection the animal behaved normally and showed no immediate bad effects; nevertheless although fed liberally it rapidly lost weight during the next few days. Second injection, five days later—6 c.c. of solution containing 0.9 gm. of ester injected into the median ear vein, the injection lasting 15 minutes. After the injection the animal appeared dull and listless. No other signs were noted for the next three hours but it died during the night.

Discussion of the animal experiments.

The feeding experiments show that the glucose esters are quite well utilized in the intestine. The injection experiments, although too few in number to allow accurate deductions, indicate that the substance is probably not well borne when injected either intraperitoneally or intravenously. In the peritoneum it seems to act as an irritating foreign body while intravenously even if we regard the death of the first animal as an accident its effects on the animal are injurious.

QUANTITATIVE OXIDASE MEASUREMENTS

By Herbert H. Bunzel[1]

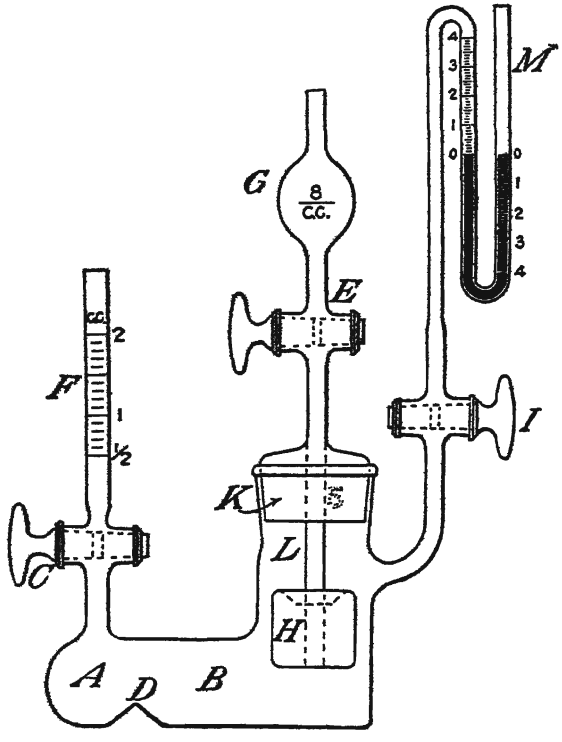
U. S. Dept. of Agric., Wash., D. C.

The very voluminous literature on the rôle and importance of oxidizing enzymes in many vital processes of plants and animals makes a thorough study of their behavior, function, and distribution necessary. They play an important part in certain pathological conditions, and in numerous industrial, and agricultural problems. As specific examples may be mentioned the work done by Woods, in the Bureau of Plant Industry, on the mosaic disease of tobacco, the work of Palladin and his school on the respiration of plants, and the casual relationship between the oxidases and color production as shown for plants by Palladin, and for animals by Gortner. They also play an important part in the darkening of tea, and the manufacture of Japanese lacquer.

Nearly all of the experiments made thus far have not been carried on quantitatively because of the lack of satisfactory methods.

The method described in this paper is based on oxygen absorption. For this reason a constant temperature is essential. The apparatus in which the oxidations are carried out is shown in the text figure. Eight cubic centimeters of the solution of the substance to be oxidized are measured in the pipette G and allowed to run into the compartment B. The plant juice, the oxidizing power of which it is desired to study, is measured in pipette F and run into compartment A. Basket H holds 1 cc. of normal sodium hydroxide to absorb the carbon dioxide formed in the process; M is a manometer charged with mercury to indicate the pressure within the oxidase apparatus. The whole apparatus is clamped to a specially constructed shaking machine. In the

[1]From the Bureau of Plant Industry, U. S. Department of Agriculture, Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations.



APPARATUS FOR MEASURING OXIDASE ACTION

air-thermostat the temperature is brought to 37° C. and maintained at that point to within 0. 1° throughout the experiment. Half an hour after the temperature of 37° is reached, all stopcocks but one are closed, and the shaking machine set in operation. The plant juice mixes with the oxidizable material and the reaction begins. From time to time the shaking is interrupted and the manometer is read. In the course of several hours the oxygen absorption is completed, as indicated by no further change of pressure within the flask. The ultimate reading expresses the oxidase content of the juice or extract with respect to the particular substance used. As a unit an oxidase solution is chosen of such a strength that one liter of it will be capable of bringing about the consumption by pyrogallol of the equivalent of one gram of hydrogen.

Hitherto pyrogallol, tyrosin. hydrochinone, guaiacol, benzidine, and alphanaphthol have been investigated. The concentration of the material to be oxidized has no effect on the end result provided it is used in excess. The carbon dioxide produced is absorbed by the alkali in the basket and may be determined at the end of the experiment by means of a special apparatus devised for the purpose. The result obtained is directly proportional, or at least nearly proportional, to the concentration of the oxidase present.

An application of the method to potato juice is given in Table 1. In each one of the experiments 8 cc. of a 1% Pyrogallol solution and 2 cc. of potato juice were used.

TABLE I.

Time of Manometric Reading P. M.	Manometric Reading a.	b.
1:30	0.00	0.00
1:40	1.00	0.90
1:50	1.40	1.20
2:00	1.55	1.42
2:10	1.62	1.55
2:20	1.76	1.70
2:30	1.80	1.80
2:40	1.80	1.82
2:50	1.82	1.83
3:00	1.82	1.85

For the sake of studying the applicability of this method to the determination of oxidases in juices of plants other than potatoes, some experiments were carried out on sugar beet leaves. The Division of Cotton and Truck Diseases of the Bureau of Plant Industry, Department of Agriculture, has for some years been investigating the curly-top disease of sugar beets. The writer was able to obtain for experimental purposes fresh samples of sugar-beet leaves affected by this disease to a striking degree, and also samples of normal beet leaves. All of the beets, of which the leaves were examined were grown in

a green-house and therefore were subjected to practically uniform conditions. The juice was obtained by grinding the leaves up in a meat chopper and pressing the juice out of the pulp through a silk cloth. The results obtained are summarized in Table II.

TABLE II.

Juice used	Manometer readings expressed in centimeters of mercury
1. Juice of normal beet leaves	1.16
2. Juice of normal beet leaves	1.07
3. Juice of diseased beet leaves	5.61
4. Juice of diseased beet leaves	4.30
5. Juice of normal beet leaves	1.10
6. Juice of normal beet leaves	1.17
7. Juice of diseased beet leaves	2.72
8. Juice of normal beet leaves	1.19
9. Juice of normal beet leaves	1.21
10. Juice of diseased beet leaves (showing slight symptoms only)	1.51
Mean absorption in experiments with juice of normal plants	1.15
Same expressed in terms of units	1.66
Mean absorption in experiments with juice of diseased plants	3.54
Same expressed in terms of units	5.09

Experiments given in Table II show a very striking difference between the juice of the normal and that of the diseased beet leaves. In all of the experiments the oxidase content as indicated by the oxygen absorption of the pyrogallol in the presence of the juice is markedly greater in the diseased than in the healthy leaves. The oxidase content of the normal leaves seems to be fairly constant, while the juice of the curly-top beet leaves shows wide variations. The leaves used in experiment three gives about five times as high a figure as normal leaves, while

the leaves chosen in experiment ten show a variation of only 25 per cent. from the normal. It is very interesting to note that the deviation in oxidase content of the pathological leaves, as measured by the method described, runs parallel with the appearance of the leaves. The plants used in experiment three showed very marked signs of curly-top, the leaves being small and shriveled, and the hairy roots abundant, while the diseased beet used in experiment 10, which showed a relatively low oxidase content, but still higher than normal, had only a slight curling of the leaves.

Although these results are quite conclusive in showing existing differences in the oxidase mechanism of the healthy and diseased sugar-beets grown in the green-house, it is not justifiable without further experimentation to apply these results to conditions in the field. Where sugar-beets are grown on a commercial scale an entirely different and widely varying environment prevails. At the suggestions of Mr. W. A. Orton of the Division of Cotton and Truck Diseases a trip was undertaken to Ogden, Utah, where tons of sugar-beets are harvested every year. The writer spent the month of August, 1911, at the beet-sugar factory of the Amalgamated Sugar Company, where all of the experiments described in this paper were carried out.

The juice of the leaves and roots was obtained by chopping and pressing out through a silk cloth as before. Table III gives a summary of the results.

TABLE III.

	Material from which juice was obtained	Activity of juice expressed in terms of units
1.	Healthy, large outer leaves	0.191
2.	Curly-top diseased, small inner leaves of same plants	0.381
3.	Root of same plant	0.252
4.	Leaves of small plant, retardation of growth, unknown, no symptoms of curly-top	0.367
5.	Upper half of root of same plant	0.086
6.	Lower half of same root	0.158

	Material from which juice was obtained	Activity of juice expressed in terms of units
7.	Leaves of large and healthy plant adjoining small one	0.201
8.	Upper half of root of same plant	0.172
9.	Lower half of same root	0.288
10.	Leaves of small plants, growth retarded by drought. No symptoms of curly-top	0.230
11.	Uppermost third of root of same plant	0.130
12.	Lowest third of same root	0.403
13.	Leaves of large and healthy plants, collected on the same field and the same time as 10	0.144
14.	Uppermost third of root of same plant	0.173
15.	Lowest third of same root	0.360
16.	Leaves of curly-top plants showing marked symptoms	0.288
17.	Uppermost thirds of roots of same plants	0.259
18.	Lowest thirds of same roots	0.475
19.	Lowest fifth of roots of curly-top plants	0.504
20.	Uppermost fifth of same roots	0.317
21.	Leaves of curly-top plants grown from beets which had been siloed and showed no symptoms of the disease in the year previous. "Trotze"	0.496
22.	Roots of same plant	0.216
23.	Leaves of plants similar to those described in 21	0.446
24.	Roots of same plants	0.183
25.	Leaves of curly-top plants with seed stem, carrying seeds	0.403
26.	Roots of same plants	0.324
27.	Leaves of "Trotze" plants but apparently healthy otherwise	0.446
28.	Roots of same plants	0.237

	Material from which juice was obtained	Activity of juice expressed in terms of units
29.	Leaves of a large healthy plant	0.256
30.	Leaves of a large healthy plant	0.266
31.	Leaves of healthy looking seed-carrying plant	0.288
32.	Uppermost third of root of same plant	0.331
33.	Lowest third of same root	0.381
34.	Leaves of plant of similar type as 31	0.230
35.	Upper half of root	0.144
36.	Lower half of roots	0.237
37.	Leaves of healthy, very young plants, leaves only 3-8 cm. long.	0.180
	Mean oxidase activity of juice obtained from leaves of healthy and developed plants (1, 7, 13, 29, 30, 31, 34)	0.225
	Mean oxidase activity of juice obtained from leaves of plants, whose growth has been retarded (2, 4, 10, 16, 21, 23, 25, 27)	0.382
	Mean oxidase activity of juice obtained from roots of healthy and developed plants (8, 9, 14, 15, 32, 33, 35, 36)	0.261
	Mean oxidase activity, of juice obtained from roots of plants, whose growth has been retarded (3, 5, 6, 11, 12, 17, 18,19, 20, 22, 24, 26, 28)	0.265

The experiments summarized in Table III fully corroborate the results obtained with curly-top diseased sugar-beets grown in the green-house. The leaves of the curly-top plants have an oxidase content about twice as great as the healthy and normally developed ones. No differences could be detected between the roots of the two kinds of plants. An abnormally high oxidase content of the leaves was also shown in other plants, the growth of which has been retarded. Whether the plants are stunted by excessive watering, drought, or for other unknown reasons, the oxidase content in the leaves is much higher than in the normal and healthy plants. The increase in oxidase concen-

tration in the leaves is not a mere function of their size, inasmuch as very young normal leaves do not exhibit this characteristic. If the condition of the plant is such that only one of its functions, the development of seed in bi-ennially-grown beets is inhibited, the oxidase content is also high. Such plants are called "Trotze." The most general conclusion to be drawn from these observations is that in sugar-beet plants where an abnormal retardation of growth has occurred, this is accompanied by an increase in the concentration of oxidases in the leaves or a change in the juice of the latter by which the pyrogallol oxidizing oxidase becomes more active.

Such an increase in the power of the juice to bring about or hasten oxidation under pathological conditions has been observed before. Woods found it in connection with a disease of tobacco, Sorauer in connection with the curly-top of potatoes. Future investigations will show whether or not the oxidases which have been studied by former investigations and by the writer are the same or directly related to those which Palladin and his school find so important with respiration in plants. If they are, it is probable that an increase in their concentration leads to increased combustion in the cells. One would then be tempted to look at such plants in a state of "fever."

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LES PHOSPHATES ET LE SON DE FROMENT DANS L'ALIMENTATION ANIMALE

PAR M. LE DR. P. CARLES, BORDEAUX

Diplôme d'Honneur de la Société d'Agriculture de la Gironde

Depuis déjà une trentaine d'années, le public s'est engoué pour l'acide phosphorique et les phosphates. Il les considère dans l'alimentation animale comme des excitants éminents de l'assimilation.

Pendant longtemps cependant on a estimé que leur rôle n'était à prendre en considération que comme appoint, et lorsqu'ils faisaient plus ou moins défaut dans l'aliment naturel. Enfin, une école a nié quelque temps que leur rôle était nul autrement qu'à l'état de combinaison organique.

En 1904, M. Cozelle, Médecin-Vétérinaire, praticien doublé d'un vrai savant dont les travaux les ont été couronnés par nos deux grandes académies et ensuite par la Société des Agriculteurs de France, a apporté de précieux arguments pour éclairer le sujet. Ses expériences bien conduites sur des veaux et des vaches laitiéres établissent nettement que l'acide phosphorique et mieux encore les phosphates, hâtent la croissance des jeunes ruminants; mais que le résultat est encore plus sensible sur les sujets en bas âge. Enfin, ces mêmes expériences démontrent que les phosphates donnés sous la forme d'os de veaux séchés et pulpés à ces jeunes ruminants sont ceux qui ont amené les résultats les plus nettement avantageux.

Cette supériorité des os sur leur poids correspondant de phosphates minéraux est des plus rationnelles. Avec es phosphates chimiques, en effet, on n'apporte à l'organisme qu'un seul et unique de ses constituants principaux; tandis qu'avec les os eux-mêmes, c'est toute la série de ces constituants connus et inconnus aussi nombreuse et variée soit-elle. On comprend

aisément qu'en fournissant à la force vitale et en proportion exacte, tout ce qu'il faut pour reconstituer l'os, elle s'en acquitte à merveille.

Cependant l'usage de poudre d'os verts, à la campagne surtout, nous parait bien aléatoire; vu qu'en se désséchant les os se putréfient vite et toujours peu ou prou et qu'il n'est pas indifféent de donner fréquemment à un herbivore surtout, un levain de putridité animale.

Par ailleurs, l'auteur apporte des preuves irréfutables de l'action heureuse des phosphates sur le volume de lait produit par les vaches laitières. Il est très sobre, il est vrai, sur les modifications qu'en éprouve la qualité, mais on conçoit mal qu'elles ne soient pas également favorables.

A côté de l'aliment phosphaté complexe qu'est l'os, on nous permettra de placer le son de froment et ses variétés. Celui-ci a l'avantage d'être de préparation facile, de conservation aisée, d'emploi presque banal.

Pour le vulgaire, le son-'est qu'un organe de protection du grain, son enveloppe adhérente, sa peau. Quand, par les procédés modernes, les meuniers l'enlèvent par grandes plaques pauvres en partie farineuses, c'est le gros son. Le nom de petits sons est réservé aux parties plus menues, plus déchirées, moins bien détachés et ou adhère un peu plus des tissues sous jacents. Enfin, les repasses sont constituées par des cassures du grain ou un éclat de son est opiniatrement resté adhérent à un fragment superficiel de l'amande farineuse. C'est une sorte de blé concassé, mais sans farine ni parties tendres centrales.

Au point de vue de l'alimentation animale, la composition de ces issues et leur rôle phosphatogène n'est pas sans intérêt.

En dehors de éléments alibiles, on y trouve d'abord une quantité notable d'oxydase, diatase soluble particulière qui a pour rôle de véhiculer l'oxygène de l'air sur les éléments avec lesquels elle est en contact immédiat par l'intermédiaire de l'eau. C'est pourquoi quand on mouille le son, toutes les parties humidifiées brunissent. C'est à cause de sa présence dans les parties du son, même les plus ténues, que les farines de queues donnent du pain bis.

Dans toutes les parties du grain où se trouve cette oxydase et dans des proportions absolument parallèles se trouve du

manganèse. Ce fait est à noter en passant, à cause de la coexistence annoncée entre ces deux substances aux allures originaires communes.

C'est probablement dans cette même région du grain que l'on trouvera tôt ou tard ces éléments rares qui paraissent nécessaires à l'évolution normale de tous les êtres vivants animaux et végétaux tels que l'iode, l'arsenic, le bore, le zinc et autres. Dans tous les cas, il est aisé de constater que c'est ici que se concentrent le fer, la chaux, la magnésie, la potasse et par dessus tout le fluor et l'acide phosphorique, etc.

Mais cet ensemble a ceci de particulier, c'est que la majeure partie de ces éléments minéraux affectent une forme organique; si bien, par exemple, que les soi-disant phosphates des cendres du son, refusent aux 3-4 au moins de se laisser précipiter par la magnésie anmomiacale, lorsque sans calcination on les a séparés du son lui-même par l'eau chlorhydrique.

Ces diverses considérations nous expliquent que physiologiquement le son est la dernière des réserves alimentaires dont la nature a doté le grain de froment pour nourrir l'embryon, puis la plantule, lorsque dans son évolution cette derniére prend déjà une part de sa nourriture dans l'air et dans le sol. Et si cette même nature lui a donné cette texture cornée et cette résistance relative aux agents de dissolution ordinaire, c'est, d'abord, à cause de son rôle physique de prorection du contenu; c'est, ensuite, pour conserver au jeune végétal un dernier morceau de pain jusqu'au jour de son émancipation maternelle.

Voilà pourquoi, lorsqu' on incinère parallèlement les diverses parties du grain de blé, telles que la meunerie moderne sait si bien les séparer, on trouve, que selon le nombre des passages du grain aux cylindres la dose des cendres forme une proportion arithmétique progressive de phosphates. Ainsi elle commence à 0. 480% pour la farine de première et elle arrive à 5.90% pour les gros sons en passant par 0.960 pour la farine entière et à 3.50% pour lesrepasses.

Or, dans ces cendres, si on dose à l'état d'acide phosphorique total les éléments phosphorés transformés, on trouve qu'ils vont de 0.175% dans la farine de première et à 3.15% dans le son en passant par 2.10 dans la repasse et 0.23 dans la farine entière.

Ces nombres traduits en *phosphate tricalcique des os* deviennent:
Pour la farine de première 0.38%—Pour le son 6.87%.
Pour la repasse 4.58% et la farine entière 0.50.

Ces considérations et ces faits traduits par des nombres nous disent pourquoi, depuis des siècles assurément, les éleveurs font inconsciemment bon usage des sons et répasses de froment pour compléter la pauvreté alimentaire de certaines pitances végétales. Ils nous indiquent comment, parmi les herbivores, les ruminants en profitent plus que d'autres; pourquoi pour les porcelest on se trouve bien de faire cuire lessons; pourquoi les repasses favorisent la quantité au moins du lait des vaches; et pourquoi, enfin, ces mêmes sons ou repasses mélangées aux patées des jeunes granivores accélèrent la croissance de leur âge et diminuent leur mortalité quand ils sont encore dans le seul duvet.

ENTRETIEN DU TISSU DENTAIRE PAR UNE ALIMENTATION APPROPRIEE

PAR M. LE DR. P. CARLES DE BORDEAUX

Pour que les dents se forment chez l'homme comme chez les autres mammifères, à l'époque de la première et de la deuxième dentition, il est indispensable évidemment que tous les éléments minéraux et peut être même une part des organiques dont elles sont formées, préexistent dans les aliments. Il faut en plus que ces éléments aient été rendus assimilables dans le tube digestif, afin que les humeurs puissent les véhiculer jusqu'à la matrice de la dent.

Comme les dents sont constituées aux 2-3 environ par des substances minérales, dont le phosphate de chaux constitue la partie prépondérante, c'est généralement ce dernier que les dentistes prescrivent dans les défauts de nutrition du système dentaire. Nous craignons qu'en le conseillant ils ne songent pas assez aux différences d'assimilabilité que ce phosphate possède selon son genre de préparation, son origine, sa constitution moléculaire organique ou minérale; pas plus qu'aux impuretésqui accompagnent bien souvent celui du commerce.[1] Ce détail mériterait cependant d'être pris en considération, car il peut constituer une cause d'inassimilabilité générale, ainsi qu'on le verra plus loin. (Expériences de Raulin).

Mais en admettant que sur tous ces points ce phosphate soit irréprochable, il n'en est pas moins vrai qu'il serait incapable à lui seul de faire des dents, puisqu'il se trouve en outre et invariablement dans ces organes de nombreuses espèces minérales différentes connues et même probablement inconnues.

Dans cet ensemble, le phosphate de chaux n'est donc qu'une dominante; et, par cela seul que ses proportions et même son assimilabilité dans l'alimentation seraient assurées, il ne s'ensuit

[1]Il contiendrait parfois jusqu'à 0.66% de plomb, ce qui rendrait dangereuse l'administration prolongée de ce sel (A. Gubler-commentaires du Codex p. 685).

pas que le recrutement des autres éléments le soit assui. Sans doute, à cause de leur quantité plus faible et même quelquefois infime, on a pris l'habitude de considérer ceux-ci comme accessoires; mais c'est là une faute, ainsi que les points suivants vont l'établir.

Raulin, de l'Ecole Pasteur a démontré le premier, avec toute la vigueur scientifique désirable, que dans un aliment complet les éléments chimiques constituants ont tous une valeur égale, indépendants de leur proportionnalité. Ceci veut dire que non seulment les espèces chimiques en minorité numérique ont autant d'influence que les autres sur la croissance normale du sujet, mais qu'elles en ont même quelquefois d'avantage. Tel est le cas du zinc, dant la présence de traces dans un aliment élémentaire complet *décuple* une récolte ordinaire d'aspergillus. Tel est, dans le sens opposé, le rôle de la présence de traces d'argent bien moindrex encore qui s'opposent *absolument* à l'assimilabilité *de l'ensemble des autres éléments constituants*[1]

Dans le même ordre d'idées, voici un autre fait cité par Pasteur lui-même[2].

Quand on veut nourrir de jeunes levures, le meilleur aliment minéral à leur fournir est représenté par les cendres de levures vieilles; mais à la condition absolue que ces cendres, aient été simplement brûlées et non pas frittées. Si, en effet, elles ont simplement chauffées au rouge jusqu'à fusion, elles deviennent aussitôt un mauvais aliment. Cette différence tient uniquement à ce qu'elles perdent une faible partie de leursalcails, sous l'influence de l'intensité du feu, l'aliment, complet avant la fusion, est, au contraire, devenu incomplet à la suite.

Cette direction d'idées a plus récemment encore été corroborée par Sachs, A. Gautier, Baumann, Robin, Bertrand. Ces savants ont démontré que les animaux supérieurs comme les inférieurs et comme aussi les végétaux ont besoin de traces de substances à actions physiologiques intenses—arsenic, iode, bore, manganse, aluminum—mais mitigées par la forme organique que la nature sait leur donner et dont la science essaie de les revêtir depuis peu.

[1]Duclaux—Chimie biologique 1883, p. 206 ou encore—Traité de microbiologie 1898 T. 1 p. 181.

[2]Duclaux—Chimie Physiologique 1883 p. 327.

Bertrand a écrit naguère[1]: Tous les éléments constitutifs de la matière vivante sont nécessaires; tous concourent à la formation des liquides et des tissus dont l'individu se compose. L'insuffisance d'un seul de ces éléments peut entrainer la diminution de tous les autres et provoquer par suite un arrêt général de la croissance. Le principe de la synergie des éléments prend donc une grande importance au point de vue du choix de certaines médications, etc.

Tous ces faits peuvent se résumer ainsi:

Dans l'alimentation, c'est l'assimilation des petits qui entraine l'assimilation corrélative des grands et non l'inverse; ou encore: En biologie, la valeur d'un corps ne se mesure pas à son taux! (Quinton)

Avant qu la science ait mis ces idées en relief, la pratique les avait déjà sanctionées à la suite de simples observations.

Ainsi, il y a déjà, long temps que les éleveurs alimentent de façon différente les animaux, suivant qu'ils veulent les mettre en chair ou en graisse.

Pour un même terrain et un même végétal, la chimie agricole moderne a démontré qu'il y a des engrais chimiques qui poussent à la seule végétation et d'autres à la fructification. Enfin, à notre instigation, certains bouilleurs de crû ont noté que si après avoir enlevé à la vendange son alcool et même son acide tartarique, on reportait tout le reste à la vigne, elle n'aurait jamais besoin d'engrais.

* * *

C'est sous l'empire de tout ce qui précède que nous avons projeté de constituer un aliment spécial pour le système dentaire. Pour les raison scientifiques largement développées ci-dessus, cet aliment doit donc contenir *sans aucune exception*, tous les éléments constituants *connus* et inconnus des dents, et autant que possible dans les proportions individuelles déterminées par la nature dans l'organe lui-même.

Pour y arriver, il faudra donc s'adresser aux dents exclusivement comme matière première et choisir celles de l'animal qui, au point de vue omnivore se rapproche le plus de l'homme. C'est pourquoi nous avons pris celles du porc.

[1]Bulletin Soté Chimique 20 Mai 1912 p. 497.

Quand l'animal est dépécé, il est aisé en faisant bouillir ses maxillaires dans l'eau d'en extraire les dents et de les priver ainsi du sable et autres impuretés qu'elles récèlent toujours. L'ébullition au surplus les stérilise une première fois.

Pour ne rien perdre de leurs éléments constituants, il semble alors qu' il n'y a plus qu' à les déssécher, à les pulvériser et arriver enfin à une poudre impalpable d'ingestion facile dans de la confiture.

Mais ces grains de poudre sont d'une telle dureté que leurs aspérités, quoique microscopiques, irritent fortement la gorge et même la muqueuse gastro-intestinale. D'ailleurs, leur texture-pseudo cornée et leur extrême ténacité les rend réfractaires à l'action des humeurs gastriques.

Au contraire, si en cet état on les soumet à l'autoclave de façon assez prolongée, l'osséine se dissout dans l'eau et il devient alors possible de les écraser presque avec les doigts. Mais la division se fait mieux alors avec l'intermède du sucre de telle sorte qu' on peut arriver aisément à un granulé au dixième de dents. Il se conserve indéfiniment.

Il est incontestable, qu'en dehors du sucre, il n'y a ainsi dans le produit rien d'ètranger; que rien de la dent primitive et stérilisée n'a ét perdu, et que les éléments constituants sont bien tels qu'ils étaient dans cette dent, avec leur forme chimique et leur invariable proportion naturelle.

. . .

L'indentité de ce dentogène peut être facilement vérifiées, voici comment: Dans un verre mettons 10 gr. de ce saccharolé et versons par dessus 50 gr. environ d'eau froide. Agitons. Tout le sucre se dissout et le liquide se transforme en bouillie laiteuse. Après un quart d'heure de repos, il s'est divisé en deux parties distinctes; un liquide limpide un dépôt abondant.

Dans le premier, se trouve une albuminoide obéisssant aux réactifs généraux de ces espèces chimiques (a. picrique, tannin, Canres). Mais elle se caractérise surtout comme gélatinoide par son incoagulabilite la par lachaleur, ainsi que par le ferrocyanure acétique et par le nitrate d'argent.

Dans le dépôt on constate la présence des carbonates, phos-

phates fluorures[1] de calcium, magnésium, fer. Tout se dissout à la minute dans quelques gouttes d'acide chlorhydrique. Avec l'acide acétique, la solubilité est ralentie, mais peu à peu il ne reste qu'un minime dépôt qui a bien son importance. Il caractérise, en effet le tissu dentaire lui-même ou plutôt l'enveloppe d'émail fluorurée, reconnaissable au microscope en ce qu'elle affete l'aspect de plaques formées par des prismes verticaux accolés parallèlement. Ce dispositif leur donne le facies d'un carré de toiture de maison.

Un pareil mélange naturel nous parait mériter le nom de *Dentogène scientifique.* Sa constitution exige qu'il soit pris au moment de la grande sécrétion gastrique, c'est à dire au cours des repas.

Les combinaisons phosphorées et fluorurées s'y trouvent à la fois sous la forme minérale et sous la forme organique.

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[1]L'expérience nous a démontré que le fluorure de calcium est très abondant dans toutes les parties de l'invoire de l'éléphant et de l'hippopotame. Nous estimons que c'est surtout à ce fluorure que ces dents doivent la finesse si remarquable de leur grain, leur dureté, leur ténacité.

Dans les dents du porc, on retrouve une part de cette répartition générale et régulière des fluorures, dans certaines grosses molaires parfois très larges chez les individus de grande espèce; mais on ne la retrove plus dans les canines et les incisives. Ici, le flourure parait se concentrer dans les parties superficielles tranchantes et piquantes. On s'en aperçoit vite en désséchant ces dents dans une étuve fortement chauffée. Comme le coefficient de dilatation est différent dans les diverses couches, elles se séparent spontanément à un moment donné. La partie superficielle, celle qui correspond à l'émail, es, beaucoup plus fluorée qu l'autre.

Quoique les défenses du porc soient creuses, elle offrent leur maximum de dureté vers la pointe; c'est là aussi que s'accumule l'émail très fluoruré. Dans les défenses si redoutables du sanglier, il doit exister un dispositif analogue.

Dans les dents humaines, on constate parallèlement que le même fluorure se porte principalement dans les parties directement agissantes. Le but de la nature est ici de leur donner plus de puissance et de dureté au point de vue de la déchirure de l'aliment, de sa mastication et de la résistance de la dent à l'usure. Enfin, le vernis fluoruré ou émail soustrait le corps de la dent à toute intrusion microbienne.

THE ORIGIN AND SIGNIFICANCE OF STARCH

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Introduction

The green leaves of plants possess the most efficient means of transforming the radiant energy of sun-light into the potential energy of carbohydrates like starch, cellulose and sugars. This process which is of fundamental importance to both plants and animals, is carried on by the chloroplasts or chlorophyll-bearing granules of the plant cell. Drawing upon the sun as their source of energy plants are the producers of a form of energy stored in carbohydrates while animals dissipate this energy in the functions of their bodies. They are spendthrifts, too, and were it not for the equilibrium maintained between these opposed functions in animals and plants the world would long since have become bankrupt for the energy so necessary for the existence of living organisms and human industries. These chloroplasts of plants provide the means of absorbing and storing for later use the incalculable amounts of radiant energy poured down upon us daily by the sun. The cellulose present to such a great extent in all living vegetation, and also in the carbonized plant remains in coal, represents one type of energy stored in carbohydrates. In starch we have another example except that it does not form such a permanent reserve for it is consumed relatively soon either by the plant producing it or by an animal. It is this *active* role of starch in its biological relations that makes it interesting. The origin of starch in the plant has offered an attractive field of study that has been worked very industriously for a long time. The biological significance of starch is something that appeals strongly to the biochemist. The industries in which starch figures are great ones and worthy of the closest study but in this place we are concerned with starch in its early history long before it has entered into the food and industries of the people.

Early Theories of Starch Formation

Before we can obtain any idea of the mechanism of the green leaf in its role of starch former we must consider the point of view of the early investigators. First of all, however, it is desirable to define the term *photosynthesis* which is used by many plant physiologists and will often appear in this paper. By photosynthesis we mean the action of the green plant in *using the radiant energy from the sun to effect the union of carbon dioxid and water thus producing gaseous oxygen and sugars* which subsequently may appear as carbohydrates or may be changed into the fats and proteins of the plant. This phenomenon has been and is still sometimes called carbon assimilation. The latter term expresses the idea correctly but does not make enough differentiation between the action of the green plant which *manufactures* its own carbon compounds and that of the lower plants and animals which can only use such compounds in assimilation after they have been elaborated elsewhere. Furthermore, the word photosynthesis clearly expresses the idea that *light* is the fundamental fact in this type of assimilation.

Priestly, to whom the chemists owe so much, found that green plants would grow in confined air rendered irrespirable by the combustion of a candle or exhalations of an animal. He said:

"Accordingly on the 17th of August, 1771, I put a sprig of mint into a quantity of air, in which a wax candle had burned out, and found that, on the 27th of the same month, another candle burned perfectly well in it. This experiment I repeated, without the least variation in the event, not less than eight or ten times in the remainder of the summer."

Later, in 1779, Ingenhoues showed that this purification of bad air by growing plants could take place *only in the light.* Next Senebier proved in 1782 that the carbon dioxid in water, in the soil humus, etc., was far too slight in amount to supply the needs of the plant and that the *atmospheric* carbon dioxid was the source of carbon dioxid for the plant. Lavoisier overthrew the phlogiston theory in which Priestly and the others believed. His methods of exact quantitative study were followed by Saus-

sure who announced in 1804 that there were definite quantitative relations existing between the intake of carbon dioxid, output of oxygen, etc. This bare outline of the early history of the study of photosynthesis will serve as an introduction to the later work which will now be treated under several heads in order to keep a clear outline before the reader.

The Formation of Starch

Decomposition of Carbon Dioxid. When an aquatic plant is illuminated the most obvious result of photosynthesis is the appearance of bubbles of gas. Upon chemical examination this gas proves to be nearly pure oxygen. By counting the number of bubbles produced in a given time one may estimate roughly the rate of photosynthetic action. By exact measurement in eudiometer tubes it is found that for every volume of carbon dioxid absorbed an *equal* volume of oxygen is set free. This is an important observation and will be referred to later. Such plants when submerged in dilute solutions of reduced dyes or venous blood cause the color changes characteristic of oxidation. The so-called bacterium method of Englemann offers a most striking means of demonstrating the production of oxygen when green plants are exposed to light. He used an air-tight preparation of a living green alga surrounded by certain bacteria which are strongly attracted by oxygen but are motionless in its absence. Now, when such a preparation is illuminated these bacteria immediately become active and all move to the centers of oxygen production which are *only* those cells in the *light.* In the darkness and in the presence of light of wave-lengths too short or too long to be visible to us the amount of oxygen set free is very small and consequently the bacteria are motionless.

The power to decompose carbon dixoid into oxygen and to build up sugars seems to be localized in the chloroplasts or green granules of the cell. For photosynthesis to go on it is necessary that we have the following intact mechanism in the leaf: the living chloroplast, a sufficient supply of carbon dioxid, light of the proper wave-length, the proper temperature and an adequate supply of water. The latter is usually ample because the evaporation from the leaves create a constant transpiration current

of water from the roots where it is absorbed through the stem to the leaf. The supply of carbon dioxid comes from the atmosphere where it is constantly present to the extent of 3 or 4 parts per 10,000. This seems to be a very small working capital but when we consider the easy access to the interior of the leaf through the multitude of little openings or stomata one realizes that while photosynthesis is taking place the internal leaf structure is a *vacuum* as far as carbon dioxid is concerned, and so the atmospheric store of this gas is ample for the purposes of the plant. However, it should be stated that an increase of carbon dioxid to ten times its ordinary amount seems to be used by the green plant to good advantage. Millions of tons of that gas are poured into the atmosphere by the respiration of all living things, the decomposition of organic matter by micro-organisms, and the combustion of fuel in the furnaces of industries and homes yet the balance is maintained by the green vegetation of the earth which decomposes this carbon dioxid to build up enormous amounts of organic matter, renewing the air at the same time with the life-giving oxygen. The water and air currents flow this way and that, thus helping in mixing and transporting the gases and keeping conditions uniform for plants both on land and in the water. In Carboniferous times green plants were in their glory because the conditions of high temperature, high content of carbon dioxid in the atmosphere and an abundant supply of water allowed them to reach an unequalled period of activity, the story of which can be read to-day in the world's coal mines.

Role of Chlorophyll. Besides the undoubtedly esthetic part played by chlorophyll in clothing the earth's vegetation with its restful green color it also plays a necessary part as the active agent in photosynthesis. In the chloroplasts this green coloring matter exists either in the form of a thin skin over the protoplasm or in granules within it. The chlorophyll may be extracted with alcohol to give a dark green solution having a beautiful red fluorescence in reflected light. Such an alcoholic solution when shaken with benzene yields a yellow alcoholic layer and benzene soluble fraction having a blue green color. The yellow substance is mostly carotene hydrocarbon crystalizing in orange plates and having the empirical formula C_{40}, H_{56}. The blue green frac-

tion has a much more complex nature and is a mixture of the so-called "chlorophyll" or cyanophyll with other closely related substances. The photosynthesic activity is associated with the blue green pigment and consequently much study has been given to it. It may be obtained in a crystalline form but probably in an altered condition. Many formulae have been given it; some investigators claiming that it contains nitrogen and phosphorus (a lecithin-like substance), and others that it contains a high percentage of magnesium.

The literature of chlorophyll is voluminous and investigators like Willstaetter, Machlewski, Stoklasa and others have all carried on series of researches upon it. Among the decomposition products of chlorophyll there are found substances nearly identical with those from haemoglobin, which is as essential for the continuance of the life of higher animals as chlorophyll is for the green plants. Any detailed discussion of the chemistry of chlorophyll would be out of place here but for many it is a fascinating chapter in modern organic chemistry.

Action of Sunlight. An alcoholic solution of chlorophyll shows a striking absorption band in the red which corresponds to wavelengths of about 640 to 670 microns. Experiments with spectra thrown on living leaves show that it is in just this region of the spectrum that the greatest formation of starch takes place. So, then, it is the energy absorbed from this region that carries on the photosynthetic transformations. The energy thus absorbed is largely turned into heat which always raises the temperature of the leaf and consequently only a small fraction of the absorbed energy is ever converted into the potential energy of carbohydrates etc. On a bright summer day when we absorb certain light rays with our skin the energy thus converted soon causes the well known unpleasant effects, and likewise when this action takes place on a photographic plate the sensitive silver salts are altered in such a manner that a permanent record of any scene may be produced at will. Some think that chlorophyll acts as a sensitizer in photosynthesis just as certain fluorescent substances do in other photochemical reactions. Others look upon the role of chlorophyll as being that of aiding in the transformation of radiant into electrical energy which then splits the carbon dioxid and water into the first products of photosynthesis.

The amount of light required for photosynthesis is not great and so upon exposure to weak illumination the process of carbon dioxid decomposition begins at once but may not become evident since the evolution of oxygen does not occur until the amount set free is in *excess* of that required for the processes of respiration. It is likely that in most conditions under which plants exist the limiting factor in photosynthesis is not lack of light but absence of sufficient carbon dioxid, water or favorable temperatures. Certain shade-loving plants thrive in a very dim illumination but in such cases the cells containing the chloroplasts are often arranged like lenses to focus the available light upon the chloroplasts. In ordinary plants the cells have many ingenious ways of focussing light upon the chloroplasts and of securing favorable alignments by means of changes of position of the chloroplasts in relation to the incident light. On a larger scale, we notice, that each leaf tries to secure the most favorable arrangement for itself, an arrangement resulting in the least shading of the leaf by others. This tendency produces "leaf mosaics" of great interest and beauty. Many plants when viewed from above (whence the most light comes) present a nearly unbroken expanse of green leaves thus enabling the plant to make the most of all the light it does receive. The plant even in strong light does not begin to form starch at once when illuminated but only after the lapse of a certain time during which, apparently, the precursor of starch has collected in sufficient quantity to start the mechanism of starch formation. The increase of dry weight of an illuminated leaf does not represent the total amount of products formed but only the quantity *remaining* in the leaf, the rest of the material produced having been translocated in diffusible form to another organ of the plant where it is laid down in the form of the so-called "secondary starch" as in potato tubers.

Nature of Photosynthetic Products. We have already seen that the volume of carbon dioxid absorbed and oxygen disengaged are nearly equal and, further, that the first distinguishable substance is starch. Now, starch has a very high molecular weight, variously estimated at from 12,000 to 30,000, and it does not seem probable that such a complicated substance should be produced at once from water and carbon dioxid. Baeyer's theory that formaldehyde is first produced and that it soon condenses to form sugars

is well known and it probably expresses correctly the nature of photosynthesis. It has been generally accepted that glucose is the *first stable* product from which starch, sugars, fats, and proteins may be constructed according to the needs of the organism. In most plants during the day this glucose is rapidly condensed to starch which fills the cells but as evening and darkness approach photosynthesis is retarded and the starch is converted back to glucose and similar easily diffusible substances which are easily translocated to other parts of the plant. Assuming that glucose is the first stable product we may write the reaction for photosynthesis as follows:

$$6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2.$$

This equation, however, does not represent the whole truth but indicates only the general trend of transformation, the important but unknown intermediate products as well as the energy relations being ignored. The heat of combustion of glucose is about 3.75 Calories and all of this energy must have come from the sun in the beginning.

It is by no means true that all plants store energy in the form of starch although many of them do so. In certain groups of plants such as the lily, orchid and amaryllis families very little if any starch is formed while in the legumes and Solanaceae large quantities are present. When starch is not produced we find substitutes in the form of cane-sugar in several plants, mannite in the Oleaceae, etc. The oils, proteins, glucosides and so on are probably not the direct result of photosynthesis but are produced later by the union of glucose with other substances or by condensation with itself to form more complex carbohydrates. The first substances produced by photosynthesis are extremely active chemically and it may well be that, at this stage and in the presence of nitrates, phosphates, and sulphates the proteins are constructed. In the green leaf many optically active substances are formed, a type of synthesis difficult to perform in the laboratory without the intervention of the experimenter or other living organism able to diffierentiate between the right and left handed modifications.

In darkness, even in the absence of chlorophyll, the plant cells can store up starch if fed with glucose, sucrose, glycerine and

many other similar substances. This shows that the photosynthetic and starch forming processes are distinct. Proteins, fats and many other types of organic materials may all be formed in darkness also. Some observers have reported that in the light the chloroplasts of certain algae seem to show a shrinking and change of their protein substance into starch. It may be that one step in photosynthesis is the disintegration of the protein of the chloroplasts to split off carbohydrate in this manner.

Artificial Photosynthesis. The idea that formaldehyde is an intermediate product of photosynthetic activity has led many investigators to see first if it really may be detected in green leaves by chemical means and secondly if it may be made to condense and produce sugars artificially. Several investigators have found that leaves do give a positive test for formaldehyde but whether formaldehyde *itself* were present can not be said. A more complex aldehyde has recently been isolated by Curtius and Franzen from certain leaves. It possesses the six carbon atom skeleton characteristic of glucose. Attempts to cause starch formation by feeding formaldehyde or its derivatives to plants have been partially successful. It is interesting that in alkaline solutions formaldehyde condenses with itself to give a sugar like glucose. Under certain conditions the silent electrical discharge breaks up carbon dioxid into formaldehyde which, in turn, may then be converted into sugars. In the presence of alkalies Stoklasa found that ultra-violet light changed a mixture of carbon dioxid and nascent hydrogen into sugars. When formaldehyde and oxalic acid were sealed in glass tubes and exposed to sunlight, those tubes only which were thus exposed were shown to contain considerable quantities of sorbose. The action of light and of the traces of alkali in the glass seemed to catalyze this reaction. Electricity and ultra-violet light seem to lower the temperature necessary for these condensations to take place. Experiments of a different type have been carried out in which a thin film of chlorophyll was deposited on water or gelatine and then this artificial leaf was illuminated and a little catalase added to decompose any hydrogen peroxide formed. Under conditions of illumination and presence of carbon dioxid the experimenters reported the formation of small quantities of formaldehyde.

All of these recent investigations show that the formaldehyde theory of sugar and starch formation has experimental ground for its existence and, at any rate, it is helpful in visualizing some of the processes of photosynthesis. Such observations also force us to consider that, after all, photosynthesis is not wholly a vital process but that under the proper conditions it may be imitated in the laboratory though in an inefficient manner.

Physical Nature of Starch

Ordinarily we see starch in the form of a white powder which gives a peculiar rustling sound when rubbed between the fingers. Under the microscope the whole appearance changes and the starch grain now takes on a characteristic form depending upon the organ and species of plant from which it came. This form is nearly constant for any given type of starch. The size of the grains varies from the large one of the Canna (visible to the naked eye) to the most minute sort. The form of the larger types like the starch from potatoes may best be described by likening them to oyster shells often with eccentric striations. In the case of corn and rice starch we do not have a simple grain but a compound structure consisting of many small grains having more or less angular faces. In polarized light the familiar black cross appears and this shows that the starch grain has a definitely organized structure of some sort.

The effect of starch on polarized light and its peculiar striated or stratified appearance have led to the publication of many theories to explain its internal structure. The layers may probably be accounted for by assuming that they represent the product of varying periods of activity on the part of the functioning chloroplasts or leucoplasts. When starch is formed in the green leaf it is produced on the chloroplast of its origin while in tubers and other storage parts it is made from glucose and maltose by the activity of the leucoplasts or colorless granules which are seats of this storing action. The layers and striations of the grain are seldom concentric because the centers of starch formation are usually not the geometrical center of these protoplasmic granules. The latter are often far smaller than the starch grain grow-

ing upon them. It must be remembered that the formation of starch from the products of photosynthesis by either the chloroplasts or leucoplasts has little to do with the photosynthetic fuction of the former but is controlled by the amount of glucose and maltose in circulation in the plant. Some authors consider that the different layers are caused by variations in the water content of the starch deposited. It was also thought for a long time that the outer envelope of the starch grain was a cellulose because of the well known insolubility of starch in cold water and the difficulty in digesting raw starch by enzyme action. The true starch or amylose was supposed to be in the interior and to imbibe water through the cellulose envelope; this causing a swelling which ruptured the envelope, yielding the familiar starch paste. Arthur Meyer believed starch was composed of sphaero-crystals consisting, in turn, of radiating needle-like crystals of two sorts, one easily soluble in water and giving a blue color with iodine and the other a substance less soluble in water like the cellulose envelope of the earlier writers. The conception of the starch grain as a sphaero-crystal is interesting and there is some experimental evidence for it. At present it is impossible to state with certainty that starch has one type of structure or the other.

The Chemical Nature of Starch

We have just seen that starch is apparently composed of two substances, one of which is water soluble and possesses all the properties commonly associated with starch while the other is more insoluble and more like cellulose in its behavior. Treatment with boiling water, acids, alkalies and digestive ferments gives first a thick colloidal solution having well marked starch reactions which decrease in intensity and finally give place to simple solutions and more active chemical properties as hydrolysis into dextrins and sugars progresses. Soluble starch is the first hydrolytic product but it is soon changed into the dextrine. The chief characteristic of soluble starch is that it dissolves in warm water to give a clear solution having the usual starch properties unchanged. This form of starch may be made by treatment with very dilute acids, alkalies, or by enzyme action, pro-

vided, of course, the reaction is arrested at the proper point. A great many interesting and industrially important starch derivatives are manufactured but they are too numerous to mention here.

The blue coloration with iodine is the commonest means of detecting starch and it is a striking and valuable test. Much study has been given it but we still lack accurate information about it. Some consider starch iodid a chemical compound, others an absorption phenomenon and still others think of it as a solid solution of iodin in the colloidal contents of the starch grain. The blue color is easily destroyed by heat but reappears on cooling and, furthermore, it is very easily changed by numerous chemicals. Not all starches stain a pure blue with iodin; some give purple and some even give red colors. This probably indicates a difference in the complexity of starches from different sources. With iodin a shade of red or brown indicates a departure from natural starch and an approach to the simpler dextrins and, finally, to the simplest and well known sugars. During digestion by diastase the starch grain is corroded and attacked more in certain portions than in others. This fact may indicate a difference in chemical nature between the different layers of the grain as already suggested.

In the classification of the carbohydrates starch is listed as a polysaccharid and it is from this word *poly* that we get the key to the whole matter. We ought to consider starch as being built of many glucose and maltose units connected in such a way that no carbonyl groups are free. This we know because, like saccharose, starch shows none of the reactions characteristic of such a group. The usual formula for starch is $(C_6H_{10}O_5)n$ in which n may be any number from 20 to 200. It is almost impossible to obtain accurate data on the molecular weight of starch but from physico-chemical studies, chemical derivatives and ultra-microscopic observations it seems likely that its molecular weight may be from 10,000 to 30,000, figures probably not often equalled even by the complex proteins. In the plant the processes of building up this complicated molecule and of breaking it down seem to be reversible and are probably under the control of enzymes. Apparently the active mass of the glucose and maltose in the food-

conducting system of the plant determines the course of this reversible reaction and determines whether its direction shall be towards the storing of starch or towards its hydrolysis into the more diffusible and immediately available sugars. The complexity of starches from different sources is a variable factor and so by starch we can only mean a general term including those substances having most of the reactions and properties commonly associated with the well known starches of commerce. More exact studies upon the chemistry of starch with the improved methods of the recent advances in chemistry ought to yield the most interesting and valuable results.

Significance of Starch in the Plant

In the earlier chapters it has already become evident that starch acts primarily as an indiffusible but easily convertible form of stored energy. The heat of combustion of starch (4.1 Cal.) is slightly higher than that of glucose but as a form of potential energy it cannot compare with the fats and oils which have an energy value of about 9 Cal. However, in many plants starch is the most abundant form of stored food and is, possibly, more easily converted into its constituents for purposes of translocation than are the fats. The proteins are more likely to appear as integral parts of the living protoplasm than to act as stores. Most of the starches with which we are familiar are nearly always prepared from some storage organ of the plant and have larger and better characterized grains than the primary starches in the leaf. The leucoplasts of the fruit pulp, tubers, etc., of the plant are the active agents in reforming starch from the translocation stream of sugars. There is a form of starch storage in which the leucoplasts do not seem to play any part. The type is represented by the somewhat temporary starch reservoirs found in pollen grains, the sheath of growing tissue, and so on. Under such conditions the starch exists in a very finely divided state and appears to be a store of a transient nature. In either form of storage the enzyme diastase seems to cause the transformation of starch into its sugar constituents and also the reverse change when circumstances demand it. The so-called translocation diastase of the

green leaf causes the change there while the secretion diastase of germinating seeds and tubers carries on a similar action in those places. The two sorts of diastase do not corrode the starch grains in the same manner nor are their other properties exactly the same. Although starch is laid up in enormous quantities in the tubers, seeds, stems and pulp of fruits, it is far from being the only polysaccharid thus stored. Glycogen has the same function in the fungi and so has inulin among plants of the Compositaceae and Liliaceae; sucrose acts likewise in sugar-cane and beets, while glucose is found in the leaves and bulb of the onion. However, starch and cellulose are the two great stores of energy in the form of carbon compounds that are produced so abundantly by nature each season.

Significance of Starch to Man and Animal

In the early history of the race our ancestors probably noticed that certain animals and birds sought much of their food in the seeds of grasses while at the same time the smaller animals dug into the earth for roots and tubers. Thus man early learned to make the starchy foods one of the main articles of his daily fare and it is true to-day that among all peoples in all climates bread from cereals or some starchy substitute is the "staff of life." Among many animals the foods of this type are the staple ration and it is only the carnivora that scorn such a diet. Upon digestion the starches are split into the sugars which are then burned in the organism to yield their energy for the maintenance of the physical activities and physiological functions of the animals. Unlike the proteins, the carbohydrates and fats are used by animals to produce heat and energy and not so much to become living protoplasm as is the case with nucleo-proteins and albumins for example. Since but little new protein is needed for the upkeep and growth of the mature plant or animal we see that the constant demands for energy supplies must be met by the sugars and fats consumed. The abundance of starchy foods eaten by men and animals is adapted to meet this necessity of energy producing material in large quantities.

