STUDIES ON PATHOGENESIS OF PASTEURELLA INFECTION IN CORTISONE-TREATED, IMMUNIZED, SUSCEPTIBLE AND CONTROL RABBITS

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**INTRODUCTION**

Pasteurellosis is a septicemic and highly fatal disease affecting various species of animals including cattle, pigs, sheep, goats and laboratory animals. It is world wide in distribution and accounts for enormous economic losses. For instance in India alone, according to Dhanda et al (1956) annual losses due to bovine pasteurellosis, commonly known as Haemorrhagic Septicaemia in this country, run into several lacks of rupees and at an average more than 33,000 deaths occur each year. With the control of Rinderpest, haemorrhagic septicaemia is the next most important disease of cattle in India.

Within the last ten years, researches into the aetiology of haemorrhagic septicaemia including "Shipping fever" conducted in various parts of the world have brought about radical changes in our outlook on the genesis of this disease. It is generally conceded that haemorrhagic septicaemia is an aetiological, host specific and distinct clinical entity ( Bain, 1959 ; Dhanda, 1959). Bain (1959) recommended that condition known as haemorrhagic septicaemia in tropics may be considered as an acute septicemic condition of cattle and buffaloes caused by *Pasteurella multocida*.

The aetiology and pathogenesis of haemorrhagic septicaemia is complex and involves multiplicity of factors. It is generally believed that the infection is endogenous. *Pasteurella* bacteria are present in the upper respiratory
passages of a small proportion of animals (Moore, 1935; Jorgensen, 1925). These may multiply and invade tissues under the influence of various environmental stressors. The influence of environmental factors on the aetiology of pasteurellosis is well illustrated by the common synonyms, such as 'Shipping fever', 'Transit fever', 'Stock yard fever', etc., used for the disease in literature.

It has been shown that one of the components of response of the animal organism to stress is marked structural and functional changes in adrenal gland resulting in increased production of cortisone (the pure synthetic substance is known chemically as 11-dehydro-17 dehydrocorticosterone-21 acetate), and adrenalin (Stumel, 1952). While the sudden increase in production of these hormones is, primarily, to counter the effect of stressing factor on the animal organism, the elevated concentration of hormones especially the cortisone, affect the defence mechanism of body in various ways. Pottinger (1939) demonstrated an interrelationship between the Autonomic nervous system and endocrine system and stated that these two systems furnish the regulating and correlating mechanism which affect the vegetative functions of the body. Adrenalin produces the effect of increase in permeability of the cell membrane and the capillaries, by stimulating the sympathetic nerve endings, which carry excitatory nerve impulses. Cortisone is a parasympathetic substance which acts on the cell and capillaries by stimulation of vagus nerve endings which carry
inhibitory impulses, causing a decrease in permeability. In normal physiological condition semipermeability of the cell membrane and capillaries is maintained by the antagonistic action of adrenalin and cortisone. Disturbance in semipermeability is caused by decrease or increase in the concentration of either cortisone or adrenalin which affects the normal concentration of metabolites and inflammatory cells between the internal and the external environment of cells and capillaries. Excessive concentration of cortisone decrease the permeability and therefore the intensity of inflammatory reaction is reduced.

The effect of cortisone on the pathogenesis of infections processes, has been extensively studied in a number of human and animal diseases, namely Staphylococcus infection (I'LIN, 1961), Enepagococcus infection (Calvallero, 1955), Poliomyelitis (Findlay, 1952), Influenza (Kasa, 1951) etc. etc. In most of the cases the hormone has been found to increase the host susceptibility and enhance the severity of infection due to its anti inflammatory property and interference in antibody production. The beneficial effects of cortisone in overcoming several physiological disorders have proved to be of paramount importance in the vast field of medicine. It has been used for the treatment of rheumatic arthritis, Addison's disease, certain corneal infections, allergic disorders, toxemias, conditions of shock and certain skin diseases.
As the stress is an important subsidiary factor in the etiology of pasteurellosis of animals, experimental study reported in this dissertation was undertaken to elucidate the effect of cortisone on experimentally infected, normal and immune animals.
REVIEW OF LITERATURE

Historical

Pasteurellosis in domestic animals has been drawing attention of many workers since long. The first report on the member of Pasteurella group of organisms was by Rivolta in 1877, who pursued his investigations on Fowl cholera. The same worker later reported that this organism caused diseases characterised by septicaemic lesions also in other species of animals.

Kitt in 1885 used the term Bacterium bipolarum multocidum to denote the organism causing the disease in cattle, sheep, goats, swine and horses. Hirstepe in 1906 studied the relationship between various species of organisms and introduced the term Bacterium Septicaemia Haemorrhagea for the aetiological agent of the fowl cholera, cattle and swine septicaemia. Lignieres on the basis of cultural and biochemical studies as well as sources of animal origin distinguished and classified the members of the genus and recommended the nomenclatures as the Pasteurella bovisenta, Pasteurella bubalisation, Pasteurella ovisenta, and Pasteurella suisenta etc. His nomenclature and classification was based almost entirely on the isolation and history of the various organisms.

Bonagarten (1911) was the first worker who on the basis of his investigations on cross pathogenicity and
cross immunity tests with strains isolated from different species of animals, opposed the distinction between the organisms of the genus *Pasteurella* proposed by Lignieres. This basis of distinction between the species was also opposed by Mohler and Eichhorn (1913), Nutyra (1925), Migge (1933) and Manninger (1934). As the *Pasteurella* strains isolated from different animals were antigenically related and possessed similar cultural and biochemical characters, Rosenbusch and Merchant (1934) suggested the name *Pasteurella multocida* for the organism. The principal diseases of animals caused by *Paste. multocida*, either wholly or in part, are Haemorrhagic septicaemia, pneumonia of sheep and goats, fowl cholera and pneumonia of swine.

**Incidence of Pasteurellosis**

The disease is world wide in distribution. Its incidence is quite high in many South East Asian countries as well as in other lands. In New Zealand heavy annual losses in cattle and sheep industries have been reported by Neilwaine (1950). In Ireland, Lynch (1949) reported a disease in calves which occurred in acute septicaemic form. Pasteurellosis is wide spread in Romania and causes heavy mortality every year (Stanatin, 1959). In Yugoslavia and U.S.S.R. the disease was reported to be sporadic and endemic, and affected various species of domestic animals (Nikajlović, 1958; Nikiporova, 1958).
In Africa the disease has been reported to cause heavy mortality in cattle (Perreau, 1960). In United States yearly losses due to Pasteurellosis amount to about $25,000,000 (King et al., 1953).

In India, Pasteurellosis is prevalent in all regions of the country. Young animals are the most common sufferers and the majority of the naturally affected animals die. The disease is found in low lying or marshy areas and has a tendency to break out with the commencement of Monsoon or during the winter rains. According to the available data in 1960, 11,603 out breaks of the disease occurred in different parts of India; 52,071 animals were affected and 32,661 died.

**Infection in domestic animals**

In cattle and buffaloes:— The disease occurs in acute, subacute and chronic forms (Lebedev, 1937) and causes heavy mortality among the affected animals. In the animals died of acute septicaemic form, the lesions are characterized by petechial haemorrhage on mucosal and serosal surfaces and enlarged lymphatic glands. Sanders (1940) reported enzootic broncho pneumonia of dairy cows which occurred mainly during warm and moist season. The disease was reported to develop due to undernourishment and infection with parasites. Broncho pneumonia in cattle caused by Pasteurella infection has been observed by several other workers (Fritsche, 1931; Stokroef, 1953);
Stevens, 1953 and Weiden Muller 1953). Shigley (1943) reported pleurisy in calves caused by the *Pasteurella* spp. Graham (1953) observed the bilateral fibrinous pneumonia usually with severe bronchitis. The *Pasteurella* has been reported to cause mastitis in the dairy cows (Lesbouyries, 1933). Tuck (1953) reported the isolation of *Past. multocida* from some cases of bovine mastitis. Pascoe (1960) reported an outbreak of the bovine mastitis due to *Past. multocida*, the disease reoccurred in dairy cows and affected large number of animals every year.

**In sheep and goats**— The disease caused by *Pasteurella* in sheep and goats is not comparable with that in the cattle and buffaloes. Montgomery (1933) reported a sporadic and enzootic form of pneumonia in sheep due to *Pasteurella* spp. Pandey (1943) isolated *Past. septica* from the cases of pneumonia in goats at a goat breeding farm. Rajgopal (1944) reported greyish white granulomatous nodular lesions of the size of millet seeds in lungs and liver of goats affected with *Pasteurella*losis. Campbell (1949) reported pneumonia in sheep associated with a *Pasteurella*-like organism, the latter may have superimposed on some other infection. Similar findings were recorded in goats by Jirna (1953), Borgman (1955) and Stamp (1955).

Nadayoshi (1957) reported *Past. multocida* as a
cause of caseous lymphadenitis in sheep. The disease occurred mostly in lambs over six weeks of age, but rarely affected adults. Smith (1960) reported peritonitis associated with pneumonia in young lambs. Smith (1943) reported that Pasteurella organisms may cause mastitis in sheep and goats, he could demonstrate the organism in the udder tissue of affected animals but never in milk secretion. An acute form of mastitis associated with the presence of the Pasteurella organism was reported by Tunnicliff (1943). The disease could be reproduced by inoculating culture via the teat canal. Simmons (1954) described the ovine mastitis caused by Pasteurella mastitidis.

In pigs: The Pasteurella causes contagious pneumonia in pigs. The chronic form of infection, is characterised by swelling of throat (Kraebehl, 1948). A form of infectious pneumonia in pigs, frequently associated with Pasteurella infection was reported by Pullar (1943) in Australia.

In rabbits: Leschenbries (1950) gave a detailed account of various forms of Pasteurella infection in rabbits. The author stated that the disease occurred mainly in acute septicaemic form and mortality varied in different forms of infection. The main lesions were reported in the respiratory tracts particularly in trachea and bronchi. Similar findings were recorded by Alexander (1952). Hogen (1959) reported a chronic form of respiratory infection
domestic rabbits. The lesions were characterised by broncho pneumonia with consolidation and necrosis of lungs; trachea was inflamed and bronchi were filled with the purulent exudate.

**Pathology of Pasteurellosis**

Moore (1925) reported *Pasteurella spp.* to be commensals on the respiratory mucous membrane of normal animals. Jorgenson (1920) confirmed these findings on the basis of his study on 250 cattle and concluded that Pasteurella produced disease only after the natural resistance of the host was lowered down due to "fatigue" or old age. Dhanda (1954) and Bain (1954, 1955) reported that the animals carry Pasteurella organisms in their respiratory tract without causing any disease. There is a speculation that accurate understanding on the epidemiology of this disease. Singh (1943) showed that animals may harbour the infection in healthy condition and that in an epidemic area 7-10% of the animals harboured infection. The strains of the organism obtained from carriers were found highly lethal to rabbits when experimentally.

Dhanda (1954) and Bain (1954) on the basis of their experimental finding reported that in any population 5-10% animals are immune to pasteurellosis. No confirmed evidence has been put forward, interrelating the harboured infection the natural acquired immunity. The carrier animals may
break down into clinical cases when subjected to some
kind of stress such as exposure to inclement weather or
chilliness etc. (Bain, 1957; King, 1958).

There is no confirmed evidence that soil or water
becomes permanently infected with pasteurella or that
there is any transmission through insects. However,
Kubiay (1934) suggested that fleas may have spread
infection amongst calves in Kenya. It is generally
concluded that natural infection is via ingestion or
inhalation. The infected serous and bloody discharges and
seces probably remain infective outside the body long
enough to be a source of danger to contact animals.

Kennedy (1949) reported the infection in calves through the
publicus.

It has been pointed out earlier (page 3) that
clinical Pasteurellosis is precipitated by various kinds
of stress. The latent infection may develop into overt
disease or the disease may manifest in severe form if
stressed stressing factor comes across. The experimentally
induced stressing effects of cortisone have been studied
several diseases and the relevant literature is briefly
viewed below.

cortisone

The cortisone (11-dehydro-17 hydroxycorticosterone
compound E) is a steroid hormone secreted by adrenal
The discovery that the administration of cortisone altered the clinical course of Rheumatic arthritis (Bench & al. 1950) and exerted beneficial effects on certain eye and skin diseases (Uvarov, 1959) has influenced almost every field of medicine. Clinical observations were extended to the vast field of inflammatory disorders and it was found that the hormone altered the course of several diseases. It thus became clear that the basic physiological mechanism involved in several diseases having the different etiological agents, was affected by this hormone. Most of the diseases in which cortisone exerts a clinical effect are entities without known, or completely acceptable etiology. The progress towards an understanding of the mechanism by which this hormone exerts its effects has, therefore, been slow.

The term 'stress' and the cortisone seem related to each other. Any type of noxious stimuli, if sufficiently prolonged or severe, cause hypertrophy of the adrenal cortex, indicating that animal undergoing stress, produces larger amount of cortisone than resting animal (Tapperman et al. 1943) and these facts produced the term 'Biological stressors' for such noxious stimuli (Hollis, 1954). The adrenal cortex hormone mediate certain physiological pattern, that are characteristic of the response to many noxious stimuli, but are not quantitatively linked to them (Ingle et al. 1949; Ingle, 1951).
Effect of cortisone on various infectious diseases.

The fact, that the hormones of adrenal cortex were instrumental in modifying the clinical course of many diseases, has brought to fore numerous findings related with several diseases. Many workers have shown that the course of Mycobacterium tuberculosis infection in laboratory animals could be modified by administration of cortisone (Engbaek et al., 1952; Cummings et al., 1952 and Bloch et al., 1951). In cortisone treated animals the infection was enhanced and a larger number of bacilli were recovered in the different organs of body as compared to those of controls. The beneficial effect of antibiotics was also rendered ineffective by the administration of this hormone (Spain, 1950). Yasano and Katsuko (1951) stated that the adults were more prone to the deleterious effect of cortisone treatment as compared to the juveniles. Michael et al. (1950) found that in the cortisone treated rats the tuberculosis infection was enhanced and the mortality rate increased. Onodera and Akao (1951) found that rabbits sensitized by tubercle bacillus followed by diagnostic dose of tuberculin when treated with cortisone showed pronounced calcification and cavity formation. Abernathy et al. (1952), while dealing with the effect of cortisone on the Brucella induced diseases, found that Brucella abortus which usually
causes mild infection in mice, caused severe disease with high rate of mortality when given cortisone.

Kass *et al.* (1951), Kass and Lundgren (1951) and Alvaless (1953) studied *Diplococcus pneumoniae* infection in mice and rabbits and reported that cortisone treatment enhanced the infection which was characterized by shortened survival, earlier deaths, greater susceptibility even in the partially immunized animals, when compared with controls. Similar findings were recorded by White *et al.* (1951) who further added that there was prolonged bacteremia in cortisone treated rabbits. Autopol *et al.* (1951) and Maistre *et al.* (1952) reported that cortisone induced spontaneous and often fatal infection in mice and rabbits with *Corynebacterium pseudotuberculosis aurium*, which usually non pathogenic for these animals.

While dealing with the course of staphylococcus infection, Kligman *et al.* (1951) and Gerlach *et al.* (1952) found that the skin lesions were aggravated and healing retarded in cortisone given rabbits. Recently I’lin (1962) reported that mice relatively resistant to staphylococcus infection, became susceptible if a preliminary injection of cortisone was given. The author added that cortisone reduced phagocytic activity of macrophages in lungs as well as delayed the leucocytic response. The effects were more pronounced with larger doses of bacteria. The marked increase in susceptibility of mice to staphylococcus
action paved the way for evolving suitable chemotherapeutic agents against this organism (Boyer et al. 1961).

Berlin et al. (1952), Gledhill (1952) and

et al. (1952), studied the effect of cortisone on

ococcuus faecalis and Streptococcus pyogenes

ections and found that cortisone produced spontaneous

aggravated artificially induced infections in mice.

added that the condition of mice pretreated with

hormone was aggravated inspite of penicillin treatment.

et al. (1951) studying the effect of cortisone on

ptococal lymphadenitis and pneumonia in mice and rats,

erved that survival time of mice was reduced and the

tected rats developed severe pneumonia. The pneunonic

ions in cortisone treated animals exhibited an accentuated

ponse of oedema, excessive bacterial population in

cted alveoli and depressed migration of leucocytes.

Schricker et al. (1961) found that Leptospira pomona

possessed low pathogenicity for guinea pigs and

duced only transient pyrexia, produced severe disease

cortisone treated animals and the leptospires remained

the circulation for 2 weeks longer than the untreated

trol guinea pigs.

Autopol et al. (1951) and Payne et al. (1955) found

after a single injection of cortisone the mortality

in mice challenged with attenuated strain of

orella pestis, was much higher than that in
untreated controls. Pasteurella pestis bacteremia was served in most of cortisone treated mice, and infection was wide spread.

The effect of cortisone on viral, protozoal and mycotic infections has also been reported in literature. Studying the effect of hormones of adrenal cortex on the course of disease caused by Influenza virus in mice, rats and embryonated eggs, Kass et al. (1951), Kilbourne et al. (1951) and Kilbourne (1952) observed an increase in mortality rate as well as increase in the virus titre in various organs of infected hosts.

Cortisone has been found to decrease the resistance to psittacotic virus, in mice and hamsters (Findlay et al. 1952; Sabin et al. 1952; Schwartzman, 1950 and Syvertsen et al. 1952). Sabin et al. (1952) demonstrated the increased susceptibility to the virus in the suckling mice.

Schmidt et al. (1951) reported his studies on the effect of cortisone on the course of primary and fully developed infection with Plasmodium cynomolgi in rhesus monkeys. This investigation had shown that repeated administration of hormone during the primary attack produced an intensification of peripheral blood infection during the post crisis phase of disease. Similar administration of cortisone during the chronic phase provoked further rudesences of remarkable severity. The parasitaemia prolonged.
There have been reports of beneficial effect of cortisone on certain infectious diseases. For instance, Rple et al. (1943) observed that cortisone delayed the death in guinea pigs infected with Clostridium welchii and 45% of animals survived the infection, which otherwise proved fatal within 24 hours.

Effect of cortisone on antibody production and immunity.

Solotorovsky et al. (1951) found that treatment with cortisone overcame the beneficial effects of vaccination in mice infected with a highly virulent strain of Mycobacterium tuberculosis. The treatment increased the susceptibility of mice to infection with Mycobacterium tuberculosis of reduced virulence. Similar findings were recorded also by Rollison and Sullivan (1957). Kass et al. (1951) observed that partially immunized mice succumbed to infection.

With the use of quantitative immunological methods it has been shown that large amount of adrenal cortical steroids cause inhibition of antibody response in rabbits Malkiel et al. 1952; Gershon et al. 1951 and Cohn et al. 1951), rats (Eison et al. 1947) and mice and guinea pigs (Hayes et al. 1952). However, Case et al. (1946) and White (1943) found that rate of antibody production to sheep erythrocytes had increased in mice, rats and rabbits by subcutaneous injection of
cortisone. Male et al. (1950) reported similar findings. Hubert (1949) and Mirick (1951) observed that cortisone did not suppress the antibody titre, but seemed to slightly increase the titre in case of human beings.

Havens et al. (1951) and Bjorneboe et al. (1951) found that administration of cortisone resulted in lowering of antipneumococcal antibody in rabbits. This occurred when hormone was administered both at the beginning and after immunization had established. Hanan et al. (1954), Altibogt et al. (1954) and Baguena (1954) recorded similar findings. Scherffarth (1953) observed that injection of cortisone in rabbits caused significant increase of the serum proteins and gamma globulins.

Third (1961) on the basis of his study on Vibrio plicae infection in rabbits presented evidence to support the view that not only the circulating antibody but also the copeptide antibody production was suppressed by cortisone treatment.

Fischel et al. (1951) demonstrated that the cortisone interferes with the synthesis of antibody but a rate of appearance of passively administered antibodies was not altered. Fischell (1966) performed experimental studies in rabbits, to determine whether cortisone would have an effect on the amount of circulating antibody after immunization has been well established. Administration of cortisone resulted in a marked
limination in the level of circulating antibody. The
author employed passive immunization and a system in
which rate of break down of antibody was minimal and the
rate of synthesis was maximal. On the basis of his two
sets of experiments, he concluded that synthesis of the
antibody protein was distinctly inhibited.

Douherty et al. (1945), Banniester (1951), Shrek
1951), Holllgren (1951) and Benson (1954), found that
cortisone decreases the number of eosinophils and
lymphocytes in the circulation. The lymphocytic reduction
as noticed in almost all lymphoid tissues. Bharadwaj
1958), however, found no alteration in the number of
lymphocytes and eosinophils in human beings given
cortisone.

Effect of cortisone on inflammation.

General.

It is generally conceded that cortisone tends to
inhibit inflammation and for this reason it is called
anti inflammatory or antiphlogistic steroid.

Mankin (1935) stated that cortisone could antago-
nise the effects of the leukotaxine in the early stages
of inflammation. Cortisone, which is a parasympathomimetic
substance, acts by stimulating the vagus inhibitory nerve.
In excessive concentration depresses the inflammatory
activity. Cortisone not only depresses the defence of
the body to bacterial and viral infections but also to a
wide variety of other irritants including burns, traumas and chemical irritants. (Osgood et al. 1950; Woods, 1950; and Spain et al. 1952). The stage of vascular reactivity to an irritant is distinctly modified (Ebert, 1950). Capillary and arterolar tone is increased, permeability to plasma proteins is diminished, endothelial swelling is reduced and adherence and clumping of the particulate elements of blood are virtually eliminated. The extent and cellular components of the exudate are greatly reduced. The number of smaller and large mononuclear cells in the exudate are usually reduced (Rebuck et al. 1951).