The greatest source of starchy food is, of course, the seeds of the various cereals which we group together as grain. The amount of

such material produced from the soil in a year is almost beyond calculation. The production of this golden flood of grain is the earth's oldest and greatest industry. Besides the starch given us in the cereals we must not forget the potato which is another staple article of diet in the whole civilized world. In different countries various starchy foods are popular such as sweet potatoes, arrow-root preparations, tapioca, sago, chestnuts, bananas, etc. From the time that man first noticed that grains were good to eat he has taken plants of this type under his special protection and given them careful cultivation. The result has been an improvement in the races of grains as judged by their yield and adaptability to varying conditions of climate. To produce these harvests the soil supplies the water and mineral nutriments while the carbon dioxid and sun-light lend their aid through no effort of man. His duty, then, is to see that the soil is kept in its most productive condition and by so doing he will have an ample supply of grain for the needs of the future.

The Industrial Importance of Starch

The observation of primitive man that the seeds of certain plants made an acceptable food was the beginning of agriculture. Another observation made sometime later was that when starchy materials were allowed to stand they underwent a peculiar transformation. The result of this change was a so-called "spirit" which was soon found to possess magic properties in making "glad the heart of man." This, then, was the origin of another vast industry whose object is the production of alcoholic materials through the fermentation of grains by enzymes and micro-organisms. Alcoholic beverages of one sort or another are known everywhere and their production goes hand in hand with the practice of agriculture. The amount of grain used by the brewing and liquor distilling industries comes to an enormous figure and is second only to that consumed as bread and various bakery products. The flour milling industries prepare starchy food for the millions, the example of the former in centralization is being followed more and more by the bakeries, especially in the larger cities. The preparation of bread in the home is becoming

less common every year and most of this work is done in large bakeries where more or less scientific methods are beginning to prevail. Various forms of natural and prepared starch are employed in large quantities in the form of specially treated foods, laundry starch, sizings, adhesive pastes and so on in great variety. Very valuable products are manufactured by heating or treating raw starch in such a way that dextrins and gums are formed. These are used as adhesives and for other purposes. The action of dilute acid upon starch yields glucose and it is upon this reaction that another great industry has been founded. Glucose has a multitude of industrial applications and it also figures in our food, sometimes under another name but tasting just as sweet. Starch and its products are valuable in many other ways than merely those already mentioned but it would be presumptuous to point them out to this Section of our Congress.

In this paper the writer has not striven to give detailed discussions of any sort for these may be found in books on plant and animal physiology but has endeavored to present many old and a few new ideas in the way that they appear to one interested in the biochemical problems of plants and animals. For those desiring a closer insight into the phenomena of starch formation a short bibliography is appended. In these works full references to the original papers in this field may be obtained.

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INFLUENCE DES IMPURETÉS GAZEUSES DE L'AIR SUR LA VITALITÉ DES MICROBES

PAR M. A. CRILLAT

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L'air que nous respirons contient des impuretés gazeuses variées. Jusqu'ici, on n'avait pas songé à étudier leur influence sur la vitalité des microbes en suspension dans l'atmosphère. Au point de vue épidémiologique, cette étude, qui peut servir à expliquer le mécanisme encore si obscur de la contagion par les germes de l'air, présente un grand intérêt.

Les expériences ont tout d'abord démontré que les microbes, dans l'état spécial où ils se trouvent dans l'air, sont extraordinairement sensibles aux moindres variations de la composition chimique de l'atmosphère. Les influences sont tantôt antiseptiques, tantôt activantes ou conservatrices, et j'ai désigné sous le nom d'ambiances favorables l'air contenant des gaz susceptibles de prolonger l'existence des microbes ou de faciliter leur reproduction. L'analyse de ces gaz a déjà démontré qu'ils renfermaient des substances alcalines gazeuses, parmi lesquelles on a distingué à côté de l'ammoniaque des amines et peut-être des alcaloides gazeux. Les ambiances favorisantes se produisent dans une foule de cas: dans la décomposition putride des substances animales ou végétales, dans le voisinage des matières fécales, dans les émanations du sol, dans l'air souillé par la respiration humaine, etc. Toutefois, la présence de ces gaz dans l'atmosphère ne suffit pas pour constituer une ambiance favorable; il faut le concours d'autres circonstances que j'ai étudiées.

D'après mes travaux, les altérations subites des matières alimentaires sont intimement liées à la formation des ambiances favorables, et j'ai vérifié cette hypothèse par une étude des causes de l'altération du lait pendant les temps d'orages.

La présence de ces gaz à l'état de dissolution dans l'eau lui communique la propriété d'être très favorable au développement

du bacille typhique. Enfin, leur introduction dans l'organisme des animaux exalte la réceptivité vis-à-vis les germes pathogènes.

L'ensemble de ces résultats obtenus montre donc l'importance de cette étude à plusieurs points de vue.

EXPERIENCES WITH DUODENAL AND STOOL FERMENTS IN HEALTH AND DISEASE

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Interest of recent years has centered on the attempt to diagnose abnormal conditions of the pancreatic gland and its secretion by testing the contents of the gastro-intestinal canal or the urine for pancreatic ferments. The stool and urine have been mainly utilized; of late years also the stomach contents after an olive-oil test meal.

It is apparent that these methods are indirect ones, involving irregular dilution of the elaborated enzymes with other body fluids.

The suggestion of both Einhorn and Hemmeter to use a tube which shall enter the duodenum offers a direct method for obtaining the external secretion of the pancreas as elaborated. Duodenal contents so collected were examined and a comparison made with the ferment analyses of the stool in the same cases.

Method. The Einhorn Duodenal Pump was utilized. It consists of a long thin rubber tubing and an attached perforated metallic capsule. The patient swallows the capsule and attached catheter to a point on the catheter marked 80 centimeters. This was done at night; in the morning two and one half hours after the patient had drunk eight ounces of milk, the duodenal content was aspirated for five minutes.

The material obtained was judged to be duodenal contents when either a radiograph showed the metallic capsule in situ or a distinct "retraction test" was noted. When the capsule occupies the duodenum the material enters the aspirator slowly; a resistance (the walls of the intestine) is felt. When the capsule, on withdrawing, enters the stomach there is a rapid gush of material, usually of an entirely different nature. This is the "retraction test."

The duodenal juice is usually golden yellow, viscid, slightly acid (due to gastric contents) or neutral; in amount ten to forty cubic centimeters. The stomach content is milky white and strongly acid.

The contents of the duodenum as obtained were diluted with twice as much distilled water; a part was immediately made slightly alkaline with sodium hydrate solution, this serving for alkaline protease test; the remainder was used for testing amylase and lipase.

The chemical methods for analzying the duodenal ferments were as follows:

For Amylase: One cubic centimeter of the duodenal juice was tested against increasing amounts,(½ to 6 cubic centimeters) of 1% soluble starch solution, the volume in each test tube being made up to 10 cubic centimeters with water. The incubation time was one hour and the persistence of starch tested for by adding a small excess of Lugol's solution. The last tube to show disappearance of starch was read and the number of cubic centimeters of starch solution used, multiplied by the dilution, was accepted as the factor.

In the earlier tests the Wohlgemuth method was employed. The method was discarded because of the inconstant results obtained by testing with only one to two drops of iodine solution.

For Lipase: To ten cubic centimeters of distilled water were added one cubic centimeter of the material to be tested, one cubic centimeter of ethyl butyrate, one cubic centimeter of toluol and a drop of phenolphthalein solution; the whole made up to 25 cubic centimeters and neutralized. After shaking forcefully for fifteen seconds, it was again brought to the neutral point A control test was always prepared with boiled duodenal contents. After incubation for 24 hours, both flasks were titrated and the amount of acid in the control subtracted from that in the test flask, and the result multiplied by the dilution.

For Protease (alkali): Mett tubes, cubes of coagulated egg albumin, Fermi gelatin tubes and the Gross-Fuld casein method were utilized.

In the stool, amylase was estimated by the Wohlgemuth-Hawk method in a slightly different form. Here again iodine was added in excess to test for the persistence of starch.

Lipase and protease tests were the same as in the duodenal tests. For all the stool analyses, a dilution of four parts of stool to fifty parts of slightly alkaline water was used. Usually no catharsis was used in obtaining the stool.

Technical Discussion of the Tests: In all the instances, the fluid was removed in the morning and immediately iced until examined in the afternoon. The acid reaction was preferable for preserving the fluid for both amylase and lipase tests; in acid reaction these ferments could be preserved for 24 - 48 hours in undiminished strength. In alkaline reaction an apparent auto-digestion took place very rapidly, probably due to the presence of trypsin.

The point was frequently raised as to whether the amylase test obtained was due to the salivary or due to pancreatic enzyme. Numerous tests of the stomach contents in these same cases showed the absence of a ferment capable, after five hours' maintenance in an acid reaction, of digesting starch. The duodenal contents, though always containing some of the same acid gastric material, rarely failed to show an active amylase; it is probable therefore that pancreatic amylase is unaffected by pepsin; salivary amylase destroyed by it.

Lipase was similarly best maintained in faintly acid medium; it was destroyed in part or totally in an alkaline medium containing other active pancreatic ferments.

Trypsin was always found in its activated state. This enzyme was best maintained in an alkaline medium.

The duodenum normally contains at least two proteases, trypsin and erepsin, the latter secreted by the duodenal mucosa as well as by the pancreas. Of the tests utilized for demonstrating the proteases, neither the Mett tubes, coagulated egg albumin cubes nor the Fermi gelatin tubes are attacked by erepsin. Casein is digested by erepsin, but in a series of experiments concentrated extracts of the duodenal and intestinal mucosa of the dog, cat, and of man digested casein in dilutions of only 1 : 10 to 1 : 140 while the active pancreatic secretion digests the same amount of casein in dilutions up to 1 : 200,000. We may conclude that though the erepsin is present, its faint proteolytic action on casein does not really affect the value of the figures

obtained, we are safe in interpreting the result as truly tryptic activity.

A similar interpretation may be held for the results of stool analyses. Where slight digestion took place in strong dilutions, the result may have been due to erepsin; where the proteolysis is complete in the much higher dilutions, the result may be interpreted as due to trypsin. Frand and Schittenhelm assert, on the basis of differential polypeptic-splitting tests, that the protease of the stool is usually erepsin and not trypsin. It is difficult to harmonize the above facts with this assertion. Further, stools which actively proteolyzed casein frequently also liquified gelatin; this could be due to trypsin only.

Results of Tests of the Duodenal Contents of a Normal Person

A male adult furnished repeated specimens.

See Table I

From a study of this table, it will readily be seen that quantitative estimates of the strength of pancreatic ferments obtained from the duodenum of a normal man vary within wide limits. In practically every instance, the three ferments tested for are found in an active state. Lipase was absent on one occasion.

Results of Tests in Cases of Interest Because of Pathological Conditions :

See Table II

The cases observed are discussed in groups.

Group A. This comprises one case of acute pancreatitis with a diffuse abscess involving the head and tail of the organ. In the duodenal contents the ferments are absent except for lipase, which is feebly present. Examination of the stool demonstrated the same conditions as in the duodenum.

Group B. Cases of Cholelithiasis (Gall-stone Disease): The ferments are here found in an active state in the duodenum. A wide range of variation is observed, yet in general the ferments are either normal or hypernormal in their activity. In case 6 the absence of amylase and lipase suggested a diseased pancreas. At operation the head of this organ was found swollen and edematous to a marked degree.

Group C. Cases of Obstructive Jaundice: The point of interest was, Is the pancreatic duct open and the pancreas secreting? In the first two instances (cases 7 and 8) this duct was evidently open and active pancreatic ferments entering the intestine.

In case 9, on first examination, the absence of all the ferments from the duodenum except a weak lipolytic ferment, and the absence of all the ferments from the stool led to the diagnosis of complete pancreatic obstruction involving all the ducts possibly emanating from this gland. On a second examination, several weeks later, the results of both duodenal and stool analyses indicated some return of pancreatic ferments in the intestine. At autopsy the head of the pancreas and the duodenum were found involved in a massive sarcomatous tumor; the ducts back of the new growth were greatly dilated and distended with fluid. It seems probable that from time to time the pressure in the ducts was sufficiently great to force pancreatic secretion through the new growth and into the intestine.

Group D represents the findings in two cases of hypertrophic cirrhosis of the liver. From the ferment analyses, the pancreas would seem to be secreting fluid of high potentiality. The examination of the stool in the one case agrees with the findings in the duodenal material.

Groups E and F. The ferments are present and active except for amylase, which is absent in two instances. The failure to demonstrate this ferment was probably due to faulty technic in the early tests.

Group G. In this group is collected the data for various gastric diseases. As far as one can judge, there is no evidence of disturbance of pancreatic secretion. It is of interest to note the absence of trypsin in the case of carcinoma of the stomach.

Case 21, one of achylia gastrica, requires a note. Repeated analyses of gastric contents showed the absence of both pepsin and rennin as well as all trace of acid. The pancreatic secretion is, however, active, all the ferments being present. Ehrman and Lederer, employing the Volhard test meal, found active pancreatic ferments in these cases. In the duodenal contents, however, obtained by me in this case, no rennin was demonstrable. It is still a question whether the human pancreatic gland secretes a ferment capable of coagulating milk.

Group H. Cases of Diabetes Mellitus: In general it may be said that these cases do not show any variation from normal figures, all the three ferments tested for being found present and active. In only one instance was the reaction for amylase weak (case 25). In the instance of case 26, on a strictly limited milk diet, the ferments were all only feebly present. A later examination, on a more full diet (oatmeal and milk) gave ferments of greater strength.

Case 27 was of interest, being a case of diabetes mellitus in a female adult with a distinct history of cholelithiasis and abdominal attacks indicating pancreatitis. The stools in this case were bulky and grayish-white and frequent. Metabolism studies indicated even on a restricted diet a loss in the stools of 54% of the fat, and 29.4% of the nitrogen intake, corroborating, so far as our studies of metabolism would indicate, pancreatic insufficiency. The duodenal findings in this case demonstrated a very scant secretion into the intestine, though a secretion of high potentiality. The stool in the same case showed active ferments though only weak protease.

REMARKS. The original intention of this study was to determine: 1, the limits of ferment activity in the normal duodenum; 2, possible variations from these normal limits in pathological cases; 3, to determine in how far the analysis of the ferments of the stool gave an indication of pancreatic activity.

In regard to establishing the strength of the ferments as normally secreted, Table I represents the limits of variations. It would seem that the pancreatic ferments show fluctuations of strength from day to day even under identical conditions, but that such fluctuations may be said to be within limits. However, the occasional failure to detect amylase or lipase in seemingly normal secretions must be noted. These ferments show the greatest variability in strength, and may apparently be occasionally absent. The protease is the most constant and is always present. In spite of the arguments against casein as a test of trypsin alone, I would hold that erepsin though present, is never sufficiently strong to interfere with the test as an index of pancreatic trypsin.

2. Of the pathological cases examined, the case of acute pancreatitis shows decided diminution in the activity of the pan-

creatic ferments. The pancreatic gland was found decidedly diseased at autopsy. In case 9 the ferments were absent from the duodenum on one occasion; also absent from the stool. The diagnosis of complete blocking of the ducts was confirmed at autopsy. In case 6 on account of the absence of two of the ferments from the duodenum, a deficient secretion of the pancreas was expected, though the duct was apparently open. At operation a patent duct, but a large swollen inflamed pancreas was palpated.

In case 27 the metabolism studies indicated pancreatic disease. The ferments were found strongly present; the amount of secretion into the duodenum was, however, very scant.

3. The variability of the same strength of the same ferments in the stool is far greater than in the duodenum. The occasional absence of ferment is a more frequent occurrence. In general, where these enzymes are strongly present in the duodenum, they are also demonstrable in the stool. The protease is here no longer reliable as an index of pancreatic trypsin. For in cases 7 and 8 and 25, though a strong reaction for trypsin was obtained in the duodenal contents, this enzyme was not demonstrable in the stool (casein and gelatin tests). However, in case 9 when the protease was absent from the duodenum it was also absent from the stool; and when it reappeared in the former, it also reappeared, though weakly, in the latter. We may conclude that a positive test for trypsin in the stool signifies an open pancreatic duct, a negative test does not necessarily imply that active trypsin is no longer being secreted into the duodenum.

The question is: Can this method of estimating the enzyme strength of duodenal contents be utilized for the diagnosis of pancreatic functional activity? It is certain that it is reliable for ascertaining the patency or non-patency of the pancreatic ducts. More experience with cases of disease in the pancreatic gland is necessary before it will be definitely known whether the method is applicable to the diagnosis of functional activity of this organ. From the few cases in the series offered, I am inclined to believe that this will be accomplished.

The results on the different days are tabulated as follows:

TABLE I

AMYLASE

3/21 1 c.c. duodenal juice hydrolyzes 6 c.c. of 1 % starch solution in 1 hr.
3/28 1 c.c. duodenal juice hydrolyzes 6 cc.
5/13 1 c.c. duodenal juice hydrolyzes 10 c.c.
5/14 1 c.c. duodenal juice hydrolyzes 9 c.c.
5/18 1 c.c. duodenal juice hydrolyzes 24 cc.
5/20 1 c.c. duodenal juice hydrolyzes 30 c.c.

Normal Average = 14.1 c.c.
Normal Limits = 6-30 c.c.

LIPASE

3/21 1 c.c. duodenal contents require 3.9 c.c. N/10 NaOH after 24 hrs.
3/28 1 c.c. duodenal contents require 3.6
5/13 1 c.c. duodenal contents require 0.6*
5/15 1 c.c. duodenal contents require 0.9
5/18 1 c.c. duodenal contents require 1.9
5/26 1 c.c. duodenal contents require 0.9

Normal average = 1.96 c.c.
Normal limits = 0.6 to 3.9 c.c.

*On one occasion no lipase was demonstrable.

ALKALI-PROTEASE

CASEIN TEST

3/21 Duodenal contents in dilution of 1:4000 digests 10 c.c. 0.1% casein sol.
3/28 Duodenal contents in dilution of 1:36,000
5/15 Duodenal contents in dilution of 1:120,000
5/18 Duodenal contents in dilution of 1:5,000
5/20 Duodenal contents in dilution of 1:36,000
5/21 Duodenal contents in dilution of 1:12,000
5/29 Duodenal contents in dilution of 1:12,000

Normal average = 1:32,000
Normal limits = 1:4000 to 1:120,000.

OTHER TESTS

	Fermi 24 hrs.	Gelatin Tubes 48 hrs.	Mett Tubes	Albumin Cubes
3/21	3.5 mm.	6 mm.	2 mm.	Slight rounding
3/28	8 mm.	11 mm.	1 mm.	
5/18				Much digested
5/20	5 mm.	10 mm.	1 mm.	All digested
5/26	8 mm.	14 mm.		
5/29	10 mm.	15 mm.		

Normal Average 7 mm. 11.2 mm.
Normal Limits 3.5-10 mm. 6-15 mm.

		Duodenum									Stool			
					Amylase	Lipase	Casein	Fermi	Mett	Cubes	Amylase	Lipase	Casein	Fermi
	Group A													
Case 1*	Ac. Pancreatitis	10 c.c.	acid	bile	0	1.8	0				0	.2	0	
	Group B													
Case 2*	Cholelithiasis	7 c.c.	acid	bile	10	0	1:58,000							
Case 3*	Cholelithiasis	15 c.c.	acid	bile	12	6.3	1:12,000							
Case 4*	Cholelithiasis	35 c.c.	acid	bile	30	10	1:1,000	3	0		0	.1	1:700	
Case 5*	Cholelithiasis	10 c.c.	neutral	bile	24	3.6	1:18,000	10	2	+	10	0.25	1:50,000	2
Case 6*	Cholelithiasis (Before oper.)	27 c.c.	acid	bile	0	0	1:5,000	10						
	Group C													
Case 7	Obstr. Jaundice New Growth	12 c.c.	neutral	0	4	2.4	1:4,000	3	½	+	0	.3	0	0
Case 8*	Obstr. Jaundice Stricture	10 c.c.	acid	0	30	0	1:4,000	3 5			½	1.0	0	0
Case 9*	Obstr. Jaundice (a)	10 c.c.	acid	0	0	0.5	0		0		0	0	0	0
	Duoden. Sarcoma (b) (b) several weeks later	5 c.c.	acid	+	4		1:600	3	0		2	0	1:200	0
	Group D													
Case 10*	Hypertrophic Cirrhosis of Liver	25 c.c.	neutral	+	27	- -	1:18,000	7	0	+	8	1.5	1:3000	0
Case 11	Hypertrophic Cirrhosis of Liver	50 c.c.	acid	+	15	1.0	1:,4000	1	0					
	Group E													
Case 12*	Retroperitoneal Hodgkin's Dis.	40 c.c.	acid	+	0	5.6	14:,000	8	1	+ +				
Case 13*	Abdominal Sarcoma	12 c.c.	neutral	+	4	3.3	1:500	4	1.5		10	.15	1:1700	0

Note: Cases marked * confirmed by operation or autopsy.

TABLE II (Continued)

					Duodenum						Stool			
					Amylase	Lipase	Casein	Fermi	Mett	Cubes	Amylase	Lipase	Casein	Fermi
	GROUP F													
Case 14	Chronic Colitis	12 c.c.	neutral	+	0	0.6	1:12,000							
	GROUP G													
Case 15	Duodenal Ulcer	20 c.c.	acid	+	20	—	1:6,000				+ +	1.5	1:5000	
Case 16	Gastric Neurosis	—	Alk.	+	+	—	+ +				0	0	1:5000	
Case 17	Gastric Neurosis	8 c.c.	acid	+	0	0	+				1			
Case 18	Duodenal Ulcer	15 c.c.	acid	+	+ + +	0	+ +				5			
Case 19	Duodenal Ulcer (hour-glass)	3½ c.c.	alk.	+	—	2.4	1:1,400	10						
Case 20	Carcinoma Stomach	7 c.c.	acid	+	9	5.4	0		0		0			
Case 21	Achylia Gastrica Simplex	4 c.c.	acid	+	3	8.4	1:4,000	3	½	rennet	6	.1	1:30,000	
	GROUP H													
Case 22	Diabetes	25 c.c.	acid	+	6	4.5	+ + +		2					
Case 23	Diabetes	9 c.c.	acid	+	9	12.6	1:4,000		0					
Case 24	Diabetes	15 c.c.	acid	+	30	2.7	1:6,000	3 7	0	+	1	0	1:700	
Case 25	Diabetes	3½ c.c.	acid	+	1½	6.0	1:4,000	2.5		+	8	4	0	
Case 26	Diabetes { Milk Diet	25 c.c.	acid	+	6	0	1:34	0						
	Diabetes { Fuller Diet		acid		6	.6	1:4,000	15	1	+				
Case 27	Diabetes	3 c.c.	alk.	+	15	1.2	1:500	5 11	1	+	2½	3	1:125	

La biophotogénèse réduite à une action zymasique

MECANISME INTIME DE LA PRODUCTION DE LA LUMIERE PHYSIOLOGIQUE: LUCIFERASE, LUCIFERINE, LUCIFERESCEINE

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Un nombre considérable d'hypothèses a été émis à propos du secret de la merveilleuse production de la lumière par les végétaux et les animaux.

Les désaccords entre les expérimentateurs sont venus souvent, presque toujours même, de ce qu'ils n'ont envisagé que des cas particuliers. C'est ainsi que les anatomistes surtout ont fait jouer chez les insectes, un rôle capital aux trachées que quelques-uns allaient jusqu'à comparer à des tuyaux de forge embrasant le protoplasme!

Ils ne songaient pas, sans doute, que l'immense majorité des êtres lumineux n'ont pas de trachées et que l'oeuf de l'insecte photogène lui-même brille avant même d'avoir été fécondé, d'une luminosité qui lui est propre, comme je l'ai jadis démontré.[1]

C'est pour remédier aux graves inconvénients des études partielles qu'à; la suite de mon ouvrage sur les ELATERIDES LUMINEUX[2] j'ai entrepris une étude générale de la question de la BIOPHOTOGENESE ou production de la lumière par les végétaux et les animaux, j'ai consulté à peu près tous les documents connus et j'ai pu combler expérimentalement ou par l'observation personnelle un grand nombre de lacunes existant dans ce beau chapitre de la physiologie générale.

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[1]De la fonction photogénique chez les oeufs du Lampyre (Bull. Soc. Zool. de France T. XII 1887).

[2]Thèses de la Faculté des Sciences de Paris et Bull. de la Soc. Zool .de France, 1886 (ouvrage couronné par l'Institut de France, grand Prix des Sciences Physiques).

Cette étude d'ensemble a présenté pour moi deux grands avantages:

1°.—J'ai pu montrer que le mécanisme intime de la Biophotogénèse est le même partout, chez les animaux et chez les végétaux.[1]

2°.—J'ai, en outre, pu choisir dans toute la série des êtres vivants ceux qui présentent le plus d'avantages au point de vue de l'expérimentation: c'est un mollusque lamellibranche, la PHOLADE DACTYLE qui nous a fourni les éléments de recherche les plus importants.

La plus grande difficulté pour les recherches d'ordre Chimique ayant trait à la biophotogénèse est l'infime quantité de substances photogènes contenue dans l'animal ou le végétal lumineux, qui, bien souvent, la consomme au fur et à mesure de sa production, comme c'est le cas des êtres où la lumière est continue (Champignons supérieurs photobactériacées).

Dès 1885, j'avais établi que chez les insectes, la production de la lumière se poursuit pendant un certain temps après que l'on a fait disparaitre toute trace d'organisation cellulaire,[2] en outre j'avais séparé deux substances qui ne brillaient ni l'une de ni l'autre au contact de l'air, quand elles étaient séparées, mais qui émettaient de la lumière quand on les mélangeait.[3] Il n'y avait pas d'oxydation *directe*, bien que la présence de l'oxygène fut nécessaire à l'exercice de la fonction photogène.

Chez l'animal entier (Pyrophorus Noctilucus) ou dans l'organisme lumineux considéré isolément, je reconnus de plus, en 1886, que l'une des deux substances photogènes se comporte comme une Zymase[4] et que, dans son essence même le phénomène ultime, fondamental de toute lumière physiologique est, en dernière analyse, réductible à un processus zymasique.

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[1]Leçons de physiologie générale et comparée, Paris 1898, et traité de physique biologique T. II Paris, Masson 1903. Dictionnaire de physiologie de Richet art. production de LA LUMIERE PAR LES ETRES VIVANTS, Alcan 1912.

[2]V. Elatérides lumineux.

[3]Loc. cit.

[4]Loc. cit.

Plus tard, j'ai pu établir que la Zymase photogène à laquelle ja'i donné le nom de LUCIFERASE, est une péroxydase et qu'elle peut, dans la réaction photogène être remplacée par un peu d'eau oxygénée ou de permanganate de potasse.

La détermination de la nature du second principe photogène, auquel, j'ai donné le nom de LUCIFERINE était particulièrement difficile à établir au moyen des insectes dont les organes lumineux sont de petites glandes à Sécrétion interne (R. Duboix).

Il n'en est plus de même avec la Pholade dactyle qui secrète extérieurement un abondant mucus lumineux et dont le siphon renferme, en outre, en réserve une forte portion de substances photogènes.

On peut résumer de la façon suivante les expériences que j'ai faites autrefois et que j'ai répétées en les complétant et en rectifiant certains points dans ces temps derniers.[1]

(a) Le siphon de la Pholade dactyle avec ses glandes lumineuses est fendu et sèché au soleil. Longtemps après cette opération (plusieurs semaines) on peut rallumer la lumière éteinte dans les glandes en humectant d'eau le siphon dessèché;

(b) Au lieu de déssècher à l'air libre les siphons, on les fend et on les enrobe, encore frais, dans du sucre en poudre fine: ils cessent de briller;

(c) Les siphons confits ainsi conservent pendant plusieurs mois le pouvoir de fournir un liquide très lumineux quand on les fait macérer dans l'eau pendant quelques instants;

(d) le sirop qui résulte de la fonte d'une partie du sucre dans le liquide rejeté par les siphons frais conservés à l'abri de la lumière a donné encore au bout de huit mois un liquide lumineux par son mélange avec trois ou quatre parties d'eau ordinaire;

(e) si l'on introduit dans une théière en grès des fragments de siphons frais ou conservés dans le sucre et que l'on verse dessus de l'eau bouillante, qui par son contact avec le vase et les fragments de siphons, tombe repidement à 70° environ, on obtient un infusum non lumineux;

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[1]Nouvelles recherches sur la lumière physiologique C. R. Ac. des Sc. t. 153 p. 690, Paris 1911.

(f) ce liquide ne brille pas par agitations en présence de l'air: C'est le liquide A;

(g) si, d'autre part, on fait macérer dans de l'eau salée tiède, en agitant de temps en temps des fragments de siphons confits, on obtient un liquide lumineux qui finit par s'éteindre et ne plus briller au contact de l'air par agitation, c'est le liquide B;

(h) si l'on melange les deux liquides A et B la lumière apparait;

(i) L'action photogène du liquide B peut être remplacée par une parcelle de permanganate de potasse ou par un peu d'eau oxygénée neutre;

(j) Si l'on chauffe à 100°, et même à une température peu supérieure à 70° le liquide A, ne donne plus aucune lumière avec le liquide B, ni par le permanganate de potasse ou par l'eau oxygénée: il s'est formé par la chaleur dans liquide A un précipité floconneux;

(k) Il se produit aussi des flocons de coagulation quand on chauffe le liquide B, mais on constate en outre que vers 60°, il perd définitivement tout pouvoir photogène;

(l) La réaction photogène s'opère donc entre deux substances coagulables par la chaleur dont l'une est détruite à 70° et l'autre vers 60°. Si l'on porte à l'ébullition le liquide ou la réaction lumineuse a commencé à se produire et où elle se continuerait à froid pendant longtemps, elle est aussitôt supprimée;

(m) les deux substances photogènes des liquides A et B présentent tous les caractères chimiques et physiques des substances protéiques;

(n) la substance active de A renferme du phosphore et présente les caractères des nucléoprotéines, je lui ai donné le nom de LUCIFERINE;

(o) l'ammoniaque liquide active fortement la réaction photogène. Dans le liquide où s'est opéré la réaction se déposent des cristaux de phosphates. Si à l'ammoniaque on ajoute du sulfate de magnésie, on constate pendant la réaction l'apparition de cristaux de phosphate ammoniaco-magnésien;

(p) Les Siphons frais, sèchés ou confits ne renferment aucune substance LOPOIDE photogène.

(q) La substance active A peut être isolée sans perdre son pouvoir photogène par précipitation à l'aide d'une solution faible d'acide picrique, dont elle doit être séparée immédiatement par filtration. Le précipité recueilli sur le filtre et repris par l'eau brille avec le permanganate de potasse;

(r) toutes les causes physiques ou chimiques qui favorisent, retardent, entravent ou suppriment les réactions zymasiques agissent de même sur le mélange de A et B

(s) Le principe actif de B jouit des propriétés générales des Zymases; il présente en outre les caractères d'une péroxydase, je lui ai donné le nom de LUCIFERASE.

Cette péroxydase n'est pas spéciale aux organismes photogènes, car on peut provoquer la lumière dans le liquide A renfermant la luciférine au moyen du sang de divers animaux à sang froid (Mollusques, Crustacés marins).

(t) Je n'ai pas, au contraire, rencontré de luciférine malgré de nombreuses recherches, en dehors des animaux photogènes.

(u) Le sirop photogène résultant du contact du siphon avec le sucre en poudre est louche; au bout de plusieurs mois de repos dans l'obscurité, on voit monter à sa surface une couche crêmeuse brun jaûnatre. On y trouve en abondance des granulations semblables à celles que l'on rencontre partout dans les organes photogènes; par leur contact avec l'eau, ces granulations prennent la forme de VACUOLIDES découvertes par moi en 1886. Ces éléments actifs *ultimes* de la matière vivante ou bioprotéon ne sont autre chose que ce que l'on a nommé depuis " mitochondries; " le nom de vacuolide que je leur ai donné il y a un quart de siècle est préférable à celui de mitochondrie, en ce sens qu'il indique nettement la nature morphologique de ces *bioultimates* d'une part et leur mode de fonctionnement d'autre part. Ils sont analogues pour toutes les *macrozymases* dont la *purpurase* est le type.[1]

En résumé: *le phénomène fondamental auquel peut être réduit, en dernière analyse toute réaction photogène chez organismes vivants résulte du conflit d'une peroxydase, la " Luciferase " avec*

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[1]V. Les vacuolides de la purpurase et la théorie vacuolidaire. C. R. ac. des Sc. T. CLIII p. 1507, 1912.

une matière protéique phosphorée la "Luciferine." Il s'agit donc d'une réaction Zymasique produisant une Oxydation INDIRECTE.

A côté de ces substances, j'en ai rencontré dans les organes photogènes du Pyrophore une autre qui joue aussi un rôle dans le fonctionnement photogénique, mais seulement un rôle de perfectionnement. C'est une substance *fluorescente* à laquelle est dû l'éclat si particulier de la lumière de ces beaux insectes des Antilles. Elle transforme des radiations obscures en radiations éclairantes, ce qui offre plusieurs avantages: 1°.—de diminuer l'énergie perdue en radiations non éclairantes; 2°.—d'éviter l'action nuisible d'une partie des rayons ultra-violets; 3°.—d'accroitre le pouvoir éclairant en lui donnant des qualités spéciales. Je lui ai donné le nom de PYROPHORINE. Je n'ai pu déterminer exactement sa nature et sa composition en raison de la très petite quantité que l'on en trouve dans les Pyrophores, mais il est probable qu'il s'agit d'une glucoside ou peut-être d'un alcaloide. L'acide acétique lui fait perdre sa fluorescence, mais l'ammoniaqué la lui restitue. On peut recommencer plusieurs fois de suite, comme si la pyrophorine formait avec l'acide acétique un sel non fluorescent. J'ai rencontré aussi, plus tard, une substance fluorescente donnant dans l'ultra-violet une belle fluorescence bleue chez un lampyride LUCIOLA ITALICA.[1]

Dernièrement M. M. Ives et W. Coblentz,[2] qui vraisemblablement ignoraient mes travaux ont trouvé également une substance présentant une belle fluorescence bleue. Chez un lampyride américain (Photinus pyralis) et ont pensé à tort que la priorité de la découverte d'un principe fluorescent chez les insectes lumineux leur appartenant.[3]

M. McDermott a signalé aussi la présence d'une matière fluorescente chez divers autres lampyrides américains.[3]

Ce dernier s'inspirant des termes de LUCIFÉRASE et de LUCIFÉRINE dont je me suis servi a proposé pour désigner le principe

[1]Rech. sur la Pourpre et s quelques pigments animaux. Arch. Zool. gén. exp. 5ème Série II, 1909.

[2]Luminous efficiency of the Firefly Bul. Bureau of Standards, t.VI n° 3 1910.

[3]De la fluorescence chez les insectes lumineux C. R. Ac. des Sc. et Sur l'Existence et le rôle de la fluorescence chez les insectes lumineux C.R. de l'A.F.A.S. Dijon 1911.

fluorescent des lampyrides américains la dénomination de LUCIFÉRESCEINE dont la terminaison rappelle celle de la FLUORÉSCEINE. Cette appellation me parait très acceptable et peut être étendue à touses les substances fluorescentes qui peuvent se rencontrer chez les animaux photogènes. On dira: les LUCIFÉRESCEINES, et si l'on voulait désigner particulièrement celle du Pyrophore noctiluque, on pourrait dire la *Pyroluciférescéine*, la *Photinoluciférescéine*, etc.

De ces diverses conditions nait une lumière spéciale dont les propriétés physiques ont été fixées exactement par moi en 1886, et les belles recherches de VERY et LANGLEY en Amérique, n'ont fait que confirmer l'exactitude des recherches que j'ai publiées en 1886 sur la lumière des PYROPHORES des Antilles. Cette admirable LUMIERE FROIDE, réalise sur toutes les autres sources, un énorme avantage puisque son rendement est presque de 100 pour 100 alors que pour nos meilleurs foyers, il n'est guère que de 4 à 5 pour cent.; d'ailleurs; d'une manière générale, l'économie des machines vivantes est bien supérieure à celle des autres.

La LUMIERE FROIDE *est la lumière de l'avenir. Celle que produisent les êtres vivants et supérieure à toutes les autres actuellement usitées et son mécanisme chimique intime est aujourd'hui connu.*

(*Recherches sur les Zymases*)

LES VACUOLIDES DE LA PURPURASE ET LA THEORIE VACUOLIDAIRE

PAR M. RAPHAEL DUBOIS

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Dans un récent et très remarquable travail[1] M. Le professeur Grynfeltt a donné une excellente description de la glande à pourpre dans laquelle on peut lire: " Les sphérules élémentaires et les boules granuleuses ont été surtout bien vues par Raphaël Dubois et répondent sans aucun doute à ce qu'il appelle les vacuolides." Mais le savant anatomiste de Montpellier n'a pas cru devoir conserver ce terme que j'avais employé[2] pour désigner les sphérules élémentaires parce que, dit-il, " dans l'esprit de M. R. Dubois les vacuolides sont les parties élémentaires du bioprotéon," tandis que pour M. Grynfeltt "ces sphérules représenteraient non le bioprotéon lui-même, mais des produits de son activité."[3]

Il m'a semblé que cette divergence de vues appelait une explication de ma part.

J'ai toujours, en effet, soutenu que mes vacuolides étaient les parties élémentaires du bioprotéon, c'est-à-dire les unités morphologiques et physiologiques les plus petites qui soient connues. Mes observations m'ayant conduit depuis longtemps[4] à admettre

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[1]Sur la glande hypobranchiale du Murex trunculus (Bibliographie anatomique, t. XXI, fasc. 4, Berger-Levrault et C°, édit Paris).

[2]Recherches sur la pourpre et sur quelques autres pigments animaux (Arch. de Zool. exp. et gén. 5è série, T. II, n° 7 1909. p. 503 et Fig. I).

[3]" J'ai donné le nom de bioprotéon à ce qu'on appelle communément matière vivante et qui n'est pour moi qu'un état particulier, transitoire, de ce principe unique, essentiellement protéique, à la fois force et matière, énergie et substance, qui, par ses innombrables et incessantes métamorphoses, donne à la nature son infinie variété et que j'ai pour cette raison appelé jadis protéon." (Leçons de physiologie générale et comparée, 1898 p. 7).

[4]Leçons de Physiologie générale et comparée, 1898, p. 74-75, Paris.

que les leucites dérivent des vacuolides[1] et, d'autre part MM. Fauré-Fremiet et Guilliermont admettant qu'ils viennent des mitochondries il est superflu de se demander si les mitochondries récentes et les vacuolides anciennes ne sont pas une seule et même chose ou, plutôt, deux formes différentes d'un même élément primordial, la granulation collçidale bioprotéonique, dont j'ai décrit et même figuré la structure et le fonctionnement physiologique dans de nombreuses publications depuis 1887[2] car deux quantités égales à une troisième sont égales entre elles.

La plupart on trait à la luciférase, mais la purpurase se comporte de même. Cette dernière, préparée par le procédé qui m'a permis de la découvrir et de l'étudier, contient en abondance les sphérules élémentaires de M. Grynfeltt, c'est-à-dire les vacuolides zymasiques de M. R. Dubois. Ces dernières ne sont pas des produits de fabrication de la cellule, mais bien au contraire, la partie active, agissante du bioprotéon des cellules purpuripares. On voit nettement au microscope que ces vacuolides absorbent les prochromogènes que j'ai appelés *purpurines* et qu'elles les transforment en chromogènes. Le passage du chromogène à l'état de pigment peut se faire dans la vacuolide ou bien en dehors d'elle, par une simple action chimique provoquée par la lumière (Murex brandaris) ou par la chaleur (Murex Trunculus). C'est le mode de fonctionnement des leucites qui est reproduit ici en plus petit.

Les vacuolides de la purpurase, comme toutes les autres naissent, évoluent et se multiplient de la même manière.

Le bioprotéon, ou matière vivante, est du protéon à l'état colloidal, c'est-à-dire composé de particules en suspension. Dans un microbe, il y en a d'innombrables quantités, suffisantes pour expliquer que ces particules ancestrales suffirtont à assurer le

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[1]Les vacuolides (C. R. de la Soc. de Biol. T. LX, p. 526) et remarque etc. (Ibid. 1906, p. 528.)

[2]Les vacuolides (C. R. de la Soc. de Biol. 8e série, t. IV 1887) Les élatérides lumineux (Bull. de la soc. Zool. de France, Fig. 7 et 8, pl. IX) anat. & phys. de la Pholade dactyle (Ann. de l'U. de Lyon, 2è Fasc., t. II, Pl. XV, 1892); la lumière physiologique (Revue gen. des Sc. p. et App. 1894, P. 532); recherches sur la pourpre et autres pigments animaux (Arch. Zool. exp. et gen. 5è série, t.II, n°7 1909, p. 503, fig. I).

fonctionnement de toute la lignée. Ces particules ne sont pas toujours visibles à l'ultramicroscope; on les voit apparaître là où il n'y avait rien l'instant avant. Ce sont les particules invisibles qui, en se développant et en se multipliant par divisions, fournissent les granulations visibles, mais dont on ne peut encore distinguer la structure; à leur tour, ces dernières deviennent les vacuolides, dont l'une des espèces les plus grosses est celle de la purpurase; à un degré développement de plus élevé, viennent les leucites. C'est pour ce dernier motif, que, dans la Note de l'Académie dans laquelle j'annonçais la découverte de la purpurase[1] j'ai dit que cette dernière était une *macrozymase.* Cette découverte d'une macrozymase, qui montre nettement que les zymases dont des ferments figurés à une grande importance d'abord au point de vue de la mèrphologie; mais ensuite et sur tout parce que *la macrozymase de la pourpre nous a permis de fournir la première explication du mode d'action des zymases pour laquelle, au lieu de faits d'observation, à la portée de tout le monde, on n'avait, jusqu' à nous, apporté que des hypothèses plus ou moins ingénieuses mais sans fondement contrôlable.*

J'ajouterai que la purpurase, suivant des influences diverses, fixation, dessiccation, coagulation, hydratation, coloration, peut prendre de multiples apparences décrites par les auteurs comme appartement au cytoplasme lui-même.

CONCLUSIONS.—*Les sphérules élémentaires décrites par M. Grynfeltt dans les cellules purpuripares sont identiques aux vacuolides de la purpurase de R. Dubois.*

La purpurase n'est pas un produit de l'activité cellulaire, elle résulte du développement de granulations bioprotéoniques actives par accorissement et multiplication. Elle *a toutes les propriétés des zymases et posédé la structure vacuolidaire. Au point de vue morphologique et physiologique ces vacuolides se comportent comme des leucites, lesquels ne sont que des vacuolides amplifiées.*

La purpurase présente, sous certaines influences, beaucoup d'apparences diverses attribuées au cytoplasme lui-même.

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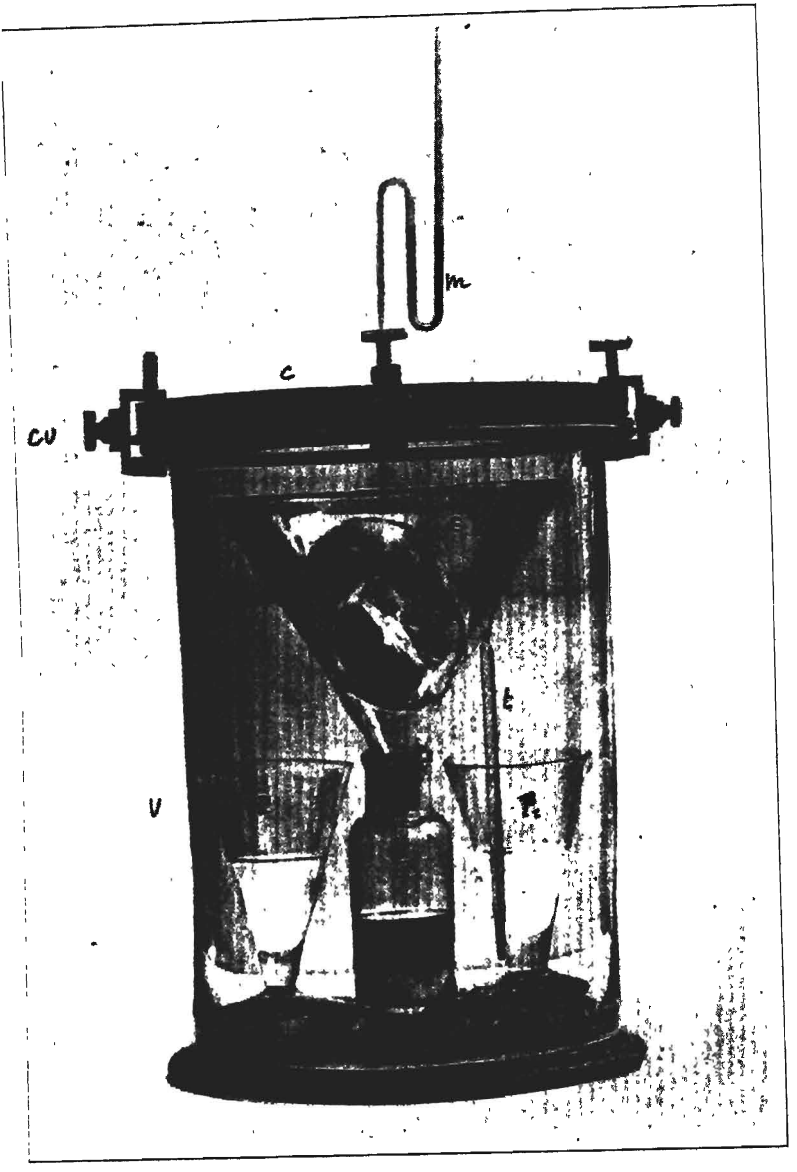
[1]Sur le mécanisme de la formation de la pourpre (Comptes rendus, t.134, 1902, p. 245-247).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PHARMACOLOGIE ET CHIMIE BIOLOGIQUE

ATMOLYSE ET ATMOLYSEUR

PAR M. RAPHAEL DUBOIS

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En raison de l'importance croissante prise en pharmacologie, par les " *intrait* " je crois devoir présenter la note suivante.

J'ai donné le nom d'Atmolyse (atmos. vapeur) à l'action qu'exercent sur la substance organisée les vapeurs des liquides organiques neutres volatils, tels que chloroforme, benzine, éthers, alcools, etc., dont l'inhalation est susceptible de produire l'anesthésie générale.[1]

Les recherches expérimentales, dont j'ai publié les résultats, principalement en 1883 et 1884 dans les Comptes rendus de la Société de Biologie, m'ont conduit non seulement à donner une explication rationnelle expérimentale et très généralement adoptée aujourd'hui du mécanisme intime d'action des anesthésiques généraux; mais encore à montrer les relations étroites, existant entre l'action du froid et celle des anesthésiques,[2] préparant ainsi, par surcroit, l'heureuse application de l'éthérification au forçage des plantes.[3]

Des principes que j'ai découverts sont nés encore d'autres applications qui, dans ces dernières années, ont pris, au point de vue de l'analyse immédiate des tissus organiques et de l'extraction de leurs principes actifs, une grande importance et donné lieu à de nombreuses publications où l'on présente comme des nouveautés ce que j'ai montré il y a un quart de siècle.

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[1]Voir influence des vapeurs anesthésiques sur les tissus vivants (Comptes rendus 1886) et Mécanisme de l'action des anesthésiques (Revue gén. des Sc. p. et app. t. II, 1891, p. 562-565).

[2]Comptes rendus, 26 mai 1912.

[3]Comptes rendus du Congrès de l'A.F.A.S.Lyons 1906.

En outre, on a confondu à tort sous les noms d'éthérolyse, de plasmolyse, d'autolyse, etc., deux procédés absolument distincts, donnant des résultats très différents. L'éthérolyse est le procédé d'extraction des sucs végétaux par immersion dans l'éther liquide, imaginé par Legrip en 1876: ce n'est pas l'atmolyse de R. DUBOIS.

L'expérience suivante montre bien la différence existant entre ces deux méthodes:

On partage deux mandarines en deux et l'on immerge deux des moitiés dans un vase renfermant de l'éther: les deux autres moitiés sont placées dans mon atmolyseur.

Ce dernier se compose d'un vase de verre cylindrique, hermétiquement fermé par un couvercle de verre muni d'un bourrelet de caoutchouc et solidement fixé par des chevalets de cuivre à vis au moyen d'un cadre arrondi de bois dur.

Dans l'intérieur est un entonnoir en verre très évasé, placé sur un flacon destiné à recevoir le liquide atmolysé; de chaque côté sont des récipients destinés à contenir des liquides générateurs de vapeurs atmolysantes, simples ou conjugués. Un manomètre indique la tension des vapeurs et un thermomètre, la température. Les tissus à atmolyser sont suspendus dans l'entonnoir, de façon à éviter le tassement et l'obstruction de la douille.

Dans le flacon d'éther renfermant les moitiés de mandarine, il se forme une couche inférieure aqueuse, colorée, amère et fortement aromatisée par l'essence des cellules de l'épicarpe dissoute par l'éther; c'est le liquide ethérolysé; au contraire, le liquide atmolysé est clair, incolore, sucré et ne contient pas d'essence, celle-ci n'ayant pas été chassée des cellules par les vapeurs d'éther employées comparativement à l'éther liquide.

On pourrait multiplier ces exemples.

Les vapeurs atmolysantes chassent des tissus principalement l'eau[1] qui entraine avec elle surtout des cristalloïdes et parfois même des colloïdes, par exemple, des zymases telles que la luciférase (Dubois), le ferment hépatique (Dastre).

[1]Voir Fonction d'hydratation (Dictionnaire de Physiologie de Richet); Paris, Alcan, 1909.

Les déplacements d'eau et de principes immédiate primitivement séparés dans la même cellule ou dans des cellules différentes peuvent donner naissance à des produits qui ne préexistaient pas à l'état normal et peuvent exercer une action toxique. C'est ce que j'ai montré en 1883 (loc. cit.) pour les graines de moutarde et les feuilles de lauriercerise atmolysées. Les expériences de Guignard[1] de Mirande,[2] d'Heckel (de Marseille),[3] les observations de Demaussy, de Pougnet, de Molisch, cités par Guérin[4] ne sont que la confirmation de mes expériences anciennes et aussi de l'identité de l'action du gel et de celle des anesthésiques généraux que j'ai, le premier, fait connaître. J'ai montré, en outre, que les vapeurs atmolysantes des anesthésiques généraux se fixent de préférence, et s'accumulent par élection dans les parties riches en lipoïdes (jaune de l'oeuf), semences végétales.[5] Cette importante remarque est, en général, à tort, attribué à M. Overton.

L'atmolyse est un phénomène osmotique se produisant par des échanges entre des liquides aqueux et des vapeurs au travers d'un septum plus ou moins dense. Les vapeurs sont d'autant plus atmolysantes pour l'eau que la chaleur spécifique des liquides qui les fournissent est moins élevée, que leur poids atomique est plus considérable et que toutes les propriétés physiques qui varient dans le même sens sont plus accentuées.