Dougherty et al. (1950) reported on the role of cortisone in regulating the inflammation and demonstrated that cortisone inhibited the allergic inflammation through an antiphlogistic action. Allison, et al. (1959) found that the cortisone suppressed the reaction of acute inflammation in its earliest recognizable phase in rabbits. Evidence was presented that anti-inflammatory effect can not be explained on the basis of its vasoconstrictive properties alone. Experimental observations supported the hypothesis that cortisone exerts a direct protective action upon endothelial cells and leucocytes and that in so doing, render them refractory to the tissue products which initiate inflammation. Selye (1953) using granuloma pouch technique as an assay method, showed
that cortisone inhibits exudate formation during a "critical period" which is at its maximum at about the third day after exposure to a topical stressor. Similar findings were recorded by Moon (1952), Ashton (1952) and Barelly (1952).

Later on Menkin (1953) observed that cortisone given intravenously accumulated at the focus of acute inflammation and suppressed the inflammatory activity. This presumably, accomplished by inhibiting capillary permeability increasing and leukocytic migration inducing actions of leukotaxine.

Capillary permeability.

Menkin (1941) concluded that cortisone inhibited the increase in permeability of capillaries when given with leukotaxine or given separately and later on extended his findings with other corticosteroids and corticotrophins (Menkin, 1943). Grahn (1943) showed that adrenal cortical extracts prevented the increase in capillary permeability as measured by the trypan blue test. Similar findings were observed by Kocha (1943). Smirnov (1955) found decreased permeability of capillaries by a single intra muscular injection of cortisone in rabbits. Hayaishi (1955) observed that cortisone treatment inhibited strikingly the accumulation of trypan blue from circulating blood into the skin area injected with leukotaxine, and such inhibiting effect
of cortisone on the capillary permeability was seen more markedly in animals treated with larger doses of cortisone.

Teilum et al. (1950) observed marked regression of the massive accumulation of plasma cells in spleen of cortisone treated rabbits. Thomas (1951) observed that cortisone inhibited the capacity of reticulo-endothelial system to fix or to remove bacterial toxin from tissues.

Effect of cortisone on phagocytosis.

Kass et al. (1951) demonstrated that the phagocytic function of large mononuclear cells was not inhibited but the capacity of such cells to dispose of the infected material seemed to be interfered with. Schmidt (1952) reported that the reduction in supply of macrophages, rather than inhibition of phagocytic activity per se was responsible for the intensification of the disease in cortisone treated animals. Grabbe (1955) observed decrease in intensity of inflammatory response to intra peritoneal injection of irritating substances. The number of macrophages as well as their phagocytic power was diminished in the presence of the pathogenic germs. The same author further observed that small doses of cortisone given daily at the rate of 0.2 mg/kg of body weight enhanced the phagocytosis of
Staphylococcus by the macrophages.

Effect of Cortisone on Repair.

The cortisone not only depresses the inflammation by affecting its early phases but also suppresses the fibroblastic activity and the formation of new blood vessels in an inflamed area.

Ragan et al. (1950), Braughan (1951) and Duke (1951) observed that reparative process, including the new capillary formation and fibrogenesis may be inhibited due to the effect of adrenal cortical hormone. Zager (1952) observed that cortisone caused delay in connective tissue repair, possibly by decreased capillary growth. Bivind (1953) demonstrated that high doses of cortisone produced delayed epithelial regeneration in experimental gastric defects in rats. The hypothesis put forward to explain the observed effect of cortisone is bi-fold; stimulating effect on gastric secretion and an impeding effect on the formation of granulation tissue, or both.

Certain investigators however, could not find any evidence of delay in the early stages of inflammation in cortisone treated animals. For instance, Lattes et al. (1953) observed that delay in appearance of elements of the inflammatory and reparative process in the skin or rats during the administration of cortisone was not apparent, histologically, in the initial phase of this process.
The available literature reviewed amply supports the view that the hormones of adrenal cortex, particularly the cortisone, modifies the course of several diseases and that its effects are manifold. In the present investigation, the study of pathogenesis of *pasteurella* infection was undertaken in an attempt to evaluate the effects of cortisone on immunity and susceptibility.
MATERIALS AND METHODS

Experimental animals

Healthy rabbits weighing 1 Kgm to 1.25 Kgm were used for experimental infection. The approximate age of the rabbits was one year. They were obtained from Amritsar and kept in scrupulously cleaned cages, each having two animals. They were housed in a well ventilated room of 10' X 10' X 5' dimensions. During winter the room was kept warm by placing a burning charcoal heater in the centre of the room during the evening and nights. The animals were given balanced ration consisting of 5 ounces of vegetable greens and 5 ounces of concentrated mixture containing 50% wheat bran, 32% crushed maize, 17% linseed cake and 1% shark liver oil.

During the period of observation clinical chart for each animal was maintained to keep a complete record of the systemic and local reactions manifested by them. Their morning and evening temperature was recorded and their faecal samples were examined for the presence of coccidia. Those showing febrile reaction were either eliminated or kept under observation until they recorded normal temperature for three consecutive days. Those who showed presence of coccidia in their faeces in numbers, were treated by inoculating
subcutaneously 0.6 ml. of 33% solution of sulphame-thione on the first day followed by 0.2 ml. on second and third days.

Cortisone

The cortisone acetate (Corlin) of Glaxo Laboratories (India) Private Ltd. was used as a stressing agent for experimental work. Each ml. of the corlin injection contained 25 mgm. of the cortisone acetate preserved in 1% of benzyl alcohol. Cortisone acetate was kept in cool and dark place.

Pasteurella culture and vaccine

Pasteurella Septica (Paste. multocida) strain 52 was used in the experiments for the study of the pathogenesis of pasteurella infection in variously treated and healthy groups of rabbits. The Paste. Septica 52 is a standard virulent strain which was isolated from a natural case and has since been maintained at the Indian Veterinary Research Institute, Nakteswar-Kumaon. The strains was kept in phase I by regular passage in healthy hill bulls. For standardisation of the dose of culture to be used in this experiment ten fold dilutions of the culture were made with sterile serum saline containing 5% rabbit serum and 0.35% sodium chloride. One ml. of each of $10^{-3}$, $10^{-4}$, $10^{-5}$ and $10^{-6}$ dilutions were inoculated intraperitoneally using one to two animals.
for each dilution. All the animals inoculated with $10^{-3}$, $10^{-4}$, and $10^{-5}$ dilutions died within 13 hours while only one of the two inoculated with $10^{-6}$ died.

One the basis of this observation it was decided to use 1 ml. of $10^{-5}$ dilution as a certain lethal dose. For infection 13 hours nutrient broth culture of the organisms in phase I was used.

Agar washed formalinised culture vaccine using **E. coli** 52 was employed for artificial immunization. For preparation of vaccine 18 hours nutrient broth culture was inoculated into the roux flasks containing yeast agar medium (prepared according to Dhanda et al. 1956), which had been incubated for 24 hours before inoculation to test the purity of medium. After 24 hours of incubation the growth was harvested with the sterile normal saline and the opacity of the bacterial suspension was adjusted to concentration equivalent to standard brown opacity tube No. 3. The material was then taken into a sterilized flask. To the culture suspension in saline, formalin (40% Formaldehyde) was added to attain a final concentration of 0.2% of formalin. After formalinising, the suspension was incubated at 37°C for 24 hours to kill the bacterial cells and tested for purity and sterility. The vaccine so formed was finally stored in the refrigerator at 4°C.
Experimental infection

The experiment was conducted in five parts in order to study the different aspects of the stressing effect of cortisone on the resistance to artificial infection. To study the effect of cortisone on the development of specific resistance after artificial immunization, a group of 12 rabbits was immunized by inoculating 1 ml. of vaccine to each of the rabbit. Animals of this group were given cortisone at the rate of 5 mg/kgm of body weight intramuscularly daily for 14 days. During the 14 days of cortisone treatment the body temperature of the animals was recorded morning and evening. During this period two rabbits died of coccidiosis. On the 14th day 9 animals were challenged by inoculating to each animal 1 ml. of $10^{-5}$ 18 hours broth culture diluted in 5% serum saline and were sacrificed in groups of 1 to 2 animals at 2, 5, 10 and 18 hours post infection (Table No. 1), for the study of macroscopic and microscopic changes. Two of the infected animals that were kept as control, and were not sacrificed died within 24 hours. The remaining one animal which served as cortisone treated control, was sacrificed for collection of the tissues.

Another group of 3 animals was used for determining the effect of two doses of cortisone acetate on
<table>
<thead>
<tr>
<th>Nature of the experiment</th>
<th>Number of animals sacrificed</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time in hours</td>
<td></td>
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<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>10</td>
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<tr>
<td>1. Immunized animals infected 14 days after cortisone treatment.</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2. Immunized animals infected after giving two doses of cortisone.</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3. Normal animals infected after giving two doses of cortisone.</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4. Immunized animals infected 14 days after immunization.</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>5. Normal infected animals.</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6</td>
<td>8</td>
<td>6</td>
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</tbody>
</table>
the course of disease in immunized rabbits. All the rabbits were immunized by giving the vaccine subcutaneously 1 cc each. The serum samples were collected regularly for determining the pastyurella agglutin in titre. The samples were kept in Refrigerator after inactivating them at 56°C for 30 minutes. During the 14 days 3 rabbits died of coccidiosis. On 14th day remaining 5 rabbits were challenged with the pastyurella culture in 10⁻⁵ serum saline as in the previous experiment. Four animals were sacrificed at various intervals (Table No. I). The remaining one which was kept as control did not die upto 48 hours, was sacrificed after that for study of pathological changes.

A 3rd group of 3 rabbits was used for determining the effect of cortisone on the course of the disease in normal healthy rabbits, of these 7 animals were given 2 doses of cortisone acetate at the rate of 10 mg/kg of body weight prior to the challenge. The first dose was given 24 hours before and other was administered simultaneously with the challenge at a separate site. One animal was kept as control and did not receive any preliminary cortisone treatment. The animals were challenged with 1 ml. of pastyurella culture in 10⁻⁵ dilution in serum saline intraperitoneally. These animals were sacrificed at various intervals (Table No. I).
For the study of the progress of infection in immunized animals, another group of seven rabbits was used. These animals were immunized by inoculating 1 ml. of vaccine to each of them. The animals of this group were not given preliminary cortisone treatment. During the course of 14 days after inoculation of vaccine two animals died of coccidiosis. On the 14th day remaining five animals were challenged with the 13 hours broth culture of Pasteurella septica. Four animals were sacrificed at various intervals as indicated in Table No. I, while the remaining one rabbit was left as control. This animal did not die of infection up to 48 hours but was later sacrificed for the study of pathological changes, if any.

Another group of 8 rabbits was taken for determining the pathogenesis of pasteurella infection in normal healthy rabbits. Seven animals were challenged with the pasteurella culture in 10^-5 dilution and the sacrifices were performed at the various intervals (Table No. I). One of the infected animal which was left as control died naturally within 13 hours.

The necropsies were performed immediately after sacrificing the rabbits and the heart blood culture was attempted on blood agar slants, nutrient broth and plain agar slant, and blood smears were made for
the study of differential leucocytic counts. After recording changes the organs to be collected were removed and transferred into 10% formal saline. The pieces of the following organs were collected for the study of pathological changes: Liver, Heart muscle, Spleen, Lungs, Trachea, Kidney and Adrenals.

Histopathological technique

The fixed tissues were washed in running tap water for 8-16 hours to remove the fixative. The washed tissues were then passed through the ascending grades of alcohol and finally dehydrated tissues were cleared in cedar wood oil. The cleared tissues were paraffinised and the blocks were made of the paraffinised tissues. The tissue sections were cut at 4-5 micron thickness and were stained by the Haematoxylin-eosin method, according to Lillie (1954). The Lillie layers Haematoxylin and 1% alcoholic eosin was used.
SECTION II
RESULTS

Presence of Pasteurella organisms in the blood circulation in infected animals

The results of cultural examination of heart blood for the presence of pasteurellae in groups of artificially infected animals subjected to different treatment and sacrificed at various intervals post infection are detailed in table No. II.

It would be seen that of the three groups of immunized animals those which were not given any preliminary cortisone treatment or those given only two doses of cortisone just before infection did not develop bacteraemia. In the third group of animals, which were given soon after immunization, cortisone for 14 days, developed bacteraemia by 10th hour which lasted till the animals died or were sacrificed.

Among the two normal groups of animals, in the group of animals given two doses of cortisone, the only remarkable difference was that those animals developed bacteraemia sooner than those which were not given cortisone. A comparison of findings in cortisone treated immunized and normal animals with the similar groups which were not given cortisone, shows that administration of cortisone decreased the ability of the animals to combat the progress of infection leading to bacteraemia and death.
**TABLE NO. IX**

Showing the presence of *pasteurella* organisms in blood circulation of artificially infected rabbits killed at different time intervals post infection

<table>
<thead>
<tr>
<th>Time intervals of sacrifice post infection</th>
<th>Results of heart blood culture after experimental infection in</th>
<th>Immunized animals infected 14 days after cortisone treatment</th>
<th>Immunized animals infected after giving two doses of cortisone</th>
<th>Normal animals infected after giving two doses of cortisone</th>
<th>Immunized animals infected 14 days after immunization</th>
<th>Normal infected animals</th>
</tr>
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<tbody>
<tr>
<td>2 hours</td>
<td>-- --</td>
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<td>5 hours</td>
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<td>10 hours</td>
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<td>++, +++</td>
<td>++, ++</td>
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<tr>
<td>18 hours</td>
<td>++, +++</td>
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<td>++, +++</td>
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<td>Control</td>
<td>++, +++</td>
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<td>++</td>
<td>++</td>
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<td>++</td>
</tr>
</tbody>
</table>

-- Negative.
++ Positive with small number of organisms.
+++ Positive with moderate number of organisms.
++++ Positive with large number of organisms.
Effect of cortisone on relative proportion of blood leucocytes

In the immunized animals treated with cortisone daily for 14 days, on the first day the neutrophils showed a sudden increase in relative proportion and were 80% of the leucocytes. After the first day the rate of increase in percentage was however, slow. On the 14th day neutrophils constituted 90% of the blood leucocytes. The proportion of lymphocytes fell from 45% on 0 day to 25% on first day and 6-8% on 14th day. The monocytes did not show any alteration from their normal proportion. The eosinophils appeared to have disappeared from the circulation after the first day.

In the rabbits treated with two doses of cortisone (one dose given 24 hours before and the other simultaneously with challenge at a different site), the relative proportion of neutrophils in blood rose from 44% to 60% at 12 hours, 70% at 24 hours and 80% at 42 hours. When the animals were sacrificed the lymphocytes decreased from their normal value of 45% at 0 hour to 35% at 12 hours and 24% at 24 hours, by 42 hours they were of 10% of the leucocytes. The monocytes, however, did not show any variation from their normal value. The eosinophils responded in a way similar to lymphocytes and by 12 hours they appeared to have completely vanished. The results of Differential Leucocytic Count are graphically represented in figures 1 and 2a.
Fig. 1a

Showing Changes in the Relative Proportion of Leucocytes in the Blood of Infected Rabbits Given Two Doses of Cortisone.

↑ - Time of Injection
↑ - Time of Inoculation of Pasteurella Culture

Key:
- - - - Neutrophils
- - - - Lymphocytes
- - - - Monocytes
- - - - Eosinophils

Percentage of the Leucocytes

Time in Hours After Cortisone Treatment.
Fig. 2a

Showing changes in relative proportion of leukocytes in immunized rabbits given cortisone daily for 14 days.

↑-Days of commencement of cortisone injection

Key:
- Neutrophils
- Lymphocytes
- Monocytes
- Eosinophils

Percentage of leukocytes

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Pasteurella agglutinin titre in immunized and immunized-cortisone-treated rabbits

The results of agglutination test indicating the agglutinin titre in immunized and immunized-cortisone-treated rabbits up to 14th day of immunization, are graphically presented in figure 3a. It may be noted that in immunized animals the average agglutinin titre attained its maximum 1:320 on the 7th day. There was a decline in titre to 1:160 on the 10th day followed by a rise to 1:320 on the 14th day.

In immunized cortisone-treated animals the rise agglutinin titre was less marked and the maximum titre did not exceed 1:80 up to the 10th day. By the 14th day the titre declined to 1:40.
Fig. 3a

Showing Average Titre of Pasteurella Agglutinins in Immunized and Immunized-Cortison-Treated Rabbits

↑ Day of Commencement of Cortisone Injection

Key —— Titre of Immunized Animals
———— Titre of Immunized-Cortisone-Treated Animals
PATHOLOGICAL CHANGES

Macroscopic changes

Macroscopic changes in animals that were sacrificed at various intervals of post infection or had died of infection naturally were generally similar with regard to nature and distribution, but varied in intensity at different stages.

At the later stages (10 hrs. and 19 hrs.) the diffuse congestion of the peritoneum and the intestines was the most striking change. In some of the animals sero sanguinous fluid had accumulated in the thoracic cavity.

Liver

In the earlier stages (2 hrs. and 5 hrs.) liver did not show any appreciable macroscopic change. In the later stages the organ appeared dark chocolate in colour and haemorrhagic foci were usually noticed. In the cortisone treated animals liver showed greyish discolouration and the characteristic striations were noticed on the surface of the organ. In three out of ten animals that had been given cortisone for 14 days, necrotic foci were noticed on the surface of the organ.

Heart muscle

The heart did not appear to show any appreciable macroscopic change in all the animals sacrificed at
different intervals. However, the heart of one of the animals from susceptible group, was coated with the white purulent exudate and the pericardium was adherent to the diaphragm.

**Spleen**

In the animals of 14 days cortisone treated group, the spleen appeared to be comparatively smaller than normal and greyish in colour. In one of the animal circumscribed, small necrotic foci were observed on the surface of the organ. In three out of ten animals of immunized group the spleen was much enlarged. In animals sacrificed at the later stages, in almost all instances, the organ appeared congested.

**Lungs**

In animals sacrificed at earlier stages apart from slight congestion there was no remarkable change in the lungs. In some of the animals sacrificed at later stages (10 hrs. and 13 hrs.), the lungs were emphysematous. In the susceptible animals, lungs appeared congested only at the later stages.

**Trachea**

In the later stages of infection mucous membrane lining of trachea was acutely congested and often haemorrhagic. The haemorrhage had occurred on the external as well as internal surface of the trachea.
Kidney

In the earlier stages kidney did not appear to show any appreciable change. Only in the later stages the cortical region was diffusely congested.

Adrenal

The adrenals were slightly congested in some of the animals of the 14 days cortisone treated group. In the most of the other cases the adrenal did not show any marked change.

Microscopic changes

Liver

Group I. Immunized animals infected 14 days after cortisone treatment

2 hours: In the periportal areas the connective tissue was infiltrated with mononuclear cells. The bile ducts did not show any appreciable pathological change. The arteries and veins appeared congested. One of the animals at this stage showed desquamation and disintegration of the cells lining the bile ducts and the lumen of bile ducts contained cysts of parasites. The hepatic cells showed early stages of degenerative changes. Some cells appeared unaffected while the changes in others varied from cloudy swelling characterized by an increase in
cytoplasmic granularity to vacuolation of the cell cytoplasm. At places small groups of necrotising cells showing nuclear changes such as pyknosis, karyorrhexis and karyolysis, were observed. The areas of necrosis were surrounded by mononuclear cells. The central veins and the sinusoids did not show any alteration from normal picture.

5 hours: In portal areas the larger bile ducts presented cysts of parasites and the high columnar epithelial cells showed degeneration and desquamation. The arteries and veins as well as capillaries and venules were slightly congested. Hepatic cells revealed degenerative changes characterized by swelling and increased granularity of the cytoplasm. In some of the hepatic cells the fatty changes were represented by the presence of variable number of cytoplasmic vacuoles. Owing to the swelling of cells, the sinusoids were relatively compressed and contained lesser quantity of blood. The central veins did not show any appreciable change.

10 hours: In the portal areas the arteries and veins were congested and appeared to contain increased number of neutrophils. In the hepatic cells degenerative changes varied from cloudy swelling to the presence of vacuoles concentrically arranged around the nucleus. The blood sinusoids were slightly congested.
12 hours: The connective tissue in portal areas was infiltrated with mononuclear cells. The arteries and veins were congested. In one of the animals at this stage, parasitic cysts were noticed in the bile ducts, which in addition showed infiltration with mononuclear cells. The connective tissue in the perportal areas of the other animal had disintegrated and split apart and showed infiltration with the mononuclear cells and neutrophils. The hepatic cells had undergone degenerative changes characterized by increase in granularity of the cytoplasm and presence of cytoplasmic vacuoles. Areas of focal necrosis were also noticed at places (Fig. 3). The blood sinusoids and the central veins were congested.

Infected control: In portal areas the connective tissue was infiltrated with mononuclear cells. The bile ducts showed degeneration and desquamation of the epithelial cells. The arteries and veins were congested. The cells in the hepatic cords had lost their normal arrangement and showed degenerative changes which varied from cloudy swelling to the vacuolation of the cytoplasm. The cellular changes were more pronounced in the middle and central zones of the lobules. Areas of focal necrosis were observed at a few places. The sinusoids and central veins were congested.
Cortisone Control: The portal areas did not show appreciable pathological changes. The cells in the hepatic cords had undergone degeneration and showed large vacuoles which had displaced the nuclei from central positions to margins. In many cells the cytoplasm was almost completely devoid of stainable material due to complete dissolution of its cytoplasmic contents (Fig. 1 and 2). Some cells had undergone necrosis as evidenced by pyknosis, karyorrhexis and karyolysis. The changes observed appeared to be essentially similar to those seen in pathological condition with heavy glycogen infiltration in liver.