Conclusions—*L'atmolyse que j'ai découverte, est le résultat de l'action osmotique exercée sur la substance organisée des tissus par les vapeurs de liquides anesthésiques. Elle ne doit pas être confondue avec l'éthérolyse, la plasmolyse, l'autolyse, etc. Elle constitue un procédé précieux d'analyse physiologique, physicochimique et aussi d'extraction de principes immédiats utilisables en thérapeutique tels que les intraits, etc.*

[1]Comptes rendus, 12 Juillet 1909.
[2]Comptes rendus, 12 Juillet 1909.
[3]Comptes Novembre 1909 et Juillet 1910.
[4]Rev. Sc. du 24 Déc. 1910.
[5]Comptes rend. de la Soc. de Biol. 19 Mai 1883 p. 376.

UEBER EINIGE CHEMISCHE REAKTIONEN DER MIKROORGANISMEN UND IHRE BEDEUTUNG FÜR CHEMISCHE UND BIOLOGISCHE PROBLEME

VORTRAG VON PROFESSOR DR. FELIX EHRLICH

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Dass Mikroorganismen wie Hefen, Schimmelpilze und Bakterien auf einer grossen Anzahl anorganischer und organisher Stickstoffverbindungen bei Gegenwart der sonst noch erforderlichen Nährsalze normal wachsen und mehr oder minder gut gedeihen können, war den Pflanzenphysiologen und Gärungschemikern seit langer Zeit bekannt. Weniger beachtet wurden aber noch vor kurzem die für den Lebensprozess so wichtigen chemischen Vorgänge bei der Stickstoffassimilation deiser kleinen Lebewesen, vor allem die weitgehenden Veränderungen, die während des Wachstums das Nährsubstrat selbst und die darin ursprünlich enthaltenen Stickstoffsubstanzen erleiden.

In Jahre 1905 habe ich zuerst bei Gelegenheit der Aufklärung der Fuselölbildung[1] auf die eigentümlichen tiefgreifenden chemischen Umwandlungen von Aminosäuren durch gärende und assimilierende Hefe nachdrücklich hingewiesen. Aus meinen Untersuchungen ging zunächst hervor, dass die Aminosäuren, die entweder direkt in der Lösung vorhanden sind oder sich durch enzymatische Prozesse aus dem Eiweiss des Nährmediums oder der Hefe selbst abgespalten haben, unter natürlichen Bedingungen und auch unter den Bedingungen der technischen Gärung die eigentlichen wichtigsten Stickstoffnährstoffe der hefe darstellen, was bis dahin keineswegs mit hinreichender Genauigkeit klargestellt war. Der exakte Beweis, dass die natürlich vorkommenden Aminosäuren wirklich von gärender Hefe aus der Lösung aufgenommen und auf Eiweiss verarbeitet werden, gelang

[1] F. Ehrlich, Zeitschrift des Vereins der Deutschen Zuckerindustrie 55, 539-567 (1905).

mit Hilfe der von mir ausgearbeiteten Gärmethode zur asymetrischen Spaltung der Racemverbindung der betreffenden Aminosäuren durch Hefe,[1] bei deren Anwendung in fast allen Fällen eine Aktivierung der vorgelegten Stickstoffsubstanz die tatsächlich erfolgte Assimilation der optisch aktiven in der Natur auftretenden Komponente durch den Hefepilz mit Sicherheit anzeigte.

Von besonderem Interesse für die Kenntnis Physiologie der Mikroorganismen war nun das durch die weiteren Untersuchungen gezeitigte Resultat, dass Hefe bei der Eiweissbildung die Aminosäuren des Nährsubstrates, auf dem sie wächst, ihrem Körperprotein nicht direkt durch Kondensation etwa nach Art der Polypeptidsynthesen Emil Fischers einverleibt, wie man bis dahin anzunehmen geneigt war. Vielmehr liess sich regelmässig beobachten, dass die gärende Hefe das Molekül der Aminosäuren bei der Assimilation spaltet, den dabei freiwerdenden Stickstoff in Form von Ammoniak für ihren Eiweissaufbau verwertet, den grössten Teil des stickstoffreien Moleküls aber in Form von Alkoholen unverwertet in der vergorenen Lösung zurücklässt. Es entsteht auf diese Weise, wie sich zeigte[2], aus Leucin inaktiver Iso-Amylakohol, aus Isoleucin optisch aktiver d-Amylalkohol, aus Valin Isobutylalkohol d.h. die Hauptbestandteile der Fuselöle der Hefegärung bilden sich aus den in grosser Menge in jedem Eiweiss vorkommenden Aminosäuren. Auf Grund dieser Befunde liess sich dann direkt eine Gärungsgleichung für die Fuselölbildung entwickeln, die folgendem Schema entspricht:

$$R.CHNH_2CO_2H + H_2O = R.\ CH_2OH + CO_2 + NH_3$$

Auf ähnliche Weise war auch die Entstehung der Bernsteinsäure bei der alkoholishcen Gärung herzuleiten nur mit dem Unterscheide, dass hier der intermediär aus der Glutaminsäure als Muttersubstanz hervorgegangene Alkahol eine weitergehende Oxydation zur entsprechenden Dikarbonsäure erfährt.[3]

Die für die Fuselölbildung aufgestellte Gleichung wurde nun direkt der Pfadfinder für die Entdeckung einer ganzen Anzahl

[1]F. Ehrlich, Biochemische Zeitschr. **1**, 8-31 (1906); **8**, 438-466 (1908).

[2]F. Ehrlich, Berichte der Deutschen Chemischen Gesellschaft **40**, 1027-1047; **40**, 2538-2562 (1907).

[3]F. Ehrlich, Biochemische Zeitschr. **18**, 391-423 (1909).

von Gärungsalkoholen denn alle α-Aminosäurenunterliegeneinem analogen Abbau durch gärende Hefe genau wie die Ursubstanzen des Fuselöls. Man kann also direkt von einer "alkoholischen Gärung der Aminosäuren" sprechen, die normaler Weise stets parallel neben der alkoholischen Gärung des Zuckers verläuft in dem Masse, wie die wachsende und gärende Hefe den Aminosäuren des Nährmediums den Stickstoff zum Aufbau ihres Körpereiweisses entzieht. Unter den neu aufgefundenen Alkoholen, die man durch Hefegärung sehr leicht und bequem präparativ darstellen kann und die je nach Herkunft mit dem Stammwort der betreffenden Aminosäure benannt wurden, sind mehrere von besonderem Interesse. Wie das Tyrosol (p-Oxyphenylaethylalkohol),[1] das Tryptophol (-Indolylaethylalkohol)[2], das Histidol und andere, deren Untersuchung noch nicht abgeschlossen ist. Offenbar haben viele von diesen Alkoholen als solche oder in Form bestimmter Ester einen hervorragenden Anteil an dem Zustandekommen des Geschmacks und Aromas der gegorenen Getränke, besonders des Bieres und Weines.

Dass die alkoholische Gärung der Aminosäuren auf enzmatische Prozesse ähnlich wie die Zuckergärung zurückführen ist, lässt sich mit grosser Wahrscheinlichkeit annehmen. Die Gesamtreaktion setzt sich scheinbar aus einer Summe von einzelnen Enzymwirkungen zusammen wie Hydratationen, Ammoniak- und Kohlendioxyd-Abspaltungen, etc., die jede für sich wohl gelegentlich beobachtet, die aber in ihrer Gesamtheit bis dahin nicht bekannt waren. Der Nachweis derartiger Enzyme gelang allerdings bisher nicht. Weder abgetötete Hefe noch Hefepressaft vermögen Aminosäuren in Alkohole zu verwandeln.[3] Est ist also anzunehmen, dass es sich hier um sehr empfindliche im Stoffwechsel der Hefe tätige Enzyme handelt, für deren Abtrennung vom Leben der Hefe unsere jetzige Methodik der Fermentforschung noch nicht ausreicht. Veilleicht ist die von C. Neuberg[4] neuerdings entdekte Carborylase, die Ketosäuren wie die Brenztraubensäure in Aldehyde und Kohlendioxyd spaltet

$$R.\,CO.\,COOH = CO_2 + R.\,CHO,$$

[1] F. Ehrlich, Ber. d. Deutsch. Chem. Ges. **44**, 139-146 (1911).
[2] F. Ehrlich, Ber. Deutsch. Chem. Ges. **45**, 883-889 (1912).
[3] F. Ehrlich, Ber. Deutsch. Chem. Ges. **39**, 4072-75 (1906).
[4] Biochem. Zeitschr. **36**, 76 (1911).

eines der vielen bei der alkoholischen Gärung der Aminosäuren wirksamen Enzyme. Die Möglichkeit eines solchen Reaktionsverlaufes, die allerdings erst noch genauer zu beweisen wäre, ist nicht unwahrscheinlich, nachdem O. Neubauer und Fromherz[1] die glatte Vergärbarkeit von Ketosäuren durch lebende Hefe zu denselben Alkoholen wie aus den entsprechenden Aminosäuren gezeigt haben

$$R . CHNH_2 . CO_2H \rightarrow R . COCOOH \rightarrow R . CH_2OH$$

und auf Grund dieser Untersuchungen die Ketosäuren, deren Isolierung auch in einen Falle gelang, als Zwischenprodukte der Bildung von Fuselöl und andern Alkoholen aus Aminosäuren auffassen. Die Ueberführung von Aldehyden in die entsprechenden Alkohole erscheint jedenfalls als enzymatischer Teilvorgang bei der alkoholischen Hefegärung nicht mehr befremdlich, nachdem C. I. Lintner und v. Liebig[2] die Hydrierung von Furfurol zu Furfuralkohol durch gärende Hefe durchführen konnten.

Als besonders bemerkenswert ergab sich dann noch bei meinen weiteren Untersuchungen, dass der Abbau von Aminosäuren zu Alkoholen durch gärende Hefe nur bei Gegenwart von Zucker vor sich gehen kann. Ohne Zucker zu vergären, ist die Hefe nicht imstande, Aminosäuren zu assimilieren oder überhaupt nur anzugreifen, und es hat sich bisher für Kulturhefen keine Substanz ergeben, welche die gärfähigen Kohlehydrate in dieser Hinsicht vollwertig ersetzen kann. Der Zucker bildet offenbar die Energiequelle, mit deren Hilfe die Hefe den Eiweissaufbau vollzeiht, und gleichzeitig das Baumaterial, aus dessen Bruchstücken zusammen mit dem aus Aminosäuren abgespaltenen Ammoniakmolekül bei der Gärung die Synthese des Körperproteins der Hefe erfolgt. Die alkoholische Gärung des Zuckers hat also scheinbar nur die Bedeutung eines allerdings sehr wichtigen Faktors bei der Plasmabildung der Kulturhefen, während die alkoholische Gärung der Aminosäure als eine notwendige Folge dieser Plasmabildung anzusehen ist, hervorgerufen durch das Unvermögen der Kulturhefen, die nach Abspaltung des Ammoniaks beim Eiweissaufbau des Organismus verbleibenden stick-

[1]Zeitschr. f. physiol. Chem. **70**, 326 (1911).
[2]Zeitschr. f. physiol. Chem. **72**, 449 (1911).

stoffreien Reste der Aminosäuren weiter für den Stoffwechselprozess aufzunnutzen.[1]

Durch ausgedehnte Versuche in grossem Masstabe, die ich mit K. A. Jacobsen ausführte, liess sich dann feststellen,[2] dass nicht allein untergärige, obergärige und Wein-Kulturhefen, sondern auch die verscheidensten wilden Heferassen z.B. des Anomalus-, Torula-, Pichlia-Typhus, Kahmhefen, etc., der gleichen Reaktion der Umwandlung von Aminosäuren in Alkohole fähig sind. Wesentlich anders verlief aber die Einwirkung von Schimmelpilzen and ihnen nahestehenden Organismen auf Aminosäuren. Hier wurden Gruppen von Mikroorganismen gefunden, wie Oidium lactis, Mucoraceen, Monilia-Pilze, etc., welche unter sonst gleichen Bedingunegn wie bei der Hefegärung Aminosäuren nicht zu Alkoholen, sondern im wesentlichen zu *Oxysäuren* verarbeiten. ent sprechend der allgemeinen Gleichung:

$$R . CHNH_2 . CO_2H + H_2O = R . CHOH . CO_2H + NH_3$$

Diese Umwandlung verläuft bei einzelnen Schimmelöuilzen wie Oidium lactis und für einzelne Aminosäuren mit aromatischem Kern derartig quantitativ, dass sie zur präparativen Darstellung der betreffenden Oxysäuren, z.B. p-Oxyphenylmilchsäure, Phenylmilchsäure, Indolmilchsäure dienen kann, die auch bei Anwendung von racemischem Ausgangsmaterial stets optisch aktiv erhalten werden. Einzelne Arten wie Monilia candida vermögen aus Aminosäuren sowohl Alkohole wie Oxysäuren zu bilden, andere dagegen wie Penicillium glaucum, Aspergillus niger und manche Mucor-Rassen bauen die ursprünglich entstehenden Oxysäuren weiter zu niedrigermolekularen Verbindungen ab. Von diesem tiefgehenden Abbau werden namentlich die Aminosäuren der Fettreihe wie Leucin, Glutaminsäure, etc., betroffen, während die Aminosäuren mit aromatischer Gruppe wie Tyrosin, Tryptophan, etc., dem Angriff einzelner Schimmelpilze viel länger Widerstand leisten. Auch hier finden sich allerdings einige Penicillium-, Aspergillus-, Arten und bestimmte

[1] F. Ehrlich, Landw. Jahrbücher 1909, V, 289-327, sowie F. Ehrlich, " Ueber die Bedeutung des Eiweisstoffwechsels für die Lebensvorgänge in der Pflanzenwelt " in Herz's Sammlung chem. u. chem.-techn. Vorträge Stuttgart 1911 Bd. XVII.

[2] F. Ehrlich-u. K. A. Jacobsen, Ber. Deutsch. Chem. Ges. **44**, 888-897 (1911).

Bakterien, die Tyrosin bis zum Verschwinden der Millon'schen Reaktion zersetzen d.h. also eine vollständige Aufsprengung des Benzol-Ringes herbeiführen.

Als eine gemeinsame sehr bemerkenswerte Eigentümlichkeit der Schimmelpilze sowohl wie der wilden hautbildenden Hefen stellte es sich nun heraus, dass diese Gruppen von Mikroorganismen ganz im Gegensatz zu den Kulturhefen Aminosäuren selbst dann angreifen und zu Oxysäuren und Alkoholen abbauen, wenn andere Substanzen wie Zucker zugegen sind.[1] Ausser Kohlehydraten können nämlich die betreffenden Organismen auch Verbindungen wie Glycerin, Milchsäure, Aethylalkohol, aber auch viele andere Substanzen der Fettreihe als Kohlenstoff- und Energiematerial für ihren Eiweissaufbau verwerten, wobei aus den Aminosäuren die gleichen Abbauprodukte wie bei Gegenwart von Zucker resultieren. So bildet aus Tyrosin die Heferasse Willia anomala Hansen Tyrosol und Oidium lactis aus derselben Aminosäure p-Oxyphenylmilchsäure in fast denselben Mengenverhältnissen, gleichgültig, ob neben Tyrosin Zucker, Glycerin oder Aethylalkohol als Kohlenstoffmaterial geboten ist. Die Willia-Hefe produziert sogar deutlich, wenn auch in geringen Quantitäten, Tyrosol, wenn nur Methylalkohol oder Amylalkohol neben Tyrosin in der Lösung vorhanden ist. In allen diesen Fällen war auch stets ein deutliches Wachstum des betreffenden Organismus wahrnehmbar. Diese Merkwürdige Erscheinung erklärt, warum bei vielen wilden Heferassen und Schimmelpilzen unter Umständen ein tiefergehender Abbau der zunächst aus den Aminosäuren gebildeten Säuren oder Alkoholen erfolgen kann, da namlich diese primären Abbauprodukte unter entsprechend ungünstigen Bedingungen leicht weitere Verwertung bei der Eiweissynthese der Organismen erfahren. Die Beobachtung, dass gewöhnlicher Alkohol bei der Ernährung hautbildender Hefen und Schimmelpilze einen vollwertigen Ersatz für Zucker bilden kann, gibt überdies eine bequeme Methode an die Hand, besonders empfindliche oder sehr leicht lösliche Stoffwechselprodukte aus Aminosäuren besser und in reinerer Form zu isolieren, da bei Verwendung von Alkohol viele häufig die Isolierung

[1] F. Ehrlich, Biochem. Zeitschr. **36**, 477-497 (1911).

störende Nebenprodukte aus Zucker bei der Aufarbeitung der Nährlösungen wegfallen.

Die eigenartigen Umwandlungen, die Aminosäuren durch Hefen und Schimmelpilze erleiden, ermutigten nun weiterhin dazu, die Einwirkung dieser Mikroorganismen auf andere Organische stickstoffhaltige Substanzen zu studieren. In dieser Hinsicht erschienen zunächst die *primären Amine* von Interesse, unter denen bekanntlich einige bei der Fäulnis von Aminosäuren auftreten. Untersuchungen, die ich gemeinsam mit P. Pistschimuka in dieser Richtung unternahmé,[1] dass diese Verbindungen von wilden Hefen und von vielen Schimmelpilzen fast quantitativ, weniger leicht dagegen von Kulturhefen analog den Aminosäuren in *Alkohole* übergeführt werden, entsprechend der Gleichung:

$$R . CH_2NH_2 + H_2O = R . CH_2OH + NH_3,$$

wobei, von den Kulturhefen abgesehen, statt Zucker ebenfalls Glycerin, Aethylalkohol, etc., als Kohlenstoffnährmaterial dienen können. So wurde Amylamin in Amylalkohol, p-Oxyphenyläthylamin in Tyrosol umgewandelt, und es erscheint besonders interessant, dass letzteres Amin, das nach Bargers Untersuchungen das giftige Prinzip des Mutterkorns bildet und für den menschlichen und tierischen Organismus so gefährliche Eigenschaften besitzt, von Hefen und Schimmelpilzen ohne jede Schädigung ertragen und sogar für den normalen Stoffwechselprozess äusserst günstig verwertet wird.

Die leichte Ueberführbarkeit der Amine in Alkohole durch viele Heferassen legt den Gedanken nahe, ob nicht etwa auch die Fuselölbildung der Hefe aus Aminosäuren einen ähnlichen Reaktionsverlauf nehmen kann, bei dem die Amine die Zwischenprodukte bilden würden zufolge des Schemas

$$R . CHNH_2 . CO_2H \rightarrow R . CH_2NH_2 \rightarrow R . CH_2OH.$$

Da bei der Fäulnis ein Abbau von Aminosäuren zu Aminen unter CO_2-Abspaltung häufig beobachtet ist, so ist die Möglichkeit eines solchen Abbaus auch bei der alkoholischen Gärung der Aminosäure nicht ohne weiteres von der der Hand zu weisen. Wenn Kulturhefen nur in geringem Masse befähigt sind, aus

[1] F. Ehrlich u. P. Pistschimuka, Ber. Deutsch. Chem. Ges. 45, 1006-1012 (1912).

Aminen Alkohole zu bilden, so spricht diese Erscheinung nicht unbedingt gegen das oben skizzierte Abbauschema, da es wohl denkbar erscheint, dass für Kulturhefen die quantitative Durchführung der Reaktion bis zum Alkohol wesentlich von der gleichzeitigen CO_2-Abspaltung abhängt, der vielleicht bei diesen Hefen die Rolle einer besonderen Energiequelle zukommt.

Neuerdings habe ich dann noch gemeinsam mit Herrn Fritz Lange das Verhalten von Hefe und Schimmelpilzen gegen *tertiäre Amine* einer eingehenden Bearbeitung unterzogen. Es erschien hier besonders von Interesse, ob solche Stickstoffverbindungen im Stoffwechsel von Mikroorganismen ausgenutzt werden können und welche Abbauprodukte dabei gebildet werden. Sehr geeignet erschienen für diese Versuche das Hordenin (p-Oxyphenylaethylt dimethylamin) $p\text{-}OH.C_6H_4.CH_2CH_2N(CH_3)_2$ und das Betain (Trimethylamidoessigsäure) $(CH_3)_3N.CH_2.COO$

Das von Léger in den Malzkeimen aufgefundene Hordenin ist interessant wegen seiner nahen chemischen Beziehungen zum Tyrosin, zum p-Oxtphenylaethylamin und auch zum Tyrosol. Das Betain bildet ein wichtiges Abfallprodukt der Zuckerindustrie in der Melasse und Melasseschlempe. Es wird in den letzten Jahren daraus in grossen Mengen technisch nach einem von mir angegenen Verfahren[1] hergestellt und in Form seines Chlorids, das in wässriger Lösung stark hydrolysiert ist, unter dem Namen Acidol oder mit Pepsin trocken gemischt als Acidol-Pepsin in der Pharmazie als Ersatz für flüssige Salzsäure heute viel benutzt. Das Betain als solches ist gegen chemische Angriffe sehr widerstandsfähig, selbst gegen Königs-Wasser. Nach den Untersuchungen vieler Physiologen wird es vom tierischen und menschlichen Organismus so gut wie garnicht ausgenutzt, und erscheint zum grössten Teil in Harn wieder. Auch über die Verwertung des Betains durch Mikroorganismen liegen bisher nur ganz vereinzelte Angaben vor, nach denen nur sicher gestellt erscheint, dass Brennerei-und Brauereihefen auf Betain nicht gedeihen können.[2]

Durch unsere Versuche haben wir nun zunächst ermittelt, dass sowohl Hordenin wie Betain vorzügliche Stickstoffnährmit-

[1]Deutsches Reichspatent No. 157173.

[2]Stanek, Zeitschr. f. d. ges. Brauwesen **30**, 566 (1907).

tel für eine ganze Reihe von Mikroorganismen bilden und zwar im wesentlichen für dieselben, die auch primäre Amine angreifen, wobei-, Alkohol ebenso wie Zucker als Kohlenstoffmaterial dienen kann. Hierunter gehören vor allem hautbildende und Kahmhefen, wie Willia anomala, Pichia farinosa und membranefaciens, Monilia candida sowie eine ganze Anzahl von Schimmelpilzen wie Oidium lactis und lupuli, Penicillium-und Aspergillus-Arten, Epicoccum purpurascens, Citromyces Pfefferianus, etc. Näher verfolgt wurde der Abbau, den die tertiären Amine durch das Wachstum der Heferasse Willia anomala erleiden. Hierbei zeigte sich merkwürdiger Weise, dass auch in diesen Fällen eine Ersetzung der Amingruppe durch die Hydroxylgruppe stattfindet, und dass bei der Assimilation von *Hordenin* fast quantitativ *Tyrosol*, bei derjenigen von *Betain* deutlich nachweisbar *Glykolsäure* auftritt zufolge der Gleichungen

$$OH \cdot C_6H_4.CH_2CH_2\ (CH_3)_2 + H_2O = OH.C_6H_4.CH_2.CH_2OH + NH(CH_3)_2$$

$$(CH_3)_3N.CH_2COO + H_2O = CH_2OH.COOH + N(CH_3)_3$$

In den Hordenin-und Betain-Lösungen, auf denen die Hefe gewachsen war, liess sich nun aber weder Dimethylamin, noch Trimethylamin nachweisen. Diese Amine scheinen durch Wasseranlagerung einen weiteren Abbau erfahren zu haben unter Bildung von Ammoniak und Methylalkohol etwa im Sinne der Gleichung

$$N(CH_3)_3 + 3H_2O = NH_3 + 3CH_3OH.$$

Auch das hierbei entstehende Ammoniak war in den Lösungen nicht auffindbar. Ganz analog wie bei der Assimilation der Aminosäuren dient offenbar das intermediär abgespaltene Ammoniak für die Eiweissynthese der Pilze, wobei wahrscheinlich gleichzeitig der nebenher gebildete Methylalkohol durch weitere Oxydation ebenfalls Verwertung findet. Hierfür liessen sich triftige Beweise aus dem Verhalten von Willia anomala gegen Trimethylamin und Ammoniak herleiten. Diese Heferasse wächst nämlich auf den anorganischen Salzen dieser beiden Basen sehr üppig, wenn ihr gleichzeitig Zucker oder Aethylalkohol geboten ist, zeigt aber auch deutliche, wenn auch nicht so starke Vegetation, sobald nur Methylalkohol als einzige C-Quelle zugegen ist. Der direkte Nachweis von Ammoniak als Zwischen-

produkt des mikro-biochemischen Abbaus tertiärer Amine gelang dann noch beim Penicillium glaucum, das schon nach kurzem Wachstum auf Betain oder Trimethylamin-Lösungen wahrnehmbare Mengen Ammoniak produziert.

Bei meinem mit F. Lange unternommenen Versuchen bin ich dann noch einen wesentlichen Schritt weitergegangen. Wenn tertiäre und auch quaternäre Aminverbindungen wie das Betain als N-Quelle für Mikroorganismen Bedeutung haben, so war zu erwarten, dass auch *ringförmige Stickstoffverbindungen* in dieser Hinsicht irgendwie in Betracht kommen mussten. In der Tat zeigte sich, dass Pilze wie Willia anomala, Oidium lactis, Pichia farinosa, Penicillium glaucum in mehr oder minder ausgeprägter Weise deutliches Wachstum auf Lösungen von Pyridinphosphat, Piperidintartrat, Cinchoninsäure, etc., zeigen. Besonders überraschend war, dass dieselben Hefen und Pilze auch teils stärker, teils schwächer normale Zellbildung auf einer ganzen Reihe von *Alkaloiden* erkennen lassen z.B. auf Coniin, Chinin, Cocain, Brucin, Nicotin. Wenn es auch hier bisher noch nicht gelang, bestimmte Stoffwechselprodukte bei Verarbeitung der Alkaloide durch die Pilze abzuscheiden, so steht doch zu erwarten, dass unter Einhaltung gewisser Konzentrationen der Nährlösungen bei manchen verhältnismässig üppig wachsenden Schimmelpilzen wie den Penicilliumarten einer solchen Isolierung wohl möglich sien wird. Vielleicht ist hiermit den organischen Chemikern ein neues Hilfsmittel an die Hand gegeben, die Konstitution mancher chemisch noch unbekannter Alkaloide oder ihrer Abbauprodukte näher dadurch zu erforschen, dass man auf den Lösungen der Alkaloide bestimmte Mikroorganismen zur Vegetation bringt und aus der Art des Wachstums und der danach isolierten Spaltungsprodukte Schlüsse auf die Bindungsform des Stickstoffs und die chemische Struktur des betreffenden Alkaloids zieht. Wenigstens haben schon eine Reihe von Vorsersuchen ergeben, dass je nach den Bindungsverhältenissen des Stickstoffs der Angriff von Alkaloiden durch Mikroorganismen sehr verscheiden erfolgt, sodass z.B. Nicotin, das einen leicht aufspaltbaren Pyrrolidin-Ring enthält, eine wesentlich günstigere Stickstoffnahrung für die Pilze bildet als Alkaloide mit fester gefügter Stickstoffgruppe wie Chinin, Cocain, etc.

Wenn auch zur Aufklärung dieser eigenartigen Beziehungen noch sehr eingehende Arbeiten erforderlich sein werden, so ermutigen doch schon die hier mitgeteilten Resultate die verschiedenen chemischenen Reaktionen, deren die Mikroorganismen fähig sind, mehr als bisher zur Erforschung organisch-chemischer Probleme heranzuziehen. In dieser Hinsicht dürfte namentlich die Eindeutigkeit interessant erscheinen, mit der in ganz heterogen zusammengesetzten organischen Stickstoffverbindungen wie primären, tertären Aminen, Aminosäuren, etc., durch sehr viele Arten von Mikroorganismen regelmässig und in zahlreichen Fällen fast quantitativ ein Ersatz des Stickstoffs durch die Hydroxylgruppe erfolgt. Zweifellos werden sich diese Reaktionen in verschiedenster Richtung noch variieren lassen und ähnlich wie jetzt zur präparativen Darstellung von manchen sonst schwer zugänglichen Alkoholen mittels Hefe und Oxysäuren mittels Oidium lactis wird man die biochemische Wirkung der Mikroorganismen noch für die Gewinnung vieler anderer organischer Substanzen vorteilhaft ausnutzen können. Das Arbeiten mit Mikroorganismen sollte daher mehr als es bis jetzt geschehen ist, zum Rüstzeug der Experimentierkunst jedes organischen Chemikers gehören. Die Einrichtungen dafür sind ja in jedem chemischen Laboratorium vorhanden oder leicht zu beschaffen, die Bereitung der Nährlösungen und die Reinzucht der Mikroorganismen sind einfache leicht erlernbare Operationen. Ueber-dies kann man hierbei vorteilhaft mit sehr geringen Mengen Substanzen experimentieren und die relativ niedrigen Temperaturen, bei denen die eigentliche Einwirkung der Mikroorganismen erfolgt, verbürgen ausserdem eine möglichst weitgehende Schonung der zu verarbeitenden Substanz und der daraus erhaltenen Produkte.

Dass die Auffindung von chemischen Reaktionen der Mikroorganismen in der Art der hier geschilderten für viele biologische Probleme von Bedeutung zu werden verspricht, geht ja schon aus den obigen Auseinandersetzungen zur Genüge hervor und bedarf daher nicht einer besonderen Hervorhebung. Man wird jetzt allmählich daran gehen können, für die Mikroorganismen mehr wie bisher einer Systematik auf chemisch-physiologischer Grundlage zu schaffen und dabei als Ausgangspunkte für das

verschiedene biologische Verhalten nicht allein die Kohlehydrate nehmen, wie bis jetzt bei den Hefen, sonedern vor allem die für die Plasmabildung so wichtigen Eiweisstoffe, ihre Spaltprodukte, die Aminosäuren, und die daraus entstehenden je nach der Gattung des Organismes verschieden gebauten Stoffwechsel-Endprodukte. Weiterhin wird dann die verschiedene spezifische Einwirkung auf andere chemische Substanzen einen Massstab für die Einteilung der verschiedenartigen Rassen und Gruppen von Hefen und Schimmelpilzen bilden können. In dieser Hinsicht sei daran erinnert, wie eigenartig und scharf die Gruppe der Kulturhefen sich von den übrigen wilden Heferassen dadurch abhebt, dass sie im Gegensatz zu diesen Hefen Amine und ähnlich konstituierte Verbindungen sogut wie garnicht für ihren Stoffwechselprozess ausnutzen können, sondern nur imstande sind, Kohlehydrate als Kohlenstoffbausteine für die Eiweisssynthese zu verwenden, während Kahmhefen alle möglichen anderen Verbindungen zu diesem Zwecke heranziehen können.

Schliesslich wird nicht zu bezweifeln sein, dass das weitere Studium der Einwirkung von Mikroorganismen in der hier skizzierten Richtung von grosser Bedeutung für die Aufklärung der Stoffwechselprozesse nicht allein in den niederen, sondern auch in den höheren Pflanzen werden kann. Hier sind es namentlich die Fragen der Entstehung der Riechstoffe und Alkaloide und ihres Schicksals in den grünen Pflanzen, zu deren Klärung Vorarbeiten auf dem Gebiete der Biochemie der Hefen und Schimmelpilze nach dem oben entworfenen Arbeitsplane sicher sehr wesentliche Beiträge liefern werden.

(Abstract)

THE CHEMICAL CHANGES TAKING PLACE IN MILK UNDER PATHOLOGICAL CONDITIONS

By Lewis W. Fetzer

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In 1907 a study was begun in the laboratory of physiological chemistry at the Maryland Agricultural Experiment Station of the chemical changes taking place in the milk of animals suffering from inflammations, other than tuberculosis, of the mammary glands. The work was done in conjunction with the veterinary department and was completed in the fall of 1909. Owing to pressure from other duties and employment elsewhere I have not been able to make a complete report upon this topic until the present time.

As we all know, there are numerous analyses of milks of abnormal composition on record, some of which are stated to be of pathological origin, and others which undoubtedly originate from animals having a mammary gland affection. In practically all instances, however, the analytical data which are presented are incomplete and almost always are confined to the constituents which are reported in commercial analysis, i.e., fat, total solids, solids-not-fat, and possibly lactose. Another feature which has not been taken into account when reporting previously recorded analyses is the stage of the disease at which the sample was obtained. This is a very important point which must be considered at all times, if the results obtained are to be utilized.

We were very fortunate in locating a barn which contained a herd in which mastitis existed. The disease had previously appeared from time to time in this barn without leaving any apparent severe effects upon the animals.

The plan followed in this work was the following one: The overseer of the dairy barn and his helpers were instructed by Dr. S. S. Buckley, the veterinarian of the Experiment Station, to report

to him any abnormality which they might note in the behavior of the animals, and furthermore, any appearance of redness, tenderness and hardness on and in the mammary glands. They were, in addition, instructed to report any change in the color and consistency of the milk and the amount of milk obtained.

Our main purpose in setting up a plan of this character was to enable us to study the milk from these animals throughout the cycle of the disease, i.e., from its inception until the time when the mammary gland appeared clinically normal and the milk had assumed its right appearance. In this connection I will say, however, that a milk that had the appearance of being normal and comes from an udder which has apparently healed is no guarantee that the milk is fit for human consumption.

On receiving the report of the dairyman or milker that such and such a cow showed some unusual peculiarity in regard to its gland or the milk therefrom, the milk was drawn in sterile Erlenmeyer flasks or sterile quart milk bottles. If the sample was taken early in the morning it was placed in a refrigerator and taken in work on the same morning as soon as the laboratory opened. If sampled in the late afternoon the milks were placed in the refrigerator and kept until the following morning, when the analysis was begun. Most of the samples of milk were obtained in the morning. In no case was a milk examined which had taken the form of a jelly or contained a compact jelly-like mass. The reason for this was that we wanted to examine the milks which were most liable to be passed on into the milk supply. The observations made were as follows:

General history of animals:

Breed of animals.
Age.
Number of calves.
Frequency of abortion.
Kind of barn kept in previously.
Whether a good milker or not.

Clinical history of animals:

Date of inception of mastitis; physical condition of mammary glands at the beginning and during the cycle of the disease;

whether one or more quarters were affected; the duration of the disease; and if the quarters affected were finally rendered worthless as far as milk production is concerned.

The Milk:

(a) Odor; consistency; whether it contained flakes or stringy pus in suspension blood, etc., or a sediment.

(b) Microscopical examination—the nature of the sediment obtained with the centrifuge.

This part of the examination was very limited, as we had only a very small laboratory force at our disposal. It consisted of obtaining a differential leucocyte count and observations as to the morphological characteristics of bacteria present.

(c) Chemical examination.

In the chemical work the observations and determinations made were as follows: Reaction, total solids, total nitrogen, total protein (nitrogen X 6.37), casein, albumin, globulin, amids, peptones, ammonia, lactose, fat, cholesterol, lecithin, ash, ash constituents; potassium, sodium calcium, magnesium phosphoric acid, chlorin, sulphuric acid and iron.

Enzym tests: Catalse, peroxidase, oxidase and reductase.

The principal changes noted in the chemical composition of the milk during the pathological process were as follows:

Acidity: Most milks at the beginning of the process showed a diminution of the apparent acidity. This in some instances went on until alkalinity set in, where it remained until the disease began to undergo resolution, after this the acidity gradually rose to its normal point again.

Total solids: In acute cases the total solids were high at the outset, but as the process went on there was a diminution of the same.

Total solids-not-fat: In some instances there was an increase, while in others no marked change took place.

Total nitrogen and protein X 6.38. Increased at the outset and remained increased until resolution took place.

Casein: Diminished in some instances and remained so until the pathological condition was eliminated.

Lactglobulin? (*Serum globulin*): Increased until resolution set in.

Albumin? (*Serum albumin*): Increased during the whole process, then back to normal.

Lactose: Diminished gradually as the process went on, then back to normal.

Fat: Diminished gradually until the fastigium of the process was reached, then increased again.

Cholesterol: Diminished gradually until the fastigium of the process was reached, and then increased again. When calculated on the basis of 100 parts of ether extract it seemed to be increased.

Lecithin: Diminished gradually, then increased gradually as resolution was taking place.

Ash: In some cases an increase took place, but only in a few instances was a very large increase apparent.

Ash constituents: The most characteristic changes taking place in the composition of the ash were an increase in the sodium and chlorin content, a corresponding decrease in the potassium content, and in most instances a decrease in the calcium and phosphoric acid content. In all probability a determination of the chlorin content of the milk, respectively in the ash, will furnish a clue as to whether the milk in question is of pathological origin or not.

THE ARYL ARSONATES: THEIR PHARMACOLOGY CONSIDERED FROM THE EXPERIMENTAL AND PRACTICAL STANDPOINTS

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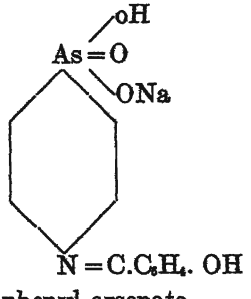
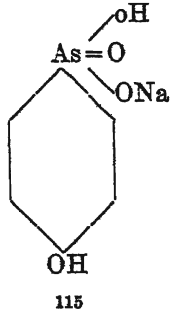
The fact that atoxyl (sodium *para*-amido-phenyl arsenic acid) can cause trypanosomes to disappear from the peripheral circulation is now generally known. Since the introduction of this substance by Thomas and Breinl in 1905[1] a number of allied substances have also been employed both on experimental animals and in practice. Breinl and Nierenstein[2] found that the following bodies had no trypanocidal action in infected animals:

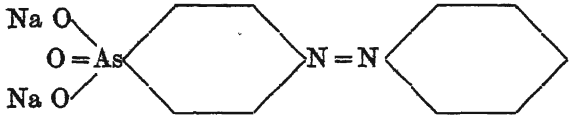
(1) Salicyl atoxyl



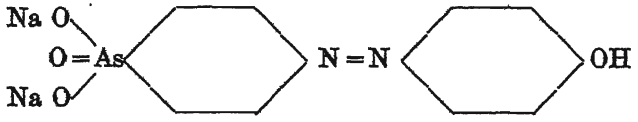
(2) Sodium *para*-hydroxy-phenyl arsenate



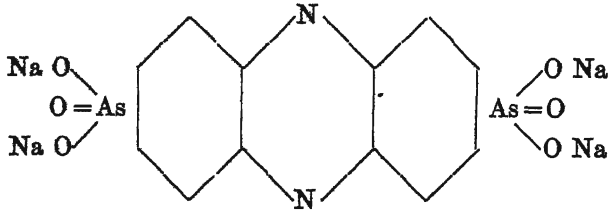
(3) Di-sodium azobenzene 4-arsenate



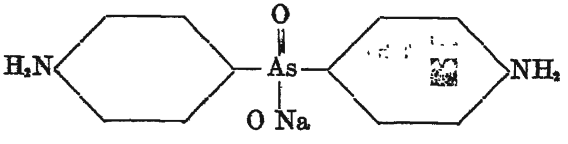
(4) Di-sodium 4-oxy-azobenzene 4-arsenate



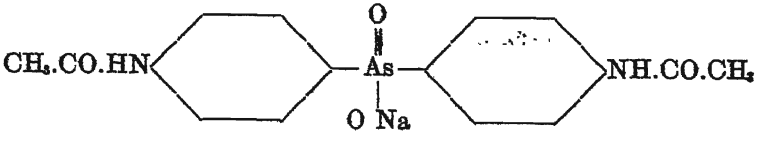
(5) Tetra-sodium phenazine 4-arsenate



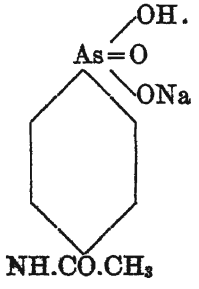
(6) Sodium di-*para*-amido-phenyl arsenate



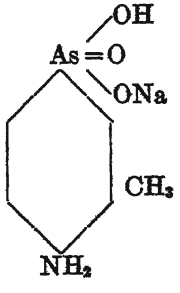
(7) Sodium di-*para*-acetyl-amido-phenyl arsenate



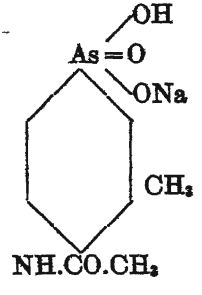
On the other hand (1) Acetyl-atoxyl



(2) Sodium 3-methyl-4-amido-phenyl arsenate (Kharsin)



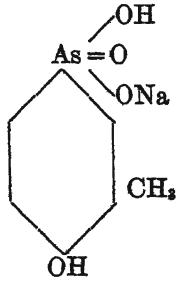
(3) Sodium 3-methyl-4-acetyl-amido-phenyl arsenate (Orsudan)



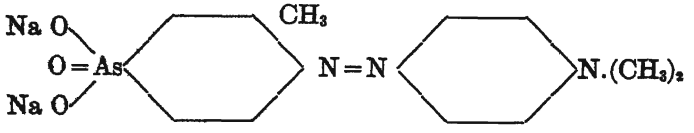
were trypanocidal for certain trypanosomes in experimental animals.

The following derivatives of Orsudan corresponding with the inactive atoxyl derivatives above enumerated had very slight trypanocidal action in the case of the first two, and none at all in the case of the two last in the list.

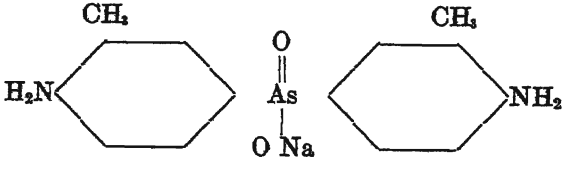
(1) Sodium 3-methyl-4-hydroxy-phenyl arsenate



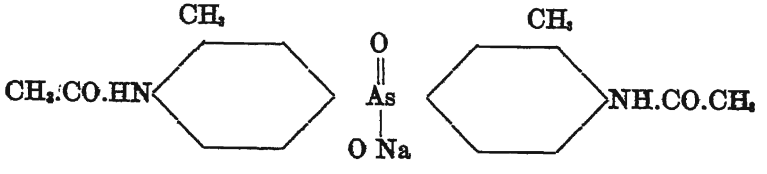
(2) Di-sodium 4-di-methylamido-2-methyl-azo-benzene 4-arsenate



(3) Sodium di-3-methyl-4-amido-phenyl arsenate



(4) Sodium di-3-methyl-4-acetyl-amido-phenyl arsenate



The acetylated compounds were found, generally speaking, to be less toxic. Acetyl-atoxyl is less toxic for animals highly susceptible to atoxyl; orsudan experimentally was without action on *T. Brucei*, but active against *T. Equiperdum* and *T. Gambiense*. Moore, Nierenstein and Todd[3] found that acetyl-atoxyl was of more value in dogs, guinea-pigs and mice infected with *T. Brucei* than the parent substance atoxyl. Salmon[4] came to a similar conclusion with regard to monkeys, fowls and rats.

Relation of chemical structures to physiological action.

An important factor in the production of a trypanocidal effect appears to be the amido group. Mesnil and Nicolle[5] and Moore, Nierenstein and Todd[6] have drawn attention to this, and the latter observers have shown a parallel phenomenon in the case of trypanocidal colouring matters.

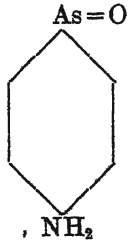
Nierenstein[7] showed that in test tube experiments, mixtures of animal serum and solutions of arsenic compounds which contain the amido group form chemical combinations, whereas in similar mixtures in which arsenic compounds without amido groups are used, no combination with serum proteins occurs.

The substances employed in his experiments were atoxyl, mono-acetyl-atoxyl and mono-benzoyl-atoxyl containing the amido group, and sodium arsenate, acetyl-benzoyl-atoxyl and sodium *para*-hydroxy-phenyl acetate, in which the amido group was either absent or substituted in respect of both the hydrogen atoms. He suggests that the amido group plays the same part as the chromogen group in a dye. The action of this group is apparently in accord with the theory put forward by Loew[8] of the interaction between amido groups with labile aldehyde groups in the living protoplasmic molecule.

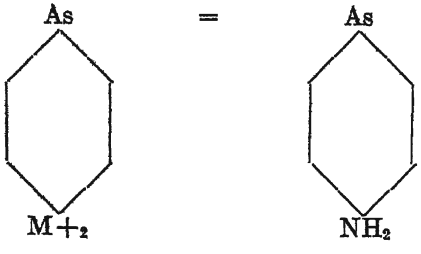
Chemical Changes in the Organism

The chemical changes in the molecule of atoxyl which take place after it has been introduced into the animal body have been variously stated. Ehrlich[9] noting the fact that atoxyl *in vitro*

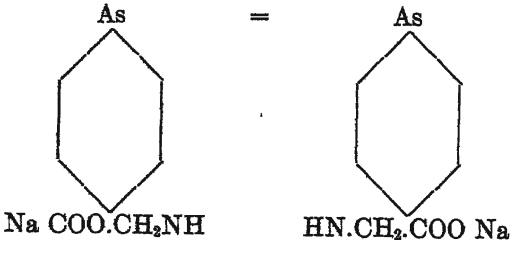
has no trypanocidal action, supposed that in the organism it was changed into a more toxic body. Two reduction products, *para*-amido-phenyl arsenic oxide



and di-amido-arseno-benzol



are trypanocidal *in vitro*, and he assumes that in the protoplasm of the trypanosome a receptor group exists, having a special affinity for the trivalent arsenic. A substitution product of diamido-arseno-benzol, namely arseno-phenyl-glycine



was prepared by Bertheim, and has been somewhat extensively used in experimental trypanosomiasis. Röhl[10] found that it was not only trypanocidal in various animals, but could be used

prophylactically in mice. It was also found to destroy *T. Lewisi*, which are not affected by other arsenic preparations. The success of this preparation, however, is dependent on the species of animal employed, and in large animals, such as donkeys, doses approaching the lethal amount could not arrest the infection. In dogs it is excreted almost quantitatively in the urine, and poisonous doses produce a marked increase in the fat and lecithin content of the blood.[11]

Levaditi and Yamanouchi[12] found that mixtures of liver emulsion, lung emulsion, or muscle emulsion with solutions of atoxyl, after incubation at 38° C. for two hours were powerfully trypanocidal. They explained this by supposing that the protein combined with the reduced arsenic, and enabled it to act on the trypanosomes after the manner of an amboceptor. Friedberger[13] supposed that the SH group in the protein molecule was the reducing agent, and obtained an analogous reaction with thioglycolic acid, which, when added to atoxyl, produced a trypanocidal substance *in vitro*. Other observers, however, have failed to confirm entirely the results of Levaditi and Yamanouchi.

Uhlnhut and Woithe[14] in 1908 obtained negative results, and Breinl and Nierenstein only occasionally got positive results when carefully repeating the original experiments. Holmes, after repeated experiments, came to the conclusion that no action such as that described by Levaditi and Yamanouchi takes place.

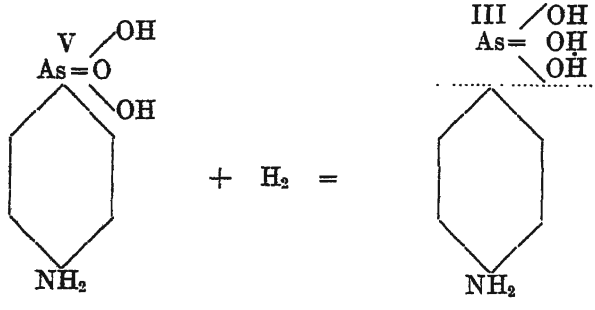
Breinl and Nierenstein,[15] however, in repeating Levaditi's experiments, were able to show that it was only when the filtrate or dialyzate of the atoxyl-liver-emulsion contained inorganic arsenic that a trypanocidal effect was obtained. Further, they showed that peroxide of Hydrogen and oxidases obtained from the liver and from vegetable sources, such as black tea, were the active agents in the production of inorganic arsenic from solutions of atoxyl. Tendron[16] and Wedemann[17] have confirmed Nierenstein's experiments by finding inorganic arsenic in the urine after the administration of atoxyl.

In contradistinction, therefore, to the theory of Ehrlich that the trypanocidal action of atoxyl *in vitro* is due to the production of a reduced trivalent arsenic compound, Breinl and Nierenstein

believe that an oxidation of the organic substance occurs, leading to the formation of inorganic arsenic, to which the effect on trypanosomes is due.

Holmes[18] states that his experiments all point to the view that a small amount of cleavage occurs, and that the therapeutic effect is due to the presence of free arsenic. Breinl and Nierenstein also found that a fermentative process occured by which atoxyl was split up into trivalent arsenic and aniline. *In vitro* this did not lead to the production of a trypanocidal substance, unless the amount of trivalent arsenic split off was sufficient to inhibit the action of the reductase.

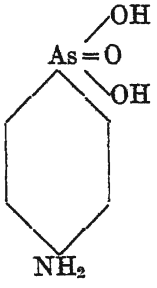
Stated fully, their view is that atoxyl when it enters the animal body is partly combined with the serum proteins by means of the amido group. This combination is then oxidized by ferment action, and the nascent inorganic arsenic exerts a trypanocidal action. At the same time, another portion of the atoxyl is reduced; the aniline is destroyed and the trivalent arsenic excreted in the faeces.



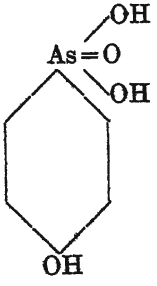
In support of this view, the following experimental evidence has been adduced, in addition to that already stated. (a)Nierenstein[19] showed that when atoxyl, mono-acetyl-atoxyl and mono-benzoyl-atoxyl were injected into rabbits and donkeys, arsenic could be detected in the serum. On the other hand, sodium arsenate and sodium *para*-hydroxyphenyl arsenate produced no arsenic in the blood serum. Acetyl-benzoyl-atoxyl, however, gave rise to arsenic in the serum, as the acetyl group is saponified and a mono-benzoyl-atoxyl is produced. About 80% of the

arsenic could be recovered from the urine, and about 40% from the faeces. Aniline could only be detected in the faeces of the horse and monkey.

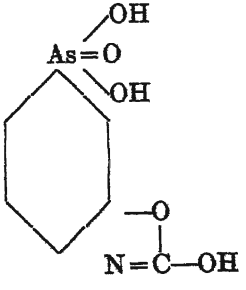
(b) The same author[20] showed that atoxyl was partly excreted in the urine as inorganic arsenic and partly as *para*-amido-phenyl arsenic acid



para-oxyphenyl arsenic acid



Oxy-carbamido-phenyl arsenic acid



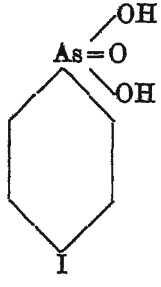
This is in accordance with the theory that atoxyl undergoes an oxidation process in the organism; a similar oxidation of Toluidine into oxycarbamido-cresol has been shown to take place.[20]

(c) Durham,[22] in 1908, published some observations on the colour of the blood in animals in the late stages of infection with *T. Brucei*. He noticed that the blood, instead of being bright red was of a "dull purplish or chocolate colour." Even after exposure to the air for a week or more, it did not regain its normal colour. He suggested that this was due to the presence of methaemoglobin and was analogous to the change in colour produced in some animals' blood by poisonous doses of dinitrobenzol. Naus and Yorke[23] have shown by careful spectroscopic examination that such blood contains partially reduced haemoglobin. Further, they were able to demonstrate that suspensions of living trypanosomes had a reducing action on haemoglobin and methylene blue, and that the incubation of living trypanosomes in the absence of air in normal defibrinated blood caused a reduction or disappearance of the oxygen combined with the haemoglobin. The carbon dioxide was not found to be correspondingly increased.