Group II. Immunized animals infected after giving two doses of cortisone

2, 5, 10 and 15 hours: In the portal areas the arteries and veins were congested. In the hepatic lobules, the cells showed varying stages of degenerative changes such as cloudy swelling and vacuolation of the cytoplasm. In the later stages blood sinuses were congested (Fig. 4). At 15 hours, the portal areas did not show any appreciable pathological change and congestion of blood vessels and sinuses was also less marked than the previous stages.
Group III. Normal animals infected with two doses of cortisone

2 and 5 hours: In portal areas the arteries and veins were congested. The cells in the lobule had undergone early stage of degenerative changes characterized by swelling and granularity of cytoplasm. The swollen cells, wholly or partially compressed the blood sinusoids. The blood sinusoids were slightly congested (Fig. 5). At 5 hours in portal areas the connective tissue and the bile ducts were infiltrated with mononuclear cells.

10 hours: In portal areas the arteries and veins were congested and contained a number of large clear vacuoles. The hepatic cells had undergone degenerative changes varying from cloudy swelling and hydropic degeneration to the vacuolation of the cytoplasm. The sinusoids and central veins were congested.

18 hours: The connective tissue in portal areas showed infiltration of mononuclear cells. The arteries and veins were congested. The cells in the peripheral zones of the hepatic lobule showed early stage of degenerative changes while changes in the cells of middle and central zones were characterized by vacuolation of cytoplasm. The sinusoids and central veins were highly congested.
Group IV. Immune animals infected 14 days after immunization

2, 5 and 10 hours: In portal areas the connective tissue and the bile ducts were infiltrated with the mononuclear cells. The arteries and veins were congested. The cells in hepatic cords, sinusoids and the central veins did not show any appreciable pathological change. At 10 hours in addition to the changes observed in earlier stages, the hepatic cells showed mild degenerative changes.

18 hours: In the portal areas the connective tissue and the bile ducts were infiltrated with the mononuclear cells. The epithelial cells of the bile ducts showed degeneration and were found desquamated at places. The arteries and veins were congested. The cells in the hepatic cords showed early stages of degenerative changes characterized by swelling of the cells and granular cytoplasm. The blood sinusoids and central veins were congested.

Group V. Normal infected animals

2 and 5 hours: In the portal areas no appreciable pathological changes were noticeable. The cells in the hepatic cords did not show any marked change except at a few places where the cells had undergone early stages of degeneration.
10 hours: In the portal areas the arteries and veins were congested. The hepatic cells showed early stages of degenerative changes characterized by granular cytoplasm and at a few places vacuoles were seen in the cell cytoplasm. The blood sinusoids were slightly congested.

16 hours: In portal areas, connective tissue fibres had separated and disintegrated. The arteries and veins were congested. The cells in the hepatic cords showed cloudy swelling. In one of the animals at this stage, the cells had undergone severe degenerative changes and at places areas of focal necrosis were observed. Blood sinusoids and central veins were congested.

HEART MUSCLE

Group I. Immunized animals infected after 14 days of cortisone treatment

2 and 5 hours: The epicardium did not show any appreciable pathological change. In one animal the blood vessels in the deeper layer of epicardium were congested. In the myocardium the muscle fibres were split apart due to haemorrhagic infiltration and showed degenerative changes, many of these at places had revealed vacuoles in sarcoplasm. The endocardium did not reveal any appreciable change.
10 hours: The epicardium had separated off from the myocardium due to the accumulation of oedematous fluid and had at places disintegrated. The blood capillaries in myocardium and endocardium were congested. The muscle fibres were in the process of degeneration and disintegration (Fig. 6). Vacuoles of various size appeared in the fibres around the nuclei while at places the fibres had undergone complete disintegration. In the blood vessel emboli appeared which were characterized by round and clear spaces.

18 hours: The blood vessels in the deeper layers of epicardium were congested and contained emboli characterized by clear spaces (Fig. 7). The myocardium showed degeneration and disintegration of muscle fibres, characterized by granularity of sarcoplasm and presence of cytoplasmic vacuoles and at places dissolution of nuclear and cytoplasmic structures accompanied with leucocytic infiltration. The blood capillaries were highly congested and areas of haemorrhage were noticeable. The endocardium did not show any appreciable change.

Cortisone control: In the myocardium the muscle fibres had, at places, separated from each other and showed degenerative changes varying from granular cytoplasm in some to the presence of cytoplasmic vacuoles in others. Some of the fibres were in the process of disintegration and dissolution.
Group II. Immunized animals infected after giving two doses of cortisone

2 hours: Smaller blood vessels in epicardium and myocardium were congested. The muscle fibres in myocardium did not show any appreciable change. The endocardium did not show deviation from normal appearance.

5 hours: The capillaries in myocardium were congested and there were focal haemorrhagic areas (Fig. 3). The muscle fibres were swollen and had lost their characteristic striated appearance. Some of the fibres showed nuclear changes such as pyknosis, karyorrhexis and lysis.

10 and 18 hours: The blood vessels in the epicardium were slightly congested. The muscle fibres showed degenerative changes and at places disintegration. The blood capillaries were slightly congested.

Group III. Normal animals infected with two doses of cortisone

2 and 5 hours: The deeper layers of myocardial fibres showed degenerative changes characterized by swelling and granular cytoplasm. The capillaries in the myocardium were congested.

10 hours: The capillaries in myocardium were congested and areas of haemorrhage were noticed which resulted in separation and disintegration of the muscle fibres. The
muscle fibres were swollen and lost their striated appearance. At places the cytoplasm of the fibres had rarified.

13 hours: The blood vessels in epicardium were highly congested and at places the fibres of connective tissue layer were split apart due to the presence of extravasated blood cells. The myocardium showed focal haemorrhages and extensive degenerative changes. The fibres had separated due to the presence of infiltrated blood elements and showed disintegration. The degenerative changes varied from granular cytoplasm to vacuolar degeneration. The capillaries in myocardium were congested. Capillaries in endocardium were congested and areas of haemorrhages were noticeable (Fig. 9).

Group IV. Immunized animals infected 14 days after immunization

2 hours: The blood vessels in the deeper layers of epicardium were congested. Areas of focal haemorrhages were noticeable in the myocardium, and around these areas the muscle fibres were degenerated and disintegrated. The fibres lost their characteristic striated appearance and were swollen and presented granular cytoplasm.

5 and 10 hours: The epicardium had at places separated apart from the myocardium and the cavity formed contained haemorrhagic exudate. The blood vessels in epicardium
were congested. In myocardium changes were similar to those seen in the previous stage.

18 hours: In the myocardium changes such as congestion, haemorrhagic infiltration of muscle fibres which showed degeneration and at places necrotic changes characterized by nuclear changes such as pyknosis, karyorrhexis and karyolysis and cytoplasmic disintegration, were more marked than the previous stages.

Group V: Normal infected animals

2 hours: The blood vessels in the epicardium were slightly congested and at a few places haemorrhage occurred. The myocardium and endocardium did not reveal any appreciable change.

5 hours: The blood vessels in epicardium and myocardium were congested. The muscle fibres did not show any appreciable change.

10 hours: The myocardium showed degenerative changes in the fibres. The regular arrangement of the fibres was disturbed and the striations were lost as the fibres were swollen and at places disintegration had occurred due to focal haemorrhagic areas.

18 hours: The blood vessels in epicardium were highly congested. The myocardial fibres showed degenerative changes which varied from increased granularity to the
presence of vacuoles in the cytoplasm. Most of the fibres lost their characteristic appearance and many of these were in the process of disintegration. Areas of focal haemorrhage were noticed in myocardium. One of the animals at this stage responded in a peculiar way. The epicardium showed well marked inflammatory reaction. The collagen fibres were heavily infiltrated with the polymorphonuclear cells and the inflammatory exudate contained also the dead and degenerated cells. The deeper layers contained large number of mononuclear cells and at places clear distinction was noticeable between the two layers of inflammatory cells. Myocardium had also undergone degenerative changes. The comparatively healthy area of myocardium was encapsulated by proliferating connective tissue. The young capillary buds were prominent in the field.

**SPLENIC**

**Group I. Immune animals infected 14 days after cortisone treatment**

2.5, 10 and 18 hours: The capsule did not show any appreciable pathological change. In the white pulp, the paucity of the mature lymphocytes was a prominent feature. The lymphatic nodules were poorly distributed in the substance of the organ. The germinal centres showed marked proliferation of the reticular cells and the
lymphoblasts (Fig. 10). Some of the lymphoblasts showed necrotic changes. The red pulp showed scarcity of the lymphocytes (Fig. 11). At some places only the skeleton of pulp, made up of reticular fibres, was noticed while at other places small necrosed areas involving both the sinuses and the "cords of billroth" were observed. The sinuses were slightly congested and contained large number of macrophages. The littoral cells lining the sinuses were swollen.

Cortisone control: In the lymphatic nodules the germinal centres showed marked hyperplastic reaction as evidenced by the presence of large number of reticular cells and lymphoblasts. The primary nodules were however not well developed. In red pulp large number of macrophages and neutrophils were observed.

Group II. Immunized animals infected after giving two doses of cortisone

2, 5, 10 and 18 hours: The lymphatic nodules showed marked hyperplastic reaction. In the germinal centres the proliferative changes involved large number of reticular cells, medium sized lymphocytes and large lymphocytes. At a few places mitotic figures were observed in the proliferating reticular cells. The corona consisted of large number of lymphocytes. The follicular arteries were ensheathed with large number of lymphocytes. The
sinuses in red pulp were congested and contained large number of neutrophils. At 13 hours the pathological changes observed in white pulp were essentially similar to those seen in the animals sacrificed at the previous stages.

**Group III. Normal animals infected after giving two doses of cortisone**

2, 5, 10 and 18 hours: Structural changes shown by animals of this group were generally similar to those seen in previous group. At 5 hours necrotic foci were observed in red pulp involving columns of Billroth and cells lining sinuses. At 10 hours lymphocytes and lymphoblasts in some of the lymphatic nodules showed necrotic changes. At 18 hours red pulp appeared congested and combined large number of polymorphs and macrophages.

**Group IV. Immunized animals infected 14 days after immunization**

2 and 5 hours: The lymphatic nodules were hyperplastic (Fig. 12). The germinal centers appeared active and contained large number of medium sized, large lymphocytes and reticular cells. The corona of the lymphatic nodules contained large number of smaller lymphocytes. The capillaries in the trabaculae were congested, and the sinuses in red pulp were congested.

10 and 13 hours: At one place the capsule was separated off from the lymphoid tissue due to infiltration of serous exudate.
The lymphatic nodules presented structural changes similar to those seen in the previous stages. The red pulp appeared shrunken and contained very little quantity of blood in sinuses.

**Group V. Normal infected animals**

2, 5, 10 and 18 hours: The lymphatic nodules did not show any appreciable deviation from normal structure. In the later stages slight to moderate congestion was observed in the red pulp and trabecular capillaries together with large number of macrophages.

**LUNG**

**Group I. Immunized animals infected 14 days after cortisone treatment**

2 and 5 hours: The bronchioles at a few places showed desquamation of the epithelial cells. Peribronchial lymphoid follicles were scarce and showed regressive changes (Fig. 13). Some of the alveoli showed the presence of serous exudate (Fig. 14) and contained neutrophils and few erythrocytes. The alveolar lobules at some places appeared ephymematous.