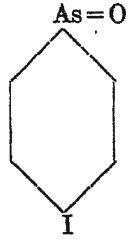
If therefore atoxyl is activated by a reduction process, it should be trypanocidal *in vitro*, which, as is well known, is not the case.[24]

(d) Mameli and Patta[25] prepared the following iodo compounds:—

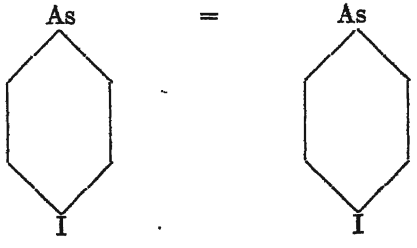
p-Iodo-phenyl arsenic acid



p-Iodo-phenyl arsenic oxide



p-di-iodo-arsenobenzol



Owing to the absence of the amido group, none of these bodies acted on *T. Brucei* either *in vivo* or *in vitro*. In the two latter, the arsenic is trivalent, and according to the reduction theory should exert a trypanocidal action.

Resistant Strains

It has been shown by Ehrlich, and is well recognized, that trypanosomes can be rendered resistant to the action of atoxyl and other drugs when these are injected into an infected animal, and that this resistance in almost all cases is specific, or holds good only for the particular drug used. It has however also been shown by Mesnil and Brimont,[26] Breinl and Nierenstein,[27] and Röhl,[28] that to a certain degree this resistance is also specific for the species of animal employed, and that atoxyl-fast trypanosomes from donkeys, for instance, lose their resistance when injected into rats.

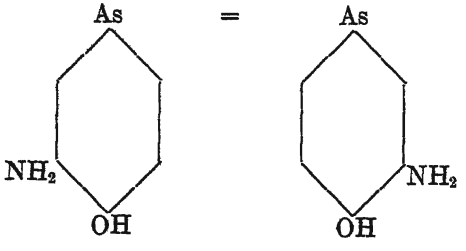
Ehrlich explains this by assuming a decreased avidity of the arseno-receptors of the trypanosomes, but strains resistant to atoxyl have been found to be influenced by arseno-phenyl-glycine and by acetyl-atoxyl. Ehrlich supposes that in the former case, the arseno receptors are not entirely put out of action, and in the latter, that there are present also acetyl receptors capable of linking the organic arsenic compounds to the trypanosomes. In fact, in his view, a number of receptors may exist in the trypanosome capable of linking it to numerous radicles.

The measure of acceptance which this hypothetical explanation gains will depend upon the importance which may be attached to bringing all the experimental results into correspondence with the " side-chain theory."

But it seems clear that the resistance acquired by the trypanosomes is a resistance not purely to the atoxyl (or other drug employed), but to the atoxyl-serum of the given species employed in the experiment.

Practical Results

The arylarsonates have been employed in the treatment of various diseases caused by Trypanosomes, both alone and in combination with other drugs. They have also been extensively employed in the spirochaetal infections, the spirochaetes, as a biological group, being held intermediate between the obviously protozoan trypanosomes on the one hand, and the bacteria—which are regarded as vegetable organisms—on the other. With regard to spirochaetes, however, recent work has been mainly concerned with the investigation of *p*-dioxy-*m*.-di-amido-benzol or salvarsan.



which has obtained a dominant position among the arsenic compounds used in the treatment of syphilis.

To deal with the practical results obtained by this preparation, alone requires a large volume; it is possible, however, and may not be without interest briefly to summarize here the position which the earlier drugs have attained as remedies for various forms of trypanosome infection in man and animals.

The most important pathogenic varieties of trypanosomes are *T. Brucei*, causing nagana or tsetse fly disease in cattle and other animals; *T. Gambiense* causing " sleeping sickness " in man; and *T. Evansi* causing " surra " in horses and other animals.

(1) *T. Brucei.* Numerous experimental results have been obtained with this organism, as it multiplies rapidly in the blood of small laboratory animals and is thus a convenient member of the group for observation. The immediate effect of the injection of atoxyl is almost always favourable, but the trypanosomes recur in the blood after a longer or shorter interval in spite of repeated injections and very few permanent cures have been obtained. In practice, and also experimentally, better results have been obtained by the combination of atoxyl with various dyes or with mercury salts. The theoretical explanation of this fact appears to be either that a certain number of trypanosomes which have been subjected to the atoxyl treatment survive and become immune to the drug, but not to some other drug, such as trypan-red or mercury; or that in the organism developmental forms of trypanosome occur which are not affected by arsenic compounds, but which are susceptible to other trypanocides.

(2) *T. Gambiense.* The long course of the infection by this parasite makes observations on the ultimate effect of drugs very difficult. In spite of earlier reports of successes by means of atoxyl in human beings, some authorities doubt whether a case has ever been cured. Mott,[29] who has recently reviewed the whole question, inclines to the view that possibly, if the diseases is treated before the organisms have invaded the subarachnoid space, a cure can take place. He describes a *post-mortem* on a case which had been very energetically treated with atoxyl, and in which there was no evidence of involvement of the central nervous system. Death had occurred from intercurrent disease

of another character. The difficulty appears to be that it is at present impossible to determine at what point, in the clinical course of the infection, the parasites (or their toxins) have already caused such damage to the nervous structures that the lesions are likely to be progressive, even if all the trypanosomes have been destroyed. Mott quotes the opinion of Hodges, based upon the observation of over 5000 cases, that the course of the disease is undoubtedly modified, if not cut short, by the administration of atoxyl and its derivatives.

(3) *T. Evansi.* Many authors have reported a certain percentage of successes in the treatment of surra in horses and mules with various forms of arsenic, sometimes combined with other drugs. The most successful results appear to have been obtained by a combination of atoxyl subcutaneously with arsenious acid by the mouth. The atoxyl has the effect of rapidly causing the trypanosomes to disappear from the peripheral circulation, and is the best method of introducing arsenic by injections, as it causes no local lesions. The continued exhibition of arsenic which is necessary in these cases can be conveniently and more effectively carried out by the inorganic form of arsenic given by the mouth. In some cases, more than 70% of animals naturally infected have been permanently cured by this method.

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[21]Fraenkel. Die Arzneimittelsynthese. Bd. II, p. 147.
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(*Abstract*)

THE UTILIZATION OF INGESTED PROTEIN AS INFLUENCED BY UNDERMASTICATION ("BOLTING") AND OVERMASTICATION ("FLETCHERIZING")

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The subjects of the investigation were two young men (laboratory assistants) weighing 63.0 kg. (J) and 58.3 kg. (F) respectively at the commencement of the experiments. The study was divided into four periods as follows, each period being seven days in length: (1) normal, (2) bolting, (3) Fletcherizing, (4) normal. A uniform diet was fed each subject throughout the course of the test. The diets as fed were as follows:

Subject "F"			Subject "J"		
Meat	185	11.21	Meat	215	13.03
Graham Crackers	150	1.93	Graham Crackers	150	1.93
Milk	650	3.31	Milk	800	4.07
Butter	150	0.16	Butter	150	0.16
Water	1800	——	Water	2100	——
Sodium chloride	1.6	——	Sodium chloride	2	——
Agar-agar	10	——	Agar-agar	15	——
Total		16.60	Total		19.18

It will be noted that meat contributed the major part of the nitrogenous portion of the diet. This meat consisted of the best "round steak" procurable, which was freed from all visible fat and connective tissue. It was then cut into approximately fifteen-millimeter cubes and cooked by being allowed to "simmer" in boiling water for two and one-half hours. After being thoroughly mixed and sampled for analysis it was placed in pint

Mason jars and sterilized at 125° C. Agar-agar was included in the diet to facilitate defecation.

During the preliminary period of the experiment the food was masticated *normally*; in the period of undermastication the diet was "bolted" with no attempt at mastication; in the period of Fletcherism the food was chewed until carried down the oesophagus by the "swallowing impulse" and in the final period normal mastication was again practiced.

Total nitrogen (Kjeldahl) determinations were made on foods, feces and urine. The feces were "separated" by means of carmine, and satisfactory differentiations were secured in every instance. All stools were examined *fresh*. *Microscopical meat residues of varying sizes were found in every stool passed during the bolting periods*. The weights of these residues in one particular stool aggregated nearly *seventeen grams*.

An attempt was made to secure data as to the actual daily output of "metabolic nitrogen" by the subjects of the experiment. To this end the suggestion of Mendel and Fine was adopted. A *nitrogen-free* diet of similar energy value to the experimental diet was ingested, agar-agar being added in sufficient quantity to bring the daily fecal output up to the level of the fecal output of the experiment proper. This diet was fed each subject for a period of four days and the average output of fecal nitrogen for the final three days of the period was taken as the "metabolid nitrogen check." This correction was then applied to the fecal nitrogen values obtained in the four periods of the experiment proper. The utilization values *corrected for metabolic nitrogen* are as follows:

Period	Utilization per cent.	
	F	J
Normal	97.0	95.5
Bolting	95.4	95.7
Fletcherizing	97.2	97.5
Normal	97.0	97.5

The data for F indicate that the protein of the diet was somewhat less efficiently utilized by this subject during the period of food bolting than during the periods of normal mastication and

Fletcherism. The uniformity of the values for the two periods in which the food was normally masticated and the practical duplication of this value when the subject Fletcherized are points to be emphasized.

In the case of J the protein portion of the diet was fully as satisfactorily utilized when bolted as when normal mastication was practiced. As he passed from bolting to Fletcherizing there was rather better utilization as is shown in the table. The improved utilization in the period of Fletcherism continued throughout the following normal period, a fact which may perhaps be interpreted as due in part to the influence of the preceding period of hypermastication. It should be mentioned in this connection, however, that the utilization value for the normal period of this subject (95.5%) is rather lower than one would expect when it is recalled that the "metabolic product" correction has been applied. Throughout the course of this normal period J was in a continual state of worry and it is quite possible that his digestive efficiency was lowered somewhat from the normal. In the bolting period he was more normal so far as mental attitude was concerned. We are inclined to believe that the utilization values for the bolting, Fletcherizing and final normal periods are dependable values whereas the value for the preliminary period of this subject we believe to have been influenced by non-experimental conditions.

Our data indicate that when meat is bolted in fifteen-millimeter cubes it is somewhat less efficiently utilized than when normally masticated or Fletcherized. However, the difference in utilization is not pronounced and cannot be considered as furnishing an experimental basis for the belief that food bolting is harmful to the organism. The protein portion of the diet was no more efficiently utilized when the food was chewed until carried down the oesophagus by the "swallowing impulse" than when it was masticated in a normal manner. In other words our data fail to show the advantages of Fletcherism or the harmfulness of food bolting.

(*Extrait*)

CHLORURE DE L'ACIDE DICHLOROARSINOBENZOIQUE. ETHERS DES ACIDES BENZARSINEUX ET BENZARSINIQUE

PAR MM. E. FOURNEAU ET K. ÖCHSLIN

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Les auteurs ont préparé à l'état de pureté le chlorure de l'acide dichlorarsinobenzoique dont la préparation avait déjà été tentée par LaCoste. Ce chlorure est distillable sans décomposition dans le vide et bout vers 189-190° sous 19 mm. C'est un liquide sirupeux qui cristallise spontanèment au bout de plusieurs semaines et immédiatement par a orcage. Il est soluble dans l'éther, le chloroforme, etc. Il se comporte comme le chlorure de benzoyle envers les alcools les phénols les aminoalcools et la quinine, etc. Les auteurs ont ainsi prémaré 1° léther benzarsinique de la quinine, soluble, a la fois, dans les alcalis et les acides en donnant des solutions insipides; 2° le produit de réduction de l'éther benzarsinique ou benzars énoquinine; 3° l'éther du gayacol et 4°, l'arsénostovaine.

Ce dernier produit est un anesthésique local don les fonctions déterminant l'anesthésie entrainent la chaine arsénicale. Il s'agit la peurent les aideurs, du premier essai rationnel de transport d'une substance active dans un tissus désigné l'avance, qui, dans le ces particulier et la substance nerveuse.

ETUDE COMPARÉE DES PRÉSURES DE L'AMANITE PHALLOIDE ET DE L'AMADOUVIER—RELATIONS ENTRE LES PRÉSURES DES BASIDIOMYCÈTES ET DES VÉGÉTAUX SUPÉRIEURS

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Les sucs de ces deux champignons sont des coagulants énergiques du lait.

Cette coagulation est une caséification diastasique. Les agents de la caséification sont très différents dans les deux espèces quant à leur localisation, leur résistance à la chaleur, leur action sur les laits cru et bouilli, l'influence de certains sels sur leur fonctionnement.

A. LOCALISATION. A l'opposé de ce que l'on observe avec les autres *Agaricacées*, la présure de l'Amanite phalloïde (amanita phalloida Fr) est beaucoup moins abondante dans les lames sporifères que dans la partie stérile du chapeau. Au contraire, conformément àce que l'on observe avec les autres *Aphyllophorées* la présure de l'Amadouvier (*Fomes fomentarius* Fr) est beaucoup plus abondante dans les tubes sporifères que dans le reste du chapeau. Ce champignon d'ailleurs constitue le matériel de choix pour établir la relation étroite qui existe entre la formation des spores et l'activité présurante du suc, chez les Basidiomycètes. Chez ce Porohydné, en effet, les tubes hyméniaux naissent par poussées successives et d'une façon telle qu'ils constituent des couches superposées très distinctes, la couche la plus ancienne, qui a perdu ses spores étant celle qui est fixée directement au chapeau, et la plus jeune qui est en voie de sporulation étant au contraire celle qui termine, en bas, la série verticale des couches superposées. Sur un amadouvier possédant trois couches de tubes, nous avonsséparé (ce qui est très facile) celles-ci les unes des autres et en avons extrait séparément les sucs. Nous avons constaté que l'activité présurante du suc de la couche la plus jeune, sporifère, étant dix, celle de la couche moyenne, est 5 et

celle de la couche la plus âgée qui a essaimé presque toutes ses spores, 2, 5 seulement: Cette dernière est même deux fois moins forte que l'activité présurante du suc rétiré de la partie stérile du chapeau qui fournit l'amadou.

B. Resistance a la Chaleur. La présure de *Amanita phalloïdes* Fr est moins résistante à la chaleur que celle de *Fomes fomentarius* Fr. La première est, en effet, complètement détruite par un séjour de 5 minutes à 65°, alors que la seconde ne perd toute action présurante qu'après un pareil temps de chauffe à 75°:

C. Action Sur les Laits Cru Et Bouilli. A fortes doses, et par suite dans le cas des caséifications rapides, les deux présures coagulent plus rapidement le lait cru que bouilli. A faibles doses, et par suite dans le cas des caséifications lentes, la présure de la phalloïde seule continue à coaguler plus rapidement le premier liquide que le second; celle de l'Amadouvier, au contraire, coagule plus lentement le lait cru que bouilli.

Cette différence est due à l'action favorisante du calcium plus prononcée dans le cas de la première diastase et à l'action empêchante des albumines et globulines du lait cru plus énergique dans le cas de la seconde.

Quelques Basidiomycètes appartemant surtout au groupe des porohydnées et qui supportent de grandes différences de température se comportent comme l'amadouvier, leurs présures coagulent mieux, à faibles doses le lait bouilli pur que le lait cru pur, elles sont dites présures du lait bouilli. Un plus grand nombre, qui ne peuvent vivre qu'entre des limites assez étroites de température, se comportent comme l'Amanite phalloïde; leurs présures à toutes doses, coagulent mieux le lait cru pur que le lait bouilli pur; elles sont dites présures du lait cru; quelques unes, plus calciphiles, sont incapables de coaguler le lait bouilli pur (*Pleurotus ostréatus* L *Armillaria caligata Viv, Clitocybe inversa Scop*); certaines mêmes (*Hypholoma sublateritium Betc*) ont un tel besoin de calcium que la quantité de cet élément dissous dans le lait cru lui-même est insuffisante pour leur permettre d'en mener à bien la caséification; elles n'agissent que sur le alit calcifié soit directement, (addition de $CaCl_2$) soit indirectement (addition de quelques molécules milligr. HCl dissolvant le phosphate de chaux en suspension). Nous les faisons néanmoins entrer

dans le type *présures du lait cru* dont elles possèdent tous les autres caractères.

D. INFLUENCE DE CERTAINS SELS SUR LA CASEIFICATION. Les chromates des métaux alcalins, les sels neutres de Nickel, de Cobalt, de Zinc, de Cadmium, de Cuivre, d'Argent et surtout ceux de Mercure d'Or et des métaux du groupe du Platine, ajoutés au lait empêchent ou retardent fortement la caséification par les sucs d'Amadouvier et des autres basidiomycètes appartenant au groupe *Présures du lait bouilli*; au contraire ils retardent faiblement, ou pas du tout, ou même accélèrent la caséification par les sucs de l'Amanite phalloïde et des autres basidiomycètes appartenant au groupe *Présures du lait cru.*

Les corps retardateurs: Lactoglobulini, lactalbumines et sels pécédents agissent non pas sur la diastase qu'ils rendraient moins active, mais sur la caséine qu'ils rendent plus résistants en formant avec elle, très probablement un complexe difficilement dédoublable.

E. PARALLELISME ENTRE LES PRESURES DES BASIDIOMYCETES ET CELLES DES VEGETAUX SUPERIEURS. Nous avons montré dans des travaux antérieurs, que les sucs présurants des végétaux supérieurs se groupent en deux classes correspondantes à celles que nous venons d'établir.

La première classe, de beaucoup la plus importante dont le type est la présure du *Vasconcelba Quercifolia* correspond à celle de l'Amadouvier; les ferments protéolytiques qu'elle renferme sont en effet, très résistantes à la chaleur, coagulent de préférence le lait bouilli et voient leur action caséifiante fortement entravée par les sels des électrolytes ci-dessus. La seconde classe, moins grande, dont le type est la présure du murier à papier (*Brounonétia papyrifera* L), correspond à celle du type Amanite phalloïde; les ferments protéolytiques qu'elle renferme sont généralement peu résistants à la chaleur—Le murier à papier fait exception—coagulent de préférence le lait cru et voient leur action caséifiante peu ou pas influencée par les sels précedents, parfois même favorisée.

Il y a donc parallélisne entre les présures des Basidiomycètes et celles des végétaux supérieurs; mais les premières sont en grande majorité des présures du lait cru et lessecondes, le plus souvent, des présures du lait bouilli.

SCHNELLES VERFAHREN ZUR BESTIMMUNG DER HARNSÄURE IM HARN

ING. CHEM. FRANZ HERLES

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Die Bestimmung der Harnsäure im Harne ist in vielen Fällen für medizinische Zwecke von Wichtigkeit. Es ist wünschenswert in manchen Krankheiten öfters die Menge dieser, im Harne ausgeschiedenen Säure zu kontrolieren. Es kommen demnach für klinische Zwecke diejenige Verfahren in Betracht, welche bei schneller und leichten Ausführung genügend richtige Ergebnisse liefern. Von den bestehenden Verfahren zur Bestimmung der Harnsäure sind als mehr oder minder genau bekannt die Metoden von Ludwig, Hopkins, Jolles und ihre zahlreichen Modificationen und Verbesserungen. Sämmtliche diese Verfahren erfordern aber mehr oder weniger Zeit zu ihrer Durchführung und Bereitung besonderer Lösungen.

Mit der Mehrazhl dieser Metoden habe ich auch entsprechende Vergleichsversuche angestellt ohne übereinstimmende Zahlen zu erhalten, manche Verfahren lieferten sogar ganz falsche Ergenhnisse.

Ich bemühte mich deshalb, da ich grössere Anzahl solcher Bestimmungen durchzuführen hatte, eine andere, möglichst rasche u. leichte, dabei aber genügend genaue Metode auszuarbeiten.

Dies gelang mir auf Grund des Prinzipes der Hopkinsschen Metode: Ausscheidung von harnsaurem Ammoniak und durch weitere einfache Manipulation mit demselben. Mein Verfahren basiert nämlich auf der direkten acidimetrischen Titration des ausgeschiedenen und ausgewaschenen Ammoniumurates. Die Harnsäure, als sehr schwache organische Säure lässt sich leicht durch stärkere Mineralsäuren aus ihren Salzen frei zu machen. Die Mineralsäuren bilden mit der betreffenden Base, mit welcher die Harnsäure verbunden war, entsprechende Salze. Der

Uiberschuss der Mineralsäure wird durch entsprechende Indicatoren genau angezeigt. Als Indicator hiezu eignet sich besonders Methylorange, welche bekanntlich durch Uiberschuss von Mineralsäure rot und nach Neutralisation mit Lauge wieder citronengelb sich färbt. Zur Titration eignet sich am besten 1/10 n Schwefelsäure. Das Verfahren empfiehlt sich wie folgt durchzuführen.

Zur Untersuchung werden 50-100 cc Harn in ein Becherglas abgemessen, 18 resp. 36 g reines pulveriges Ammoniumchlorid zugesetzt und bis zur Auflösung desselben gerührt. Nach einer halben Stunde kann schon durch Papierfilter filtrirt werden. Nach vollständigem Abfliessen der Mutterlauge wird dreimal nach einander mit gesätigter Salmiaklösung ausgewaschen und zwar am besten, dass jedesmal das Filter mit der Waschflüssigkeit voll gefüllt und vollständig abfliessen gelassen wird.

Der Niederschlag sammt Filter wird sodann in das Becherglas, in welchem der Niederschlag gebildet wurde, gegeben, ca 50 cc destilirtes Wasser zugesetzt, zum Kochen erhitzt und 2 Tropfen Methylorangelösung zugesetzt, damit nur schwache Gelbfärbung entsteht. Mehr Tropfen von diesem Indicator zuzusetzen ist zu vermeiden, da hiebei eine rotgelbe Färbung entsteht, welche die Erkennung des Uiberganges der Reaktion sehr erschwert. Nachher wird 1/10 n Schwefelsäure im Uiberschuss bis zur kenntlichen Rotfärbung zugesetzt und mit 1/10 n Lauge bis zur Eintretung der gelben Färbung zurücktitriert. Zur Gewinnung gehöriger Geläufigkeit in Erkennung dieses Farbenumschlages und Gewinnung vollständig sichtiger Resultate empfiehlt es sich nochmals die Säure bis zur Rotfärbung zusetzen und mit der Lauge zurücktitrieren.

Die verbrauchten cc der 1/10 n Säure, multipliciert mit dem Faktor 0.01682 ergeben die Menge der Harnsäure in g in der abgemessenen Harnmenge.

Ist der untersuchte Harn nicht mehr klar, muss der eventuell ausgeschiedene harnsäurehältige Niederschlag in Lösung gebracht und die ausgeschiedenen Phosphate abfiltrirt werden. Hierbei wird wie folgt verfahren: Nach gehörigem Durchmischen werden 100 cc Harn in Becherglas abgemessen, einige Tropfen Phenolphtaleinlösung zugesetzt, erwärmt und tropfenweise Aetznatron bis zur dauernden Rotfärbung zugesetzt, eine Weile gekocht,

damit ein Teil der Lösung verdampft und alles in ein 100 cc Kölbchen gebracht und mit destillirtem Wasser nach Abkühlung zu 100 cc nachgefüllt werden kann. Sonach wird durch trockenes Filter filtrirt und vom Filtrate 50 cc zur Bestimmung der Harnsäure nach der oben angegebenen Metode entnommen.

Mit diesem Verfahren wurden zahlreiche Kontrolversuche sowie im Harne als auch in reinen Harnsäurelösungen ausgeführt, welche sehr befriedigende Resultate ergeben haben. Da die käufliche reine Harnsäure zu diesen Versuchen nicht genügend rein war, wurde dieselbe zu diesen Versuchen wie folgt gereinigt: 1 g der käuflichen Harnsäure wurde in 50 cc Wasser suspendirt, einige Tropfen Phenolphtaleinlösung zugesetzt, erwärmt und tropfenweise Aetznatron bis zur dauernden Rotfärbung zugesetzt, welches wieder durch ein Tropfen Salzsäure entfärbt wurde. Nachher wurde die Flüssigkeit filtrirt und das reine Filtrat nach Zusatz von 2 Tropfen Methylorangelösung durch einen kleinen Uiberschuss von konzentrirter Salzsäure (bis zur eintretenden Rotfärbung) zersetzt, die ausgeschiedene Harnsäure durch das Ludwigsche Filtrationsrohr abfiltrirt, mit destillirtem Wasser zum vollständigen Verschwinden der Chlorreaktion ausgewaschen, bei 40° C. im Wassertrockenschrank und nachher über Schwefelsäure zur Gewichtskonstanz ausgetrocknet. Auf diese Weise gereinigte Harnsäure wurde zu den Kontrolversuchen verwendet. Zu diesem Zwecke wurde eine bestimmte Menge Harnsäure abgewogen, in destillirtem Wasser suspendirt, mit titrirter Aetzlauge unter Zusatz von Phenolphtalein neutralisirt, auf bestimmtes Volumen gebracht und von dieser Lösung immer nötige Mengen entnommen um nach Zusatz von bestimmter Menge destillirten Wassers den im Harne vorkommenden Harnsäuremengen entsprechende Lösungen zu bekommen.

I

Versuche mit unreiner Harnsäure (in 100 cc):

1. Verwendet 0.10 g, gefunden 0.0841 g
2. Verwendet 0.02 g, gefunden 0.0168 g
3. Verwendet 0.10 g, gefunden 0.0841 g

II

Versuche mit gereinigter Harnsäure (in 100 cc):

4. Verwendet 0.04 g, gefunden 0.0390 g
5. Verwendet 0.08 g, gefunden 0.0782 g
6. Verwendet 0.20 g, gefunden 0.1952 g
7. Verwendet 0.20 g, gefunden 0.1952 g
8. Verwendet 0.30 g, gefunden 0.2938 g
9. Verwendet 0.01 g, gefunden 0.0097 g

Versuche mit Bestimmung der Harnsäure im Harne führten zu ebenso günstigen Ergebnissen. Die Harnsäuremenge wird gewöhnlich auf 1 L oder auch auf in 24 Stunden entleerten Harn gerechnet angegeben. Da die tägliche Harnmenge öfters schwer anzugeben ist und die Berechnung auf 1 L Harn bezogen nicht einwandfrei sein kann, da sehr von der momentanen Konzentration des Harnes abhängend, dürfte einer Erwägung wert sein, ob nebstdem auch die Harnmenge auf Trockensubstanz des Harnes bezogen nicht angegeben werden sollte. Die Trockensubstanz könnte zu diesem Zwecke entweder refrektometrisch oder aus dem specifischen Gewichte nach Uiberführung in Saccharometergrade (nach der Ballingschen oder Brixschen Tabelle) bestimmt werden. Bei zuckerhältigen Harnen müsste die Harnsäuremenge auf die zuckerfreie Trockensubstanz bezogen werden. Die prozentische Harnsäuremenge in der Trockensubstanz könnte "Harnsäurequotient" benannt werden. So z.B. enthält ein Harn 0.037% Harnsäure. Das specif. Gewicht—1.0199, entsprechend 5.06% saccharometrischer Trockensubstanz. Es sind demnach in 100 T. Trockensubstanz 0.73 T. Harnsäure enthalten, oder der Harnsäurequotient—0.73%.

THE UTILIZATION OF INDIVIDUAL PROTEINS BY MAN AS INFLUENCED BY REPEATED FASTING

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A study was made of the utilization of *meat*, *milk*, *glidine*, *plasman* and *gluten*, substances of which the nitrogenous portion is made up for the most part in each instance by some particular individual protein. The utilization of a standard "cracker-milk-butter-peanut-butter" diet was studied for comparison. In order that a uniform basis for the study of the utilization might be secured the subject (58 kg. man) was fasted for a period of two days previous to the ingestion of the various experimental diets. A series of eight such fasts was made, water being taken in uniform quantity daily.

The standard diet furnished 12.12 grams of nitrogen and 2500 calories of energy. For a period of two days immediately following each fast this diet was increased 50% thus furnishing 18.18 grams of nitrogen and 3750 calories of energy. Following this increased diet the normal nitrogen and calorific level was again assumed for a three-day interval. At this point the subject again fasted for two days. In brief the experimental plan consisted of a series of two-day fasts separated by five-day feeding intervals the diet for two days immediately following the fast being increased 50% above that fed during the next three days. No attempt was made to separate the feces of the two-day fasting intervals. On the basis of previous fasting experiments in this laboratory it has been found that the output of feces by fasting men even yields approximately 0.1 gram of nitrogen per day. This correction was applied in this case.

The utilization and nitrogen balance data for the experiment are summarized below.

UTILIZATION VALUES (PER CENT.)

Diet	Days	Source of Protein Fed								
		Milk	Glidine	Standard I	Standard II	Plasmon I	Plasmon II	Standard III	Gluten	Meat
150%	2	94.2	97.5	96.5	92.2	96.3	95.8	93.8	90.5	97.4
100%	3	94.3	96.5	85.5		94.5			86.0	96.5
Nitrogen Balance for 5 days Grams N	5	+7.8	−1.3	+1.4	— —	±0.0	+5.0	— —	−9.7	+11.3

An examination of the data indicates that meat and glidine were utilized more efficiently than the other nitrogenous foods fed, whereas plasmon, milk, standard diet and gluten follow in the order given. It will be noted that in practically every instance, no matter what the character of the protein, there was more complete utilization during the two-day period of high diet immediately following the fast, than during the subsequent three-day interval on the normal nitrogen level.

From a consideration of the nitrogen balances we find that the greatest nitrogen gains were made upon meat and milk. The standard diet, plasmon I, glidine and gluten were next in order. The nitrogen balance for plasmon II is not comparable with the other nitrogen balances inasmuch as it relates solely to the two days during which the 150% diet was fed. There was of course always a pronounced retention of nitrogen during this period no matter what the character of the ingested protein. It will be observed that the most pronounced plus balances were obtained when proteins from *animal sources* (meat and milk) were fed.

The proteins of vegetable origin, e.g., glidine and gluten yielded minus balances. This speaks for the greater efficiency of the animal proteins. It is also an interesting fact that the nitrogen of milk was a much more satisfactory nutritive medium than was the dried milk protein preparation (plasmon).

The fact that utilization values are not necessarily reliable indexes of efficiency is demonstrated through the data for meat and glidine. The protein from these two sources was equally well utilized. However when we examine the nitrogen balances we observe that the meat yielded a *plus* nitrogen balance of 11.3 grams whereas glidine gave a *minus* balance of 1.3 grams. It is evident therefore that so far as digestion and absorption are concerned there is apparently no margin of choice between meat and glidine. However, when the question of the retention of their nitrogen for the use of the organism is concerned the evidence is strongly in favor of the meat.

A word of explanation should be offered regarding the low utilization values for gluten. It so happened that there was a delay in the arrival of the gluten flour and it was necessary to feed it before its composition could be determined by analysis. The nitrogen value as recently determined by Mendel and Fine (14%) was therefore made the basis of our calculations. Subsequent analyses (a dozen or more) indicated that our gluten contained *less than* 7% *of nitrogen*. Therefore instead of feeding the same quantity of nitrogen in the form of gluten as was fed in the form of the other proteins we were feeding *less than one-half as much nitrogen*. For this reason the utilization values for gluten are in no way comparable with the other utilization values. It might be well to mention the fact that we made a starch determination on the gluten flour as purchased and found 50.7% *of starch*. This particular specimen can hardly be considered a satisfactory flour for the use of diabetics.

PURINE CATABOLISM IN THE MONKEY

By Andrew Hunter and Maurice H. Givens

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While the urine of man contains considerable amounts of uric acid with almost negligible traces of allantoin, it is the latter substance which for the lower mammals forms the principal product of purine catabolism. The apparently unique position occupied in this respect by the human species makes it of interest to ascertain the fate of purine material in apes and monkeys. Our contribution to the problem is at present limited to observations upon a female guenon monkey (*Cercopithecus callitrichus*), weighing 4.7 kilograms. We have already[1] reported results showing that in this animal the allantoin-purine ratio is of the same order as in the lower mammals. The present communication deals with later experiments which confirm and amplify our earlier conclusion.

The monkey was maintained for ninety-six days on a diet of milk, peanuts, and bananas, the urine being regularly collected in two-day periods. Of the forty-eight urine samples thus obtained twenty-six represented the normal excretion on the (presumably) purine-free regime selected. The allantoin nitrogen of these twenty-six controls varied between 20 and 32 milligrams; in twenty cases it lay between 26 and 31 milligrams; the average of all was 27.7. Purine nitrogen ranged from 6.7 to 13.9 milligrams; in twenty cases from 9.1 to 12.1; average, 11.0. This purine output appeared to consist mainly of bases; at any rate uric acid was never isolated from the normal urine. Of the total allantoin-purine nitrogen of individual samples allantoin accounted for a minimum of 64 and a maximum of 82 per cent. Each of these extremes was exceptional ; on all but six occasions the ratio lay between 71 and 76; its mean value was 72.

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[1]Proc. Amer. Soc. Biol. Chem., II, p. 73, 1912; Journ. Biol. Chem., XI, p. xxxix.

The results of the oral or subcutaneous administration of Sodium nucleate, sodium urate, and allantoin are summarized in the table. (The figures are milligrams of nitrogen; for sodium nucleate, of which 2 gram doses were given they represent nitrogen of the purine ring only.)

Substance	Amount given	Method	Amount recovered		Total	Percentage recovered
			Uric acid	Allantoin		
Sodium nucleate	140	oral	3.5	12.7	16.2	12
	140	oral	5.6	30.1	35.7	26
Sodium urate	32.9	oral	9.3	0.0	9.3	28
	34.4	oral	0.0	1.4	1.4	5
	65.9	oral	1.2	4.6	5.8	9
	66.6	subcut.	28.9	37.6	66.5	100
Allantoin	45.8	oral		0.0		0
	96.3	oral		22.3		23
	98.0	oral		13.5		14
	33.5	subcut.		25.1		75
	34.1	subcut.		27.9		82
	70.4	subcut.		63.1		90

The feeding experiments with nucleic acid demonstrate the conversion of the purine nuclei into allantoin with uric acid as an intermediate product. The proportion in which these appear is such as might have been expected, allantoin accounting for 78 and 84 per cent. of the recovered purine nitrogen. The total amount of the latter is however but a fraction of that administered. The result of feeding uric acid is even less illuminating; very little indeed reappears in the urine, and the allantoin excretion is not appreciably affected. How the deficit in either case is to be explained—whether by destruction in the intestine, or by failure of absorption—we have not yet been able to decide.

One must of course reckon with the possibility of allantoin being not a terminal but an intermediate product. The small percentage of ingested allantoin recoverable in the urine would harmonize with such a view. The injection experiments seem to negative it completely. The quantity of allantoin recovered unchanged after subcutaneous introduction is practically as great as if it had been directly dissolved in the urine. Injected uric acid is likewise completely accounted for, more than half being converted into allantoin. One seems forced meanwhile to conclude that in the intermediary metabolism of the monkey allantoin is indestructible, and that it constitutes not only the principal but also the final product of purine destruction.

THE RELATION BETWEEN CHEMICAL CONSTITUTION AND PHYSIOLOGICAL ACTION AS EXEMPLIFIED BY THE GLYOXALINES, ISO-QUINOLINES AND ACID AMIDES

By H. A. D. Jowett, D.Sc., F. L. Pyman, D.Sc., and F. G. P. Remfry, D.Sc.

Wellcome Chemical Works, Dartford, Kent, England

At the Eighth International Congress of Applied Chemistry we discussed the relation between chemical constitution and physiological action, and entered into particulars with respect to three classes of chemical compounds, namely, the Arylarsonic Acids, the Alkamine Esters and the Tropeines. Little has been added to our knowledge of the physiological action of the two last mentioned classes of compounds since then, but much progress has been made by Ehrlich and his collaborators in the case of the organic derivatives of arsenic. Briefly, it has been shown that the arylarsonic acids yield on reduction successively arylarsenious oxides and arsenoaryls. A very large number of these compounds have been physiologically examined, and have led eventually to the production of 3:3′-diamino-4:4′-dihydroxyarsenobenzene, which has since been largely employed in the treatment of syphilis and other protozoal diseases.

The success attending the use of organic arsenic compounds has naturally led to the production and physiological examination of other organo-metalloidal and organo-metallic compounds. For instance, several investigations have been carried out with a view of preparing aryl antimony compounds for use in medicine. None of these, however, has reached a satisfactory conclusion; and this has been due partly to the physical unsuitability, such as insolubility, of many of the compounds prepared, which has hindered or prevented their physiological investigation, and

partly to the fact that such as could be satisfactorily tested failed to have the desired action.

Another development of work on these lines was suggested by the combined treatment of syphilis with sodium p-aminophenylarsonate and mercurials, or with a mercury salt of p-aminophenylarsonic acid. It was thought that the introduction of one or more mercury residues into the aromatic nucleus of phenylarsonic acids might give rise to valuable therapeutic compounds. A considerable series of oxymercury derivatives of phenylarsonic acids were therefore prepared,[1] a typical example of such compounds being disodium 3-oxymercury-4-aminophenylarsonate (HOHg.) (NH_2) C_6H_3.AsO $(ONa)_2$, which is a derivative of sodium p-amino-phenylarsonate. These compounds contain mercury in a non-ionised condition. They do not coagulate albumin, and are satisfactory on the whole as regards toxicity and suitability for hypodermic injection, but the results obtained on physiological examination have been disappointing.

In connection with organo-metalloidal compounds, von Wassermann's recent use of an eosin-selenium compound in experiments with cancerous mice has aroused widespread interest, and it will be interesting to follow the further developments of this work.

On this occasion, we propose again to deal with the relation between Chemical Constitution and Physiological Action in certain selected fields in which we have been working, namely, glyoxalines, *iso*quinolines and acid amides.

The investigation of the glyoxalines has proceeded in two directions of physiological interest dealing respectively with synthetic substances allied to 4(or 5)-β-aminoethylglyoxaline and pilocarpine. Our knowledge that aminoethylglyoxaline is a base of physiological importance is due to the extended investigations of Barger and Dale, who have shown that it is one of the active principles of ergot.

In the course of the last few years, great progress has been made in the elucidation of the bases to whose combined effect the properties of ergot are due, and it is now known that besides

[1]Wellcome and Barrowcliff (*Eng. Pat.* 12,472 of 1908).

the alkaloids ergotinine and ergotoxine, a number of other bases also contribute towards it.

These bases are derived from amino-acids by the loss of the elements of carbon dioxide, and are derivatives of ethylamine; the two most important are p-hydroxyphenylethylamine derived from tyrosine, and aminoethylglyoxaline from histidine. One of the most important and thorough examinations of the pharmacology of any series of compounds has recently been carried out by Barger and Dale in the study of the relation between chemical structure and sympathomimetic (adrenine-like) action of the phenylalkylamines and their phenolic derivatives, the class to which p-hydroxyphenylethylamine belongs.

Their results are too profuse to receive consideration in detail, but we may draw attention to certain analogies which exist between the aminoalkyl derivatives of benzene, and those of glyoxaline, which latter are more fully described in the special part of this paper.

In the case of the phenylalkylamines, the optimum structure for physiological effect is present when the benzene ring and amino-group are separated by a chain of two carbon atoms, and another optimum condition is the presence of two phenolic hydroxyls in the 3:4 positions relative to the side chain.

Similarly it has been found that only those aminoalkylglyoxalines have any pronounced physiological activity, in which the glyoxaline ring and amino-group are separated by a chain of two carbon atoms. Here again the presence of an acidic grouping in the ring is an optimum condition; in this case it is of course the imino-group which so functionates in the place of the hydroxylic substituents of phenylalkylamines.

A second line of work in connection with the derivatives of glyoxaline has been followed in attempts to prepare bases of pilocarpine-like action. The results of such experiments, however, have been entirely negative and serve to show that like other groups of compounds, such as the tropeines, the glyoxalines only become possessed of physiological activity under conditions difficult to determine.

Glyoxalines which are very closely related chemically vary enormously in physiological action, and a good example of this

is seen in the reduced activity of *iso*pilocarpine as compared with its stereo-isomeride pilocarpine.

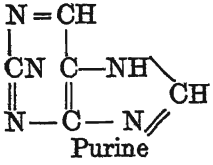
The pharmacology of the *iso*quinoline derivatives, a section which comprises a large number of well-known alkaloids, is, of course, a large subject, and we will consequently limit ourselves to a discussion of the relation between Chemical Constitution and Physiological Action in bases of the cotarnine type, of which there are now a number of known examples. The conclusion to which we are led in this case is that the property of causing contraction of the uterus is common to those 2-alkyl-3:4-dihydro-*iso*quinolinium bases (that is bases of the cotarnine type) which contain methoxy- or methylenedioxy-groups. At the same time we desire to qualify this statement by pointing out that only a comparatively small number of such compounds have been prepared and physiologically examined. Experience teaches that an apparent relation between Chemical Constitution and Physiological Action often appears to exist between a small number of closely allied substances, but on extending the field of enquiry somewhat wider, it is frequently found that no satisfactory generalisation can be deduced.

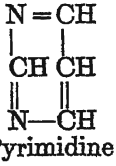
The third section of this paper deals with recent work on the relation between Chemical Constitution and Hypnotic Action, and for this purpose only those compounds containing the acid amide radicle, - CO - NH -, have been described. Under this heading such well-known groups as barbituric acid, urethane and their allied compounds can be included, and it was found advisable to limit the paper in this manner on account of the wide range of the subject.

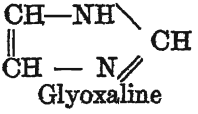
Glyoxaline Derivatives

The organic bases which occur in nature contain nitrogen combined in many different ways. Of the ring compounds with one nitrogen atom mention may be made of the pyridine, pyrollidine, quinoline, *iso*quinoline and indole derivatives. Rings containing more than one N-atom also occur, thus a large and important class of naturally occurring substances—the purine derivatives—

contain a double ring system, each containing two nitrogen atoms, the two rings being the pyrimidine and glyoxaline rings.

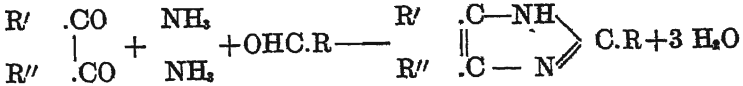

Purine


Pyrimidine


Glyoxaline

Until the last decade, however, the simple glyoxaline ring had not been recognised as a constituent of physiologically important compounds, although glyoxaline itself and some of its simpler derivatives had long been known. The first recognition of the simple glyoxaline ring in a naturally occurring compound was made in the case of the alkaloid *iso*pilocarpine; shortly afterwards the important amino-acid histidine was shown to be a glyoxaline derivative, and quite recently 4(or 5) β-aminoethylglyoxaline and ergothioneine, another glyoxaline derivative, have been isolated from ergot.

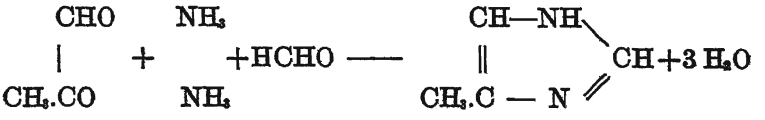
The occurrence of glyoxaline derivatives amongst natural products is susceptible of a ready explanation. Glyoxalines it is well-known are readily formed when ammonia is added to a cold aqueous solution containing an aldehyde, R.CHO, and a compound R′.CO.CO.R″, where R, R′, and R″ may be hydrogen as in the case of glyoxal itself



and many alkylglyoxalines have been prepared in the laboratory in this way. Moreover, it has been shown[1] that when an aqueous solution of glucose, formaldehyde and zinc hydroxide-ammonia is left exposed to light for some time 4(or 5)-methylglyoxaline is produced, and it seems probable that in this reaction the glucose

[1]Windaus and Knoop (*Ber.*, 1905, **38**, 1166).

is first degraded to methylglyoxal, which then enters into combination with the ammonia and formaldehyde as depicted below:

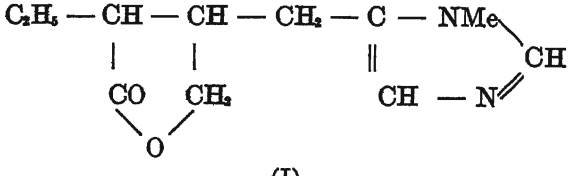


It is interesting to note that this reaction takes place under normal conditions of temperature and pressure, and requires only such reagents whose formation in nature can readily be understood.

Before going on to describe the synthetic experiments made with the view of preparing substances of physiological activity, we propose to give a short account of the recent researches on the naturally occurring glyoxaline derivatives.

Pilocarpine.

Pinner and Schwarz[1] first suggested that pilocarpine was a glyoxaline derivative, and proposed the constitutional formula (I)



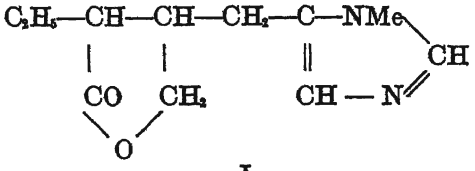
(I)

This formula was based on

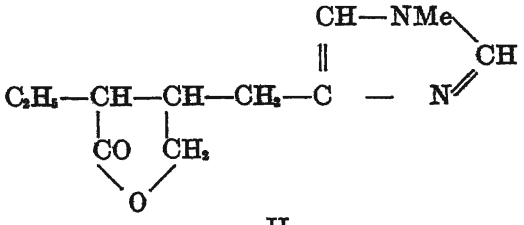
1. The constitution of homopilopic acid, which had previously been determined by Jowett[2].
2. The composition of the fragment left on subtraction of the homopilopic residue from the empirical formula of pilocarpine.
3. The formation of methylurea on oxidation, and
4. Certain analogies shown to exist between pilocarpine derivatives and glyoxalines.

[1] *Ber.*, 1902, **35**, 2441.
[2] *J. C. S. Trans.*, 1901, **79**, 1331.

Definite proof that pilocarpine is a glyoxaline derivative was furnished by Jowett[1] in the next year. By distilling *iso*pilocarpine with soda lime he isolated and identified 1-methylglyoxaline, 1:4(or 1:5)-dimethylglyoxaline and 1:4(or 1:5)-methylmylglyoxaline. For the purpose of comparison, Jowett and Potter[2] prepared what they believed to be a homogeneous 1:4(or 1:5)-dimethylglyoxaline by methylating 4(or 5)-methylglyoxaline, but came to the conclusion that this was not identical but isomeric with the dimethylglyoxaline from *iso*pilocarpine. As there was no evidence to show which of the two dimethylglyoxalines was the 1:4 isomeride and which the 1:5, Jowett put forward for *iso*pilocarpine the two alternative formulae (I) and (II) of which (I) is identical with that suggested by Pinner and Schwarz, and represented pilocarpine as a stereo-isomeride.



I



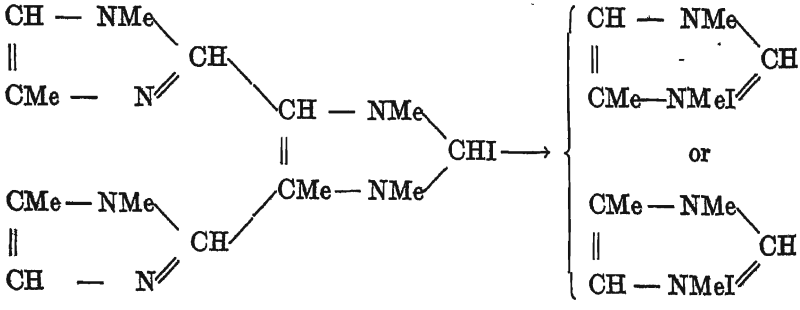
II

Pinner[3] regarded pilocarpine and *iso*pilocarpine as structural isomerides—derivatives of the two 1:4 and 1:5-methylglyoxalines corresponding with the formulae (I) and (II), but Jowett[4] was able to show that the alkaloids are not structural but stereo-isomerides, since they are mutually interconvertible by means of alcoholic potash.

[1] *J. C. S. Trans.*, 1903, 83, 438.
[2] *J. C. S. Trans.*, 1903, 83, 464.
[3] *Ber.*, 1905, 38, 1510.
[4] *J. C. S. Trans.*, 1905, 87, 794.

Recently Pyman[1] has repeated the methylation of 4(or 5)-methylglyoxaline, and isolated the two isomerides 1:4 and 1:5-dimethylglyoxaline. The latter proved to be identical with the dimethylglyoxaline obtained by Jowett by the distillation of *iso*pilocarpine with soda lime. *Iso*pilocarpine has therefore the formula (I).

Further evidence was also obtained against the view that the difference between pilocarpine and *iso*pilocarpine depends on structural isomerism as represented by Pinner. Thus, it was found that 1:4 and 1:5-dimethylglyoxaline yield one and the same methiodide, doubtless owing to tautomeric changes in the sense of the following scheme:



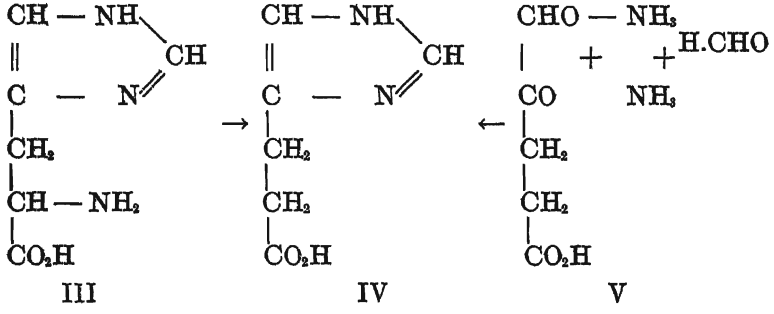
By analogy therefore if pilocarpine and *iso*pilocarpine owed their isomerism solely to structural causes, in the sense that they were 1:4 and 1:5-methylglyoxaline derivatives, they should also yield one and the same methiodide. This is, however, not the case, pilocarpine giving an amorphous methiodide, whereas *iso*-pilocarpine methiodide is crystalline. These facts, therefore, afford further evidence against Pinner's view of the isomerism of pilocarpine and *iso*pilocarpine, and consequently strengthen Jowett's position.

Histidine.

Histidine is an amino-acid which occurs as a degradation product of most albumins. It is readily prepared by hydrolysing

[1] *J. C. S. Trans.*, 1910, **97**, 1814.

haemoglobin. Pauly[1] first suggested the constitution of histidine (III) which is now known to be correct, but it was Knoop and Windaus[2] who first proved that this amino-acid is a glyoxaline derivative by degrading it to β-glyoxaline-4(or 5)-propionic acid (IV) which they also prepared synthetically by the action of formaldehyde and ammonia on glyoxylpropionic acid (V).



III IV V

The recent synthesis of histidine by Pyman will be referred to later.

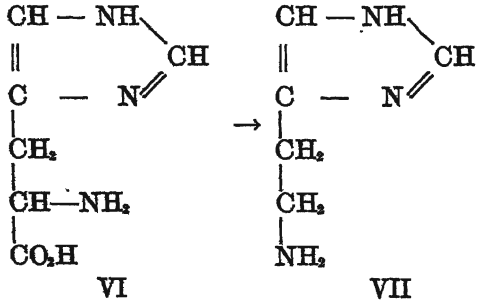
4(or 5)-β-Aminoethylglyoxaline.

This base was first prepared synthetically[3] by the degradation of β-glyoxaline-4(or 5)-propionic acid (IV) by Curtius' method some years before it was recognised as a naturally-occurring compound. Recently, however, it has been shown to be present in certain preparations of ergot,[4] and to have very great physiological activity.[5]

4(or 5)-β-Aminoethylglyoxaline (VII) can be prepared from histidine (VI) in the laboratory by the elimination of carbon dioxide by bacterial action,[6] and it seems reasonable to suppose that it is produced in this manner in nature. It can also be

[1] *Zeitsch. physiol. Chem.*, 1904, **42**, 513.
[2] *Beitr. chem. Physiol. Path.*, 1905, **7**, 144.
[3] Windaus and Vogt (*Ber.*, 1907, **40**, 3691).
[4] Barger and Dale (*J. C. S. Trans.*, 1910, **97**, 2592).
[5] Dale and Laidlaw (*J. physiol.* 1910, **41**, 318).
[6] Ackermann (*Zeitsch. physiol. Chem.*, 1910, **65**, 504).

prepared from histidine by removing carbon dioxide by purely chemical means, such as the use of acids at a high temperature.[1]

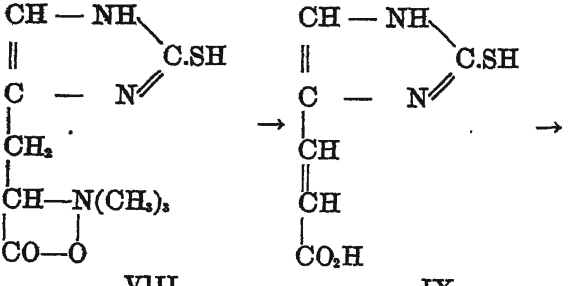


VI VII

The most convenient method for its preparation, however, is the synthetic method to be described.

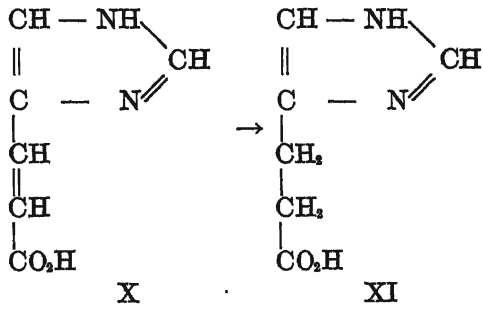
Ergothioneine.

This was isolated from ergot by Tanret[2] who determined its composition, $C_9H_{15}O_2N_3S$. Barger and Ewins[3] have recently shown that this compound is β-2-thiolglyoxaline-4(or 5)-propiobetaine (VIII) in the following manner. On boiling the base with strong aqueous potassium hydroxide, trimethylamine was removed, and β-2-thiolglyoxaline-4(or 5)-acrylic acid (IX) resulted; on oxidation with nitric acid this gave β-glyoxaline-4 (or 5)-acrylic acid (X) and the latter compound furnished β-glyoxaline-4(or 5)-propionic acid (XI) on reduction; the last two acids mentioned were identical with snythetically prepared specimens.



VIII IX

[1]Ewins and Pyman (*J. C. S. Trans.*, 1911, **99**, 339).
[2]*J. Pharm. Chim.* 1909 (VI), **30**, 145.
[3]*J. C. S. Trans.*, 1911, **101**, 2336.



X XI

Ergothioneine has no marked physiological action. It is an interesting addition to the comparatively small number of plant principles containing sulphur, and is the first example of a 2-thiolglyoxaline to be found in nature.

Iodated Proteins.

It will be observed that apart from pilocarpine and the allied alkaloids the glyoxaline derivatives which have hitherto been isolated from natural sources are derivatives of or nearly related to histidine. The latter is widely distributed in nature in combination with other amino-acids, entering into the composition of most albumins, and it has recently been suggested that naturally-occurring iodated proteins may contain the iodine fixed in the glyoxaline nucleus of histidine residues.

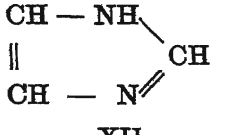
The active principle of thyroid glands, for instance, has been shown[1] to be an iodated globulin, and in view of its physiological importance, the nature of the iodine-bearing group in this compound, iodothyrin, has been the subject of investigations by Pauly and Gundermann.[2] These authors reviewed the various amino-acids which are formed by the hydrolysis of proteins, and showed that histidine is the one which can most readily fix iodine permanently when treated with gentle iodating agents, such as iodine and alkali. They therefore prepared and tested a number of iodated and brominated glyoxalines, and found that the halogenated glyoxalines (unlike the halogen free bases) caused

[1] Baumann (*Zeitschr. physiol. Chem.* 1895, **21**, 319).

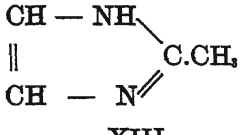
[2] *Ber.*, 1908, **41**, 3999; 1910, **43**, 3243 and *Arch. expt. Path. Pharm.* 1911, **65**, 259.

a marked acceleration of the pulse and breathing frequency; this is of particular interest since the characteristic pharmacological action of thyroid gland and iodothyrin is the acceleration of the pulse-frequency.

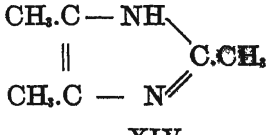
With regard to toxicity, whilst glyoxaline (XII), 2-methylglyoxaline (XIII) and 2:4:5-trimethylglyoxaline (XIV) had little effect on dogs in doses of 1.0-gram, fractions of this amount proved toxic in the case of the halogenated bases.



XII

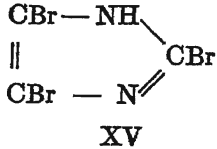


XIII

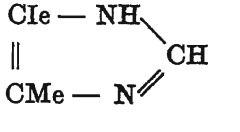


XIV

The most toxic compound tested was 2:4:5-tribromoglyoxaline (XV) of which 0.2-gram given per os was sufficient to kill a 6½-kilo dog in 2 hours. Of the iodated glyoxalines 2(or 5)-iodo-4-methylglyoxaline (XVI) was the most toxic 0.3-grams per os killing a medium weight dog in 10 hours, then followed 4:5-diiodo-2-methylglyoxaline (XVII) of which the lethal dose was 0.4-grams and 2:4:5-triiodoglyoxaline (XVIII) of which the lethal dose was 0.6-grams or more.



XV



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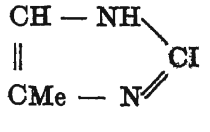
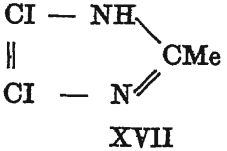
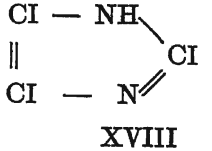


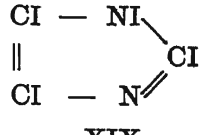
XVI



XVII



XVIII

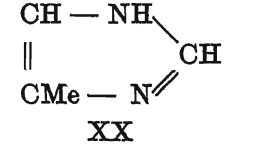


XIX

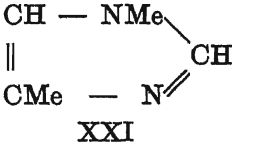
1:2:4:5-tetraiodoglyoxaline (XIX) and tetraiodohistidineanhydride had little or no pharmacological action probably owing to their slight absorption from the intestine.

Synthetic Glyoxaline Derivatives and Their Physiological Action.

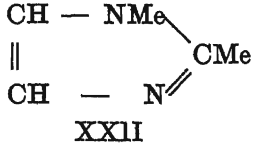
Until quite recently synthesis in the glyoxaline series has been confined almost exclusively to the preparation of glyoxalines with simple substituents such as alkyl-groups. A few of these appear to have been physiologically tested; thus 4(or 5)-methylglyoxaline (XX) is described as toxic.[1] Further, 1:4-dimethylglyoxaline (XXI) and 1:2-dimethylglyoxaline (XXII) are said to have no pilocarpine-like action,[2] whilst 1-ethyl-2-methylglyoxaline (XXIII) (oxalethylin) is stated to show a surprising similarity in its action to that of atropine.[3]



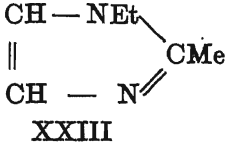
XX



XXI



XXII



XXIII

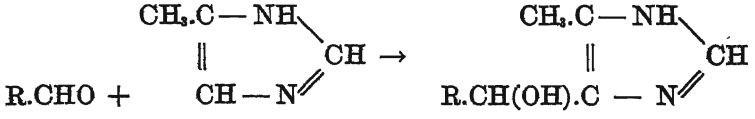
and apart from the fact that considerably larger doses are necessary to cause all the characteristic effects of atropine. In view of this statement we have prepared a quantity of 1-ethyl-2-methylglyoxaline by Radziszewski's method[4] and Dr. H. H. Dale who has tested it finds that 4-drops of 4% solution instilled into the eye of a cat produced no trace of mydriatic action; 100-mgms. did not paralyse the heart vagus or the action of the chorda tympani on salivary secretion, and the only trace of atropine-like action exhibited was seen in its antagonistic action to that of pilocarpine when directly applied to the frog's heart.

Within the last few years, however, glyoxalines with longer side chains have been synthesised; Knoop and Windaus' synthe-

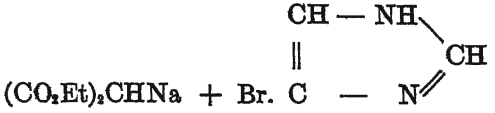
[1] Kowalewski (*Biochem. Zeitschr.*, 1909, **23**, 1).
[2] Jowett (*J. C. S. Trans.*, 1903, **83**, 466; 1905, **87**, 406).
[3] Schulz (*Ber.*, 1880, **13**, 2353).
[4] *Ber.*, 1883, **16**, 489.

sis (in 1905) of β-glyoxaline-4(or 5)-propionic acid from glyoxylpropionic acid and their degradation of the former compound to 4(or 5)-β-aminoethylglyoxaline have already been mentioned.

Further the discovery[1] that 4(or 5)-methylgloyxaline can be readily prepared in quantity from glucose has led to a number of interesting researches, [2], [3], [4] in which the base has been condensed with variou aldehydes to yield alcohols in accordance with the following scheme:



In 1905 after clearing up the constitution of pilocarpine, Jowett[5] suggested the preparation of pilocarpine-like compounds by the condensation of brominated glyoxalines with substances such as ethyl sodiomalonate.



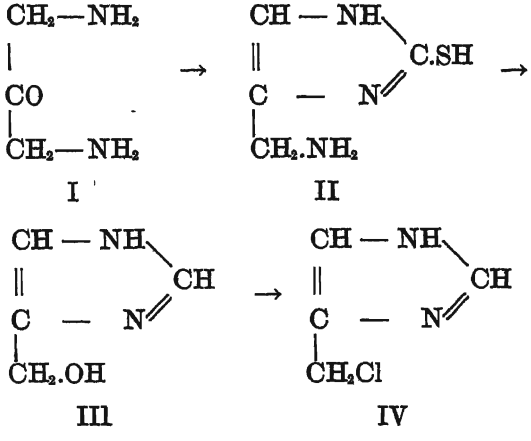
but at the time brominated glyoxalines were not readily accessible and no actual condensation experiments were carried out.

Some years afterwards Pyman[6] prepared quantities of several glyoxalines brominated in the ring, but found that they would neither react with compounds of the type of ethylsodiomalonate, nor would they react with magnesium to form glyoxaline magnesium bromides, and were therefore useless for synthetic purposes. Later, however, the same author[7] devised a method by which 4(or 5)-chloromethylglyoxaline could readily be prepared.

Diaminoacetone dihydrochloride (I) was condensed with one molecule of potassium thiocyanate and the resulting product

[1]Windaus and Knoop (*Ber.*, 1905, **38**, 1166).
[2]Gerngross (*Ber.*, 1909, **42**, 398; 1912, **45**, 509).
[3]Windaus (*Ber.*, 1909, **42**, 758).
[4]Ewins (*J. C. S. Trans.*, 1911, **99**, 2052).
[5]*J. C. S. Trans.*, 1905, **87**, 405.
[6]*J. C. S. Trans.*, 1910, **97**, 1814; 1912, **101**, 530.
[7]*J. C. S. Trans.*, 1911, **99**, 668.

(II) treated with nitric acid yielding 4(or 5)-hydroxymethylglyoxaline (III); this on treatment with phosphorous pentachloride gave 4(or 5)-chloromethylglyoxaline (IV).



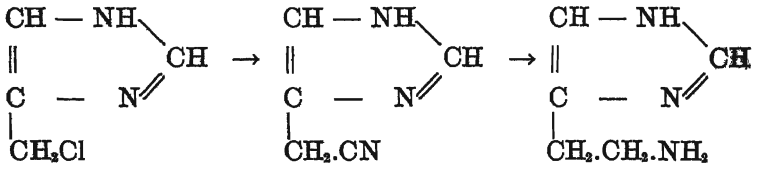
The latter compound contains the chlorine atom in a very reactive condition and may be employed for the introduction of the glyoxaline methyl ($C_3H_3N_2.CH_2$) group into organic compounds in the same way that benzyl chloride is used for the introduction of the benzyl group.

Mainly by the use of this compound it has been possible to carry out the following researches:

1. Synthesis and physiological examination of various aminoalkylglyoxalines.
2. Synthesis of histidine.
3. Synthesis and physiological examination of various glyoxaline derivatives containing carboxylic groups.

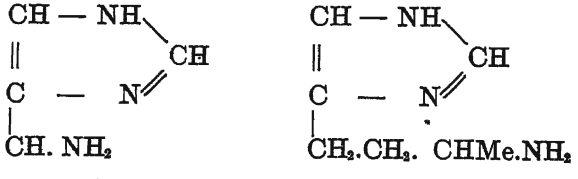
1. *Synthesis and Physiological Examination of Various Aminoalkylglyoxalines.*

In the first instance, 4(or 5)-β-aminoethylglyoxaline (VI) was synthesised from this compound by replacing the chloro-group by the cyano-group forming 4(or 5)-cyanomethylglyoxaline (V), which was then suitably reduced.



IV V VI

Physiological examination of these compounds showed that Nos. (II), (III), (IV) and (V) were almost devoid of stimulant action on the uterus, and had only trivial pressor effects on the blood pressure, whilst 4(or 5)-β-aminoethylglyoxaline (VI) has a very powerful motor effect on the isolated uterus, and a well marked depressor effect upon the blood pressure. The preparation of homologous aminoalkylglyoxalines might therefore be expected to lead to interesting results. Barger and Dale[1] in dealing with the relationship between the chemical constitution of the amines and their physiological action, have shown that the activity varies greatly with the length of the side-chain; in the fatty series the maximum of activity is attained at hexylamine, whilst the most active phenylalkylamine is phenylethylamine, having a fatty side-chain of two carbon atoms. It appeared, therefore, of interest to determine the optimum length of side-chain for physiological effect in the aminoalkylglyoxalines.[2] For comparison with 4(or 5)-β-aminoethylglyoxaline, 4(or 5)-aminomethylglyoxaline (VII) and 4(or 5)-γ-aminopropylglyoxaline were required, but since the latter was not readily accessible its methylhomologue, 4(or 5)-γ-aminobutylglyoxaline (VIII) was prepared and tested in its place.



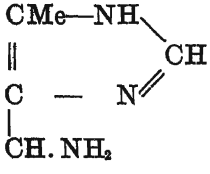
VII VIII

[1] *J. Physiol.*, 1910, **41**, 19.

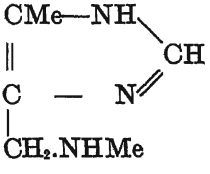
[2] Pyman (*J. C. S. Trans.*, 1911, **99**, 2172).

Neither of these bases had any physiological action at all comparable with that of No. (VI), both of them only producing very weak motor effects on the uterus, and very faint pressor effects on the blood pressure.

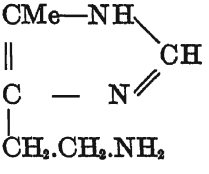
A similar relation obtains in the homologous series derived from 4(or 5)-methylglyoxaline.[1] Here again the aminomeyethyl derivatives, 4(or 5)-methyl-5(or 4)-aminomethylglyoxaline (IX) and 4(or 5)-methyl-5(or 4)-methylaminomethylglyoxaline (X) are physiologically almost inactive, whilst the ethylamine derivative (XI) has considerable physiological activity.



IX



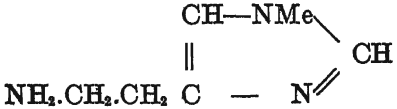
X



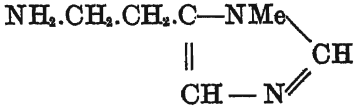
XI

This base, 4(or 5)-methyl-5(or 4)-β-aminoethylglyoxaline produced a fall in blood pressure similar to, though somewhat less powerful than, that of 4(or 5)-β-aminoethylglyoxaline when injected intravenously. Its motor effect on plain muscle however, was far less than that of the last mentioned base.

The next point investigated was the effect of substituting the imino-hydrogen atom in (VI) by the methyl-group. The two isomeric *N*-methyl derivatives 1-methyl-4-β-aminoethylglyoxaline (XII) and 1-methyl-5-β-aminoethylglyoxaline (XIII) were accordingly prepared,



XII



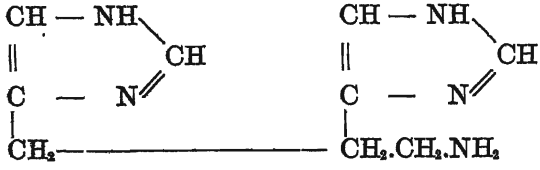
XIII

and tested. Their physiological action, however, was negligible compared with that of the parent compound (VI).

β-γ-Bis[4(or 5)-glyoxaline] propylamine (XIV) which may be regarded as an aminoethylglyoxaline containing a glyoxa-

[1]Ewins (*J. C. S. Trans.*, 1911, 99, 2054).

linemethyl substituent was also tested physiologically; the effect of this base on the uterus and blood pressure is barely perceptible.



XIV

The nature of the results obtained by the investigation of these aminoalkylglyoxalines is readily seen from the following table in which the bases are arranged according to their chemical constitution:

Ref.No.	Base	Physiological Activity
(VII)	$C_3H_3N_2.CH_2.NH_2$	Slight
(IX)	4:5-Me. $C_3H_3N_2.CH_2.NH_2$	Slight
(X)	4:5-Me. $C_3H_3N_2.CH_2.NHMe$	Slight
(VI)	$C_3H_3N_2.CH_2.CH_2.NH_2$	Very great
(XI)	4:5-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Considerable
(XII)	1:4-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Slight
(XIII)	1:5-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Slight
(XIV)	$C_3H_3N_2.CH_2$	
	$C_3H_3N_2.CH.CH_2.NH_2$	Slight
(VIII)	$C_3H_3N_2.CH_2.CH_2.CHMe.NH_2$	Slight

Amongst the aminoalkylglyoxalines tested therefore only those two (VI) and (XI) have any pronounced physiological action in which

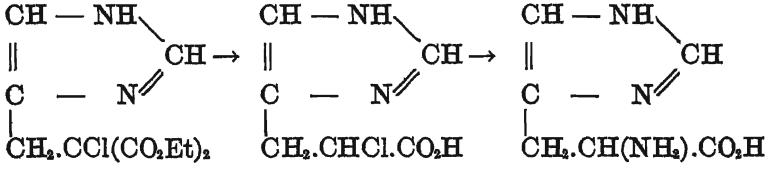
(1) the amino-group and the glyoxaline complex are connected by a chain of two carbon atoms, and

(2) the imino-group of the glyoxaline complex is free.

2. *Synthesis of Histidine.*

4(or 5)-chloromethylglyoxaline readily reacts with compounds of the type of ethyl sodiomalonate forming condensation products, and histidine has been synthesised by this means as follows:[1]

4(or 5)-chloromethylglyoxaline was condensed with ethyl sodiochloromalonate to give ethyl 4(or 5)-glyoxalinemethylchloromalonate (XV); this on hydrolysis with hydrochloric acid gave α-chloro-β-glyoxaline-4(or 5)-propionic acid (XVI) which when treated with strong ammonia under pressure gave racemic histidine (XVII); the latter was then resolved into its optically active components by fractional crystallisation of the acid tartrates.



XV XVI XVII

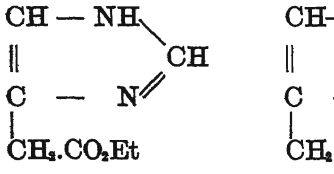
3. *Synthesis and Physiological Examination of Various Glyoxaline Derivatives Containing Carboxylic Groups.*

Starting with 4(or 5)-chloromethylglyoxaline it has been possible to synthesise a large number of compounds having this much in common with pilocarpine that they contain the glyoxaline complex, and an esterified carboxyl-group.[2] It may be stated at the outset that none of the compounds prepared had any pilocarpine-like action, and most of them were physiologically inactive.

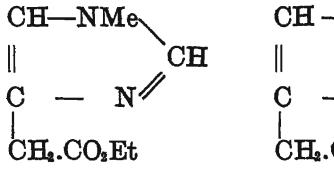
In the first place a number of ethyl esters were prepared and tested. These were ethyl glyoxaline-4(or 5)-acetate (I), ethyl 1-methylglyoxaline-4-acetate (II), ethyl 4(or 5)-glyoxalinemethylmalonate (III), ethyl 4(or 5)-glyoxalinemethylmethylacetoacetate (IV), ethyl 4(or 5)-glyoxalinemethylchloromalonate (V) and 4(or 5)-glyoxalinemethylchloromalonamide (VI).

[1]Pyman (*J. C. S. Trans.*, 1911, **99**, 1386).

[2]Pyman (*loc. cit.*).



I



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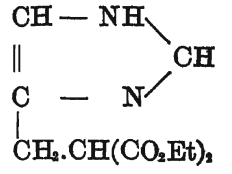
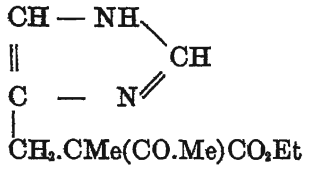
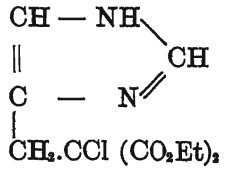


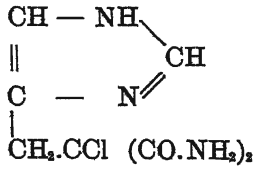
III



VI



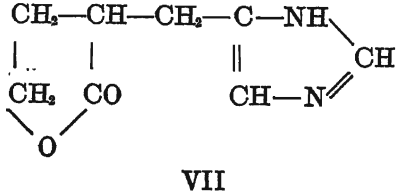
V



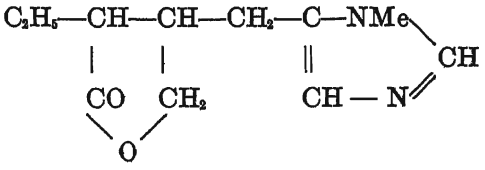
VI

The fact that none of these substances had any pilocarpine-like action showed that the presence of an esterified carboxyl-group in a glyoxaline derivative was not sufficient to confer the physiological properties characteristic of this alkaloid. It was thought, however, that if the carboxyl-group were internally esterified forming a lactonic complex as in pilocarpine, the desired effect might be produced. The lactone of α (β-hydroyethyl)-β-glyoxaline-4(or 5)-propionic acid(VII) was therefore synthesised.

This compound has certain constitutional features in common with pilocarpine (VIII); thus both contain a glyoxaline residue connected through a methylene group with a butyryl lactone residue, though the point of attachment to the lactone ring

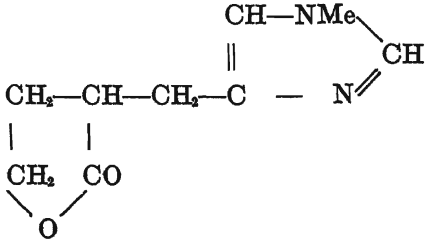


VII

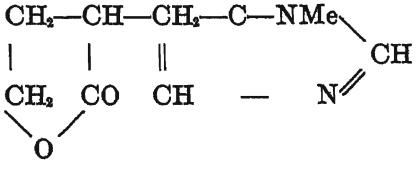


VIII

is different in the two cases. The two bases also differ in that the former is not methylated, and when it was found that the lactone (VII) was physiologically inactive, a quantity was methylated with a view to the preparation of the two isomeric N-methyl-derivatives (IX) and (X)



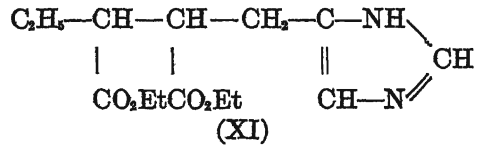
IX



X

Only one of these was isolated in a pure state, and this also was found to be physiologically inactive.

Another series of experiments, which cannot be described in detail here, was carried out with the object of synthesising pilocarpine, and resulted in the preparation of substances containing the skeleton of this alkaloid. Of these ethyl α-4(or 5)-glyoxalinemethyl-β-ethylsuccinate (XI) was submitted to physiological examination but likewise proved to be inactive.



(XI)

All attempts to synthesise glyoxaline derivatives of pilocarpine-like action have therefore hitherto been uniformly unsuccessful.

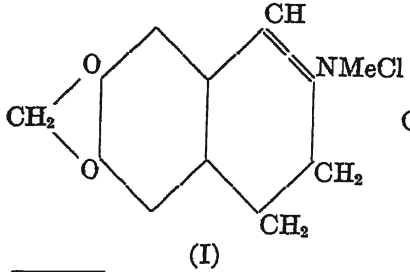
*ISO*QUINOLINE DERIVATIVES.

The constitution of many members of the large and important group of alkaloids containing the *iso*quinoline ring have been known for a long period, but it is only within the last few years that synthetic methods have been developed for their preparation. During the latter period, however, much work has been done in this field, and we may recall in particular the syntheses of laudanosine[1] papaverine,[2] berberine[3] and of narcotine.[4]

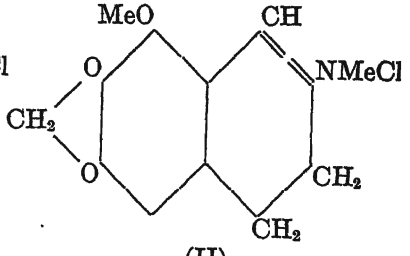
Besides the naturally-occurring alkaloids of the *iso*quinoline series, a considerable number of less complex bases have also been studied. Some of these have been prepared synthetically, whilst others are best obtained by the partial degradation of natural *iso*quinoline alkaloids.

We propose in this paper to deal with a special group of bases of the latter class, namely, the 2-alkyl-3:4-dihydro*iso*quinolinium bases with which we have been more particularly concerned.

Until the year 1909 only three bases of this type were known, namely, hydrastinine (I), cotarnine (II) and cotarnamic acid (III), a compound derived from cotarnine by hydrolysis.

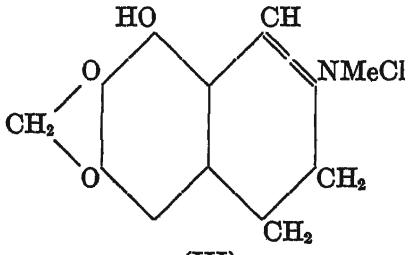


(I)



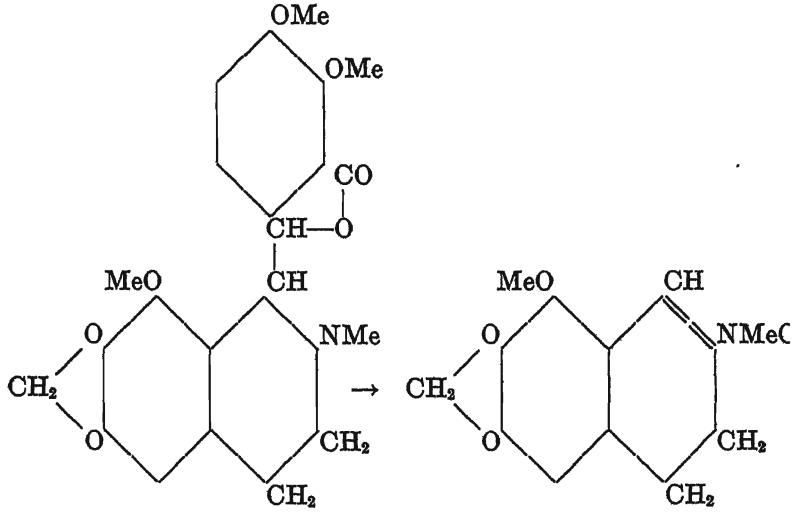
(II)

[1]Pictet & Finkelstein (*Ber.*, 1909, **42**, 1979).
[2]Pictet & Gams (*Ber.*, 1909, **42**, 2943).
[3]Pictet & Gams (*Ber.*, 1911, **44**, 2480).
[4]Perkin & Robinson (*J. C. S. Trans.*, 1911, **99**, 775).

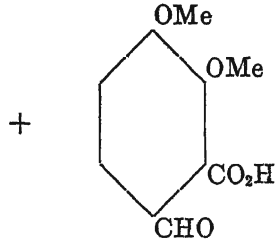


(III)

The only practical method at that time existing for the preparation of hydrastinine and cotarnine was the oxidative fission of hydrastine and narcotine (IV) respectively, when in each case opianic acid (VI) was obtained as a bye product. The following scheme depicts the oxidation of narcotine to cotarnine (V), and serves equally well to show the preparation of hydrastinine from hydrastine, when the methoxyl-groups in the 8-position of the *iso*quinoline rings are removed.

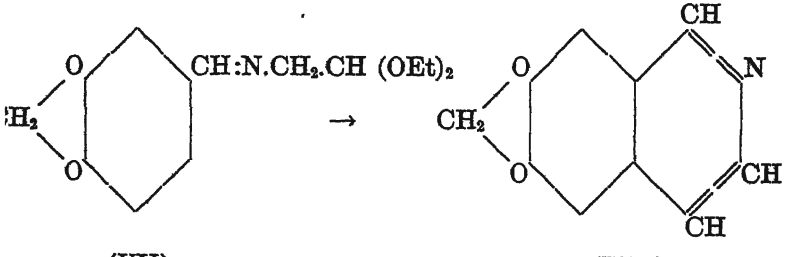


(IV) (V)

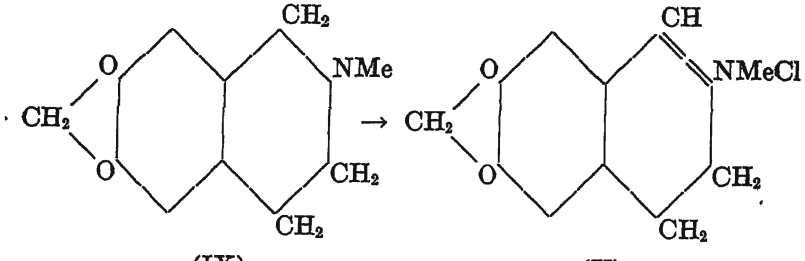


(VI)

No other method was known for the preparation of cotarnine, but hydrastinine had been synthesised in another way, which was not however, suitable for its preparation in quantity. This method[1] consisted in condensing 3:4-methylenedioxybenzylideneaminoacetal (VII) to 6:7-methylenedioxy*iso*quinoline (VIII) reducing a methyl salt of this base to hydrohydrastinine (IX) and oxidising the latter to hydrastinine (X)



(VII) (VIII)



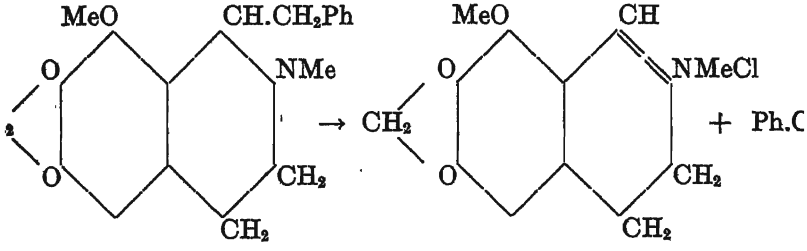
(IX) (X)

[1]Fritzsch (*Annalen*, 1895, **286**, 1).

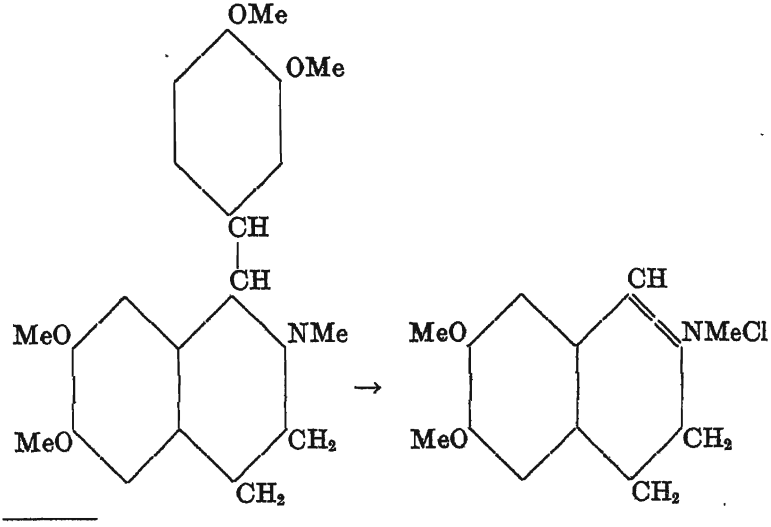
Since no other bases of the type of narcotine and hydrastine were known, cotarnine, hydrastinine and cotarnamic acid remained up till this time the only representatives of the 2-alkyl-3:4-dihydro*iso*quinolinium bases.

In 1909, however, Pyman[1] found that 1-benzyl-2-alkyltetrahydro*iso*quinolines in general gave on oxidation 2-alkyl-3:4-dihydro*iso*quinolinium bases together with the aldehyde corresponding with the substituted benzyl group: thus

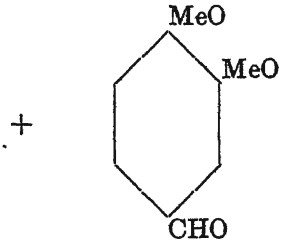
(1) 1-benzylhydrocotarnine gave cotarnine and benzaldehyde



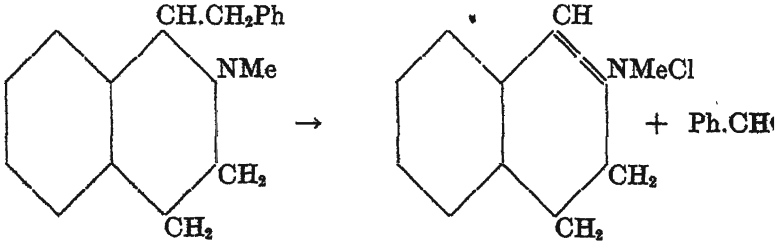
(2) laudanosine (1-veratryl-6:7-dimethoxy-2-methyltetrahydro*iso*quinoline) gave 6:7-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride and veratraldehyde,



[1] *J. C. S. Trans.*, 1909, **95**, 1266, 1738.



(3) and in the simplest possible case, 1-benzyl-2-methyltetrahydro*iso*quinoline gave 2-methyl-3:4-dihydro*iso*quinolinium chloride and benzaldehyde.

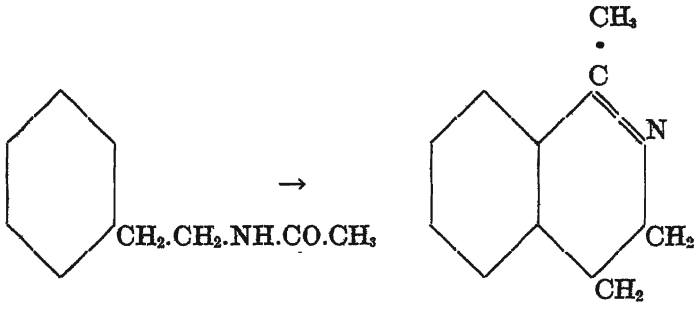


The discovery of this general method has led to important results, for it has been applied not only to the preparation of a considerable number of new 2-alkyl-3:4-dihydro*iso*quinolinium bases, with which we shall deal later, but has also been utilised in the synthesis of cotarnine by Salway, the synthesis of hydrastinine by Decker and the preparation of hydrastinine from berberine by Freund.

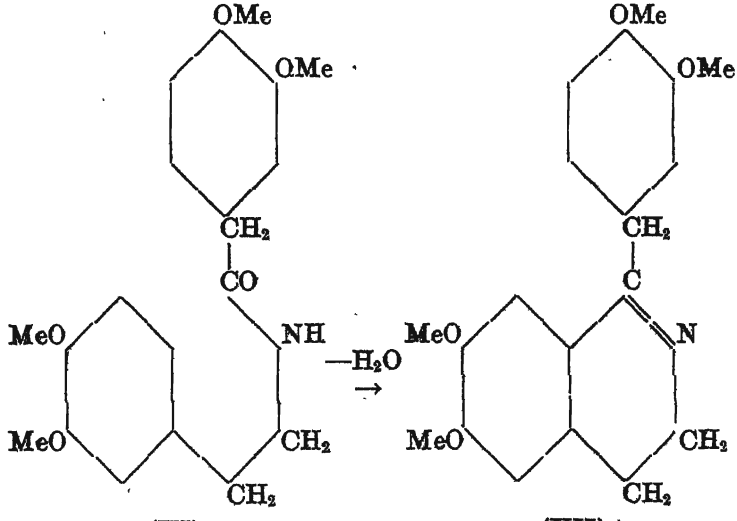
By means of this reaction, 2-alkyl-3:4-dihydro*iso*quinolinium bases can readily be prepared from the corresponding 1-benzyl-2-alkyltetrahydro*iso*quinolines, and it is therefore of interest to note how the latter are obtained.

In the first place, Bischler and Napieralski[1] in 1893, described a general method for the formation of *iso*quinoline derivatives consisting in the internal condensation of the acyl-derivatives of phenylethylamines.

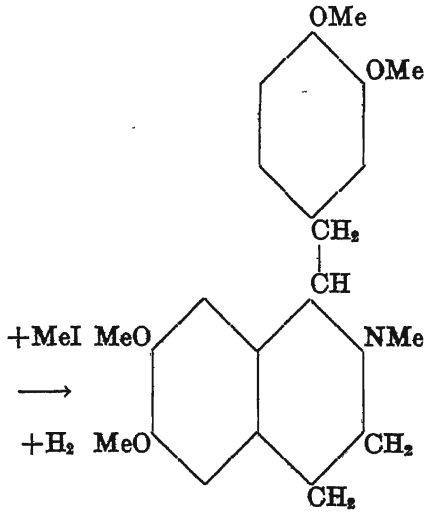
[1] *Ber.*, 1893, **26**, 1903.



Recently, the process has been thoroughly investigated by Pictet and Kay[1] and Decker and Kropp,[2] and has been developed into an excellent method for the preparation of 1-benzyl-2-alkyltetrahydro*iso*quinolines and similar bases. For instance, Picet and Finkelstein[3] were able to synthesise laudanosine (XIII) by first carrying out the internal condensation of homoveratroylhomoveratrylamine (XI) which they had prepared synthetically, then methylating the *iso*quinoline base (XII) so produced and reducing its methochloride.



[1] *Ber.*, 1909, **42**, 1973.
[2] *Ber.*, 1909, **42**, 2675.
[3] *Ber.*, 1909, **42**, 1979.

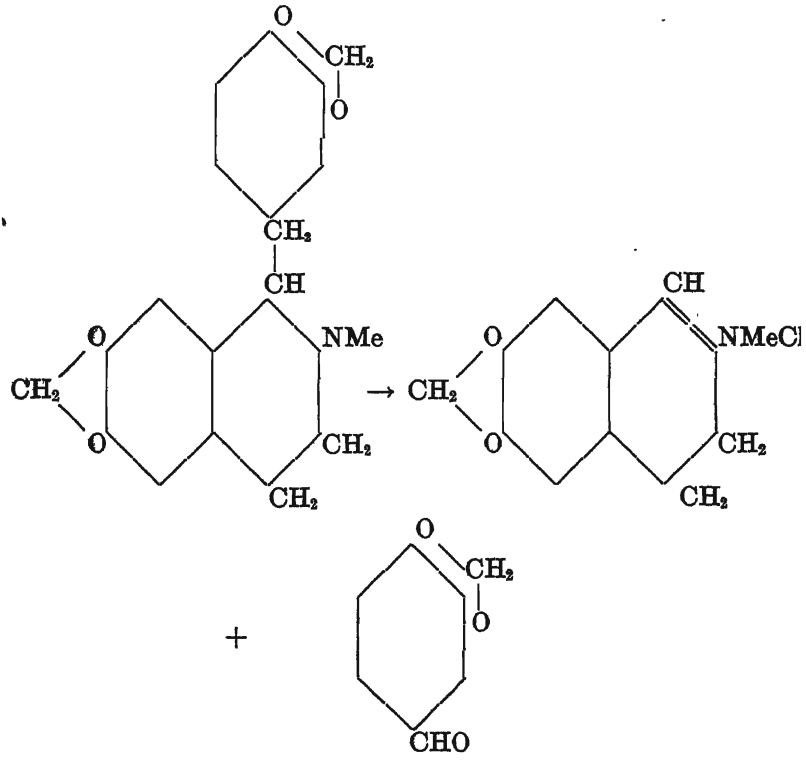


(XIII)

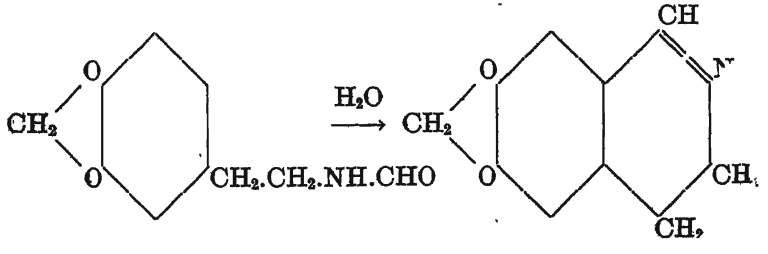
This method was applied by Salway[1] in 1910 to the synthesis of 1-benzylhydrocotarnine, and since this compound, as Pyman had previously shown, yields cotarnine on oxidation, the synthesis of cotarnine was thus effected.

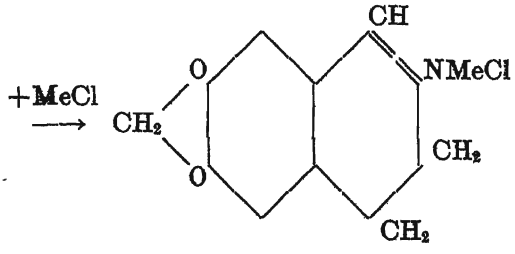
Decker[2] subsequently synthesised hydrastinine in a similar manner by first preparing 1-homo-piperonylhydrohydrastinine, and then splitting it by oxidation according to the following scheme:

[1] *J. C. S. Trans.*, 1910, **97**, 1208.
[2] *Chem. Zeit.*, 1911, **35**, 1077.



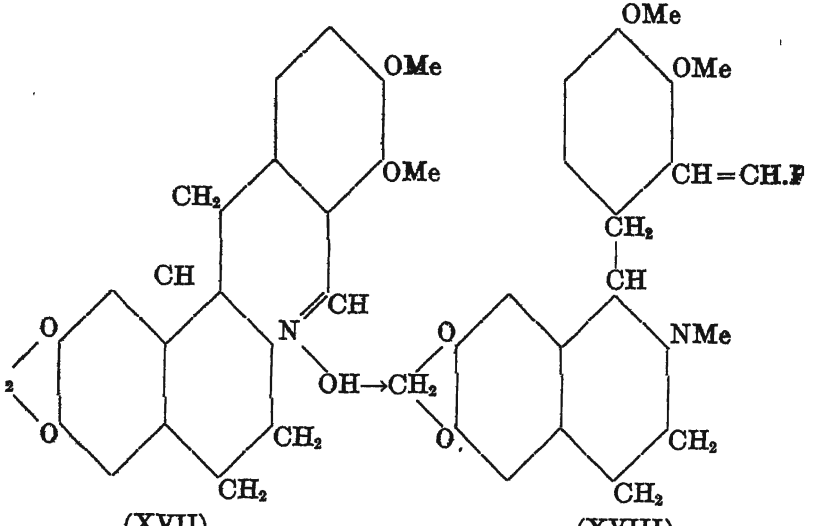
The same author has also described a method for the syntheses of hydrastinine and cotarnine by the internal condensation of the formyl-derivatives of the corresponding phenylethylamine; thus formylhomopiperonylamine (XIV) gave rise to 6:7-methylenedioxy-3:4-dihydro*iso*quinoline (XV) of which the methochloride is hydrastinine chloride (XVI), but this method gave poor yields owing to the reaction proceeding mainly in another direction.





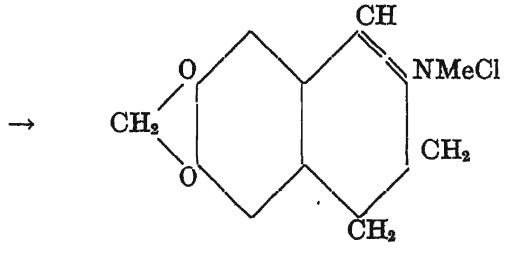
(XVI)

The general method for the preparation of 2-alkyl-3:4-dihydro-*iso*quinolinium bases by the oxidation of 1-benzyl-2-methyl-tetrahydro*iso*quinolines has recently been utilised by Freund[1] in the preparation of hydrastinine from berberine (XVII); in this process the latter alkaloid is converted by an interesting series of reactions into a compound of the formula (XVIII), which in accordance with the general rule yields the corresponding 2-alkyl-3:4-dihydro*iso*quinolinium base—in this case hydrastinine (XIX)—on oxidation.



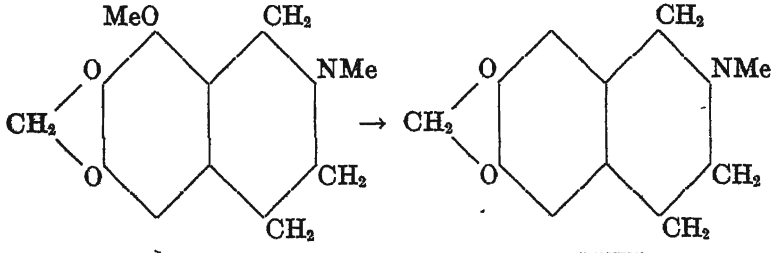
(XVII) (XVIII)

[1] *Chem. Zeit.* 1911, **35**, 1090.



(XIX)

Besides hydrastine and berberine, another naturally-occurring alkaloid, namely, narcotine may be used as a source of hydrastinine by means of the following process.[1] Narcotine is oxidised to cotarnine, and this reduced to hydrocotarnine (XX) by known methods; the latter base is then strongly reduced by means of sodium and alcohol, when the methoxyl-group is replaced by hydrogen and hydrohydrastinine (XXI) results.



(XX) (XXI)

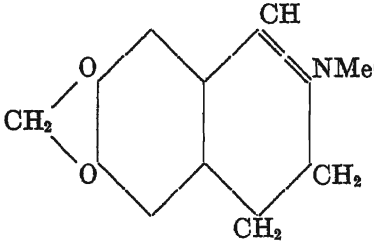
The latter base readily yields hydrastinine on oxidation.

Having now given some account of the methods applied to the synthesis of the previously known alkaloids cotarnine and hydrastinine, we propose to deal with a number of new 2-alkyl-3:4-dihydro*iso*quinolinium bases. All these have been prepared by the general method already described, that is the oxidation of the corresponding 1-benzyl-2-alkyl-tetrahydro*iso*quinoline.

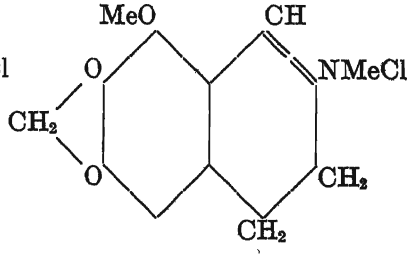
Hydrastinine (A) and cotarnine (B) have long been used in therapeutics as haemostatics particularly in abnormal uterin

[1]Wellcome, Pyman & Remfry (*Eng. Pat.* 23,736 of 1911).

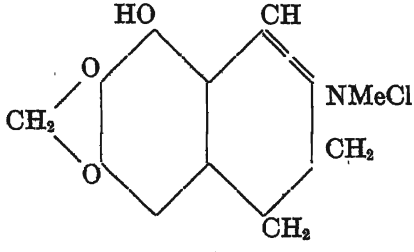
conditions. They cause contraction of the isolated uterus of cat, rabbit or guinea-pig. Hydrastinine occasions a rise of blood pressure, and cotarnine a fall succeeded by a very slight rise. Cotarnamic acid (C) in which the methoxyl-group of cotarnine is replaced by a hydroxy-group produces a minimal rise of blood pressure, but has no significant action on the uterus or other organ.



(A)



(B)



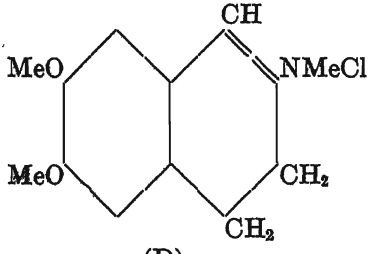
(C)

The new bases described below were prepared by one of us[1] except where otherwise stated, in an endeavour to produce an improved uterine haemostatic.

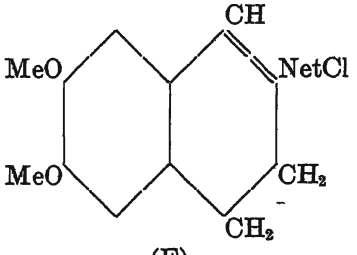
The physiological action of the most important of the new bases, 6:7-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (D) has already been fully described by Laidlaw[2] who has shown that this compound produces a well-marked contraction of the uterus, and a rise of blood pressure due to vaso-constriction and increased cardiac output; its action appears to be similar

[1]Pyman, (*J. C. S. Trans.*, 1909, **95**, 1266, 1738; 1910, **97**, 264).
[2]*Biochem. J.* 1910, **5**, 243.

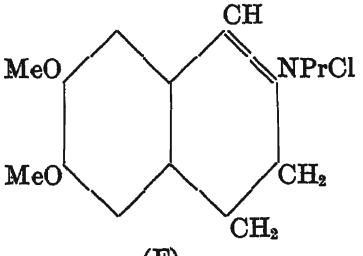
to that of hydrastinine. Clinical reports have shown that it is of great value in abnormal uterine conditions. It is slightly more toxic than cotarnine.



(D)



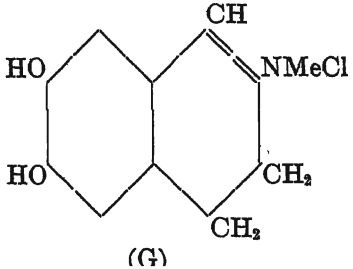
(E)



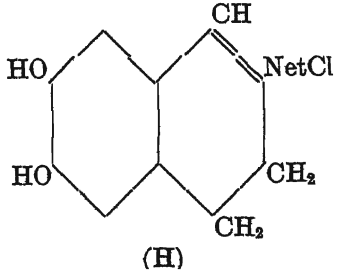
(F)

A considerable number of bases differing only slightly from (D) in chemical constitution has been prepared. In the first place, the compounds (E) and (F) in which the methyl-group on the nitrogen atom is replaced by the ethyl- and propyl-groups respectively were made; of these (E) proved to be very similar in its general action to (D), but was considerably more toxic. In its action on the blood pressure, (E) resembles cotarnine causing a fall, succeeded by a very slight rise.