10 and 18 hours: The damaged bronchioles contained the exudate composed mainly of desquamated epithelial cells and neutrophils. At places alveoli contained exudate consisting large number of neutrophils. The
capillaries in the interalveolar septa were highly congested and contained large number of neutrophils and few macrophages. The changes in the peribronchial lymph follicles were similar to those seen in the previous stage.

**Cortisone control**: The bronchial epithelial cells exhibited degenerative changes and had desquamated. At places the alveoli were engorged with the serous exudate and red blood cells.

**Group II. Immunized animals infected after giving two doses of cortisone**

*2 and 5 hours*: The bronchioles did not show any appreciable change except that the peribronchial lymph follicles were hyperplastic. The blood vessels were highly congested. The alveoli at places, contained the serous exudate, neutrophils and red blood cells. The capillaries in the interalveolar septa were highly congested.

*10 and 13 hours*: The epithelial cells lining the bronchioles showed desquamation and the lumen of the bronchioles was engorged with hemorrhagic exudate. The blood vessels and the interalveolar capillaries were congested which however, at 13 hours did not show any appreciable change from the normal structure.
Group III. Normal animals infected after giving two doses of cortisone

2 and 5 hours: The bronchioles and the alveoli did not show any marked change. At 5 hours the epithelial cells lining some of the bronchioles had desquamated. The blood vessels and the interalveolar capillaries were congested. Some of the alveoli showed the presence of red blood corpuscles.

10 and 13 hours: The changes in bronchioles were similar to those seen at 5 hours. At some places the bronchioles were filled with haemorrhagic exudate (Fig. 15). Some of the alveoli appeared emphysematous. The interalveolar capillaries were engorged with the blood and contained large number of neutrophils. Some of the alveoli were engorged with the red blood cells.

Group IV. Immunized animals infected 14 days after immunization

2.5, 10 and 13 hours: The epithelial lining of the bronchioles did not show any appreciable pathological changes. The peribronchial lymph follicles showed hyperplastic reaction (Fig. 16). Some of the bronchioles contained serous exudate in the lumen. The interalveolar capillaries were highly congested and at places extravasated erythrocytes were noticed in the alveoli. At 10 and 13 hours the bronchioles showed desquamation and disintegration of the lining
epithelial cells. Some of the bronchioles contained the fibrinous exudate, alveolar changes and the capillary congestion were not prominent as in the previous stages.

**Group V. Normal infected animals**

**2.5 and 10 hours**: The bronchioles showed desquamation of epithelial cells and the presence of haemorrhagic exudate in their lumina. The blood vessels and alveoli did not show any marked change. At 10 hours, the blood vessels and interalveolar capillaries were congested.

**18 hours**: The changes in bronchioles were similar to those observed in the previous stages. The alveoli at places contained the serous or haemorrhagic exudate. The interalveolar capillaries were highly congested and the septas were swollen due to congestion and increased cellularity of the endothelial cells of capillaries.

**TRACHEA**

**Group I. Immunized animals infected 14 days after cortisone treatment**

**2, 5, 10 and 18 hours**: The epithelial layer lining the trachea did not show any appreciable change from normal. The blood vessels in the submucous coat appeared to be congested. In one animal sacrificed at 10 hours red blood cells together with a number of leucocytes
were seen on the luminal surface of epithelial cells.

**Cortisone control:** The trachea of the cortisone treated control rabbit showed slight congestion of blood vessels in submucosa and desquamation of epithelial cells at places.

**Group II. Immunized animals infected with two doses of cortisone**

2,5,10 and 18 hours: At the earlier stages the goblet cells showed hyperplasia with the degenerative changes and desquamation of the cells at places (Fig. 17). The blood vessels were congested. Serous exudate was noticeable under the lamina propria. At 18 hours the wall of trachea was thickened due to the infiltration of serous exudate in between the cartilage and the mucous membrane. Lamina propria showed leucocytic infiltration at places.

**Group III. Normal animals infected after giving two doses of cortisone**

2,5,10 and 18 hours: The epithelial lining did not show any deviation from normal structure. The blood vessels were slightly congested and haemorrhagic exudate was seen in the lumen of trachea. At 10 hours haemorrhagic and serous exudate was seen in the connective tissue as well as in the lumen of the trachea. At 18 hours the cells lining the epithelium showed degenerative changes and had desquamated and disintegrated
at a few places. In the submucosal connective tissue mononuclear infiltration was noticed (Fig. 18). Other changes were similar to those seen at 10 hours stage.

Group IV. Immunized animals infected 14 days after immunization

2.5.10 and 18 hours: The epithelial lining and the blood vessels did not reveal any appreciable change, haemorrhage had occurred on the luminal surface of the epithelial cells. At 10 hours cells lining the epithelium were disintegrated and desquamated. At 18 hours the changes appeared to be slightly more pronounced than that of the previous stages (Fig. 19). Haemorrhagic patches on the epithelial surface were also noticed.

Group V. Normal infected animals

2.5.10 and 18 hours: At 2 hours there was no appreciable change from normal structure. At 5 and 10 hours the epithelial layer showed desquamation of its cells and was covered with haemorrhagic exudate. In the submucosa the blood vessels were congested. At 18 hours, in addition to the changes described above, the epithelial cells showed degeneration, desquamation and disintegration. Haemorrhage was noticed in the connective tissue.
KIDNEY

Group I. Immunized animals infected 14 days after cortisone treatment

2.5 and 10 hours: The capsule did not show any appreciable pathological change. The glomerular tufts were swollen and showed increased cellularity. In a few animals, small eosinophilic masses could be seen in the intercapillary spaces of the tufts. At places the epithelial cells of the peritubular layer of Bowman's capsule were swollen. At a few places extravasated erythrocytes were noticed within the Bowman's capsule. Erythrocytes could also be seen in the interstitial tissue of kidney. The interlobular blood vessels were slightly congested. The proximal and distal convoluted tubules showed degenerative changes characterized by granular cytoplasm (Fig. 20). At places their nuclei also had undergone pyknotic and karyorrhectic changes. In the medulla epithelial cells lining the collecting tubules appeared swollen and presented a wide clear space around the nuclei (Fig. 21). Some of the collecting tubules contained hyaline casts in their lumen.

18 hours: The capsule at few places had disrupted due to subcapsular haemorrhages. At some places albuminous mass was noticed in the intercapillary spaces of glomerular tufts (Fig. 22). The intertubular blood
vessels and capillaries in medulla were highly congested. Other changes were generally similar to those seen in the previous stages.

Cortisone control: Some of the renal corpuscles showed the presence of small eosinophilic hyaline masses in between the glomerular tufts, and bowman's capsule. The cells of proximal and distal convoluted tubules were swollen and presented granular cytoplasm. Homogenous casts were noticeable in a few of the tubules in cortex and medulla. In medulla the capillaries were congested.

Group II. Immunized animals infected after giving two doses of cortisone

2,5,10 and 18 hours: In the early stages the glomerular tufts were swollen and highly congested. The hypertrophy and hyperplasia in the endothelial cells of glomerular tufts were noticeable. At some places the extravasated cells were present in the subcapsular spaces. The cells of tubules in cortex and medulla showed cloudy swelling. At 10 and 18 hours, in addition to changes described above, the interlobular blood vessels in cortex and capillaries in medulla appeared slightly congested (Fig. 23).

Group III. Normal animals infected after giving two doses of cortisone

2 and 5 hours: The glomeruli did not show any appreciable pathological change. The capsule in one of
the animals at this stage showed extensive haemorrhagic infiltration. The connective tissue fibres had separated due to accumulation of erythrocytes.

In the cortex the interlobular arteries were congested and focal areas of haemorrhages were observed in the interstitial net work (Fig. 24). The proximal and distal convoluted tubules showed early stages of degenerative changes. At places dissolution of cell cytoplasm had occurred together with the nuclear changes such as pyknosis and karyorrhexis. The capillaries in medulla were congested.

10 and 18 hours: The glomeruli were swollen and congested, and showed increase in cellularity. At places the extravasated erythrocytes were observed in the subcapsular space of Bowman's capsule. The blood vessels in cortex and medulla were congested. The cells of tubules in the cortex and medulla showed changes varying from cloudy swelling to necrotic changes.

Group IV. Immunized animals infected 14 days after immunization

2.5 and 10 hours: The glomeruli did not show any marked change except increase in cellularity at a few places. The cells of proximal and distal convoluted tubules showed mild degenerative changes characterized by swelling of cells and granular cytoplasm. Some of the cells of collecting tubules showed hydropic degeneration.
18 hours: The glomeruli were congested and swollen. 
The interlobular blood vessels in the cortex and 
capillaries in medulla were congested. Areas of focal 
hemorrhage were also observed. Other changes were 
similar to those seen in the previous stages.

**Group V. Normal infected animals.**

2, 5, 10 and 18 hours: The glomeruli were swollen and 
congested. The interlobular blood vessels were congested.

At 10 and 18 hours the glomeruli were markedly congested, 
swollen and showed increase in cellularity of the 
endothelial cells. The capillaries in medulla were 
congested. Serous exudate was noticeable in the inter-
tubular spaces. The tubules in the cortex and medulla 
showed cloudy swelling.

**ADRENAL**

**Group I. Immunized animals infected 14 days after** 
cortisone **treatment**

2, 5 and 10 hours: The zona glomerulosa did not show any 
appreciable pathological change. The parenchymal cells 
of zona fasciculata, particularly in the deeper layers 
did not show the presence of characteristic vacuoles 
which represent lipid droplets, but were compact in 
arrangement and their cytoplasm tended to be more 
escinophilic in character than normal cells. The sinusoids
in the cortex were congested and the endothelial cells lining the sinusoids were swollen. The cells in the medulla showed degenerative changes which were characterized by granularity, and at places dissolution of cytoplasm. At 10 hours the cells of zona glomerulosa were also involved and showed granular cytoplasm (Fig. 25). A few of the cells showed nuclear changes characterized by pyknosis and karyorrhexis.

18 hours: The cells in zona reticularis were swollen and showed granular cytoplasm and cell boundaries of some of the cells appeared disorganized. The cortical sinuses were highly congested (Fig. 26).

Group II. Immunized animals infected after giving two doses of cortisone

2.5 and 10 hours: The parenchymal cells in zona fasciculata showed degenerative changes characterized by granular and vacuolar cytoplasm. Some of the cells had undergone hydropic degeneration characterized by wide clear space around the nuclei of swollen cells (Fig. 27). The blood sinuses in the cortex were congested. At 10 hours degenerative changes in zona glomerulosa were more pronounced than the previous stages, and at some places complete dissolution of the cytoplasm was noticed.

18 hours: The intensity of pathological changes appeared to be milder than that seen in early stages. The cells in
Zona fasciculata and zona reticularis also presented granular cytoplasm (Fig. 23).

Group III. Susceptible animals infected after giving two doses of cortisone

2, 5, 10 and 13 hours: The cells in zona fasciculata were devoid of characteristic vacuoles representing lipid droplets. Owing to the absence of lipid substance the cytoplasm of these cells appeared more eosinophilic than that of normal cells. At places the cells in zona fasciculata and reticulata showed cytoplasmic changes such as granular cytoplasm. The sinususes were congested.

Group IV. Immunized animals infected 44 days after immunization

2, 5, 10 and 13 hours: The cells in cortex and medulla did not show any appreciable pathological change. The blood sinususes were highly congested and contained large number of neutrophils and mononuclear cells. At 10 and 8 hours, the cells of zona fasciculata and zona reticularis showed mild degenerative changes characterized by granular cytoplasm.

Group V. Normal infected animals

2, 5, 10 and 13 hours: The cortex and medulla did not present any appreciable change. At 10 hours, the sinususes are found congested. At 13 hours the cells in zona fasciculata and zona reticularis showed early stages of degenerative changes characterized by swelling and amularity of cytoplasm.
DISCUSSION

Information regarding the nature of modifying effect of cortisone on the course of different infectious diseases is limited. The present investigation to study the effect of cortisone administration or rabbits experimentally infected with Pasteurella multocida, was undertaken because stress which brings about increase in production of corticosteroid hormones is one of the important factor in aetiology of animal pasteurellosis and also probably influences the course of disease in naturally affected animals.

After artificial infection variation in the duration of time interval shown by different groups of animals for the development of bacteraemia was an interesting finding. Those susceptible animals which were given two doses of cortisone about the time of artificial infection developed bacteraemia by 2 hours. The susceptible animals developed bacteraemia by 5 hours post infection and bacteraemia persisted till death.

The early development of bacteraemia in cortisone treated susceptible animals reflects the inhibition of humoral and cellular mechanism of inflammation and that of phagocytosis (Crabbe 1955). The failure of immunized animals to develop bacteraemia may be due to several factors. According to Opit (1921, 1929) at the site of inoculation
allergic inflammation is produced which results in blockage of lymphatics and thus prevents the organism to gain access into circulation. Further the antibody molecules are fixed to antigens on bacterial surface and the bacterial cells are held in situ.

In the immunized animals treated with 2 doses of cortisone no significant difference was observed with regard to the development of bacteraemia presumably on the 14th day when titre of antibodies in immunized animals was at its maximum two doses of cortisone did not seem to affect adequately the concentration of antibodies and the protection conferred. Fischell (1951) explained similar finding on the basis that cortisone affects the antibody production but not the catabolism of antibody.

The immunized infected 14 days after cortisone treatment developed bacteraemia in 10 hours. These animals had shown a poor immunogenic response as compared to those animals which were not given cortisone. Inability of these animals to localise the infection and to prevent the development of bacteraemia was probably due to inadequate concentration of antibodies at the site of inoculation, inhibitory effect of cortisone on phagocytosis and poor mobilization of polymorphonuclear leucocytes. The decrease in activity of polymorphonuclear leucocytes seems to be the major cause in production of bacteraemia in cortisone treated animals. The oxygen intake which is an initiative
for the phagocytosis and the digestion of the bacterial organisms by the neutrophils is inhibited by the action of cortisone. The pseudopodial activity together with the ability of phagocytes to adhere to capillary wall and diapedesis and migration into the area of allergic inflammation is interfered with the action of cortisone (Gall, 1954; Michael, 1951). These observations are similar to those of Glasser (1951) on streptococcal infections and Germuth (1952) on pneumococcal infections. These findings also tend to support the contention of Payne (1955) that the mechanism of natural defence against pasteurella infection is primarily cellular and depends on the activity of reticuloendothelial system in conjunction with the mobilization of polymorphs.

There was significant rise in percentage of neutrophils in the blood by 24 hours of cortisone infection but after that the rate of rise was slow. The observed increase in proportion of neutrophils may be due to increase in survival time of neutrophils or the apparent increase in number may be due to relative decrease in the number of lymphocytes, caused by cortisone administration. The proportion of monocytes did not alter in the cortisone treated animals.

The lymphocytes recorded a marked decrease in percentage between 1 and 12 hours of cortisone treatment. From 12 hours to 24 hours the rate of decrease was not as prominent as from 1 to 12 hours. From 24 hours to 42 hours
there was again marked fall and at the terminal stage
dvalues as low as 10% were recorded. Decrease in number
of lymphocytes as a result of cortisone treatment has been
reported by numerous workers (Bannister 1951; Shrek 1951;
Denson 1954). Ramiharat (1945) found that lymphopenia
occurred within 30 minutes of cortisone injection. It
has been stated that lymphopenia is brought about by the
dissolution of lymphocytes in blood. This is a phenomenon
of rapid energy starvation taking place in lymphocytes
during a period when it has an obligatory high metabolic
rate. Barron (1952) and Blecher (1958) explained the
lysis due to the inhibiting effect of cortisone on the
utilization of glucose and protein synthesis, thus
utilizing its own cellular protein. In the later stages
lymphopenia may be due to the decrease in the rate of
delivery of normal number of lymphocytes in circulation.

No eosinophils were found in the peripheral blood
at 12 hours and subsequent stages. The findings are
contrary to those of Bharadwaj (1953) who could not
observe any noticeable depressing effect of cortisone
on lymphocytes in human beings.

It is believed that cortisone inhibits the
formation of antibodies. In the present investigation,
immunogenic responses were studied in immunized and
immunized-cortisone-treated animals. In immunized animal the
agglutinin titre increased gradually and recorded highest values on 7 and 14 days after immunization. In the cortisone treated immunized group the titre was comparatively much lower to that of immunized group. Similar findings have been recorded by number of other workers such as Björneboe (1951), Hanan (1954) and Fagraeus (1961).

**Macrosopic and Microscopic changes**

On post mortem examination of infected animals the congestion of the abdominal viscera and the peritoneum was a constant finding. However Smith (1959) did not record this gross change in young lambs infected with pasteurella culture. It is probable that the diffuse congestion of abdominal viscera observed in this study was due to the fact that pasteurella culture was inoculated intra peritoneally while Smith (1959) inoculated the culture intravenously.

In cortisone treated animals the liver was soft, fragile and with fine necrotic spots. The enlargement of the liver as reported by Payne (1955) was however not noticed. Lungs did not show any appreciable macroscopic changes. Smith (1959) reported fibrinous pneumonia in pasteurella infected lambs. Spleen in susceptible animals was congested in later stages while in immunized animals the organ was appreciably enlarged. Increase in number of plasma cells may partially account for the enlargement
of the organ. Similarly the smaller size of spleen in cortisone-treated-immunized rabbits may be due to atrophy of spleen pulp and malpighian bodies. The necrotic foci in spleen as observed by Payne (1955) were not recorded in the present experiments.

The trachea was inflamed and haemorrhagic. This finding was similar to that recorded by Lesboujries (1950), Alexander (1952) and Hagen (1959).

The nature and extent of microscopic changes observed in different groups of animals varied considerably. Liver showed marked changes in almost all animals in different groups. The changes were early in onset and were more pronounced in the susceptible group treated with 2 doses of cortisone. In the susceptible group of animals given two doses of cortisone, the changes were more or less similar to those seen in the immunized-cortisone-treated animals. In the immunized animals the changes were quite marked in the early stages post infection and by 8 hours the structural changes were mild and inconspicuous.

No appreciable difference in pathological reaction could be observed between immunized and immunized animals given two doses of cortisone. The changes in liver were most prominent in immunized animals treated with cortisone for 14 days. The changes were confined to hepatic cells which appeared to be heavily infiltrated with glycogen and in some animals showed vacuolar cytoplasm due to the
conservation of glycogen. Long (1940), Fraley (1959) and several others have reported glycogen conserving effect of cortisone resulting in accumulation of abnormal quantities in the liver cells. The glycogen appeared preponderance of empty spaces with in the cytoplasm, indicated the position occupied by glycogen before commencement of processing the tissues. Hanes (1950) concluded the presence of glycogen in the liver cells on the similar basis. It has been stated that decrease in permeability of liver cells caused by cortisone, results in glycogen storage in liver cells (Silvette, 1932).

Cortisone also inhibits the oxidation of glucose and thus a state of tissue hunger is maintained which may suggest a possible cause for the production of fatty changes in liver and degenerative changes in certain other parenchymatous organs of the body. Glycogenic infiltration and fatty changes recorded by Hartroft (1951) and Moran (1961), have been confirmed in the present study but formation of fatty cysts in liver as reported by Moran (1961), were not observed. It may be mentioned that Moran (1961) used high doses of cortisone (12.5 mg/kg) and fatty cysts were recorded on 21st day. However, it can be stated that marked fatty changes were noticeable only in advanced stages of cortisone treatment. The congestive changes in cortisone treated immunized animals were in no way similar to those of immunized animals but were comparable to those
of immunized animals but were comparable to those of susceptible animals. Focal necrotic areas were found in some of the cortisone treated animals and earlier, similar findings were recorded by Payne (1955).

The changes in bile ducts and the connective tissue of portal areas were relatively more prominent in cortisone treated group. The bile ducts in some animals were extensively damaged and studded with the parasitic cysts. Extensive tissue damage caused by activation of subclinical coccidial infection even in animals pretreated with sulphaemazine, may be due to general decrease in resistance caused by cortisone treatment. The susceptible group treated with sulphaemazine did not develop any coccidial infection.

In the myocardium the changes were more pronounced in the cortisone treated susceptible animals than the other groups of animals. In the immunized animals given no cortisone and those given two doses of cortisone no appreciable difference was noticeable. However, congestion of blood vessels was more pronounced in immunized animals treated with two doses of cortisone. In the immunized animals treated with cortisone for 14 days the degenerative changes in muscle fibres were more prominent than any other immunized group. However the haemorrhage in myocardium and endocardium was not a prominent feature in
cortisone treated animals as in the susceptible animals, probably due to the depressing effects of cortisone on the diapedesis of erythrocytes.

In the spleen of susceptible animals, no appreciable change was noticed except the congestion of red pulp in the later stages. The spleen of susceptible animals given two doses of cortisone, did not show any remarkable feature. In immunized animals the changes in spleen were hypertrophy of malpighian corpuscles and hyperplasia in the germinal centres which were surrounded by packed zones of lymphocytes. These finding are similar to those of Schmidt (1951) and Payne (1954). Bjorneboe (1950), in addition recorded an increase in number of mononuclears in the red pulp. In the sinuses there was a large number of phagocytes with engulfed particles. In the immunized cortisone treated animals the spleen was smaller in size and there was marked atrophy of the lymphatic nodules. In one animal areas of necrosis in red pulp were observed. The germinal centres were active. Schmidt (1951) recorded similar finding in the primate malaria infection. Bjorneboe (1950) did not record the active germinal centre in cortisone treated immunized animals, which was noticed in the present study. It seems that hyperplastic changes in immunized animals are not effected by cortisone but only the small lymphocytes are affected in lymphatic nodule as well as in red pulp. Areas of necrosis were not
prominent at any stage except in one animal, and in this respect findings differ from those of Payne (1955) who could demonstrate severe necrosis in spleen.