Then, the corresponding dihydroxy-bases (G) and (H) were prepared from (D) and (E) respectively by hydrolysis:

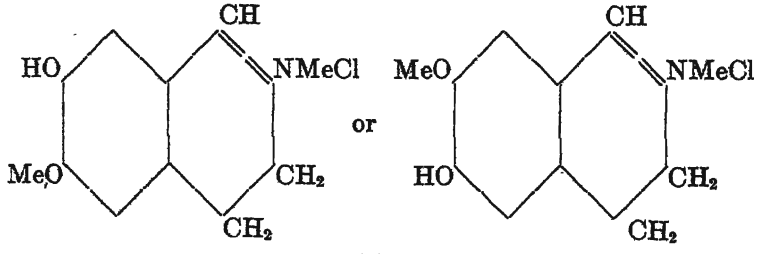


(G)



(H)

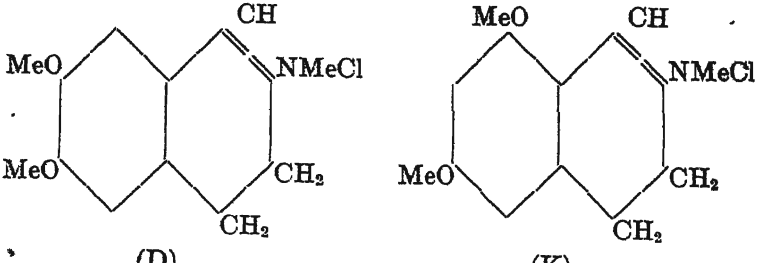
Of these, (G) was physiologically examined, but produced only a minimal rise of blood pressure, and had no significant action on the uterus, thus behaving like cotarnamic acid (C), the hydrolytic product of cotarnine. 6(or 7)-Methoxy-7(or 6)-hydroxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (J) which has one of the two formulae given below:



(J)

represents an intermediate stage in the hydrolysis of (D) to (G), one methoxyl being replaced by hydroxyl; this compound causes contraction of the uterus and a slight rise of blood pressure.

Salway[1] has recently prepared 6:8-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (K), which is isomeric with (D), differing only from it in the position of one of the methoxy-groups as is shown below.

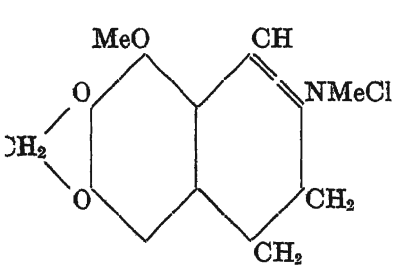


(D) (K)

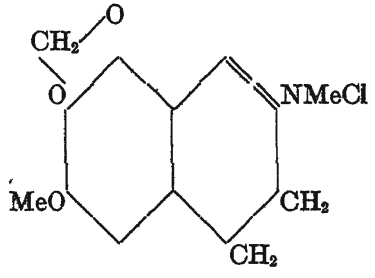
Laidlaw has shown that (K) closely resembles (D) in its action on the isolated uterus, and is considerably less toxic than either (D) or cotarnine. It further resembles (D) in producing a rise of blood pressure accompanied by slowing of the heart beat when injected into the blood stream of a cat.

[1] *J. C. S. Trans.*, 1911, 99, 1320.

Salway[1] has also prepared *neo*cotarnine (L) an isomeride of cotarnine (C) having the relation to it shown below:



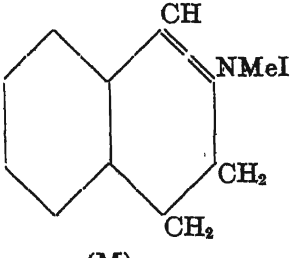
(C)



(L)

but unfortunately no account of its physiological action has yet been published.

The simplest possible example of this type of substance, namely, 2-methyl-3:4-dihydro*iso*quinolinium iodide (M) has also been prepared and tested.



(M)

Its action, however, is peculiar in that it produces in doses of 10 to 20 milligrams a marked rise of blood pressure superficially similar to that produced by adrenine.

It will be clear from a consideration of these results that the property of causing contraction of the uterus is common to all those 2-alkyl-3:4-dihydro*iso*quinolinium bases tested which contain only methoxy- or methylenedioxy-groups as substitutes; where these are replaced by hydroxy-groups (except in the case of J) this property appears to be lost or at least seriously diminished.

[1] *J. C. S. Trans.*, 1910, **97**, 1208.

Acid Amides

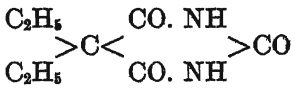
The title of this paper being the relationship between chemical constitution and physiological action in acid amides, we would explain at the outset that we use here the term " acid amides " in a rather wider sense than is usual and include under this heading substances containing the — CO — NH — group, such as urethanes and barbituric acids. The large majority of hypnotics are found to contain an NH_2 group and Fränkel[1] has brought forward evidence to show that even when several ethyl-groups are present in the molecule ăn unsubstituted NH_2 group is often necessary to impart to it hypnotic properties. Thus he regards ethyl urethane as an ester of an acid amide and not as an amino acid, for the reason that if an amino-acid, then the next higher gomologue, ethyl glycollate, should have hypnotic properties, but as a fact has none. In the light of this reasoning several large classes of hypnotic substances can fairly be brought within the scope of the present paper. With the theory of narcosis we are not specially concerned as that is more a question for the physiologist than the chemist. Suffice it to say that the principal one has been that devised by Overton and Meyer, which, put briefly, states that the more soluble a substance is in fats (lipoid substance) and the less in water, i.e., the greater the ratio of the solubility fat: water, the higher will be its hypnotic power.

Thus a compound having a large solubility ratio will be a stronger hypnotic than one having a smaller ratio.

Although giving results agreeing very closely with those found in practice, it has not held undisputed sway, and several new theories have been put forward of late years. For them we would refer readers to the original papers or to an excellent condensed exposition of the subject in Fränkel's Arzneimittelsynthese, 3rd edition 1912, page 510.

The trend of modern investigation has followed rather closely on those lines which have proved most successful in the past and the splendid results obtained by the use of diethylbarbituric acid (I)

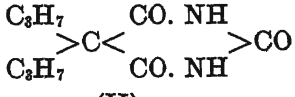
[1] *Arch. expt. Path. Pharm.* 1908, suppl. 181.



(I)

have led to the production of an enormous number of closely allied derivatives in the hope that an even safer and more effective hypnotic could be thus obtained.

Dipropylbarbituric acid (II) is the only derivative in which a change in the alkyl groups has effected an increase in hypnotic power. This, however, has been found to be almost too powerful in its action and consequently dangerous for general use.

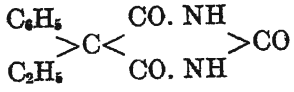


(II)

In order to obtain an hypnotic of the same order of activity as diethylbarbituric acid, but one of which it was necessary to take less in order to produce the same effects, the sodium derivative of this acid was introduced. It was expected that its much greater solubility in water would enable it to exert a prompter action and in this way have the same effect as a larger dose of the free acid. These expectations, however, were not realised in practice, for very little real difference was found in the rapidity with which sleep was induced by the two compounds.

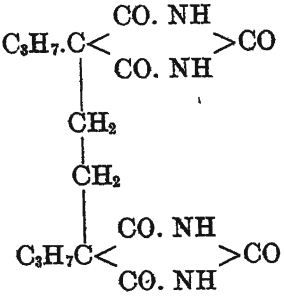
In the light of the Overton-Meyer theory this is not to be wondered at as the sodium derivative is probably quite insoluble in lipoid substances and hence, before action can take place, has to be decomposed and the free diethylbarbituric acid liberated by the acids of the body.

Dibenzylbarbituric acid has already been proved inactive, but just as acetamide (which also has no hypnotic effect) is endowed with slight hypnotic properties by the introduction of a phenyl radicle as in phenylacetamide, $C_6H_5.CH_2.CO.NH_2$, so inactive ethylbarbituric acid is said to be converted to a safe and active hypnotic when transformed into the phenyl-derivative (III).



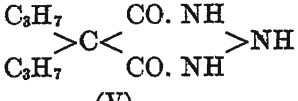
(III)

The joining together of two molecules of propylbarbituric acid by an ethylene linkage as in ethylene bis-5-propylbarbituric acid[1] (IV) resulted in an inactive compound although it may be regarded as ethylpropylbarbituric acid substituted on the β-carbon atom of the ethyl radicle by a molecule of propylbarbituric acid.



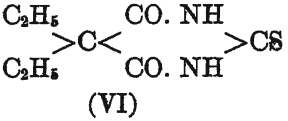
(IV)

Any attempt, however, to interfere with the barbituric acid ring dooms the product to failure as far as useful hypnotic properties go, for example, dipropylmalonylguanidine (V) is inactive.



(V)

Diethyl-*N*-methylbarbituric acid is very poisonous although still a strong hypnotic and the same occurs in the case of diethylmalonylthiourea (VI).

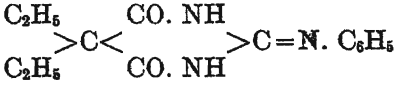


(VI)

[1]Remfry (*Trans.*, 1911, 99, 623).

The poisonous properties imparted by the methylation of the imino-group are analogous to the case of methyl benzamide (C_6H_5. CO. NH. CH_3) which has a strychnine-like action whilst benzamide itself has a slight alcohol-like narcotic effect. Similarly *N*-methylphenacetin is much more poisonous than phenacetin.

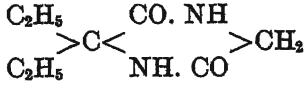
Einhorn[1] has described a great number of what may be regarded as derivatives of diethylmalonamide, in which the carbon atom joining the two nitrogen atoms has had different groups attached to it, such as



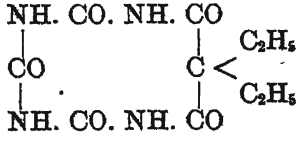
These, however, all proved to be inactive.

Other examples of substances which differ only slightly in the construction of the ring, but still contain at least two ethyl or propyl radicles attached to one carbon atom and are yet inactive, are given in the following:

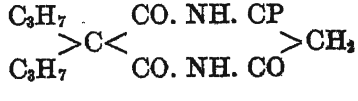
Diethylketopiperazine[2]



Diethylmalonylcarbonyldiurea

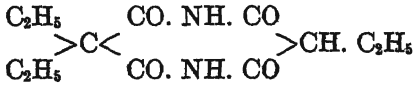


Dipropylmalonylmalonamide[3]

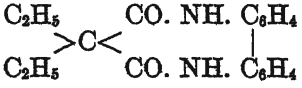


[1] *Annalen*, 1908, **359**, 145.
[2] Rosenmund (*Ber.*, 1909, **42**, 4470).
[3] Remfry (*Trans.*, 1911, **99**, 618).

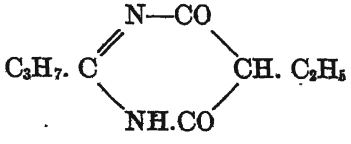
Diethylmalonylethylmalonamide[1]



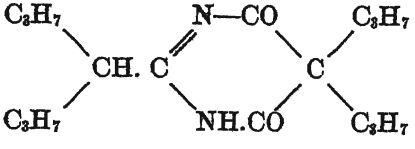
Diethylmalonylbenzidine[1]



4:6-*diketo*-2-*propyl*-5-*ethyltetrahydropyrimidine*[1]



4:6-*diketo*-5:5-*dipropyl*-2-∝ -*propylbutyltetrahydropyrimidine*[1]



This last substance is a good example to show that multiplicity of alkyl-groups is of no avail when the nucleus of the compound is incorrect. In all these cases however, inactivity may be due to a possible insolubility in lipoid substance in the light of the Overton-Meyer hypothesis.

Many compounds described in recent literature have been prepared evidently (from their formulae) on the chance of their possessing hypnotic properties, but no reference can be found to the results of physiological tests. In all such cases we are forced to conclude that the substances were either inactive or had such small activity that it was of no use pursuing that course further. Belonging to this class are a large number of compounds pre-

[1]Remfrey (*Trans.*, 1911, **99**, 618)

pared by Clemmensen and Heitman,[1] all containing two ethyl radicles attached to one carbon atom and of the general formulae

$R_2 : C(OH)\ CO.\ NH$ $R_2 : C(OH)\ CO.\ NH.\ CO.\ NH_2$

$R_2 : C(OH)\ CO.\ NH > CO$ and

$(R = C_2H_5)$

They also prepared diethylthiohydrantoin, which, however, would only be expected to have the same slight hypnotic power as diethylhydrantoin itself, and at the same time to be more toxic.

An example of the small structural change which is sufficient to deprive a substance of all hypnotic properties is afforded in the urethane as well as in the barbituric acid series.

Acetyl urethane (VII) which is as active as urethane itself, only differs materially from tertiary amyl allophante (VIII) in having a CH_3 in place of an NH_2 group, yet, whilst the former is possessed of moderate hypnotic powers, the latter is a perfectly inert substance.

Et. O. CO. NH. CO. CH_3 (VII) $(Me)_2$. Et C. O. CO. NH. CO. NH_2 (VIII)

It is curious that ethyl cinnamoylcarbamate $C_6H_5.CH:CH.\ CO.\ NH.CO_2C_2H_5$, where the NH_2 group of urethane is substituted by an aromatic in place of a fatty acid radicle, should prove quite inactive. Urethanes have not received much attention of late years; a great number of derivatives have already been prepared and no very satisfactory results having been obtained, has made it apparent that this substance is not a favourable groundwork to build on and hence it has been shelved for more promising subjects. A single exception to this is found in amylenehydrate-carbamate

$(Me)_2$ Et C. O. CO. NH_2

which has been found[2] to have about twice the hypnotic power of amylenehydrate itself and to give good results where no very drastic treatment is necessary. This substance affords another example of the fact that alkyl-groups alone do not necessarily

[1] *Am. chem. journal*, 1908, **40**, 280.

[2] Huber (*Med. Klinik.*, 1911, 1234).

endow a compound with hypnotic properties as can be seen by comparing its formula with that of tertiary amyl allophanate.

Besides urethanes other acid amides are deprived of their hypnotic properties when substituted in the amido-group.

Ethyl cinnamoylcarbamate can be regarded as a substituted cinnamamide and in this case we have another active acid amide neutralised by the entrance of a carbonic ester into the amido radicle. It was shown many years ago[1] that most aromatic acid amides possess hypnotic properties in varying degree, but if one or both H atoms of the amido-group are substituted by an alkyl radicle then the substance becomes more like ammonia and strychnine in its action. Now, however, it has been demonstrated that other substituents as well as alkyl-groups are capable of depriving aromatic acid amides of their hypnotic properties, and it seems as if almost any substituent in the NH_2 group were sufficient for that purpose.

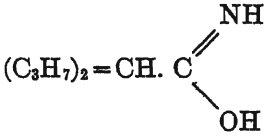
Carbonyl dicinnamamide (Dicannamoyl carbamide) $(C_6H_5.CH : CH.CONH)_2 = CO$ is quite inactive.[2]

A like result was found when the two active hypnotics cinnamamide and acetophenone are combined in cinnamoyl-p-aminoacetophenone $C_6H_5.CH : CH.CO.NH.C_6H_4.CO.CH_3$; also in the combination of bromisovaleramide, which has narcotic properties,[3] and acetophenone.[4] These compounds also afford further proof of the fact observed by Hildebrandt[5] that when p-aminoacetophenone was combined through the NH_2 group with an aldehyde, the hypnotic power was decreased unless the aldehyde possessed a free hydroxyl group, when an increase was observed.

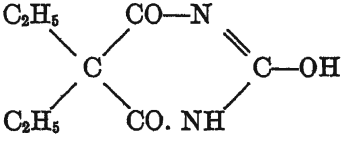
Fuchs[6] has advanced the theory that the presence of an OH group, as well as alkyl radicles, is necessary in an hypnotic substance in order to act as an anchor. This conclusion is arrived at in consideration of the fact that whilst diethyl-, ethylpropyl-

[1]Nebelthau (*Arch. expt. Path. Pharm.* 1895, **36**, 451).
[2]Remfry (*loc. cit.*).
[3]Eckhart (*Arch. expt. Path. Pharm.* 1907, **57**, 339).
[4]Remfry (*loc. cit.*).
[5]*Arch. expt. Path. Pharm.* 1905, **53**, 87.
[6]*Zeit. f. angew Chem.* 1904, **17**, 1505.

and dipropyl- etc., ketones are inactive the corresponding ketoximes are occasionally strong hypnotics. Also dipropylacetamide, which on being dissolved in alkali goes into its tautomeric iminoether form



is found to be more powerful in its action than such substances as chloral hydrate, sulphonal, amylenehydrate, etc., and is only surpassed by diethylbarbituric acid. This latter is also considered to act in the form



If this theory of the anchoring hydroxyl-group is correct it accounts satisfactorily for the loss of hypnotic properties by aromatic acid amides when both H atoms of the amido-group are replaced by alkyl radicles, it being impossible for the tautomeric iminoether form to be assumed. The acceptance of this theory moreover forces one to the conclusion that only in the case where no substitution at all occurs in the NH_2 group can a change to the tautomeric form take place, otherwise it is probable that some at least of the mono-substituted acid amides would have exhibited hypnotic properties.

Dipropylacetbromamide $(C_3H_7)_2 = CH.\ CO.\ NH.\ Br$[1] is a further case in point. This compound is quite inactive whilst bromdipropylacetamide $(C_3H_7)_2 = CBr.\ CO.\ NH_2$ possesses considerable activity, only slightly less than that of the diethyl-compound which has found practical application.

Although having no close connection with acid amides it is of interest to note several cases which have appeared lately to show

[1] Fuchs (*loc. cit.*).

the varying results obtained by the introduction of alkyl-groups into a compound. Glycerol is quite inactive, as is also the trialkyl ether where the three alkyl groups are similar. When, however, one differs from the other two, or when all three are dissimilar, then it is claimed that substances having hypnotic properties are produced[1] as for example glycerin-α-ethyl-α-propyl-β-methylether.

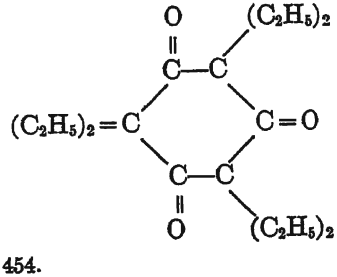
Ortho ketone ethers of the general formula $\begin{matrix} R_1 & & OC_2H_5 \\ & >C< & \\ R_2 & & OC_2H_5 \end{matrix}$

were made by Reitter and Hess,[2] which from the number of alkyl radicles and the general resemblance to ethyl ether might well be expected to have proved successful, yet turned out to be entirely without physiological action whatever. This appears somewhat strange in view of the fact that trioxyethylmethane (ortho formic ethyl ether) has been recommended

$$CH(OC_2H_5)_3$$

by Chevalier[3] as an antispasmodic where it evidently acts as a sedative or very mild hypnotic. The inactivity of the former compound may however, be due to insolubility in lipoid substance (Overton-Meyer theory).

A further example is given by Fränkel[4] who showed that the introduction of ethyl radicles into phloroglucin to the fullest extent, i.e., hexaethylphloroglucin, was without power to endow this substance



[1] D. R. P. 226, 454.
[2] *Ber.*, 1907, **40**, 3024.
[3] *Rep. de pharmacie*, 1907, **6**, 271.
[4] *Arch. expt. Path. Pharm.*, 1908, **58**, 181.

with narcotic properties, a compound being obtained having only a strychnine-like action.

In conclusion it may be mentioned that a close connection seems to exist between narcotics and local anaesthetics.

Gros[1] has compared the action of these two classes of substances and concludes that, to all intents and purposes, it is the same in both cases. He therefore considers that local anaesthetics are nothing more than strong hypnotics. For the purposes of comparison the narcotics employed were chloroform, paraldehyde, chloral, amylene hydrate, ethyl propionate, amylacetate, acetophenone, phenyl and ethyl urethane, whilst the local anaesthetics comprised alypin, cocaine, eucaine, stovain, tropacocain, nirvanin, holocain and subcutin.

Chloral has also been used in medical practice as a general anaesthetic but was found to be dangerous. Lately, however, another substance which, like chloral, was first employed as a hypnotic has been very successfully used in producing general anaesthesia. This substance is methylpropylcarbinolurethane, which was first introduced as a hypnotic about the year 1900. In 1910 its use as a general anaesthetic by intravenous injection

$$\text{Me Pr CH. O. CO. NH}_2$$

was first described,[2] since when several reports have appeared, the latest being by Page.[3]

It is therefore possible to attack the problem of the true manner in which hypnotics act from two points, and the fact that hypnotics also act as local and general anaesthetics irrespective of their volatility may help to elucidate the matter.

Finally, we should like to express our best thanks to Drs. H. H. Dale and P. P. Laidlaw of the Wellcome Physiological Research Laboratories, who have co-operated with us throughout the work and conducted most of the physiological experiments herein recorded.

[1] *Arch. expt. Path. Pharm.*, 1910, **62**, 380; 1910, **63**, 80; 1911, **64**, 67.

[2] Siehkovski (*Russki Vrach St. Petersbury* 1910, **9**, 1447).

[3] *Lancet*, 1012, **182**, 1258.

SUR LES ELEMENTS MINERAUX CONTENUS DANS LA CASEINE DU LAIT

PAR M. L. LINDET

Paris

Nous connaissons bien mal l'état dans le quel se présentent certains éléments minéraux, quand nous les rencontrons associés à des matières protéiques. On dit communément par exemple que la caséine du lait renferme du phosphate de chaux, parceque l'analyse permet d'y déceler du phosphore et du calcium; dans la caséine précipitée par la présure, le phosphore, exprimé en P_2O_5, représente de 3,50 à 3,55 % de la caséine sèche, alors que le calcium, exprimé en CaO, représente de 3,10 à 3,80%; si ces éléments formaient, à l'intérieur de la molécule protéique, du phosphate de chaux, celui-ci aurait une formule intermédiaire entre le phosphate bicalcique et le phosphate tricalcique.[1]

Je voudrais démontrer, dans ce mémoire, qu'une partie seulement du phosphore, environ la moitié, est à l'état de phosphate, probablement tricalcique, et que l'autre est engagée, à l'état d'acide phosphorique encore, dans une combinaison, hydrolisable par les alcalis. Quant à la chaux en excès par rapport à celle qui forme le phosphate de calcium, elle sature la fonction acide de la caséine; mais cette saturation n'est que partielle; car, comme je l'indiquerai plus loin, on peut faire absorber à la caséine, plus de 7% de chaux, comme on peut lui faire absorber de l'alumine, du zinc, etc. Il est probable que le phosphate de calcium est lui-même dissous par cette fonction acide; nous avons, M. L. AMMANN et moi, (Ann. de l'Institut national agronomique, 1906, p. 283) montré que l'on peut saturer du

[1]Les dosages d'acide phosphorique et de chaux ont toujours été obtenus en attaquant la caséine par l'acide nitrique fumant, puis par l'acide sulfurique jusqu'à décoloration; on reprenait ensuite par l'eau et par l'ammoniaque; on acidulait par l'acide acétique, pour éliminer ensuite la chaux au moyen de l'oxalate; puis on dosait l'acide phosphorique à l'état de phosphate ammoniaco-magnésien.

caséinate de chaux par l'acide phosphorique sans que le liquide se trouble, c'est à dire sans que le phosphate formé se dépose. Il est également possible que le phosphate de chaux soit soluble dans le caséinate de chaux, bien que je n'aie pu jusqu'ici réaliser cette solubilisation, en partant du phosphate précipité; car il convient de remarquer que la caséine, précipitée, par la présure, est entièrement soluble, sans dépot de phosphate de chaux, dans l'ammoniaque et même dans la résorcine concentrée.

I. Je traite la caséine précipitée par la présure au moyen d'une solution acétique faible, et j'enlève de cette façon la chaux combinée à la fonction acide, et le phosphate de chaux, et j'obtiens un résidu décalcifié, qui renferme encore à peu près la moitié du phosphore que la caséine contenait primitivement.[1]

Les résultats de l'épuisement acétique de la caséine, provenant de l'emprésurage, sont consignés dans le tableau suivant:

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

	P_2O_5	CaO	ce qui représenterait: Phosphate de chaux	Chaux en excès enlevée par l'acide acétique
1er Épuisement	1,01	2,47	2,20	1,28
2e Épuisement	0,40	0,79	0,85	0,34
3e Épuisement	0,14	0,33	0,30	0,17
4e Épuisement	0,05	0,10	0,10	0,05
Résidu	1,88	0,00	0,10	0,05
	3,48	3,69	3,45	1,84
au lieu de	3,55	3,80		

[1]Les liquides acétiques dissolvent malheureusement de la caséine; on les en débarassait au moyen de sulfate de bioxide de mercure, et dans les liquides, additionnés de citrate d'ammoniaque et d'ammoniaque, on ajoutait le chlorure de magnésium; on s'assurait que le précipité mercurique ne renfermait pas d'acide phosphorique, en le reprenant par l'acide nitrique fumant.—J'ai obtenu également l'élimination de la caséine dissoute en chauffant les liqueurs en autoclave, en présence du formol.

Si tout le phosphore de la caséine s'y trouvait à l'état de phosphate de chaux, il n'y aurait aucune raison pour que l'acide étendu ne l'enlève pas en même temps que toute la chaux; quand on attaque en effet du phosphate tricalcique par de l'acide acétique éten du, l'acide phosphorique et la chaux se dissolvent, à tout moment, en quantités équivalentes. Nous dirons donc que l'acide acétique a fait disparaitre le phosphate de chaux (3,45% de la caséine) et la chaux combinée à la fonction acide de la caséine (1,84%).

La substitution de l'acide acétique à la présure dans la coagulation de la caséine détermine la précipitation d'une caséine pauvre en chaux, que l'on peut appauvrir davantage par un lavage à l'acide étendu; mais comme dans le cas ci-dessus, il reste du phosphore insoluble dans l'acide acétique étendu; celui-ci, compté en $P_2 O_5$, a représenté, dans mes expériences, sensiblement le même chiffre que précédemment (de 1,80 à 2,00%). Le fait est d'ailleurs connu des fabricants de caséine, qui, suivant l'usage au quel est destiné le produit, caillent le lait écrémé, soit par la présure, soit par l'addition d'un acide minéral, soit par l'action biologique du ferment lactique; j'ai trouvé dans le commerce une caséine, provenent de l'acidification lactique, qui conservait encore une quantité de phosphore, représentant 1,80% de $P_2 O_5$.

J'ai été d'ailleurs à même de vérifier ce fait, en recherchant l'action de l'acide phénique sur le lait; je pensais que cet acide phénique, dont j'ai montré les propriétés, dissolvantes vis à vis de la chaux (Bin Soc. chimique. 1910, P. 435) serait capable de déplacer la chaux combinée à la fonction acide de la caséine; l'expérience a été négative. Mais elle n'a pas été inutile; car elle confirme ce qui vient d'etre dit: Deux portions d'un même lait, dont l'une avait été additionnée d'acide phénique, ont été caillées par la présure, et l'on a récolté les sérums; le lendemain, on a coagulé par la chaleur chacun d'eux, et on a dosé l'acide phosphorique et la chaux dans les coagulums et dans les liquides. Dans le coagulum du sérum phéniqué, il y a eu plus d'acide phosphorique et plus de chaux que dans le coagulum du sérum témoin, parceque ce sérum s'était acidifié du jour au lendemain, et que l'acide lactique produit avait enlevé du phosphate de

chaux et de la chaux; le complément de ces deux éléments se retrouve dans les liquides séparés du coagulum ainsi que le montre le tableau suivant:

		Rapporté au litre de lait : en grammes :	
		P_2O_5	CaO
Coagulum	du sérum témoin	0,263	0,304
	du sérum phéniqué	0,279	0,349
Liquide séparé du coagulum	du sérum témoin	0,713	0,284
	du sérum phéniqué	0,700	0,245
Total dans le sérum primitif	témoin	0,976	0,588
	phéniqué	0,979	0,594

II. Pour rechercher l'état chimique que le phosphore affecte dans le résidu insoluble, j'ai eu recours, comme je l'ai dit plus haut, à une hydrolise ménagée en présence des alcalis ou des alcalino-terreux, et j'ai été frappé tout d'abord de la facilité avec la quelle ceux-ci dissocient, même à froid, la molécule de caséine. Mais ce qui nous intéresse en l'espèce, c'est que le phosphore de la caséine, qui restait insoluble dans l'acide acétique étendu, est dès lors facilement décelé à l'état d'acide phosphorique.

Si, par exemple, on traite par un lait de chaux de la caséine décalcifiée, et si on filtre, on obtient une solution qui renferme de la caséine, du phosphate de chaux et de la chaux en excès, et qui représente, comme nous l'avons appelé, M. L. Ammann et moi (loc. cit.) une solution de phosphocaséinate de chaux. Cette chaux en excès, abstraction faite de la chaux que l'eau dissoudrait naturellement, a représenté, dans mes expériences, de 7,30 à 7,75% de la caséine; elle est fixée par la fonction acide de la caséine. En outre, cette solution qui se décompose, qui se dégrade, en fonction du temps et de la température, donne naissance à de l'ammoniaque et aux produits que Schutzemberger a isolées, en chauffant des matières albuminoides à 180° en présence de la Baryte. L'addition d'acide acétique en excès dans une semblable solution précipite de la caséine non décomposée, en quantité d'autant plus grande que la dégrada-

tion a été moins accentuée. Mais ce qui frappe surtout, c'est que cette caséine ne renferme plus de phosphore, et que le phosphate de chaux, dissous dans l'acide acétique étendu, est passé dans les liqueurs. Les chiffres du tableau suivant indiquent la marche du phénomène:

	Durée du contact de la chaux avec la caséine	Caséine non dégradée % (1)	Caséine dégradée %	Azote de l'ammoniaque dégagée % de l'azote total	Acide phosphorique contenu dans la caséine précipitée
A 20-25°	24 heures	79,2	20,8	"	0
	48 heures	75,9	24,1	2,03	0
	96 heures	69,2	30,8	3,06	0
	10 jours	60,4	39,6		0
A 35°	48 heures	63,2	36,8	"	0

La soude ne dégrade pas et n'hydrolise pas l'acide phosphorique aussi vite que la chaux; quand on a soin de ne mettre que la quantité nécessaire de soude pour dissoudre une caséine à 1,80% d'acide phosphorique, on obtient, dans trois précipitations successives à l'acide acétique, des caséines qui renferment encore 1,56, 1,06, 0,78% d'acide phosphorique. A chaud, la dégradation de la caséine est plus rapide, et après un chauffage d'une heure à 120°, on ne précipite plus de caséine par l'acide acétique.

L'ammoniaque est, vis à vis de la caséine, encore moins énergique que la soude, et j'ai pu, en dissolvant à froid de la caséine à 1,80% d'acide phosphorique, avec le minimum d'ammoniaque, et pendant le minimum de temps, et en précipitant trois fois par l'acide, obtenir le même taux d'acide phosphorique. Mais un chauffage de cinq heures au Bain-Marie a fourni une caséine précipitée, qui ne renfermait plus que 1,30 d'acide phosphorique.

Je reviens à l'action de la chaux: On peut mettre, en évidence d'une façon plus élégante, cette action dissolvante de la chaux vis à vis de l'acide phosphorique que la caséine retient. La solution de phosphocaséinate de chaux, telle qu'elle a été préparée plus haut, est chauffée en autoclave, à 120°, pendant une heure;

(1) J'ai compté comme caséine non dégradée celle qui était précipitée par l'acide en excès, et celle que l'acidité acétique dissolvait normalement dans le liquide.

la dégradation de la matière protéique se produit; mais la caséine non dégradée, qui représente, dans ce cas, de 25 à 38% de la caséine primitive, se coagule, emprisonnant tout le phosphate primitif (P_2O_5 = 3,66% du coagulum) et un excès de chaux (CaO = 11,70% du coagulum), tandis que les liqueurs, qui renferment les matières azotées dégradées, en même temps que la chaux en excès, sont exemptes de phosphore. J'ai mesuré la dégradation de la caséine, dans ce cas, en dosant l'ammoniaque dégagée;[1] celle-ci, comptée en azote, a représenté de 13,5 à 26,0% de l'azote total.

Ce coagulum a été alors épuisé par de l'acide acétique étendu qui a enlevé très facilement le phosphate de chaux formé et la chaux en excès, en sorte qu'il est resté, comme le montre le tableau ci-dessous, une caséine sans calcium ni phosphore; cette caséine comme la précédente, se dissout dans la chaux, renferme 15,55% d'azote, etc.

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

				ce qui représenterait:	
		P_2O_5	CaO	Phosphate Chaux	Chaux en excès, enlevée par l'acide acétique
1.	Épuisement	3,16	10,80	6,90	7,36
2.	Épuisement	0,30	0,54	0,65	0,19
3.	Épuisement	0,04	0,11	0,10	0,05
	Résidu	0,00	0,01	"	"
		3,50	11,46	7,65	7,60
	au lieu de	3,66	11,70		

Le fait que nous ne pouvons isoler le phosphore à l'état d'acide phosphorique sans dégrader la caséine mise en oeuvre, constitue-t-il une objection sérieuse contre le préformation de cet acide phosphorique dans la molécule de caséine? Je ne le crois pas. La dislocation de la matière protéique, dans la réaction de Schutzemberger, se produit sans oxydation; nous avons eu re-

[1]Le ballon était muni d'un tube à boules, contenant de l'acide sulfurique titré; de plus les liquides du ballon étaient saturés par de l'acide acétique; puis l'ammoniaque en était chassée en présence de Magnésie.

cours à une réaction moins énergique encore, puisque nous l'avons produite à la température ordinaire; dire que les réactifs employés ont été de nature à oxyder le phosphore métalloïdique équivaudrait à conclure que dans la lécithine, dans la phytine, etc., le phosphore peut n'etre pas à l'état d'acide phosphorique, puisque c'est par une saponification que l'on en sépare celui-ci. J'admets donc que, dans ces expériences, le phosphore qui a été retiré par l'action des alcalis se trouvait, préalablement à tout traitement, sous forme d'acide phosphorique.

J'ai à plusieures reprises cherché à réaliser cette sorte de saponification sous l'influence des seuls éléments contenus dans la caséine. Puisqu'une partie de la chaux de la caséine est combinée à sa fonction acide, ne peut-on pas, en faisant bouillir du lait, détacher cette chaux de l'acide faible que représente lacaséine, et la porter sur la molécule phosphorique saponifiable? Pour celà, je traitais du lait cru et du lait bouilli, puis refroidi, par une même quantité d'acide acétique; celui-ci dans le premier cas, devait dissoudre les phosphates naturels du lait, ainsi que le phosphate de chaux de la caséine, et, dans le second cas, en outre de ces phosphates, le phosphate de chaux formé par saponification. Je n'ai réussi qu'incomplètement, à cause de la faible alcalinité du lait; mais j'ai toujours eu, avec le sérum du lait cuit, plus d'acide phosphorique qu'avec le sérum du lait cru, ainsi que le montre le tableau suivant:

	Acide phosphorique dosé dans le sérum (en grammes)		Acide phosphorique du lait cuit pour un d'acide phosphorique du lait cru
	du lait cru	du lait cuit	
I	0,870	0,930	1,07
II	1,240	1,436	1,16
III	1,051	1,111	1,06
IV	1,106	1,260	1,14

III. La caséine que l'on précipite par la présure n'est pas la seule matière albuminoïde que l'on puisse extraire du lait; quand on chauffe le sérum qui s'égoutte de l'emprésurage, on obtient une matière albuminoïde qui semble, d'aprés les résultats que M. L. Ammann et moi avons fait connaitre (loc. cit.) un mélange de caséine et d'albumine. Le coagulum renferme du

phosphore, qui, compté em P_2O_5, représente de 4,86 à 6,17%, et du calcium, qui, compté en CaO, représente de 5,71 à 7,52%. Il est donc plus riche en éléments minéraux que la caséine provenant directement de l'emprésurage. J'ai épuisé également ce coagulum par l'acide acétique étendu; mais il est resté, comme dans le cas précédent, du phosphore non dissous:

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

				ce qui représenterait:	
		P_2O_5	CaO	Phosphate de Chaux	Chaux en excès, enlevée par l'acide acétique
1.	Épuisement	3,92	5,43	8,55	0,80
2.	Épuisement	1,27	1,84	2,75	0,36
3.	Épuisement	0,29	0,30	0,60	0,00
	Résidu	0,73	0,00	"	"
		6,21	7,57	11,90	1,16
	au lieu de	6,17	7,52		

Je n'ai pu appliquer à ce coagulum épuisé par l'acide acétique étendu la méthode que j'ai décrite plus haut pour en extraire le phosphore résiduaire à l'état d'acide phosphorique, parceque la matière, qui avait été coagulée par la chaleur ne se redissolvait qu'incomplètement dans un lait de chaux.

IV. J'ai voulu substituer à l'acide acétique étendu pour la dissolution du phosphate de chaux et de la chaux en excès dans la caséine d'emprésurage, le citrate d'ammoniaque ammoniacal. Ce réactif a laissé dans le résidu insoluble, une quantité de phosphore inférieure à celle que l'acide acétique a laissée; mais il convient de remarquer que l'on agit en milieu alcalin, et que l'alcali est capable de saponifier une partie de l'acide phosphorique, comme le fait la chaux:

POUR 100 DE CASÉINE SUPPOSÉE SÈCHE:

		P_2O_5	CaO	ce qui représente: Phosphate de Chaux	Chaux en excés, enlevée par l'acide acétique
1er	Épuisement	1,26	2,40	2,75	0,91
2e	Épuisement	0,36	0,72	0,80	0,28
3e	Épuisement	0,33	0,23	0,70	0,00
4e	Épuisement	0,21	0,10	0,45	"
5e	Épuisement	0,13	0,03	0,15	"
	Résidu	0,85	0,00	"	"
		3,14	3,48	4,85	1,19
	au lieu de	3,85	3,80		

C'est encore cette action saponifiante de l'ammoniaque qui permet d'expliquer le fait suivant: Quand on cherche à précipiter, en présence de caséine, par exemple dans du lait écrèmé, l'acide phosphorique à l'état de phosphate ammoniaco-magnésien, on n'obtient, au bout de 24 heures, que 30% environ du phosphore contenu dans la caséine ou dans le lait; la caséine gène la précipitation; mais celle-ci se continue lentement, au fur et à mesure que la caséine se dégrade en produits moins visqueux et que la combinaison phosphorique se saponifie, et au bout de six mois on peut recueillir jusqu'à 81,9% du phosphore total, alors que 50% environ était, dans la caséine primitive, à l'état de phosphate de chaux.

Ce phénomène semble dépendre, non de la quantité de caséine dissoute dans la liqueur, mais du rapport de l'acide phosphorique dissous à la caséine dissoute; car, en précipitant une même liqueur, concentrée ou étendus d'eau et d'ammoniaque, de façon à avoir la même quantité d'alcali, j'ai obtenu, après le même temps, la même quantité de phosphate ammoniaco-magnésien.

Nous conclurons donc de cette étude que l'acide phosphorique et la chaux forment trois groupes d'éléments minéraux: de la chaux combinée à la fonction acide, du phosphate de chaux, probablement tricalcique, et de l'acide phosphorique, retenu par la molécule protéique, et susceptible d'en etre détachée par hydrolise ou saponification.

L'étude du soufre contenu dans la molécule de caséine fera l'objet d'une étude ultérieure.

(*Extrait*)

LA QUESTION DE L'ACIDE SULFUREUX DANS LES VINS BLANCS

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L'auteur frappé par les contradictions qui existent entre les résultats des recherches sur l'action physiologique de l'acide sulfureux contenus dans les vins blancs, entreprises à Bordeaux par une commission d'étude nommée à cet effet, et les conclusions du rapport présenté par M. le Prof. Gautrelet, rapporteur, a repris l'examen détaillé des tableaux d'expériences et a pu établir ainsi, dans sa communication en reproduisant des tableaux comparatifs formés des chiffres pris à même le rapport de M. Gautrelet; en soulignant, d'autre part, certains passage des commentaires d'expériences, que les conclusions du rapport de Bordeaux ne sont *nullement celles qui découlent naturellement des expériences* dont l'auteur analyse l'essence au cours de sa communication.

En se basant sur les résultats purement expérimentaux de la commission bordelaise, l'auteur en arrive à conclure que les expériences de Wiley semblent bien plutôt *confirmées* qu'infirmées par le rapport, et il émet le voeu que *l'usage de l'acide sulfureux dans les vins soit étroitement réglementé en attendant qu'il ait été établi par une commission internationale de chimistes et de médecins, quelles sont les doses de cet antiseptique qui peuvent être tolérées par tous les organismes humains et pendant une longue durée correspondant à une absorbtion habituelle de vin sulfité.* (Les expériences de Bordeaux sont une heureuse initiative, sans doute, mais incomplètes, écourtées et où l'auto-sugestion semble avoir joué un trop grand rôle.)

(La santé publique et le commerce français trouveront l'un et l'autre leur compte à ce qu'il ne puisse plus être émis de doute à l'égard de nos produits nationaux.)

THE INFLUENCE OF HYDROXYL AND CARBOXYL GROUPS ON THE PHARMACOLOGICAL ACTION OF NITRIC ESTERS

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As a pharmacological group the nitric esters belong to the class of vaso-dilators. Their chief action is exerted on unstriped muscle fibre, and especially on that of the blood vessels. Nearly all the nitric esters so far investigated cause a fall of blood-pressure owing to dilatation of the arterioles, and when administered in small doses this is almost their sole effect. This action, consequently, forms a convenient test for determining the pharmacological activity of any member of the group, and it has been the one employed in this investigation. The experiments were made on anæsthetised rabbits and cats. The blood-pressure was taken from the common carotid artery. The injections were made into one of the facial veins (rabbits) or into the external jugular vein (cats).

The substances employed in the investigation were: glycerol-dinitrate, methyl-glycerol-dinitrate, tetra-methyl-mannitol-dinitrate, di-methyl-mannitol-tetranitrate, mannitol-pentanitrate, ducitol-pentanitrate, and the nitric esters of tartaric and ethyl-tartaric acids, of citric and ethyl-citric acids, and of lactic and ethyl-lactic acids. The glycerol-dinitrate was prepared according to the method of Will;[1] the mannitol-pentanitrate and dulcitol-pentanitrate were obtained by reducing the corresponding hexanitrates by means of pyridin;[2] the remainder were made by nitrating in the ordinary way the corresponding alcohols, acids or alkyl compounds by means of a mixture of nitric and sulphuric acids kept cool by a freezing mixture.[3] As

[1] Ber. XXXXI p. 1107 (1908).

[2] Wigner, Ber. XXXVI p. 794 (1903).

[3] I am indebted to the kindness of my colleague Professor Irving for the methyl-glycerol, Di-methyl-mannitol, and Tetra-methyl-mannitol from which the nitric esters were made.

most of the nitric esters are but slightly soluble in water, diluted alcohol was frequently used to prepare the injections.

The presence of hydroxyl or methoxyl groups appears to diminish very considerably the vaso-dilating action of this group of substances. Glycerol dinitrate and methyl-glycerol dinitrate, for example, are much less powerful than nitroglycerine, and the loss of effect with increase of methoxyl groups is even more marked in the compounds of mannitol. Thus in one experiment (exp. I) in which 0.01 g. glycerol dinitrate reduced the blood-pressure from 81 Mm. Hg. to 52 Mm. Hg., one-twentieth this dose of nitro-glycerin caused a fall from 80 Mm. Hg. to 58 Mm. Hg.; and in another experiment (exp. II) the dose of tetramethyl-mannitol dinitrate causing a minimal effect—a fall of 2-3 Mm. Hg. was found to be 0.002g., whereas a similar effect was produced by 0.0003g. of dimethyl-mannitol tetranitrate. This dose of mannitol pentanitrate, although not given in this particular experiment, produces a decided fall of blood-pressure. Obviously the effect is not merely due to the smaller number of nitrate groups since the loss of activity is much greater than this will explain.

When compared with completely nitrated alcohols containing the same number of nitrate groups, most of the esters containing a hydroxyl or methoxyl group are less active. The exception occurs in the case of the glycerol dinitrates which seem to be at least equal in activity to the glycol dinitric esters (glycol dinitrate, propylene-glycol dinitrate, trimethylene-glycol dinitrate) I have tried (exp. III). Tetramethyl-mannitol dinitrate, however, is less active than these, and dimethyl-mannitol tetranitrate is much less active than erythritol tetranitrate. Mannitol pentanitrate and dulicitol pentanitrate are also less active than arabitol pentanitrate or erythritol tetranitrate or glycerol trinitrate (exp. IV-VI).

The following experiments will serve to illustrate these remarks. To economize space the blood-pressure before the injection and the lowest blood-pressure reached after the injection are alone given. And for the same reason in most cases only a portion of the experiment is described. The series of injections given, however, is consecutive, the injections left out

being for the most part repetitions of the substances mentioned in different series or in different doses.

Exp. I. Rabbit. Ether

	Fall of Blood Pressure
1.0Cc. 1 per cent. Glycerol Dinitrate	from 81 to 52Mm.Hg.
0.5Cc. 0.1 per cent. Glycerol Trinitrate	from 80 to 58Mm.Hg.

Exp. II. Cat. 2850g. Chloroform then ether

	Fall of Blood Pressure
1Cc. 1/500 Methyl-glycerol Dinitrate	from 158 to 116Mm.Hg.
1c. 1/500 Tetramethyl-mannitol Dinitrate (partly suspended)	from 151 to 149Mm.Hg.
1Cc. 1/100 Tetramethyl-mannitol Dinitrate (in 29% alcohol; partly suspended)	from 150 to 128Mm.Hg.
1Cc. 1/3000 Dimethyl-mannitol Tetranitrate (in 12.5% alcohol, partly suspended)	from 142 to 139Mm.Hg.
1Cc. 1/50 Methyl-glycerol Dinitrate (in 14% alcohol, partly suspended)	from 137 to 72Mm.Hg.
1Cc. 1/500 Dimethyl-mannitol Tetranitrate (in 75% alcohol)	from 126 to 67Mm.Hg.
1Cc. 1/500 Glycol Dinitrate	from 130 to 101Mm.Hg.

Exp. III. Rabbit. 2000g. Ether

	Fall of Blood Pressure
1Cc. 1/500 Propylene-glycol Dinitrate	from 66 to 50Mm.Hg.
1Cc. 1/500 Glycol Dinitrate	from 62 to 50Mm.Hg.
1Cc. 1/500 Trimethylene-glycol Dinitrate (partly suspended)	from 62 to 51Mm.Hg.
1Cc. 1/500 Glycerol Dinitrate	from 65 to 50Mm.Hg.
1Cc. 1/500 Propylene-glycol Dinitrate	from 64 to 54Mm.Hg.

Exp. IV. Cat. 2950g. Ether

	Fall of Blood Pressure
1Cc. 1/10000 Mannitol Pentanitrate (in 20% alcohol)	from 123 to 117Mm.Hg.
1Cc. 1/10000 Glycerol Trinitrate (in 20% alcohol)	from 120 to 97Mm.Hg.
1Cc. 1/10000 Erythritol Tetranitrate (in 20% alcohol)	from 120 to 105Mm.Hg.
1Cc. 1/10000 Mannitol Pentanitrate (in 20% alcohol)	from 116 to 113Mm.Hg.
1Cc. 1/10000 Arabitol Pentanitrate (in 20% alcohol)	from 112 to 101Mm.Hg.

Exp. V. Rabbit. 1450g. Ether

	Fall of Blood Pressure
1Cc. 1/5000 Dulcitol Pentanitrate (in 20% alcohol)	from 54 to 49Mm.Hg.
1Cc. 1/5000 Dulcitol Hexanitrate (in 30% alcohol)	from 49 to 35Mm.Hg.
1Cc. 1/10000 Arabitol Pentanitrate (in 20% alcohol)	from 43 to 32Mm.Hg.
1Cc. 1/5000 Dulcitol Pentanitrate (in 20% alcohol)	from 56 to 50Mm.Hg.
1Cc. 1/5000 Mannitol Pentanitrate (in 20% alcohol)	from 52 to 44Mm.Hg.
1Cc. 1/10000 Glycerol Trinitrate (in 20% alcohol)	from 48 to 34Mm.Hg.

Exp. VI. Rabbit. 2250g. Chloroform

	Fall of Blood Pressure
1Cc. 1/5000 Mannitol Pentanitrate	from 76 to 54Mm.Hg.
1Cc. 1/20000 Erythritol Tetranitrate	from 76 to 56Mm.Hg.
1Cc. 1/5000 Glycerol Trinitrate	from 77 to 49Mm.Hg.
1Cc. 1/5000 Mannitol Pentanitrate	from 78 to 58Mm.Hg.

The influence of the carboxyl group on the vaso-dilating action of nitric esters is still more marked than that of the hydroxyl group. The nitric esters of tartaric, citric, and lactic acids, neutralised with sodium bicarbonate, produced, when injected intravenously, no fall of blood-pressure whatever, and the nitric esters of methyl-citric and methyl-lactic acids caused a fall only after the lapse of several minutes.

Exp. VII. Rabbit. 1850g. Ether

	Fall of Blood Pressure
2Cc. 1/10 Tartaric Acid Dinitrate (neutralised)	no effect
0.8Cc. 1/20000 Erythritol Tetranitrate	from 80 to 47Mm.Hg.

Exp. VIII. Rabbit. 1850g. Chloroform

	Fall of Blood Pressure
1Cc. 1/200 Ethyl-tartaric Acid Dinitrate (25% alcohol)	no effect.
1Cc. 1/200 Ethyl-citric Acid Nitrate (10% alcohol)	no fall for 3 mins. then gradual fall from 98 to 72Mm.Hg. at 8 mins.
1Cc. 1/200 Ethyl-lactic Acid Nitrate (25% alcohol)	no fall for 3 mins. then gradual fall from 87 to 68Mm.Hg. at 11 mins.
1Cc. 1/5000 Mannito Pentanitrate	from 76 to 53 Mm.Hg.

THE PHARMACOLOGICAL ACTION OF BROM-STRYCHNINES

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Three brom-strychnines have been described—two mono-derivatives and one di-derivative. The first monobromstrychnine, $C_{21}H_{21}O_2N_2Br$, was prepared simultaneously by Shenstone[1] and Bechurts[2] and was obtained by the action of equi-molecular proportions of bromine and strychnine hydrochloride (Shenstone) or hydrobromide (Bechurts) in aqueous solution. It forms rhombic crystals melting at 222° C. (Bechurts). Later Loebisch and Schoop[3] by the action of bromine on strychnine in strong sulphuric acid, obtained a product crystallising in needles arranged in rosettes and giving different colour reactions from those given by the monobromstrychnine of Shenstone and Bechurts. This substance they regarded as a new monobromstrychnine and termed it β monobromstrychnine to distinguish it from the monobromstrychnine previously obtained. Still later a monobromstrychnine was obtained by Martin[4] in colourless needles melting at 199° C. but no colour reactions of this substance are given.

Dibromstrychnine, $C_{21}H_{20}O_2N_2Br_2$, was first described by Bechurts[5] who obtained it, along with monobromstrychnine and apparently some perbromide, by the action of four atoms of bromine (as bromine water) on one molecule of strychnine hydrobromide in aqueous solution. It formed rhombic crystals, which when heated to 230° C. decomposed and gave off red brown fumes. Its solution in dilute alcohol when warmed on the water-bath quickly became acid; aldehyde and hydrobromic acid were

[1]Journ. Chem. Soc. XLVII p. 139 (1885).
[2]Ber. XVIII p. 1236 (1885).
[3]Monatsh. f. Chem. VI p. 855 (1885).
[4]Bull. Soc. Chim. de Paris (3) XXXI p. 386 (1904).
[5]Ber. XVIII p. 1237 (1885).

given off and monobromstrychnine formed. More recently Bechurts[1] has described this reaction of two equivalents of bromine on strychnine as resulting in the formation of monobromstrychnine hydrobromide and bromstrychnine dibromide. An excess of bromine produced monobromstrychnine tribromide. A year previous to this Martin[2] by a method similar to that used to prepare his monobromstrychnine obtained what he describes as a dibromstrychnine as small colourless crystals melting at 130-131° C. which became coloured on exposure to light. More recently Ciusa and Scagliarini,[3] by the action of bromine on strychnine in glacial acetic acid have obtained what they regard as strychnine dibromide which is said to exist in two modifications, an unstable form crystallising from alcohol in colourless needles united into rosettes, and a stable form obtained by repeated recrystallisations or better by fusion of the first form and differing from it in melting at 260° C. and crystallising in large monoclinic crystals. Like the dibromstrychnine of Bechurts it proved to be easily converted into monobromstrychnine.

In view of the unsatisfactory state of this subject it may be of interest to publish some pharmacological experiments on brominated products of strychnine made ten years ago.

Mono-Brom-Strychnines. The two monobromstrychnines were prepared according to the methods of Shenstone and of Loebisch and Schoop respectively, and, for purposes of pharmacological investigation, were converted into the hydrobromides. The bases crystallised in different forms and gave somewhat different colour reactions. Crystallised from hot absolute alcohol the α monobromstrychnine separate as large crystals, the β monobromstrychnine as amorphous globules. The latter, however, readily crystallised from hot water forming long prisms for the most part united into rosettes. When dissolved in concentrated sulphuric acid and a crystal of potassium bichromate was added the α variety showed a very transient blue colour passing quickly through green to a light brownish-yellow; the β variety gave a

[1]Arch. d. Pharmaz. CCXLIII p. 493 (1905).
[2]Bull. Soc. Chim. de Paris (3) XXXI p. 388 (1904).
[3]Atti del. Accad. dei Lincei (5) XIX p. 555 (1910).

more permanent and deeper blue which passed through purple to a light red.

Pharmacological. The bromstrychnine isolated by Shenstone was investigated pharmacologically by Lauder Brunton[1] who, however, merely states that "In the pithed frog it causes clonic convulsions, which, like those of strychnine, may be brought on by a slight touch, jar, or external irritation." Unfortunately the dose given is not mentioned. Loebisch and Schoop[2] after administering 0.0016g. α–monobromstrychnine (Bechurts) to a frog, observed increased sensitiveness in three minutes, tetanus in five minutes, and death in thirty minutes. After the same dose of β– monobromstrychnine they noticed at first diminution in reflex excitability, and after eight minutes fibrillary twitchings of the muscles. Tetanus occurred later and death followed thirty minutes after the administration.

My experiments show that both monobromstrychnines behave, pharmacologically, like a weak strychnine. Of the two the β compound seemed to be slightly the more powerful; it induced convulsions somewhat earlier than the α compound, but in some cases these early convulsions were more transient than those obtained with the α variety. I have not observed the preliminary diminution in reflex excitability described by Loebisch and Schoop with the β modification, nor do any of my experiments show, as their experiment does, the later appearance of convulsions in the β as compared with the α compound.

The following table gives the time of onset, in minutes of the first convulsion with different doses (calculated to one gramme body-weight of frog) of the hydrobromide in Rana temporaria.

Dose	α -monobromstrychnine	*B* -monobromstrychnine
0.003Mg. p. g.	30′	15′
0.005Mg. p. g.		10′
0.006Mg. p. g.	13′	
0.05Mg. p. g.	5′	3′

Di-Brom-Strychnine(?) This substance was prepared for me by my friend H. A. D. Jowett, D.Sc. for the purposes of another re-

[1]Journ. Chem. Soc. XLVII p. 144 (1885).
[2]Monatsh. f. Chem. VI p. 861 (1885).

search, and was made by adding bromine to strychnine in glacial acetic acid, decomposing the perbromide with ammonia, and crystallising the precipitate formed from alcohol. The small almost colourless crystals have remained apparently unchanged for the last ten years. A Carius determination showed that it contained two bromine atoms, and one of these was found to be broken off by dissolving the substance in nitric acid and adding silver nitrate at ordinary temperatures so that the substance was probably monobromstrychnine bromide. Like the dibromstrychnine of Bechurts a solution in diluted alcohol heated on the water-bath acquired an acid reaction; but the change to monobromstrychnine must have been relatively slow since the product, after heating for half an hour, produced a pharmacological effect more closely resembling that of the parent substance than that of monobromstrychnine. The substance decomposed on heating and consequently had no definite melting point. When subjected to the ordinary strychnine reaction it gave a very transient purple passing into reddish-yellow.

Pharmacological. In the paper already cited, Ciusa and Scagliarini state that they have studied the physiological action of monobromstrychnine and the two strychnine dibromides described by them, but I have been unable to find any description of these experiments. And, as far as I am aware, the pharmacological action of a brominated strychnine containing more than one atom of bromine has not previously been described.

The substance I have investigated differs markedly from the monobromstrychnines in pharmacological action. It is not only much less toxic but it produces, in frogs at least, paralytic symptoms of peripheral origin. In rabbits no obvious paralysis was observed.

When 0.01Mg. per gramme body-weight was injected into the dorsal lymph sac of a grass frog there appeared in fifteen to twenty minutes, slight depression and the animal remained on its back for a short time when placed in that position. From this state of lethargy it gradually recovered without showing any other symptoms. After an injection of 0.025Mg. per gramme body-weight the animal commenced to sink on to the table in three minutes and when laid on the back was unable to turn over.

Gradual recovery from this state of paralysis occurred and then the animal developed a condition of increased excitability, a slight tetanic convulsion being produced by hitting the table. This condition was observed in one case on the following day. Still larger doses produce more obvious convulsive symptoms. After an injection of 0.05 Mg. per gramme body-weight into the dorsal lymph sac the animal manifested the same paralytic symptoms but six minutes after the injection slight twitches of the limbs were observed on hitting the table and a few apparently independent twitches of the toes occurred fifteen minutes after the administration. Otherwise the animal lay as if paralyzed. Four hours after the injection the frog had almost recovered, slight increased excitability being alone present.

To determine whether the paralytic symptoms were of central or peripheral origin the right thigh of a frog was ligatured and an injection of 0.3Mg. per gramme body-weight made into the dorsal lymph sac. Three minutes after the injection the animal commenced to sink on the table. Respiratory movements were still present but failed later. Six minutes after the injection the right limb become tetanic on touching any part of the body, the left limb merely gave a momentary twitch and then remained lax. This condition continued, except that on repeated stimulation the left limb often failed to twitch, until the frog was pithed fifteen minutes after the injection. The irritability of the cut sciatic nerves to electrical stimulation (one accumulator cell) was then determined, with the following result.

Left sciatic. Secondary coil 24—5Cm.
= slight contraction of toes.

Left sciatic. Secondary coil 0Gm.
= marked contraction of leg.

Right sciatic. Secondary coil 47Cm.
= decided contraction of leg.

Since the muscles reacted to weak stimulation it would seem from this experiment that the paralysis is due to depression of the nerve-endings.

As already stated no symptoms of paralysis were observed in rabbits. After injecting subcutaneously 5Mg. per kg. no unequivocal symptoms occurred. The animal became quieter after eight minutes and there was a slight fall in the frequency of the respirations and the heart beats. Forty minutes after the first injection a second injection of 15Mg. per kg. was given. This caused slightly increased reflex excitability which commenced eleven minutes after the injection and continued for about forty minutes. Twenty minutes after the administration a slight tetanic attack was produced by hitting the table. No other symptoms were noticed.

The pharmacological evidence would seem to show that, whatever the constitution of this supposed dibromstrychnine may be, both bromines form an integral part of the molecule. The difference in action between this substance and the monobrom-strychnine hydrobromides can scarcely be explained on any other grounds. It is true that relatively slight modifications of strychnine, such as the formation of strychnine oxide or the conversion to isostrychnine, would produce similar effects, but such an assumption is unnecessary and it is improbable that such a change in the strychnine molecule would be brought about by the method used in preparing this substance. It is well known that various strychnine derivatives, e.g. methyl-strychnine, are predominently paralytic in action, and strychnine itself, in large doses, exerts a paralysing influence on the motor nerve-endings of frogs. To what portion of the strychnine molecule, if indeed any, this paralysing influence is due, has not been determined, but it is of interest to point out that in this so-called dibrom-strychnine, it is mainly the convulsant action of strychnine which has been lost rather than a new action which has been acquired. In other words, strychnine, administered in the doses necessary in the case of the di-brominated compound, exerts a depressant action on motor nerve-endings.

In this connection it is also of interest to note that each bromine atom causes a uniform fall in the convulsant power of the substance. Thus the minimal dose per gramme body-weight of

frog, injected into the dorsal lymph sac, necessary to produce the slightest convulsive symptoms was found to be, for—

strychnine	0.00035Mg.
monobromstrychnine	0.003Mg.
dibromstrychnine(?)	0.025Mg.

Summary. The two monobromstrychnines act like strychnine but are 8–9 times weaker.

Dibromstrychnine(?), although it also possesses a convulsant action, produces in frogs mainly paralysis due to depression of the motor nerve-endings.

RELATIONS DE LA PLANTE AVEC LES ÉLÉMENTS FERTILISANTS DU SOL: LOI DU MINIMUM ET LOI DES RAPPORTS PHYSIOLOGIQUES

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Le rendement d'une culture effectuée sur un milieu nutritif naturel ou artificiel se règle, dit-on, sur la substance alimentaire la moins abondante par rapport aux besoins de la plante.

Voilà l'énoncé d'une loi connue en agriculture sous le nom de *loi du minimum.*

Mes recherches sur le développements du maïs en milieu aseptique m'ont permis de constater que cette loi est une conception purement spéculative.

Les relations d'un végétal avec son substratum nutritif sont subordonnées, comme je vais le montrer, à des conditions multiples qui obéissent à une loi que j'appellerai la *loi des rapports physiologiques.*

Soit par exemple le milieu suivant, tableau I,

Phosphate de potassium neutre	1.	Chlorure de zinc	0.05
Sulfate de magnésium + 7 aq.	0.2	Silicate de potassium	0.05
Sulfate ferreux + 7 aq.	0.1	Carbonate de calcium	2.
Chlorure de manganèse + 4 aq.	0.05	Eau distillée	1000.

qui, additionné de 1 p. 1000 de nitrate de sodium ou d'un sel azoté de richesse équivalente en azote, constitue une solution nutritive dans laquelle le maïs accomplit son évolution complète jusqu'à la maturation des graines.

On y fait pousser des plantes jusqu'à ce qu'elles aient atteint un poids en moyen de 10 gr. A partir de ce moment on les place dans une solution incomplète pourvue d'un seul élément nutritif.

La plante vit alors sur les réserves de matières minérales qu'elle a empruntées à la solution mère. Dans ces réserves les divers éléments présentent entre eux les rapports les plus favorables au développement du végétal lui-même. L'élément en solution

vient troubler ces rapports; les chiffres du tableau II montrent jusqu'à quel point son influence s'exerce sur l'évolution de la plante, l'eau distillée étant prise comme terme de comparaison. Les poids de matières sèches sont déterminés au moment où la plante peut être considérée comme morte.

TABLEAU II

Nature de l'aliment azoté de la solution mère	Substance introduite dans la solution incomplète en gr. p. 1000.	Poids sec de la plante calculé au moment de l'introduction de la solution incomplète en gr.	Poids sec de la plante à la fin de l'expérience en gr.	Gain de matières sèches en gr.	Rapport du gain au poids sec initial	Durée de l'expérience en jours.
Nitrate d'ammonium	No_3NH_4-0,5	13,976	40,10	26,224	1,87	49
Nitrate de sodium	No_3Na-1	7,947	27,38	19,433	2,44	48
Sulfate d'ammonium	$So_4(NH_4)_2$-0,5	9,751	15,79	6,039	0,61	40
Chlorure d'ammonium	NH_4Cl-0,5	7,315	13,36	6,045	0,82	39
Nitrate de sodium	$Po_4HK_2$1	12,002	33,015	21,013	1,75	60
Nitrate d'ammonium	Eau distil.	10,084	39,65	29,566	2,94	47

Les substances minérales nutritives des solutions incomplètes, bien qu'offertes à des concentrations favorables au développement des plantes, c'est-à-dire à des concentrations physiologiques, arrêtent l'évolution du végétal parce que les rapports des divers éléments minéraux de la plante et de la solution ne répondent plus aux éxigences de la nutrition de la cellule vivante. C'est l'eau distillée, où ces rapports ne sont pas altérés, qui donne les meilleurs résultats et de beaucoup.

. . . . .

On peut procéder d'une autre manière pour mettre en évidence les influences des solutions incomplètes. Au lieu d'opérer avec des plantes déjà bien développées, on utilise des plantes qu'on a fait germer dans l'eau distillée.

On observe alors des résultats variés parmi lesquels les suivants seuls nous intéressent.

Toutes les solutions constituées par un seul élément nutritif qui ne renferme ni soufre ni fer provoquent une chlorose plus ou

moins intense du maïs. Les plantes placées dans l'eau distillée conservent leur couleur verte pendant toute la durée de l'expérience. Le nitrate de potassium à 0.5 p. 1000 décolore les feuilles de la plantule formées après l'immersion des racines dans la solution incomplète; on peut constater que l'élément soustrait à la plantule par voie d'exosmose est le fer; une solution de nitrate ferrique à 0 g. 2 p. 1000 fait apparaître la chlorophylle à l'endroit où des gouttelettes déposées sur le limbe des feuilles abandonnent aux cellules du parenchyme des traces de fer.

On voit ainsi que les solutions incomplètes agissent sur les plantes supérieures suivantes des procédés variés.

. . . .

Les végétaux inférieurs se prêtent très bien aussi à ces démonstrations.

Comme les divers éléments du liquide Raulin présentent entre eux des rapports physiologiques très favorables au développement de l'*aspergillus niger*, c'est ce dernier que j'ai utilisé.

J'ai déterminé d'abord les limites de concentration que l'aspergillus peut supporter, en le cultivant sur des solutions Raulin de concentration $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 8, 16 dans lesquelles l'acidité et la teneur en sucres restent constantes et de même valeur que dans 1.

Le tableau III donne les résultats comparatifs de ces essais. Durée des cultures à 30° 4 jours.

TABLEAU III

Concentration	$\frac{1}{4}$	$\frac{1}{2}$	1	2	4	8	16
Poids du mycélium en gr.	0 473	0.737	1.094	1.057	0.1960	0.842	0.872

Prenons maintenant les concentrations extrêmes $\frac{1}{2}$, 1 et 16, et portons dans $\frac{1}{2}$ ou 1, la concentration de l'élément Azote à 16, en multipliant le nitrate d'ammonium par le coefficient 16. Si les renseignements fournis par les végétaux supérieurs se confirment, les milieux $\frac{1}{2}$ + 16 Az. et 1 + 16 Az. donneront un poids de mycélium inférieur à celui qu'on récoltera sur les milieux $\frac{1}{2}$, 1 et 16, le sucre et l'acidité restant constants.

Les résultats sont consignés dans le tableau IV.

TABLEAU IV

	Durée des cultures en jours	Concentration	Poids du mycélium en grammes
	3	$\frac{1}{2}$	0.466
I.	3	$\frac{1}{2}$ + 16 Az.	0.217
	3	16	0.723
	4	$\frac{1}{2}$	0.646
II.	4	$\frac{1}{2}$ + 16 Az.	0.481
	4	16	0.830
	3	$\frac{1}{2}$	0.475
III.	3	$\frac{1}{2}$ + 16 Az	0.316
	3	16	0.991
IV.	3	1	0.758
	3	1 + 16 Az.	0.386
	3	16	0.972
	4	1	0.905
V.	4	1 + 16 Az.	0.495
	4	16	1.109

Ces résultats sont, comme on le voit, en contradiction avec *la loi du minimum* et obéissent à la *loi des rapports physiologiques.*

Dans la pratique agricole l'emploi irraisonné des engrais minéreaux solubles peut conduire à des abaissements de rendement, si l'on ne tient pas compte de ces données. Je préciserai d'ailleurs, bientôt, les règles qui doivent présider à leur incorporation à la terre.

(*Abstract*)

SOME NEW COMPOUNDS OF THE CHOLINE TYPE

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This paper reviews briefly the substance of parts of a preliminary paper previously published under the same title and reports upon methods and procedure involved in the preparation of the following new compounds and upon some of their properties: the acetyl-, pheyl-acetyl-, propionyl-, benzoyl-, vareryl-, monobromisocapronyl-, and palmityl derivatives of α-methylcholine chloride ($Cl.N\begin{cases}(CH_3)_2\\ CH.\,CH_2\,OH\\ CH_2\end{cases}$) and their platinum and gold salts; the phenylacetyl derivatives of β-methylcholine ("β-homocholine"—$Cl.N\begin{cases}(CH_3)_2\\ CH_2.\,CHOH\\ CH_3\end{cases}$) and of "$\gamma$-homocholine "chloride ($Cl.N\begin{cases}(CH_3)_2\\ CH_2.\,CH_2.\,CH_2OH\end{cases}$) and their platinum and gold salts.

The synthesis of β-dimethylcholine chloride ($Cl.N\begin{cases}(CH_3)_2\\ CH_3.\,COH\\ CH_3\quad CH_3\end{cases}$) and of $\beta.\beta$-methylethylcholine chloride ($Cl.N\begin{cases}(CH_3)_2\\ CH_2.COH\\ CH_3\quad C_2H_5\end{cases}$), also

the preparation of their platinum and gold salts, were described in the preliminary paper. Similar development of β. β-methyl-

phenylcholine chloride ($Cl.N\begin{matrix}(CH_3)_2\\ CH_2.COH\end{matrix}$ $\begin{matrix}\\ \\ \end{matrix}$) and its platinum

CH_3 C_6H_5

and gold salts is here reported. The work will be continued.

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THE RELATION OF THE HYPOPHYSIS TO GROWTH AND THE EFFECT OF FEEDING ANTERIOR AND POSTERIOR LOBE

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As observed by the clinician, there are two clinical entities both disturbances of growth, ascribed to disturbed functioning of the hypophysis. One is acromegaly with over growth of bone, the other Fröhlich's Syndrome with delayed development adiposity and genital atrophy. When the former occurs in early life gigantism results, when it first appears after maturity, enlargement of only certain portions of the bony skeleton is observed. The other type, when the disturbance appears in early life, causes delayed skeletal development, with adiposity and failure of sexual development, when it appears after maturity, adiposity and sexual atrophy.

Pierre Marie in 1886 first called specific notice to the relation between acromegaly and the hypophysis, although Carl von Langer in 1872, in an anatomical study of giants, referred to a certain type with enlarged sella turcica. The observations of Marie have been confirmed until at the present time, it is generally conceded that acromegaly is due to a disturbance of the hypophysis, and according to Sternberg 40 per cent. of the pathologic giants have enlargement of the hypophysis. Regarding the exact nature of the disturbance in the hypophysis in acromegaly, there is still considerable difference of opinion. The weight of evidence, however, favors the view that it is due to hypersecretion of the anterior lobe. The pathologic condition most frequently associated with acromegaly is an adenomatous development of the

anterior lobe with increase in the specific secretory cells. In some instances where enlargement of the anterior lobe is lacking, increase in the specific secretory cells may still be demonstrated, and in addition hyperplasia of the pharyngeal hypophysis should be considered. There is reported in the literature, malignant tumors of the hypophysis with acromegaly. Lewis, who has reviewed these cases, believed that in the majority and possibly all of these the tumor was an adenoma. Although, it is too early to state positively that acromegaly is due to hypersecretion from the anterior lobe, it must be admitted that the weight of evidence supports this view.

The condition of underdevelopment, adiposity, and genital atrophy, first described by Frohlich and referred to as the Frohlich syndrome, all admit is associated with disturbances at the base of the brain, more especially tumors, and has been referred to as cerebral adiposity. Whether in all of these cases the hypophysis is involved, either directly or indirectly by either increased intercranial or intercerebral pressure, has not been determined. On account of its position the hypophysis is especially liable to injury from internal hydrocephalus and it is quite possible that any cerebral disturbance causing increased pressure in the ventricle may compress and disturb the hypophysis. There is one case on record, where a bullet wound of the hypophysis, was followed by adiposity (Madelung). Adiposity may be associated with acromegaly, according to Crenzfeld in 1.6% of cases. In five of the recorded cases of adiposis dolorosa with autopsy, the hypophysis has been abnormal (Lyon). Cases are also on record where removal of the hypophyseal tumor is followed by disappearance of the adiposity (Von Eiselberg).

Assuming that adiposity may arise from disturbances of the hypophysis, it is still to be determined what portion of the gland is responsible for these changes. Fisher believes it is due to hyposecretion of the posterior lobe, and in addition to certain theoretical considerations presents some autopsy findings, where as the result of pressure, the posterior lobe was flattened and had undergone brown atrophy. It can be readily conceived, however, that any pressure involving the posterior lobe must also compress the anterior portions. In all of Fisher's evidence there is little

that is actually convincing, that the posterior lobe plays a role. A more tenable view is that hyposecretion of the anterior lobe is responsible for the adiposity. Zollner in a case of this type found a carcinoma of the anterior lobe. In the five cases of adiposis dolorosa, referred to, with hypophyseal involvement, two showed round cell infiltration of the anterior lobe (Price), one with glioma evidently of the posterior lobe (Burr), one marked increase in size of the anterior lobe from connective tissue hyperplasia and alveolar sarcoma (Guillain), and finally Dercum's case with carcinoma involving both anterior and posterior lobes. Although few deductions may be drawn from the above evidence, on the whole it favors disturbance of the anterior lobe. Most convincing, however, is the experimental evidence. In dogs, removal of the posterior lobe is not followed by any serious consequence, the animal recovers and later fails to show any anomalies of growth, Paulesco, Cushing, Ascola. Where a considerable portion of the anterior lobe of a young dog is removed, and the animal recovers from the effect of the operation, he later develops the Frohlich syndrome of delayed development, adiposity and failure of sexual development, Aschner, Cushing, Casselli, Ascola. This evidence is most suggestive and points strongly toward hyposecretion of the anterior lobe as the cause of the adiposity and sexual atrophy. The question may properly be raised whether the adiposity is due directly to the lessened secretion of the anterior lobe or is secondary to the genital atrophy as Tandler and Grosz have shown that castration in dogs leads to increased deposits of fat and this phenomena is observed in women after removal of the ovaries. It might be argued that it would be exceedingly difficult on the basis of hyposecretion of the anterior lobe to explain the occasional presence of adiposity in acromegaly. Such adiposity is very infrequent according to Creutzfeld in 1.7% of cases, while hypoplasia and gential atrophy were noted in 36.4% of the cases. The greater frequency of the sexual disturbance would lead us to suspect that it and the adiposity were due to separate factors. Here, again, the possibility of the adiposity being secondary to the genital atrophy must be considered its inconstant associations being due to the varying degrees of sexual hypoplasia in the various cases. To those, who believe that the adiposity is due to

the hyposecretion of the posterior lobe, its presence in acromegaly is explained by pressure of the enlarged anterior lobe upon the posterior lobe.

Summarizing it may be said that acromegaly is probably due to increased activity of the anterior lobe. Adiposity, if due directly to disturbances of the hypophysis, is most probably due to hyposecretion of the anterior lobe.

Metabolism is acromegaly. The metabolism in acromegaly has now been studied in a dozen or more cases. Retention of nitrogen is very frequent, often reaching considerable amounts. In some of these cases calcium and phosphorous metabolism are unchanged, in others retention of both have been reported. Seven cases from five different observes all show a retention of nitrogen, five of phosphorous, and five of calcium. Oberndörffer has recently reported two cases and reviewed the literature on this subject. He was unable to detect any variation from the normal in his two cases, and questions whether the results of others are really conclusive on account of the great variation in elimination of normal individuals. Before the work on this phase of subject can be accepted, more extensive studies should be carried out.

Metabolism in animals after the administration of hypophysis. Thompson and Johnson fed dogs upon the entire dried gland of horse, calf and sheep hypophysis and found that they lost in weight and excreted an excessive amount of N and P (Ca and Mg. not determined). They also reported more marked results when glands of young animals were used; Malcomb gave dogs 2–3 gms. daily of dried anterior lobe, for a period of five days, a total of 15 gms. the animal showed slight retention of N. and slightly increased output of P_2O_5 and Ca; after giving posterior lobe (10 gm.) slight increased output of P_2O_5 and marked increased output of Ca. When he gave fresh entire glands 25 gms. daily, there was scarcely any change in the Ca or Mg. output. Franchini injected rabbits, intravenously, daily, with an amount of extract equivalent to one entire hypophysis, this was followed by greater elimination of Ca Mg and P_2O_5 in both urine and feces. The loss in P_2O_5 being less marked than that of Ca or Mg. The animals lost in weight and finally died. The X-ray failed to show any change in the bony skeleton. Some of the animals tolerated

the injection well—others showed marked dyspnea vomiting and diarrhea. Franchini conclusion that hyperpituirtrism leads to loss in weight and failure of development is scarcely justified on account of the severe reaction following the intravenous injection. Oswald gave dogs 2–3 gms. daily of dried hypophyseal extract obtained from Merck (portion of gland not specified) and was unable to detect any change in N or P_2O_5 elimination.

Benedict and Homans working with hypophysectomized dogs and determining carbon dioxide production as an index of total metabolism, found it markedly reduced. The results of these various findings are so at variation, that they throw little light on the disturbance of metabolism following administration of dried hypophysis and may be practically omitted as furnishing definite evidence.

Feeding Experiments. Comparatively few satisfactory feeding experiments have been reported. In some, no attempt has been made to separate the anterior from the posterior lobe. Others have administered the extract subcutaneously or intravenously, Cerletti, Franchini, Delille, Caselli. When given in this way it frequently gives rise to such marked constitutional disturbances, as vomiting and diarrhea, with finally intestinal ulceration, that it is impossible to draw any conclusion regarding the actual effect of the hypophyseal extract. After this method of administration, Cerletti and Franchini report loss in weight and delayed bone development. The entire hypophysis was used and the animals treated for only a few days. Caselli injected young dogs and rabbits with the glycerin extract and did not notice any effect on growth. Delille injected extracts of the entire hypophysis into 4 rabbits for a period of 14 months and reported increased deposits of fat.

Only two references have been obtained of feeding experiments, where the animals received preparations of the hypophysis by mouth for a considerable period of time.

Sandri fed rats on hypophysis exclusively for a period of two months, the controls receiving an exclusive meat diet. While this is an unsuitable diet, Sandri reports that the animals thrived. He found that those fed on the anterior lobe showed greater gain in weight than the controls; when we consult the actual figures,

we find that these differences are so slight that they can scarcely be considered as significant. The controls during the two months gained on an average 10 gms., those fed on the posterior lobe 7 gms., those fed on the anterior lobe 12 gms. Variation of this extent may occur in any group of feeding experiments continued over a period of three months.

Schaefer has conducted the most satisfactory feeding experiments, using, however, only the anterior lobe. Four young rats were fed small amounts of the dried anterior lobe, mixed with bread and milk. The controls received powdered testicle or ovary with bread and milk. The amount consumed by each group of animals was accurately determined. The feeding experiment was continued for about three months. At the beginning, the average weight of the group fed on hypophysis was 44.25 gms. and that of the controls exactly the same. At the end of the feeding, the average weight of those fed on hypophysis was 160 gms. and of the controls 131 gms. During the first six weeks of the feeding, there was little difference between the two groups, during the last six weeks those fed on hypophysis made the more rapid gain. These results would appear to be conclusive, but they are not sufficient in number, to eliminate error.

In undertaking this investigation, it was decided to carry through several series of animals with controls. Young white rats were selected. Each rat was placed in an individual cage, ground cracker was pressed into tablets, each of the same weight. It was then determined how much of this food each rat would consume daily. Although there were some individual differences, it was possible to determine with reasonable accuracy, the daily ration. Having determined this point, Cracker tablets of the requisite weight were made, and to each was added a weighed amount of the hypophysis, or in case of the control meat, and each animal received the same amount daily. Occasionally for a few days, a rat might not eat this whole tablet, if so a note was made of this fact. However, the ration was so arranged that with rare exception, it was consumed daily; and no doubt some of the animals would have eaten more, but the fact that they gained in weight and appeared on the whole healthy would indicate that they were properly fed. By this method, each rat

received and consumed the same amount of food daily, containing the same amount of substance to be tested. The animals were weighed each week.

The ox hypophysis were obtained perfectly fresh from Armour and Co. The anterior and posterior lobes were separated; chopped up fine and dried in a blower at a temperature of approximately 100° F. The dried glands were then powdered and a weighed amount added to the powdered cracker and pressed into a tablet. Three series were fed in this way for about three months each; at the end of the time, the rats were killed and X-rays taken to detect any changes in the bony skeleton. The first series consisted of 9 rats. Three received, daily, .2 gm. of dried anterior lobe, three the same amount of posterior lobe and three controls the same amount of meat. The feeding was continued for 79 days. The second series consisted of 8 rats; four received .4 gms. anterior lobe and the other four as controls received the same amount of dried meat. This group were fed for 90 days. The third series of nine young rats were divided into three groups; one group received .3 gm. daily of beef, another the same

TABLE I—*Series I*

Number of animals	Food daily	Average weight at beginning	Average weight at termination	Change in weight	Period of feeding
		gms.	gms.	gms.	
3	Dried Beef .2 gm.	52.2	91.8	38.6	78 days
3	Dried Anterior lobe .2 gm.	54.2	92.3	37.9	78 days
3	Dried Posterior lobe .2 gm.	58.1	102.6	44.5	78 days
	Series II				
4	Dried Beef .4 gm.	58.	95.2	37.2	90 days
4	Dried Anterior lobe .4 gm.	66.6	107.3	40.7	90 days
	Series III				
3	Dried beef .3 gm.	131.6	144.3	12.6	67 days
3	Dried Posterior lobe .3 gm.	115.3	121.5	6.2	67 days
3	Dried Thymus .3 gm.	118.6	135.3	16.7	67 days

amount of posterior lobe, and the other the same amount of dried thymus. This series was kept under observation for 67 days. By repeating the experiment in this manner, it was thought that some sources of error might be eliminated.

It is interesting to note, that animals consuming the same amount of food daily and apparently enjoying equally good health, should show such marked variation of gain in weight. The minimum gain in weight of the controls in Series I was 30.6 gms., the maximum 44.4 gms. In series II the minimum gain in weight of the control rats was 32 gms., the maximum 38.5 gms. In series III, where the rats were $\frac{2}{3}$ grown and, therefore, not so suitable for the test in both the control and those fed on posterior, one of the animals lost 5 gms.

As will be seen by the table in the first series, the controls and those fed upon the anterior lobe showed practically the same gain in weight. Those fed upon the posterior, gained on an average of 6 gms. each more than the controls. When we consider the individual animals, one of those fed on the posterior lobe gained less than one of the controls, the other two gained more than the controls and each animal fed on the posterior lobe gained more than those receiving the anterior lobe. In series III, however, the animals receiving the posterior lobe gained less than the controls and much less than those animals receiving thymus. In series I, animals receiving anterior lobe gained slightly less than the controls, while in series II, they gained somewhat more than the controls. The X-ray pictures of all these animals failed to reveal any variations in the bony skeleton.

Only one conclusion can be drawn from these feeding experiments, viz., that at least in this series of tests neither anterior nor posterior lobes had any effect on the weight or growth of the animal. The experiment was conducted in such a manner that serious causes of error were excluded. The amounts administered were sufficient to give results, as it would be equivalent to 230 gms. daily to the average man—on the other hand, it was not sufficiently large to have a deleterious effect, as the animals so fed gained the same in weight as the controls. Doubling the dose of anterior lobe did not modify results. It must be admitted, however, that this does not prove that disturbed secretion of the

hypophysis may not modify growth. In the feeding experiment, the digestive fluids may destroy the active substances responsible for these changes. Again feeding preparations by mouth can scarcely be considered as analagous, to the continuous secretion occuring in actual life.

Summarizing the entire field of the role of the hypophysis in the growth of the individual. In acromegaly where there exists abnormal development of certain portions of the body especially in their bony structures, there is apparently hypersecretion of the anterior lobe. In the Frohlich syndrome of adiposity and failure of sexual development, it is thought by many that there is lessened function of the posterior lobe. Experimental evidence suggests hyposecretion of the anterior lobe. Regarding studies in metabolism in patients with acromegaly, there is again nothing conclusive, and more work must be carried out upon this subject before it can be accepted that there is a lessened katabolism than in the normal individual.

Turning to the results of partial removal of the hypophysis in animals, only one point, having a direct bearing upon this subject, seems to have been determined, viz: that partial removal of the anterior lobe, when performed upon young animals, modifies growth and sexual development in such a manner as to resemble very closely Frohlich syndrome. Removal of the posterior lobe, apparently, has no effect upon growth. This is a distinct contradiction to those who believe lessened function of the posterior lobe is responsible for the Frohlich syndrome. Feeding experiments, on animals, fail to furnish any definite evidence that the administration of either the anterior or posterior lobe has any effect on growth.

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THE INFLUENCE OF THE CHEMICAL CONSTITUTION OF CERTAIN ORGANIC HYDROXYL AND AMINIC DERIVATIVES ON THEIR GERMICIDAL POWER

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The method employed in estimating the germicidal powers of various organic substances was that devised by Martin and Chick (*Journ. of Hygiene*, 1908, 8, No. 5. Nov. p. 654); it consisted in making a comparison of the concentrations of the substance and of pure phenol required to kill an equal number of organisms of the same species in a constant volume of the disinfectant solution (5 cc) during a constant period of time (15 minutes) and at a constant temperature (20° C).

All test-tubes, pipettes and flasks were first sterilized and different amounts of a standard solution of the substance under examination were introduced into a series of test-tubes, and sterilized water was then added so as to make up each volume to 5 cc. so that the tubes contained a constant volume of solutions containing different concentrations of the substances. A series of phenol solutions was similarly prepared and the two sets of tubes were immersed in a thermostat at 20°. When the tubes had attained this temperature five drops were added to the first dilution from a standard capillary pipette (1 drop = 0.02cc) of a 24 hours' culture of the organism, obtained by inoculating 6 cc. of broth with a standard loopful of agar culture.

The inoculation of the disinfectant solutions proceeded at one minute intervals and at the 15th minute two tubes containing 10 cc. of glucose broth were each inoculated with two loopfuls of the contents of the first tube taken out by means of a standard platinum loop (a loopful of broth weighing about 0.004 gram). The other tubes of the reacting solutions were also subcultured at one minute intervals so that in each case the disinfectant acted for 15 minutes.

The subculture tubes were inoculated at 37° for 96 hours and then examined when the presence or absence of growth was referred to the corresponding dilution. The average of the lowest concentration of the substance which killed and the highest concentration which failed to kill was compared with the corresponding mean phenol concentration and the latter divided by the former gave the carbolic acid co-efficient of the substance.

The organisms used throughout the work were *Staphylococcus pyogenes aureus* and *Bacillus typhosus*. The broth was made according to the following recipe: Brand's meat juice 10 cc, salt 5 grams, peptone 10 grams, glucose 10 grams in 1 litre of tap water. The reaction of the broth was kept constant being + 6 to + 7 to phenolphthalein (Eyre's notation).

The important difference between this method of standardizing disinfectants and the Rideal-Walker method was the selection in the former of a constant reacting time for the germicides, namely 15 minutes.

I. Compounds Containing Hydroxyl Groups

1. *The aliphatic alcohols.*

Jalan de la Croix (*Archiv. f. exp. Pathol.* 1881, p. 175) found that a 1 in 21 aqueous solution of ethyl alcohol prevented the growth of bacteria in broth, but 22 p.c. solutions were required to kill them. Stronger solutions of alcohol (83 p.c.) were necessary to kill spores.

Koch (*Mittheil. a. d. K. Gesundh.* 1881, Vol. 1) found that anthrax spores were not killed by immersion for 110 days in absolute alcohol and in its 33 p.c. and 50 p.c. dilutions. A 1 p.c. solution impeded and an 8 p.c. solution completely arrested the development of anthrax spores.

Fowler (*Journal of the Royal Army Medical Corps.* 1907, July, "Some disinfectant values") found that ethyl alcohol possesses a carbolic acid coefficient of only 0.03 when tested on *B. typhosus.*

There is evidence, therefore, that alcohol possesses feeble germicidal properties. Nevertheless it has sometimes been

employed for antiseptic purposes in surgery, but its chief value is as a vehicle for the application of other therapeutic substances.

All the following alcohols, with the exception of amyl alcohol are freely miscible with water. The carbolic acid coefficients of these compounds were determined on *Staphylococcus py. aureus* in the absence of organic matter.

Alcohol	Concentration required to kill in 15 minutes (parts per 1,000)		Carbolic acid coefficients
	Alcohol	Phenol	
Methyl alcohol	350	9.0	0.025
Ethyl alcohol	350	9.5	0.027
Ethyl alcohol (with *B. typhosus*)	325	8.5	0.026
Propyl alcohol	140	9.0	0.064
iso-Propyl alcohol	210	8.5	0.040
n-Butyl alcohol	41	9.5	0.250
Trimethylcarbinol	190	10.5	0.055

iso-Amyl alcohol (a saturated ($\frac{1}{2}$%) solution at 20° failed to kill in 15 minutes).

The germicidal powers of the foregoing alcohols are considerably less than that of phenol so that the action of the latter must be largely determined by its benzene nucleus. The equality in germicidal powers of methyl and ethyl alcohols is an exception to the general tendency for this action to increase as the homologous series is ascended. This abnormality in the case of methyl alcohol corresponds with the anomalies observed in regard to its chemical and physical properties.

A comparison of the results obtained with *n*-butyl alcohol and trimethylcarbinol shows that the primary alcohol is much superior to the isomeric tertiary alcohol in germicidal power.

Normal propyl alcohol also exceeds its isomeride, *iso*propyl alcohol in germicidal power.

It will be noticed that in the foregoing series of alcohols those members having the higher specific gravity, heat of combustion and boiling point have the greater germicidal power. The phe-

nomenon of germicidal action may be determined by those constitutive influences which determine the physical and chemical properties of substances.

2. The Phenols and Their Homologues and Derivatives

Alcohol is sometimes employed for dissolving the phenols so as to obtain them in a form suitable for disinfecting and antiseptic purposes. For example, there are two non-official preparations of thymol containing ethyl alcohol, liquor antisepticus, and liquor thymol. Kronig and Paul (*Zeitsch.* fur. *Hygiene* 1897, 25, 2, p. 1.) showed that alcohol decreased considerably the germicidal action of phenol on anthrax spores and a solution of phenol in absolute alcohol had very little germicidal power. This inhibiting effect is probably due to the greater solubility of phenol in alcohol, which alters the distribution of this germicide between water and the bacterial proteins with the result that the spores absorb considerably less of the phenol.

In practice the destruction of non-sporing pathogenic organisms is generally the object in view, and it is important to know what effect alcohol has on the germicidal action of phenols on such organisms. Non-sporing microorganisms are more sensitive to the germicidal action of alcohol than are spores, and it was accordingly of interest to ascertain how far the contribution of alcohol towards the bactericidal action of the phenols counterbalanced the inhibiting influence of alcohol on the partition coefficient.

In investigating the effect of known percentages of alcohol on the germicidal action of phenol the experiments were carried out in the manner already described, except that the various dilutions of phenol were made up with sufficient alcohol to bring the percentage of this solvent to the desired extent. The large error involved in the bacteriological test obviated any necessity for a correction for the contraction in volume produced by mixing water and alcohol.

The following table gives the concentrations of phenol required to kill a constant number of *B. typhosus* or *Staphylococci* in 15 minutes at 20° in the presence of various concentrations of alcohol.

Disinfectant	Organism	Percentage of Alcohol					
		0 water	5	10	15	20	30
Phenol in 1000 parts of solvent	*B. typhosus*	8.5 mean of 4 expts.	8.5	7.0 mean of 2 expts.	6.5	4.75 mean of 3 expts.	1.4 mean of 3 expts.
Phenol in 1000 parts of solvent	*Staphylococcus*	9.5		7.5			

The action of the alcohol on the above non-sporing organisms was sufficiently appreciable to overcome within 15 minutes its depreciating effect on the action of the phenol, so that the presence of this solvent led to an apparent increase in the germicidal efficacy of the phenol, a 0.15 p. c. solution of phenol in 30 p.c. alcohol doing the same amount of disinfection as a 0.85 per cent. aqueous solution. The changes produced by alcohol on the germicidal efficiency of resorcinol and thymol were next investigated.

ORGANISM. *B. Typhosus*

Disinfectant	In Water		In 30 p.c. Alcohol	
	Concentration Killing in 15 mins. at 20°	Carbolic Acid Coefficient	Concentration Killing in 15 mins. at 20°	Carbolic Acid Coefficient
Phenol in 1000 parts solvent	8.5	1	1.1	7.7
Resorcinol in 1000 parts solvent	28.0	0.3	5.0	0.22
Phenol in 1000 parts solvent	7.5	1	1.3	5.6
Thymol in 1000 parts solvent	0.325	23	0.18	7.2

Although 30 p.c. alcohol increases considerably the apparent germicidal effect of resorcinol and thymol on *B. typhosus*, yet as the increase is less marked than in the case of phenol, the car-

bolic acid coefficients of these two substances were reduced by the presence of alcohol of this concentration. Although the use of alcohol cannot be recommended for solutions of phenols required to destroy pathogenic sporing organisms yet this solvent may be employed with advantage in the disinfection of non-sporing organisms, particularly as the phenols are less caustic in alcoholic than in aqueous solution.

As alcohol affects the germicidal powers of different substances to a varying extent, different conclusions may be reached with regard to their efficacy, depending on the medium employed in the process of disinfection, and these irregular variations should be borne in mind in considering the possible relationship between chemical constitution and germicidal power.

Influence of Orientation on the Germicidal Action of the Dihydroxybenzenes

Organism—*B. Typhosus.* Temperature 20°

Dihydroxybensene	Concentration of substance required to kill in 15 minutes $x/1000$	Concentration of phenol required to kill in 15 minutes $x/1000$	Carbolic Acid Coefficient
Resorcinol	26.0	7.50	0.29
Catechol	17.0	8.25	0.48
Quinol	7.5	8.50	1.1

The minimum effect is produced by the meta-isomeride and the maximum by the para-compound, the ortho-derivative giving an intermediate value of the carbolic coefficient. It is of interest to note that the two isomerides containing hydroxyl groups in sympathetic positions (ortho and para) exert a greater germicidal action than resorcinol in which these groups are in the apathetic meta-position with respect to each other.

The Nitrophenols

The only nitrophenols which appear to have been previously employed as germicides are picric acid (2:4:6—trinitrophenol) and the potassium salt of dinitro-*o*-cresol [$C_6H_2(No_2)_2(CH_3)$ OK.]

Jalan de la Croix (*Arch. f. expt. Pathol.* Jan. 27, 1881) found that 1 in 1000 aqueous solutions of picric acid killed bacteria in infusions of egg-white. Cheron (*J. de Therapeut, Gubler*, 1880, p. 121) used a saturated solution of this compound for purposes of disinfection in hospitals. Koch (*l.c.*) found that 1 in 10,000 dilutions of picric acid impeded the development of anthrax spores, but that 1 in 20,000 dilutions were not sufficient to arrest growth.

Potassium diunitro-*o*-cresoxide has been employed chiefly as an insecticide and a fungicide.

The nitrated derivatives of phenol are not very soluble in cold water but solution was facilitated by gentle warming.

ORGANISM. *Staphylococcus py. aur.* Temperature 20°

Substance	Concentration of substance killing in 15 minutes $x/1000$	Concentration of phenol killing in 15 minutes $x/1000$	Carbolic acid coefficient
p-Nitrophenol	4.6	10.5	2.3
Potassium *p*-nitrophenoxide	20.0	10.5	0.52
m-Nitrophenol	2.7	9.5	3.5
Picric acid	1.4	10.5	7.5
Picric acid with *B. Typhosus*	1.0	8.5	8.5

Potassium *p*.nitrophenoxide contained 2 H_2O of crystallization, and allowing for this the carbolic acid coefficient of dry salt, $No_2.C_6H_4.OK = 0.52 \times {}^{213}/_{177} = 0.62$.

The successive introduction of nitro-groups into the phenol molecule produces a progressive increase in the germicidal power of the substance but the practical application of these nitro-compounds is hindered by their poisonous and staining properties. In this series the meta-isomeride is a more powerful germicide than the para-compound. The alkali salt is far less efficacious than the free nitro-derivative, this influence of salt formation will be plainly noticeable in the following series of aromatic hydroxycarboxylic acids.

The following table gives a comparison of the carbolic acid coefficients of the nitrophenols and cresols, the organism employed being *Staphylococcus*.

Nitrophenols	Coefficient	Cresols	Coefficient
1 : 2	—	1 : 2	2.1
1 : 3	3.5	1 : 3	2.0
1 : 4	2.3	1 : 4	2.4

Although *p*-cresol and *p*-nitrophenol have practically the same bactericidal power, *m*-nitrophenol is considerably more active than *m*-cresol.

Saturated solutions of *o*-nitrophenol (0.3 p.c.) and of 2 : 4-dinitrophenol (0.1 p.c.) failed under the prescribed experimental conditions to kill *Staphylococcus py. aur.* in 15 minutes.

COUMARIN, THE COUMARIC ACIDS AND THEIR ALKALI SALTS

The three coumaric acids (hydroxycinnamic acids) combine in their molecular structure the chemical constitutions of cinnamic and salicylic acids and have accordingly been suggested as substitutes for the latter acids in the therapeutic application of these substances. (*British Medical Journal*, 1905, *i.* 1143). The acids have been employed for this purpose in the form of their sodium salts, which are freely soluble in water. The salt of the ortho-acid appeared to be more physiologically active than that of the para-acid whereas the salt of the meta-acid, exerted a more powerful action than either of these substances.

Coumarin is so sparingly soluble in cold water that it was for the purpose of the test, converted into its soluble sodium salt; in these circumstances it dissolves in aqueous sodium hydroxide, forming sodium coumarinate, the *cis*-isomeride of sodium *o*-coumarate which has the *trans*-configuration.

ORGANISM: *B. Typhosus* (24 *hours' culture*)

Substance	Concentration of substance killing in 10 minutes	Concentration of phenol killing in 10 minutes	Carbolic acid coefficient
Sodium *o*-coumarate	1 in 10	1 in 105	0.095
Sodium *o*-coumarinate	1 in 10 failed to kill in 10 minutes		
Sodium *p*-coumarate	1 in 10 failed to kill in 10 minutes		

The results show that the germicidal action of these sodium salts is very feeble, the ortho-coumarate being the most active. This feeble action of soluble alkali derivatives of aromatic phenolic compounds is noticeable in the foregoing case of potassium *p*-nitrophenoxide.

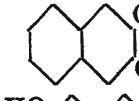
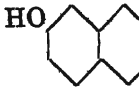
o-Coumaric acid and coumarin are freely soluble in 30 p.c. alcohol and their germicidal action was compared with that of phenol in the same medium.

Substance	Organism	Concentration of substance required to kill in 10 minutes	Concentration of phenol required to kill in 10 minutes	Carbolic acid co-efficient
o-Coumaric acid	*B. coli.*	1 in 500	1 in 620	0.80
o-Coumaric acid	*B. typhosus.*	1 in 500	1 in 680	0.73
Coumarin	*B. typhosus.*	1 in 550	1 in 620	0.56

The Dihydroxynaphthalenes

Although B-naphthol and certain of its sulphonic acids have found employment as germicides the effect of the dihydric naphthols on pathogenic organisms has not hitherto been examined.

1 : 5-Dihydroxynaphthalene is so sparingly soluble in water that its action could not be ascertained in aqueous solution. Two of the 3 isomerides having both their hydroxyl groups in B-positions, namely 2 : 3-dihydroxynaphthalene (m.p. 161°) and 2 : 7-dihydroxynaphthalene (m.p. 190°) were taken for the test, these compounds being sufficiently soluble in cold water, and, as in the foregoing experiments, the carbolic acid coefficient was determined in the absence of added organic matter, *B. typhosus* being taken as the test organism.

Dihydroxy-naphthalene	Concentration of the substance killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic acid coefficient
	1.9 in 1000	8.5 in 1000	4.4
	2.8 in 1000	8.0 in 1000	2.8

The isomeride containing the hydroxyl groups in contiguous positions is the more active, and both compounds are greatly superior to phenol in bactericidal power.

The dihydroxynaphthalenes have not hitherto been suggested for germicidal purposes, and although the cost of preparing the 2 : 3-isomeride would militate against its employment in this direction yet the 2 : 7-isomeride has been prepared economically on a manufacturing scale as an intermediate product in the formation of organic coloring matters. Naphthalene is sulphonated with 5-6 parts of concentrated sulphuric acid for 4 hours at 140°; the resulting naphthalenedisulphonic acid is converted successively into its calcium and sodium salts. The latter is fused with 2 parts of sodium hydroxide and 0.5 part of water, the melt is acidified with dilute sulphuric acid, and, after expelling the sulphur dioxide by means of wet steam, the solution is cooled when 2 : 7-dihydroxynaphthalene separates in almost colorless crystals and is purified by further crystallization from hot water.

It will be noticed that the two dihydroxynaphthalenes examined above greatly exceed the three dihydroxybenzenes in their germicidal action.

II. The Organic Amines

1. *Aliphatic Amines*

Koch (*l.c.*) found that 5 p.c. solutions of trimethylamine in water did not kill anthrax spores in 12 days.

The mixture of aliphatic amines from the interaction of herring-brine and lime has been used for the sterilization of sewage, for which purpose Klein stated that it was very efficient. It consists largely of trimethylamine and under the name of "Aminol" has been introduced as a general disinfectant.

The aliphatic amines used were all freely soluble in cold water. Their germicidal powers determined with *B. Typhosus* are given below.

Amine	Concentration of amine killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic coefficient
Ethylamine	7.09 in 1000	9 in 1000	1.27
Ethylene-diamine	Between 2 p.c. and 30 p.c.	8.5 in 1000	Between .03 & .4
iso-Amylamine	3 in 1000	8.5 in 1000	2.8
n-Heptylamine	0.35 in 1000	8.5 in 1000	24.3

The aliphatic amines therefore possess considerable germicidal power which increases with the size of the alkyl group in the amine molecule and which, when *n*-heptylamine is reached, attains a very high value.

The apparently high germicidal power was found *not* to be due to the inhibitory effect of the traces of amine carried over into the sub-culture tubes, as when the contents of the broth-tubes were sub-cultured into a second series of tubes in which the concentration of the amine did not exceed in any experiment .00000025 p.c., the carbolic coefficient was not affected.

Ethylenediamine is much feebler than ethylamine in germicidal power.

The germicidal powers of the fatty amines are compared in the following table with those of the corresponding alcohols:

	Organism.	*B. Typhosus*	
Amine	Coefficient	Alcohol	Coefficient
Ethylamine	1.27	Ethyl alcohol	0.026
iso-Amylamine	2.80	*iso*-Amyl alcohol	Under 1.7

The aliphatic amines are therefore considerably superior in germicidal power to the corresponding alcohols.

2. *Aromatic Amines*

Angus Smith (*Disinfectants*, Edinburgh, 1869) examined the germicidal power of aniline and regarded it as a disinfectant of moderate efficiency.

Fischer (*Mittheil a.d. K. Gesundt.* Vol. II) has found that a solution of aniline in water disinfected tubercular sputa in 24 hours.

Many of the aniline dyes have been shown to possess inhibitory and germicidal powers. Stilling (*Lancet*, 1890, Vol. XI. p. 965) showed that dilutions of 1 in 500 to 1 in 1000 of the methyl-violets prevented the growth of moulds on bread and 2 in 1000 dilutions prevented the souring of milk.

Prioux (*Internat. J. of Microscopy, and Nat. Science*, Vol. III, part 18) showed that 1 in 500 to 1 in 2000 dilutions of the methyl violets arrested the growths of *B. typhosus* and *B. coli*.

Fowler (*l.c.*) determined the germicidal power of methylene-blue on *B. typhosus* and found that it possessed a carbolic acid coefficient of 1.5.

Pyridine has been used mixed with oil of peppermint in the treatment of diphtheria and the injection of its aqueous solutions has been beneficial in gonorrhoea (Helbeig, *Mod. Mat. Med.* p. 65). Blyth showed that *Staphylococcus py. aur.* was killed by 1 p.c. solutions of pyridine and its homologues derived from bone-oil.

May found that magenta base (consisting largely of rosaniline) exceeds phenol in germicidal power and is moreover less toxic and more readily diffusible (*J. Amer. Medical Association*, 1912, 8 (16) April 20th).

With the exception of pyridine, the aromatic amines used were not freely soluble in water but solution was accelerated by gentle warming.

ORGANISM. *B. Typhosus*

Substance	Concentration of substance killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic Acid Coefficient
Aniline	15 in 1000	8.5 in 1000	0.57
o-Toluidine	7.5 in 1000	7.5 in 1000	1.00
m-Toluidine	6.5 in 1000	8.5 in 1000	1.30
p-Toluidine	6.0 in 1000	7.5 in 1000	1.25
Pyridine	48 in 1000	8.5 in 1000	0.18
ac-Tetrahydro-*B*-naphthylamine	1.6 in 1000	8.5 in 1000	5.3
o-Phenylenediamine	a saturated solution (2 p.c.) failed to kill in 15 minutes		under 0.42
m-Phenylenediamine	a 4 p.c. solution failed to kill in 15 minutes		under 0.2
p-Phenylenediamine	a saturated solution (3 p.c.) failed to kill in 15 minutes		under 0.3
Tolylene-1:4-diamine	a 4 p.c. solution failed to kill in 15 minutes		under 0.2

The germicidal power of aniline is therefore considerably less than that of phenol. The toluidines exceed aniline in germicidal power, indicating that the introduction of a methyl group into the benzene nucleus of aniline increases bactericidal action. The relative positions of the amine—and methyl—groups has some effect on germicidal power, which is greatest when the groups are in the meta- or para-position to one another.

Of all the monacidic amines examined, whether aliphatic or aromatic, pyridine is the feeblest in germicidal action.

The results obtained with aniline and phenylenediamines and with the toluidines and tolylene—2.4-diamine show that the entrance of a second amino-group into the benzene nuclei of aniline and the toluidines leads to considerable decrease in germicidal power. The aliphatic diamine, ethylene-diamine, is also weaker than ethylamine in bactericidal power.

In the following table the germicidal efficiencies of the toluidines and cresols are compared.

ORGANISM. *B. Typhosus*

Toluidines	Carbolic Coefficient	Cresols	Carbolic coefficient
Ortho.	1.00	Ortho.	2.6
Meta.	1.30	Meta.	2.6
Para.	1.25	Para.	2.6

The cresols are therefore consistently superior to the toluidines in germicidal power.

In the following table the germicidal powers of the dihydroxybenzenes and diaminobenzenes are compared.

ORGANISM. *B. Typhosus*

Amines	Coefficient	Phenols	Coefficient
o-phenylenediamine	Under 0.42	Catechol (1 : 2)	0.48
m-phenylenediamine	Under 0.2	Resorcinol(1 : 3)	0.29
p-phenylenediamine	Under 0.3	Quinol (1 : 4)	1.1

The dihydroxybenzenes are therefore superior to the diaminobenzenes in germicidal power.

The superiority in germicidal efficiency of phenol to aniline, of the cresols to the toluidines and of the dihydroxybenzenes to the diaminobenzenes indicates that the substitution of the amino-group for the hydroxy-group in the benzene nucleus is accompanied by a decrease in germicidal power. In the aliphatic series, on the other hand, the substitution of an amino-group for the hydroxy-group leads to a great rise in germicidal efficiency.

The germicidal powers of the aliphatic and aromatic amines are compared below.

ORGANISM. *B. Typhosus*

Amine	Carbolic Coefficient
Ethylamine	1.27
*iso*Amylamine	2.8
n-Heptylamine	24.3
ac-Tetrahydro-*β*.naphthylamine	5.3
Aniline	0.57
o-, *m*-, & *b*-Toluidines	1.00; 1.30; 1.25
Pyridine	0.18

It is seen that ethylamine exceeds aniline and is approximately equivalent to the toluidines in germicidal power whilst *iso*amylamine and *n*-heptylamine surpass very considerably the aromatic amines in efficiency. High germicidal power in the amines thus seems to be produced not by the presence of groups of an acidic nature such as phenyl and tolyl but by the presence of alkyl groups on which the strong basic properties of the aliphatic amines largely depend.

It is possible that the fatty amines owe their high germicidal efficiency in aqueous solution partially to the presence of hydroxyl ions liberated through ionisation of the alkylammonium hydroxides which are formed by the combination of the amines with water—

$$C_2H_5 \cdot NH_2 + H_2O = C_2H_5 \cdot NH_3 \cdot OH$$

$$C_2H_5 \cdot NH_3 \cdot OH = C_2H_5 \cdot NH_3^+ + OH^-$$

From this point of view, however, the feeble germicidal action of the strong base, ethylenediamine is certainly exceptional.

ac.—Tetrahydro-*β*-naphthylamine has a high carbolic acid coefficient which is noteworthy in connection with the chemical relationships of this amine. Its amino-group is attached to a fully hydrogenized ring; the substance is a strong base, non-diazotisable, absorbing carbon dioxide from the air and soluble in water. The high germicidal power of the base is also of interest

when considered in conjunction with the powerful fever-inducing action of this substance on the higher animals. The replacement of hydrogen in ammonia by radicles of an acidic nature such as phenyl or tolyl, gives rise to substances of feeble germicidal action. Other acidic groups such as succinyl have a similar effect. A 2 p.c. solution of succinimide failed to kill *B. typhosus* in 15 minutes, thus indicating a carbolic acid coefficient of less than 0.4.

The Effect of Age on the Germicidal Power of Aromatic Amines

Most of the aromatic amines gradually become highly colored on exposure and the following comparison has been made of the germicidal powers of aniline and *o*-toluidine in the colored and colorless (redistilled) condition.

Organism. *B. Typhosus*

	Carbolic coefficient	
Amine	Colorless	Colored
Aniline	0.57	0.69
o-Toluidine	1.00	1.20

The effect of exposure is therefore to increase slightly the germicidal powers of these amines.

This fact is of interest in relation to the work of Thalhimer and Palmer (*Journal of Infectious diseases*, Vol. IX, 1911, p. 172) who have recently shown that phenol which had become colored either by age or by exposure to sunlight also possesses a higher germicidal power than fresh colorless phenol.

The authors desire to express their thanks to Dr. C. J. Martin, F.R.S., for laboratory facilities given at the Lister Institute and to the Government Grant Committee of the Royal Society for a Grant which has partly defrayed the cost of the chemicals employed in this investigation.

DOSAGE ET MOYEN DE CARACTERISER DE PETITES QUANTITES D'ALCOOL METHYLIQUE DANS LE SANG ET LES TISSUS

PAR MAURICE NICLOUX

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L'alcool méthylique qui ne présentait jusqu'ici au point de vue pharmacologique qu'un intérêt des plus restreints se trouve aujourd'hui à l'ordre du jour du fait de la récente " épidémie de Berlin (Janvier 1912)."

Sans entrer dans le détail d'expériences que je poursuis en collaboration avec M. Placet sur la toxicité comparée de l'alcool mèthylique et de l'alcool éthylique et sur l'élimination de ces deux alcools, j'indiquerai ici brièvement comment il est possible de doser et de caractériser de petites quantités d'alcool méthylique dans le sang et les tissus.

I° Dosage dans le sang et les tissus. Le sang ou les tissus sont additionnés de 6 à 8 fois leur poids d'une solution saturée d'acide picrique, les tissus coupés et réduits en menus morceaux au sein de la dissolution picrique. On distille dans l'appareil de Schloesing-Aubin. En raison de la présence de l'acide picrique il n'y a pas production de mousse gênante et lorsqu'en a recueilli le ⅙ de volume total mis à distiller l'alcool se trouve entièrement réuni dans le distillat. Pour l'y doser il suffit d'employer la méthode de dosage par le bichromate de potasse que j'ai publiée il y a seize années, et dont maints auteurs ont reconnu la simplicité et l'exactitude. Elle s'applique en effet parfaitement au dosage de l'alcool méthylique. En employant une solution de bichromate à 1gr. par litre cette solution est telle que 1cc. correspond à 5cc. d'une solution à 0,5 pour 1000 d'alcool méthylique.

II° Procédé pour caractériser l'alcool méthylique. Dans le distillat qui provient de la distillation du sang ou des tissus, on effectue en vase clos l'oxydation de l'alcool méthylique que l'on veut caractériser par le bichromate de potasse et on recueille et

dose l'acide carbonique produit. Dans ces conditions on possède tous les éléments pour déterminer avec exactitude le rapport $\frac{CO_2}{O_2}$: CO_2 étant mesuré comme il vient d'être dit, O_2 se déduisant avec la plus grande facilité de la quantité de bichromate employé pour arriver au terme de l'oxydation. Or le rapport $\frac{CO_2}{O_2}$ est spécifique, il est égal à 0,915 pour l'alcool méthylique. Si le chiffre obtenu expérimentalement pour la détermination du rapport $\frac{CO_2}{O_2}$ coïncide avec 0,915 aux erreurs d'expérience près, on peut affirmer que l'on se trouve bien en présence d'alcool méthylique et de cet alcool seul.

IL CALCIO E IL MAGNESIO DEL CERVELLO IN DIVERSE CONDIZIONI FISIOLOGICHE E FARMACOLOGICHE

PROF. IVO NOVI

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Sommario.

1 = Il Calcio nel cervello del cane oscilla da gr. 0,0143 a 0,031; il Magnesio da 0,0143 a 0,0167 per cento di sostanza fresca.

2 = L'età ha una grande influenza sul contenuto di Calcio nel cervello. Nei cani la quantità massima si ha nel feto e nel neonato, la minima prima del divezzamento e nell'età avanzata si ritorna alla quantità inziale. Cosi avviene nell'uomo. Nelle cavie invece il Calcio è in quantità minima nei feti, si raddoppia quasi pochi giorni dopo la nascita, continua a crescere per un mese e si mantiene costante fino all'età adulta per accrescer si infine nella vecchiaia, nella quale diviene anche il decuplo.

3 = Introduzioni di NaCl nello stomaco, sotto cute, nella vena, nelle carotidi in soluzioni isotoniche ed ipertoniche sottraggono fino al 50% del Calcio al cervello.

4 = Il Magnesio si mantiene sempre costante nel cervello in tutte le età e in tutte le condizioni sperimentali accennate.

5 = Consiglio le cure clorurate nell'arteriosclerosi prima delle lesioni renali, e le diete declorurate nell'osteo malacia e rachitismo.

LA IMPORTANZA FISIOLOGICA DEL MANGANESE NELL'ORGANISMO ANIMALE

PROF. GUIDO M. PICCININI

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SOMMARIO

1. Il Mn somministrato agli animali, produce non solamente un aumento del Ferro nel sangue, come gli altri agenti ematogeni, ma anche un aumento del Ferro della riserva minerale (fegato e *mil za*). Tra Ferro e Manganese, nei rapporti dell'assimilazione del Ferro, esiste la legge del minimo.

2. Il Mn colloidale produce un aumento dell'ossigeno mobile del sangue e ve lo mantiene per lungo tempo.

3. Il Mn colloidale attenua la virulenza della tossina difterica.

4. Il Mn non deve più essere considerato come un componente accidentale dell'organismo, ma bensì come un elemento costante del corpo animale perchè dotato di due azioni importantissime: la prima; come elemento costitutivo cellulare, la seconda, come elemento *at* tivatore delle ossidazioni.

5. Forse esistono nel corpo umano delle ossidasi manganiche, cioè dei fermenti solubili con lo scheletro metallico dato dal Manganese.

6. Nelle cure ricostituenti dovrebbe essere sempre regola fisiologica la somministrazione associata di Fe e di Mn.

(*Abstract*)

THE ENZYME ACTIVITIES INVOLVED IN CERTAIN PLANT DISEASES

By Howard S. Reed

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The present work deals with the processes involved in the decay of apples by certain fungi, principally the bitter rot of apples, caused by *Glomerella rufomaculans*.

Previous work has shown that several fungi and phytopathogenic bacteria produce cytolytic enzymes which break down the cell walls of their host plants and bring disorganization of the tissues. In some cases a thermostable toxin has been found. In the case studied by de Bary this substance was shown to be oxalic acid.

Glomerella rufomaculans is a fungus which causes a characteristic decay of apples. It causes the pulp to turn brown and lose its tissue organization. After several weeks the tissues shrivel, becoming changed into a hard persistent mass which may resist further disorganizing agents for some time.

The author has shown that when decayed apple pulp is mashed and extracted with water under aseptic conditions, enzymes may be demonstrated in the extract. Oxidizing and reducing exzymes have been shown to exist in such extracts. By the addition of an excess of alcohol to the extracts it was possible to obtain a precipitate containing amylase, invertase, erepsin, and amidase.

More active enzymes were obtained by cultivating the fungus upon sterile nutrient solutions and making an acetone-ether preparation from the mycelium thus obtained.

Amylase was formed by the fungus when cultivated upon any solution, but its production was stronger when starch was the only carbohydrate furnished for the nutrition of the fungus.

The extracellular amylase was weaker than the intracellular form. A small amount of acid favors the action of the amylase, while alkali slightly retarded. Invertase was present in all preparations both as an extracellular and as an intracellular enzyme. Cytase is probably not abundant in the apples affected with bitter rot but it was formed when the fungus was cultivated upon celluose. Inulase was present but weak. Zymase was not present.

In every case an intracellular enulsin was formed which acted upon arbutin, amygdalin, and salicin. No extracellular emulsin was found.

A lipase capable of hydrolysing ethyl acetate and ethyl butyrate was found. Hydrolysis appeared to be greater in the case of ethyl acetate.

Three enzymes capable of acting upon proteins or their cleavage products were found. Protease was identified by its action upon fibrin and a commercial protein. Erepsin was identified by its ability to form tryptophane from peptone and casein. Amidase was identified by the formation of ammonia from alanin and asparagin.

An enzyme splitting hippuric acid into glycocoll and benzoic acid was demonstrated in the enzyme powder.

The thermal death point of the emulsin lay between 55° and 65° C. The death point of invertase and erepsin lay between 70° and 75° C.

The enzymes here demonstrated are such as have the ability to break down certain important constituents of the tissues upon which the fungus grows.

SUR LA NUTRITION MINÉRALE DU BACILLE TUBERCULEUX

PAR B. SAUTON

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Les divers milieux proposés pour la culture du bacille tuberculeux contiennent tous de la glycérine et un acide amidé. Ils diffèrent profondément par leur composition minérale: le chlore, le sodium, le calcium, le magnésium, le fer, le mangnèse, le zinc, etc. ne figurent pas dans toutes les formules.

Je me suis proposé de déterminer les éléments utiles pour la culture du bacille de Koch et je résume dans le présent travail les premiers résultats obtenus.

Le bacille tuberculeux est cultivé sur un liquide nutritif constitué de produits très purs. Après 20 jours à l'étuve à 38° on pèse le poids de récolte obtenu comparativement sur le milieu complet et sur le même milieu dépourvu de l'élément, dont on veut étudier l'influence.

J'étudie donc uniquement l'influence des éléments sur le poids de récolte obtenu, en laissant de coté l'étude de leur action sur la virulence du microbe et sur la production de la tuberculine.

Les produits employés sont soumis à plusieurs cristallisations successives. La glycérine est purifiée par distillation sous pression réduite. Les divers constituants du milieu sont dissous dans de l'eau soigneusement distillée. Le liquide, neutralisé par l'ammoniaque, est réparti par portions de 100 cc entre des matras de 250 cc, puis stérilisé à 120°.

La formule du milieu nutritif est la suivante:

Asparagine	4 gr. 0/00	Phosphate de potassium	0.5 0/00
Glycérine	60 0/00	Sulfate de magnésium	0.5 0/00
Acide citrique	2 0/00	Citrate de fer ammoniacal	0.05 0/00

Ce milieu, parfaitement limpide, est ensemencé après neutralisation et stérilisation par une portion de voile provenant d'une

précédente culture agée de 8 jours. Le germe employé est un bacille d'origine bovine (BB, LA, ou LP de l'Institut Pasteur).

Après 20 jours de culture, on stérilise l'autoclave à 120°. On filtre sur filtre caré, on lave, on dessèche, on pèse la récolte.

Le poids de récolte obtenu varie de 0 gr. 9 à 1.25 par cc. de liquide. Dans les mêmes conditions, la récolte sur bouillon glycériné est de 0.6 environ. Elle est de 0.35 environ sur le milieu artificiel de Proskauer et Beck. Ce dernier milieu n'est favorable qu'à la condition de renfermer à l'état d'impureté le fer, qui ne figure pas dans sa composition et qui est des éléments nutritifs importants pour la culture du bacille tuberculeux.

Dans le milieu nutritif, utilisé dans le présent travail, le citrate d'ammoniaque n'intervient que pour empêcher la précipitation des phosphates. L'utilité des autres éléments ressort des résultats suivants:

Poids sec après 20 jours

Liquide complet	1.15
sans soufre	0.12
sans phosphore	pas de culture
sans magnésium	0.03
sans potassium	pas de culture
sans fer	0.35

Le potassium ne peut pas être remplacé par le sodium, le lithium, le caesium, ni le rubidium. Avec ce dernier métal, on obtient pourtant un début de culture soit que le rubidium puisse être utilisé comme aliment de misère, soit qu'il agisse par le potassium qui l'accompagne à l'état d'impureté.

De même le fer ne peut pas être remplacé par le mangnèse. Les éléments les plus voisins au point de vue chimique sont donc nettement distincts au point de vue biologique.

Au cours de ces premiers essais, il n'y a jamais eu augmentation du poids de récolte par addition de chlore, de calcium, de mangnèse, ou de zinc au milieu de culture.

Pour étudier l'influence du calcium, on substituait, aux matras de verre si facilement attaquables par les réactifs, des capsules de porcelaine. Le calcium n'était décelable dans aucun des produits employés. L'addition de divers sels de cet élément au milieu de

culture ne s'est jamais traduite par une augmentation du poids de récolte obtenu.

L'absence de zinc dans le milieu nutritif a été constatée d'une part par les réactifs chimiques et, d'autre part en utilisant la sensibilité bien connue de l'A. niger pour cet élément. Dans ce but, le milieu non neutralisé par l'ammoniaque était additionné de sucre, puis divisé en deux portions; dans l'une de ces portions on ajoutait 0.04% de sulfate de zinc. On ensemençait ces liquides par des spores d'A. niger. La différence très notable du poids des récoltes indiquait nettement l'absence de zinc dans le milieu nutritif. L'addition de cet élément au liquide qui en était dépourvu ne s'est jamais traduite par une augmentation du poids de récolte du bacille tuberculeux.

Néanmoins, certains éléments pouvant agir à des doses infinitésimales je ne considère pas comme définitifs les résultats obtenus en ce qui concerne les substances dont une première étude n'a pas démontré l'utilité, et je me propose de continuer ces recherches.

SUBCUTANEOUS ABSORPTION OF THYMOL FROM OILS

By W. H. Schultz and Atherton Seidell

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While determining the toxicity of thymol dissolved in various media it was observed that the number of milligrams necessary to cause death varied greatly with the solvent used. Furthermore there appeared to be a relation between the solubility in a given medium and the rate with which the toxic symptoms developed. A series of experiments was therefore planned with the object of ascertaining why, for instance, thymol is more toxic when dissolved in liquid petrolatum than it is when dissolved in olive oil. The necessary solubility and distribution data for comparison with the results of the present experiments upon the subcutaneous absorption of dissolved thymol are presented in detail in another paper from this laboratory. (Section VIII b. Pharmaceutical Chemistry.)

When properly injected a solution of thymol in oil is retained in pocket-like enclosures, the walls of which for most practical purposes serve as an animal membrane through which the thymol must pass. When pure olive oil or pure liquid petrolatum is injected alone so as to be enclosed in one or several of these pockets it is absorbed very slowly, especially the liquid petrolatum. At the end of 90 hours as much as 80 to 90 per cent. of the olive oil can be recovered and liquid petrolatum itself remains for many days as a mass underneath the skin of mice, for the most part where first injected. If, however, oil containing thymol is injected and subsequently withdrawn and analysed the amount of thymol recovered decreases gradually with the increasing interval between injection and collection. Furthermore the amount of thymol recovered will also vary with the solvent used, being less for petrolatum than for olive oil, hence it seemed likely that the

retention of the thymol by oily solvents is proportional to its solubility in the oil tested. The determination of the thymol in the recovered samples of injected oil was made by steam distillation and titration of the distillates by the recently described bromine-hydrobromic acid methold.[1]

The plan of the experiments was as follows. Four sets of mice, *a*, *b*, *c*, *d*, which had been raised upon the same diet, and under the same conditions, were injected subcutaneously in the dorsal region. At first care was taken to have the individuals of each group weigh the same to within a gram, but later it was found that a variation of several grams did not materially alter the results, the main factors seemed to be the time element, the kind of pocket formed and especially the region in which the oil pocket was located. Each mouse received one cubic centimeter of the oil solution. Each cubic centimeter of solution injected contained for sets *a*, *b*, and *c*, respectively, 20, 40 and 60 milligrams of thymol in olive oil, while set *d* was injected with liquid petrolatum containing 20 milligrams of thymol per cc. of solution.

At varying intervals of time after the injection a mouse was chloroformed, the oil pocket carefully exposed by an incision into the skin and the unabsorbed oil drawn into an all-glass syringe, graduated in tenths of a cubic centimeter. The oil thus obtained was estimated to one-hundredths of a cubic centimeter and the sample carefully transferred with the acid of about one half a cubic centimeter of carbon tetrachloride to a distilling flask arranged for steam distillation, about 200 cc. of water were added and the aqueous solution distilled with a current of steam into three 250 cc. glass stoppered bottles. The first, second and third distillates collected in this way were each titrated separately, thus assuring complete removal of the thymol. The quantities of thymol recovered by this method were in all cases somewhat greater than used in the experiment. This constant error is probably due to small amounts of volatile constituents of the oil which react with the bromine used for the titration in a manner somewhat similar to thymol. Since the quantity of oil was kept constant, a correc-

[1]Seidell, Am. Chem. Jour. 47, 520, 1912.

tion of the apparent excess of thymol is not necessary and the conclusions drawn from the experiments are not affected by this constant error.

The experimental data as summarized in Tables 1, 2 and 3 have been plotted on cross-section paper and average curves constructed (Fig. 1). The points corresponding to the different sets of mice are shown differently and indicate, for all except the liquid petrolatum experiments, that the individual variations are much greater than the differences between the adjoining curves. In spite of this, however, the general directions of the three olive oil curves probably indicate in a general way the rate of absorption of thymol from this oil.

Analysis of the data lead to the following

TABLE No. I. Absorption of Thymol from Olive Oil. Subcutaneous Injection in Mice

Thymol Solution = 20 Mgs. per 1 cc. Olive Oil. Amount injected = 1 cc.

Mouse No.	Mouse Wt. in Gms.	Time Hrs.	cc. Oil Recovered	Gms. Thymol Found	Gms. Thymol per cc. Recovered Oil	Mg. Thymol Absorbed
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0261	——
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0242	——
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0247	——
Blank	—— 1 cc. Pure Olive Oil				0.0020	——
	1 cc. Pure Olive Oil 18 hrs.		0.78	0.0026	0.0033	——
96	23	$\frac{1}{4}$	0.74	0.0176	0.0238	1.2
94	18	$\frac{1}{2}$	0.89	0.0194	0.0218	3.2
103	24	$1\frac{1}{2}$	0.9	0.0192	0.0213	3.7
93	18	2	0.79	0.0144	0.0183	6.7
98	27	$3\frac{1}{4}$	0.93	0.0178	0.0191	5.9
95	25	4	0.92	0.0180	0.0196	5.4
97	23	5	0.9	0.0178	0.0198	5.2
104	24	6	0.77	0.0111	0.01446	10.5
123	25	$13\frac{1}{4}$	0.5	0.0090	0.0180	7.0
99	18	17	0.76	0.0111	0.0142	10.8
124	32	$19\frac{1}{3}$	0.72	0.0086	0.0119	13.1
105	29	$22\frac{1}{2}$	0.91	0.0156	0.0172	7.8
		24	0.60	0.0036	0.0059	19.1
		46	0.90	0.0080	0.0096	15.4
100	23	47	0.78	0.0047	0.0061	18.9
106	23	$71\frac{1}{2}$	0.74	0.0033	0.0045	20.5

TABLE No. 2. Absorption of Thymol from Olive Oil. Mice—Subcutaneus Injection

Thymol Solution = 40 Mgs. per 1 cc. Olive Oil. Amount injected = 1cc.

Mouse No.	Mouse Wt. in Gms.	Time Hrs.	cc. Oil Recovered	Gm. Thymol Found	Gm. Thymol per cc. Recovered Oil	Mg. Thymol Absorbed
Blank 1 cc. Olive Oil Solution					0.0450	—
134	24	½	0.92	0.0409	0.0444	0.6
133	25	¾	0.92	0.0391	0.0425	2.5
130	31	1	0.88	0.0347	0.0395	5.5
112	31	1	0.81	0.0307	0.0375	7.5
135	25	2	0.83	0.0368	0.0443	0.7
131	25	2	0.94	0.0385	0.0409	4.1
125	24	2½	0.84	0.0344	0.0410	4.0
115	31	4½	0.90	0.0359	0.0400	5.0
127	29	4½	0.91	0.0325	0.0357	9.3
136	31	6	0.90	0.0290	0.0322	12.8
121	26	12	0.72	0.0245	0.0340	11.0
111	—	17¼	0.80	0.0195	0.0241	20.9
114	21	20⅔	0.72	0.0199	0.0276	17.4
126	21	94⅓	0.82	0.0065	0.0079	37.1

Thymol Solution = 60 Mgs. per 1 cc. Olive Oil. Amount injected = 1 cc.

Blank 1. cc. Olive Oil Solution					0.0641	—
113	32	¾	0.91	0.0553	0.0607	3.3
109	24	2⅓	0.85	0.0459	0.0541	10.0
110	24	5	0.82	0.0428	0.0534	10.6

TABLE No. 3. Absorption of Thymol from Liquid Petrolatum. Mice—Subcutaneous Injection

Thymol Solution = 20 Mgs. per 1 cc. Petrolatum. Amount injected = 1cc.

Mouse		Time Hrs.	cc. Oil Recovered	Gm. Thymol Found	Gm. Thymol per 1 cc. Recovered Oil	Mgs. Thymol Absorbed
No.	Wt. in Gms.					
Blank 1 cc. Petrolatum Solution					0.0239	—
117	25	½	0.72	0.0154	0.0215	2.5
118	28	1	0.94	0.0182	0.0194	4.6
119	26	2½	0.905	0.0144	0.0159	8.1
120	26	5	0.91	0.0113	0.0124	11.6
128	30	11½	0.92	0.0069	0.0075	16.5
129	29	12	0.85	0.0051	0.0060	18.0
122	25	24	0.95	0.0043	0.0046	19.4
116	27	40	0.95	0.0016	0.0017	22.3

Conclusions: (1) The rate of absorption is greatest during the first hour following subcutaneous injection. The rate of absorption per unit of time gradually diminishes so that the curve is roughly parabolic. For one reason or another some mice absorb slowly whereas others absorb rapidly. Indeed if a sufficient number of experiments be performed it is possible to plot curves of the rate of absorption, one of which will represent the rapid type and the other the slow type of absorption. This variation is independent of season, of diet or of environment, it seems to be due to an inherent difference in the mouse itself and is probably congenital. It is illustrated by the sets of high and low points plotted in connection with both curves *o* and *x* of Fig. 1. The curves representing the rapid and slow types of absorption each show minor deviations above or below a mean rate of absorption. These deviations, however, are mainly accounted for by the character of the injection pocket, its position underneath the skin and certain other factors of technique. Curves *o*, *x*, and □, therefore, represent the mean values for olive oil containing, respectively, 20, 40 and 60 milligrams of thymol per cubic centimeter of solution.

(2) The rate of absorption of thymol injected subcutaneously is decidedly influenced by the amount of thymol contained in one cubic centimeter of olive oil. When mice are injected with olive oil containing 20 milligrams of thymol per cubic centimeter of solution a curve like *o* Fig. 1 is obtained. If a 40 milligram solution be employed a curve slightly steeper and on a higher level is obtained whereas with a 60 milligram solution the curve is still steeper. The higher doses, however, are so toxic that considerable influence is apt to be exerted by the absorbed drug acting as a general depressant. Not only are the cells of the membranous injection pocket affected by the thymol, but the current of circulating body fluids is greatly retarded so that those mice which very early show marked signs of depression may after a long interval, five to fifteen hours, yield oil that contains considerable thymol, showing that absorption had been retarded because of the various physiological factors mentioned.

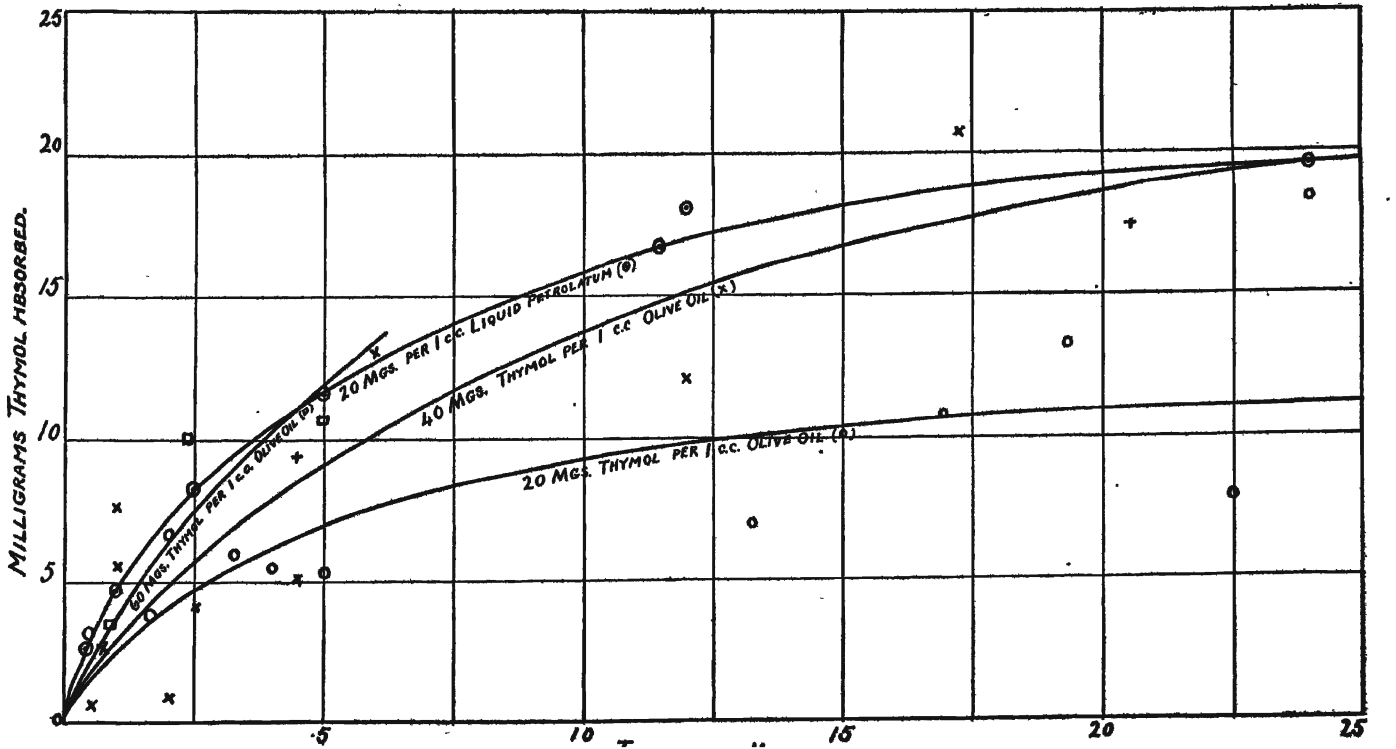
Absorption of thymol from liquid petrolatum containing 20 milligrams of thymol per cubic centimeter of solution is of unusual interest. (1) The absence of individual variations from the observed rate of absorption is noteworthy. (2) This mineral oil is very slowly absorbed from underneath the skin of white mice; it is apparently but slightly acted upon by the body fluids or enzymes. (3) Liquid petrolatum dissolves at 37° C. only 0.39 as much thymol as does olive oil at the same temperature. A solution of liquid petrolatum containing 20 milligrams of thymol per cubic centimeter is therefore more nearly saturated with thymol than is olive oil of the same concentration, hence diffusion ought to be more rapid from the liquid petrolatum solution. Actual experiment demonstrates the correctness of this assumption. Within certain limits, therefore, when equal volumes of different inert oils contain equal amounts of thymol the rate of subcutaneous absorption is proportional to the relative saturation of the solvent with thymol.

Conclusions

1. Thymol when dissolved in oil and injected underneath the skin of white mice is absorbed from the oil much more quickly than the oil itself is absorbed, the rate of thymol absorption increasing with the concentration of thymol in a given oil.

2. The rate of absportion from oils varies with the oil used and with the partition coefficient between thymol in oil and water. Within certain limits, when equal volumes of the solution of thymol in different inert oils contain equal amounts of thymol the rate of subcutaneous absorption is proportional to the relative saturation of the solvent with thymol.

3. Thymol is absorbed more rapidly at the beginning of the experiment than it is some hours later. This change in rate is probably due to a diluting of the injected solution, to local action of the thymol, and to the general action that it has upon the cardiac and respiratory apparatus.


MILLIGRAMS THYMOL ABSORBED.
25
20
15
10
5
5
10
15
20
25
20 MGS. PER 1 c.c. LIQUID PETROLATUM (⊙)
40 MGS. THYMOL PER 1 c.c OLIVE OIL (x)
20 MGS. THYMOL PER 1 c.c. OLIVE OIL (o)
60 MGS. THYMOL PER 1 c.c. OLIVE OIL (⊙)

THE DETERMINATION OF THYMOL IN DOG FECES

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It has frequently been observed during the examination of feces for hook-worm eggs that samples from patients having received large doses of thymol may show undoubted evidence of the presence of this drug in an unaltered state. Since it has long been known that thymol is eliminated through the urine in combination with glycuronic and other acids,[1] it appeared of interest to ascertain what proportion comes through the alimentary tract unchanged. A satisfactory quantitative method for the determination of thymol having been devised by one of us[2] it was decided to attempt to apply this method to the determination of thymol in dog feces.

On account of the requirements of the method, that the thymol solution be neutral and contain no substance which is acted upon by bromine, it was decided to take advantage of the volatility of thymol with steam in order to separate it from the fecal material. Experiments were therefore made for the purpose of ascertaining the proper conditions for the steam distillation of the samples of feces in order that the least possible amount of interfering substance would be obtained in the distillate. It was found that distillation from an acidified mixture gave an acid distillate and from an alkaline medium a more or less strongly alkaline one. A double steam distillation from first an acid and then an alkaline mixture was then adopted and, as might be expected, gave a practically neutral distillate; blank determinations showed that the alkaline medium which was used, viz. magnesium oxide suspended in water, did not retain an appreciable quantity of thymol. When, however, determinations were made upon mixtures of feces and known amounts of thymol, high results were

[1] Blum, Z. physiol. Chem., 16, 514-24. 1892.

[2] Seidell, Am. Chem. Jour., 47, 520. 1912.

invariably obtained. After many experiments it was ascertained that the cause of the trouble was the hydrogen sulphide which passed readily through the second distilling flask containing the magnesium oxide in suspension. In attempting to retain this hydrogen sulphide it was found that the addition of lead nitrate was quite effective, but if used in the first distilling flask containing the acid mixture the thymol was also held back to a greater or less extent. When used in the second distilling flask with the aqueous suspension of magnesium oxide it exerted no influence whatever upon the thymol and completely retained the hydrogen sulphide.

There are, of course, in feces very small amounts of certain phenols and phenol-like compounds, for instance, indol, skatol, etc., which might be expected to yield bromine substitution products very much as thymol. Blank determinations run with feces show, however, that although a certain amount of hydrobromic acid is formed when these distillates are treated with bromine vapor, the necessary correction is small.

The details of the method which our experiments have finally led us to adopt are as follows: The apparatus consists of an ordinary steam generator made from an empty ether can, and two 500 cc. distilling flasks connected in series for steam distillation. The distillate from the second flask passes into a condenser and is received directly into the 250 cc. glass stoppered bottle in which the titration is to be made. Three of these bottles should be provided to collect the first, second and third distillates, each of which should come over in about 20 to 30 minutes and measure about 150 cc. A convenient amount of feces to use is about 20 grams and, as will be seen from the following table, the amount of thymol should be approximately 0.2 gram in order that a suitable amount of the standard thiosulphate be required for the titration. In all of the determinations shown in Table No. 1 the thymol was added in the form of 1.0 per cent. solution made by dissolving it with gentle warming in just a little more than the calculated amount of normal sodium hydroxide to form the sodium salt of thymol and diluting with the necessary amount of water. The feces were in some cases the hard white lumps and in others darker and softer masses. In each case the first flask contained in addition

to the feces and thymol about 100 cc. of water and 3 cc. of phosphoric acid solution, made by diluting the ordinary 85 per cent. H_3PO_4 syrup with an equal volume of water. The second distillation flask in all cases contained approximately five grams of magnesium oxide suspended in about 100 cc. of aqueous 1.0 per cent. $Pb(NO_3)_2$ solution. The distillate was immediately treated with about 1 cc. of CCl_4, and then the bromine vapor was poured in, a little at a time, with alternate shaking and addition of bromine until the mixture retained a distinct red brown color. It was then allowed to stand in a dark place about one-half hour five cc. of CS_2 and 5 cc. of 20 per cent. KI solution were added and the bottle well shaken, standard thiosulphate solution was run in until the pink color of the iodine in the CS_2 layer was just discharged, an additional amount of KI solution was added and if no further liberation of iodine occurred the reading on the burette was taken. Five cc. of 2 per cent. KIO_3 solution were then added and after thorough shaking the titration with thiosulphate was continued until the iodine color was just discharged for the second time. The completion of the reaction may be tested by a further addition of KI and KIO_3 solutions. The difference between the first (which should be from about 5 to 15 cc. 0.1 n thiosulphate) and second reading corresponds to the hydrobromic acid formed by the action of the bromine on the thymol. The calculation is made on the basis of two molecules of HBr per one of thymol; 1 cc. 0.1 n thiosulphate is, therefore, equal to 0.0075056 gram thymol.

TABLE No. 1. Showing the Results of the Determination of Thymol when Mixed with Dog Feces in Various Proportions.

Composition of Sample		Distillates Obtained		Gm. Thymol Recovered	
Gms. Feces	Gm. Thymol	No.	Volume		
—	0.2	1st	150	0.194	
20	0.2	1st	150	0.192	
20	0.05	1st	120	0.051	0.063
		2nd	120	0.012	
20	0.1	1st	130	0.096	0.115
		2nd	110	0.010	
		3rd	150	0.009	
20	0.5	1st	120	0.343	0.444
		2nd	110	0.076	
		3rd	110	0.025	
80	0	1st	110	0.0143	0.027
		2nd	140	0.0089	
		3rd	120	0.0036	
50	0.2	1st	125	0.153	0.224
		2nd	140	0.047	
		3rd	150	0.024	
10	0.2	1st	160	0.169	0.186
		2nd	145	0.017	
5	0.2	1st	90	0.178	0.182
		2nd	120	0.014	
	0.40	1st	100	0.311	0.380
		2nd	110	0.057	
		3rd	100	0.012	
—	0.40	1st	100	0.380	0.399
		2nd	125	0.015	
		3rd	140	0.004	

Although the results shown in Table 1 are not entirely as satisfactory as could be desired, further work upon the improvement of the method was not done since preliminary experiments upon the feces of dogs to which small doses of thymol had been given showed that only insignificant amounts of thymol were present.

PROTOCOLS OF EXPERIMENTS

1. Dog No. 6, Wt. 6.95 kilograms, amount of thymol given = 1.0 gram.

Samples of Feces Time	Amt. Gms.	Apparent Gas. Thymol in sample	Correction per 45 Gms. Feces	Thymol Recovered
Before administration of thymol	56.0	0.028		
5 ½ hrs. after administration of thymol	45.0	0.100	—0.022 =	0.078
24 hrs. after administration of thymol	40	0.029	—0.022 =	0.007
				0.085

The correction per 45 gms. feces is obtained from the blank determination made upon the feces obtained before the administration of the thymol. Since the total amount of administered thymol was 1.0 gram, it is apparent that not more than 8.5 per cent. of it came through the alimentary tract unchanged.

2. A second experiment with Dog No. 6 was made four days later. The amount of thymol given was 0.5 gram. The total feces was collected in several portions during the first 25 hours after administration of the thymol and amounted to 49 grams. The apparent thymol recovered from this quantity was 0.046 gram and this figure corrected for the blank determination as shown in the previous experiment is 0.046 - 0.22 = 0.024 gram thymol recovered from 0.5 gram administered or approximately 5 per cent.

3. Dog No. 26 was given 0.5 gram thymol at 10 o'clock A. M. but vomited 4 - 5 hours afterwards. The combined vomit was distilled and found to contain approximately 0.05 gram thymol. The administered thymol as corrected for this amount is therefore 0.45 gram.

Samples of feces Time	Amt. Gms.	Apparent Gm. Thymol in sample	Correction per 45 Gm. Feces	Thymol Recovered
3 hrs. after administration of thymol	15	0.012	0.007	0.005
23 hrs. after administration of thymol	44	0.040	0.022	0.018
				0.023

From these results it is seen that about 5 per cent. of the thymol came through the alimentary tract.

Although these experiments are not as numerous as desirable they show conclusively that when small doses of thymol are given only insignificant amounts are eliminated unchanged with the feces.

Preliminary experiments with the *urine* from dogs which had received thymol showed that all of the drug excreted in this manner is in firm combination, probably with glycuronic acid. Such urines were found to yield no appreciable amount of thymol from neutral solution, by steam distillation, but did so when considerable free acid was used in the distilling flask. Quantitative results, however, have so far not been obtained since the distillation method has not been developed to the extent of eliminating certain interfering substances that are distilled with thymol. These experiments are still in progress and it is hoped to improve the technique so that it will be possible to account for nearly all of the ingested thymol.

SUR LA RÉSISTANCE DE LA PEROXYDASE A L'AMMONIAQUE ET SUR SON ACTIVATION PAR CONTACT AVEC L'ALCALI

PAR M. J. WOLFF

Paris

J'ai constaté dans les jeunes pousses d'orge l'existence d'une peroxydase très active et particulièrement résistante à l'action de la chaleur, attendu qu'elle n'est détruite qu'après plusieurs minutes d'ébullition. Comme les autres peroxydases connues, celle des pousses d'orge est détruite presque instantanément par de faibles doses d'acide sulfurique et phosphorique,[1] et résiste, sans être sensiblement affaiblie, à des doses équivalentes de bases alcalines.

J'ai étudié cette action des alcalis et j'ai vu que si des doses un peu massives de soude sont capables de détruire la peroxydase au bout de quelques heures, des quantités équimoléculaires d'ammoniaque n'attaquent l'enzyme qu'avec une extrême lenteur.

EXEMPLE: Je mets en contact d'une part 1 cc. de macération diastasique avec 3 cc. de soude normale; d'autre part 1 cc. de la même macération avec 3 cc. d'ammoniaque normal. Je constate alors qu'au bout de 8 à 10 heures l'enzyme a été complètement détruit par la soude. La peroxydase qui a été laissée en contact avec l'ammoniaque conserve ses propriétés pendant 8 à 10 jours. Au bout de ce temps elle est affaiblie, mais nullement détruite.

Ce fait inattendu m'a suggéré l'idée d'étudier à l'aide d'expériences plus délicates ce qui se passe lorsqu'on laisse en contact la peroxydase avec l'ammoniaque, et de suivre les modifications qui peuvent survenir au cours de cette action.

Pour étudier les différentes phases du phénomène, je me suis servi comme réactif du gayacol en présence d'eau oxygénée et

[1] Il n'est pas indifférent de remarquer que la peroxydase résiste mieux à de faibles doses d'acide phosphorique qu'à de faibles doses d'acide sulfurique.

j'ai toujours exécuté la réaction dans un milieu renfermant un faible excès de phosphate acide de potassium, en prenant comme mesure l'intensité de la coloration produite et la rapidité de son apparition. C'est ainsi que j'ai pu observer un ralentissement considérable de la réaction tout à fait au début de l'expérience, par comparaison avec un témoin sans alcali; puis par des prélèvements opérés d'heure en heure sur le même mélange de peroxydase et d'ammoniaque, j'ai vu l'activité augmenter de plus en plus à mesure que le contact se prolongeait. La marche du phénomène montre que l'activité perdue au début est regagnée, puis considérablement dépassée.

Les diverses phases du phénomène peuvent se résumer ainsi:

1°. Au moment où la peroxydase entre en contact avec l'ammoniaque, il y a une perte considérable de l'activité primitive.

2°. A mesure que le temps de contact s'accroît, l'activité s'accroît.

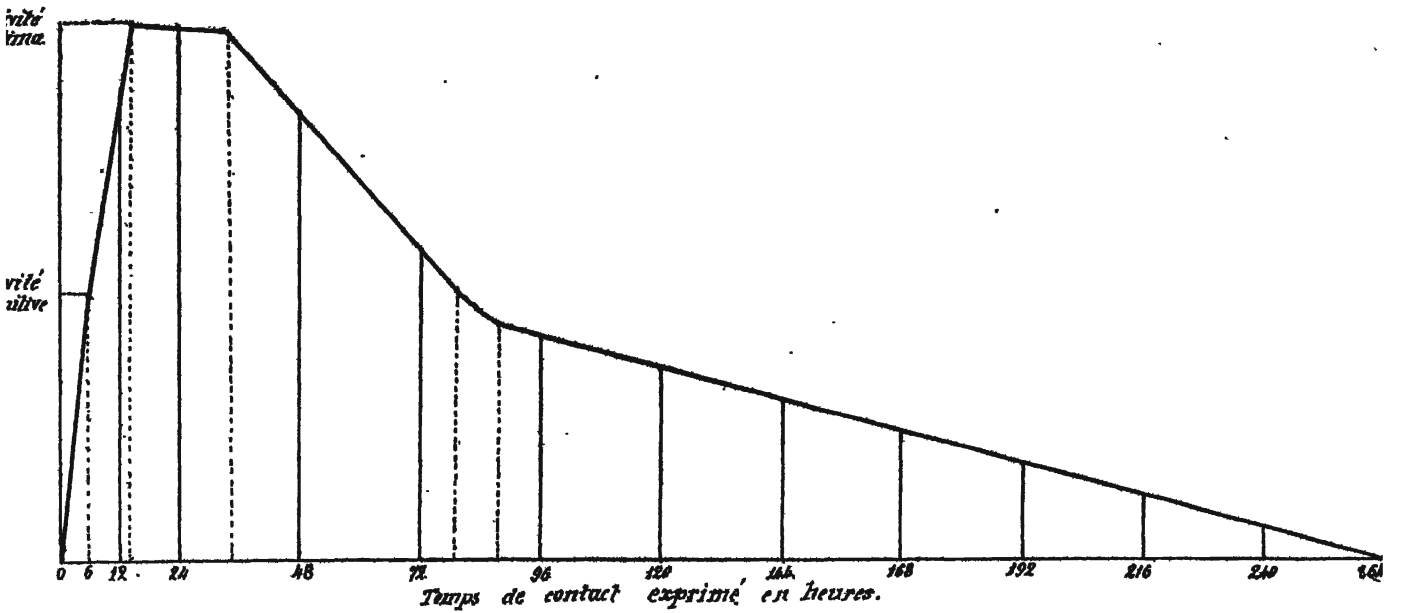
3°. Au bout de 4 à 5 heures, cette activité, a repris sa valeur primitive.

4°. A partir de ce moment, l'intensité de la réaction produite continue à s'accroître et l'activité atteint son maximum vers la 14e heure; cette activité représente environ le double de l'activité primitive de la peroxydase.

5°. L'activité de la peroxydase reste ensuite sensiblement constante pendant quelques heures, puis elle décroît lentement.

6°. Au bout de 11 jours, l'activité est de nouveau très affaiblie, et elle est comparable à ce qu'elle était à son début, au moment du premier contact (en 1).

On peut essayer, au moyen de ces données, de représenter grossièrement le phénomène d'activation par une courbe. Si on adopte pour 0 l'instant précis où l'activation commence, et si l'on porte les temps de contact en abscisses et les activations en ordonnées, ce que l'on peut faire si l'on observe que l'activité maxima est le double de l'activité primitive ou normale, on aura le tracé suivant:



Courbe des vitesses de réaction

On voit par la forme de la courbe que la marche du phénomène se rapproche beaucoup de ce que l'on observe habituellement dans les actions diastasiques. La diminution lente de l'activité est le résultat de la destruction progressive de la peroxydase.

Il est bon de faire remarquer que le contact de 1 cc. de peroxydase avec une solution *décinormale* de soude peut donner lieu à des phénomènes analogues, mais la destruction de l'enzyme étant beaucoup plus rapide avec la solution *décinormale de soude* qu'avec la *solution normale d'ammoniaque*, on ne les observe que sous une forme très atténuée. Avec les acides sulfurique et phosphorique, même très étendus, ces phénomènes d'activation ne se produisent pas; tout au moins je n'ai pu les observer.

Dans les expériences que je viens de décrire, je me suis servi comme réactif du gayacol. Lorsqu'on s'adresse à d'autres réactifs tels que le pyrogallol ou l'hydroquinone, le phénomène ne se passe pas tout à fait de la même façon; en effet, on observe *une activation immédiate de la peroxydase*, lorsqu'en présence de celle-ci et d'un excès de phosphate acide, on introduit dans le milieu une petite quantité de soude ou d'ammoniaque. Un contact plus ou moins prolongé de l'enzyme avec l'ammoniaque n'a pas pour effet d'augmenter l'intensité de la réaction comme cela a lieu dans le cas du gayacol. Je n'ai pu jusqu'ici m'expliquer ces différences. Toutefois, je crois utile d'attirer l'attention sur leur importance. On se rend compte, en effet, par cet exemple, que la substance qui subit l'action de l'enzyme est aussi sensible aux influences du milieu que l'enzyme lui-même.

Enfin, il se dégage de toutes ces expériences que les bases alcalines, soit qu'elles agissent à l'état libre, soit à l'état combiné, sont un des facteurs principaux des phénomènes d'activation analysés dans ce travail.