In susceptible animals the changes in lungs were not prominent. This organ showed congestion only in the later stages. There was thickening of the interalveolar septa due to engorgement of neutrophils in the capillaries. Pneumonic changes were not recorded and it was only at few places that neutrophils and erythrocytes were noticed in the alveoli. Smith (1959) recorded fibrinous pleurisy and acute pneumonic changes in lambs. Hagen (1959) reported broncho pneumonia in pasteurella infected rabbits. Alexander (1952) and Lesboujries (1950) demonstrated the haemorrhagic infiltration only in bronchi. The bronchioles in the later stages were filled with haemorrhagic exudate and the bronchial epithelial lining was desquamated. In some way the findings resemble those recorded by Alexander (1952). In the cortisone treated animals lungs did not exhibit any significant change. The emboli characterized by round clear spaces were recorded only in few animals. Moran (1961) described lacy appearance of lungs in cortisone treated animals and found emboli which were not surrounded by inflammatory cells.

The trachea in the susceptible animals showed marked congestion, haemorrhage and at later stages desquamation of
lining epithelial cells. Alexander (1952) recorded similar findings. In immunized animals there was marked congestion. The immunized cortisone treated animals showed mucoid degeneration and hypertrophy of goblet cells.

The kidney of susceptible animals showed congestion of glomeruli together with mild degenerative changes in the tubules. In immunized animals severe congestion with haemorrhagic areas was noticeable feature. In the cortisone treated animals degenerative changes were quite prominent in the tubules of cortex and medulla. Moran (1961) recorded the development of lipaemia and the severe fatty changes in kidney together with fatty cysts. Absence of such marked changes in the present study is probably due to the smaller doses of cortisone used in experiments. Moran (1961) correlated the fatty changes of kidney with the severe fatty changes and fatty cysts of liver. Aluminoius casts were recorded by Moran (1961) and were also observed in the present study. Sigaund (1954) reported damage to the glomeruli and development of nodular lesions in kidney. He also reported fat emboli in glomeruli due to the lipaemia.

In susceptible animals the adrenal showed mild degenerative changes and congestion of sinuses only at later stages. In immunized animals, in early stages there was an acute congestion of sinuses and degenerative changes involved particularly the zona glomerulosa and zona fasciculata. In
the cortisone treated susceptible or immunized animals
degenerative changes in zona glomerulosa and zona fasciculata were more pronounced. The changes in zona reticularis
were not marked. However, Moran (1961) did not record any
change in zona fasciculata but described severe fatty
changes with the formation of fatty cyts in the zona
reticularis.

Dougherty (1947) recorded depletion of lipids in
the cells of zona fasciculata due to cortisone administration.
This work explained and confirmed his findings by showing
lack of adrenal cholestrol during the cortisone treatment.
These findings indicate that depletion of lipids results
in compact appearance of cells of zona fasciculata due to
the high concentration of mitochondria, ribonucleic acid
and alkaline phosphatase (CLARK Vol. 29, 1961). It has
been further stated that conversion of clear cells into
compact cells is a characteristic feature of zona fasciculata under the stress.
SUMMARY

The present study was undertaken to evaluate effect of cortisone on the pathogenesis and course of experimental infection with Pasteurella multocida in immunized and susceptible rabbits. For determining the effect of cortisone on the course of pasteurellosis in immunized animals, two groups of animals were used. To the first group of 10 immunized rabbits 5 mg/kg of cortisone was administered intra muscularly daily, from the day of immunization upto 14 days. To the second group of 5 immunized animals cortisone was given only in two doses; one given 24 hours before and the other simultaneously with experimental infection. The animals of both the groups were infected on the 14th day with one ml. of $10^{-5}$ dilution of 18 hours broth culture of Paste. multocida by intra peritoneal route. Five rabbits which served as the control group were infected with pasteurella culture 14 days after the immunization, without any preliminary cortisone treatment.

To study the effects of cortisone in susceptible animals a group of 8 rabbits was treated with 2 dose of cortisone; one 24 hours before and the other simultaneously with challenge. The other group of 8 animals was infected with pasteurella culture without any preliminary cortisone treatment. Necropsies were performed and the heart blood cultures were attempted in nutrient broth and blood agar media.
Blood smears were made for differential leucocytic counts and blood was also collected for serological study. The following tissues were collected for the study of pathological changes: Liver, Heart muscle, Spleen, Lungs, Trachea, Kidney and Adrenals.

After the intra peritoneal inoculation bacteraemia had developed by 5 hours in the susceptible and 2 hours in the susceptible animals treated with two doses of cortisone. In the immunized animals, bacteraemia could not be detected at any of the stages up to 18 hours. In the immunized animals treated with two doses of cortisone also, bacteraemia did not develop but in the immunized animals treated with cortisone for 14 days, bacteraemia developed at 10 hours and persisted till the death.

Differential leucocytic counts in the cortisone treated animals revealed a sudden rise in the proportion of neutrophils in the blood which was marked at 12 hours, and the relative proportion of neutrophils rose from 44% to 80% at 42 hours. The lymphocytes showed a decline in the proportion and at 42 hours, they decreased from their normal value of 45% at 0 hour to 10% at 42 hours. The eosinophils could not be detected in circulation after 12 hours. The monocytes did not show any variation in percentage.

The agglutinin titre in the immunized animals recorded its maximum 1:320 on the 7th day. In the immunized-
cortisone-treated animals the titre did not exceed
1:80 and by 14th day the titre equalled to 1:40.

Macroscopic changes observed in infected rabbits
were not pronounced. Slight congestion of peritoneum
and abdominal viscera was noticed in early stages. The
liver presented haemorrhagic foci in the later stages
of infection in susceptible group of animals, while the
organ was soft, fragile and greyish in colour in cortisone
treated rabbits. Lungs did not show any macroscopic change
except slight congestion in the later stages. The trachea
was inflamed and haemorrhagic in the later stages of
infection. The spleen in the cortisone treated immunized
animals appeared comparatively smaller. In the immunized
animals the spleen was slightly enlarged and congested.
The heart muscle, kidney and adrenals did not appear to
show any remarkable macroscopic change except that slight
congestion was noticed in the kidney and adrenal in the
later stages of infection.

On microscopic examination the liver in the suscep-
tible animals revealed congestion of the blood vessels
in portal areas and cells showed early stages of degener-
ative changes. The changes were slightly more pronounced
in susceptible animals treated with two dose of cortisone
and at the early stages of infection in the immunized
animals and immunized animals treated with two doses of
cortisone. In the immunized-cortisone-treated animals
the hepatic cells appeared to contain glycogen. Fatty changes were noticeable in a few animals. Bile ducts were damaged and infiltrated with mononuclear cells. The heart muscle showed congestion of blood vessels in epicardium, myocardium and endocardium in the susceptible animals and susceptible animals given two doses of cortisone. The changes were quite severe in immunized animals in early stages. In cortisone treated immunized animals the muscle fibres showed degenerative changes.

The spleen of susceptible animals showed congestion and presence of large number of macrophages in the sinuses. The intensity of these changes was more pronounced in cortisone treated susceptible rabbits. In immunized animals the malpighian corpuscles exhibited hyperplastic reaction in the germinal centres with large number of reticular cells and lymphoblasts surrounded by thick zone of lymphocytes. In the early stages of infection in immunized animals, the trabecular blood vessels and sinuses were congested. In immunized-cortisone-treated animals the germinal centres in lymphatic nodules were active and contained large number of reticular cells and lymphoblasts, but the purity of the lymphocytes in the corona was a noticeable feature. In one of the animals medulla showed excessive depletion of the cells, with a few necrosed areas.
The lungs did not show any remarkable pathological change. The alveoli, only at few places, were partially filled with fibrinous exudate. The bronchioles showed desquamation of the lining epithelial cells and in a few animals haemorrhagic exudate was noticed in the bronchioles. The trachea in the later stages showed congestion of blood vessels and haemorrhagic exudate, in the connective tissue and on luminal surface of the epithelial cells. The lining epithelial cells had desquamated at the later stages.

The kidney showed congested glomeruli with increased cellularity of endothelial cells in the later stages. The blood vessels in cortex and medulla were congested and the tubular cells showed mild degenerative changes. In the immunized animals the congestion of blood vessels was quite marked. In immunized-cortisone-treated group the degenerative changes in tubules were more pronounced than any other group.

The adrenal showed mild degenerative changes in the cells of zona glomerulosa and zona fasciculata in the susceptible group of animals. In immunized animals the sinuses were acutely congested and the lining endothelial cells appeared swollen. In cortisone treated immunized animals the cells of zona fasciculata appeared compactly arranged.
Findings of the present investigation tend to show that administration of cortisone lowers the resistance of rabbits to pasteurella infection by interfering the development of humoral defence and probably also by inhibiting the cellular defence mechanism.
SECTION III
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LEGENDS TO PHOTOMICROGRAPHS
**Fig. 1**  Liver.
Showing swelling and vacuolar degeneration of hepatic cells due to glycogen infiltration.
Nuclear changes are also marked.
Cortisone control. Group I animals.
H.E. (x 140)

**Fig. 2**  Higher magnification of Fig. 1
(x 560)
Fig. 3  Liver.
Showing foci of coagulative necrosis associated with general disorganisation.
18 hours post infection.
Group I animals.
H.E. (x 140)

Fig. 4  Liver.
Showing acute congestion of sinusoids and blood vessels in portal area.
2 hours post infection.
Group II animals.
H.E. (x 140)
Fig. 5  Liver.

Showing slight congestion of
sinusoids.

2 hours post infection.

Group III animals.

H&E (x 140)
Fig. 6
Heart.
Showing congestion of endomysial capillaries, granular degeneration in myofibrils, hypertrophy of sarcosomal nuclei and presence of hyaline thrombus in blood vessel in epimysium.
10 hours post infection.
Group I animals.
H.E. (x 560)

Fig. 7
Heart.
Showing acute congestion of blood vessels in sub epicardial connective tissue.
18 hours post infection.
Group I animals.
H.E. (x 140)
Fig. 8  Heart.
Showing congestion of blood vessels in endomysium, haemorrhage in epimysium and granular degeneration of myofibrils.
5 hours post infection.
Group II animals.
H.E. (x 320)

Fig. 9  Heart.
Showing congestion of blood vessels and thrombus in the endocardium.
18 hours post infection.
Group III animals.
H.E. (x 140)
Fig. 10  Spleen.
Showing hyperplastic reaction of lymphoblasts around the central arteriole.
5 hours post infection.
Group I animals.
H.E. ( x 320 )

Fig. 11  Spleen.
Showing depletion of reticular cells and necrotic areas.
2 hours post infection.
Group I animals.
H.E. ( x 140 )
**Fig. 12**

Spleen.
Showing follicular hyperplasia and reactive germinal centre in white pulp.
5 hours post infection.
Group IV animals.
H.E. (x 110)

**Fig. 13**

Lung.
Showing primary bronchioles with atrophy of peribronchial lymphatic follicles.
18 hours post infection.
Group I animals.
H.E. (x 30)
**Fig. 14**

Lung.

Showing serous exudate in alveoli and slight thickening of interalveolar septa.

2 hours post infection.

Group I animals.

H&E (x 30)

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**Fig. 15**

Lung.

Showing haemorrhage in the lumina of bronchioles.

18 hours post infection.

Group III animals.

H&E (x 30)
**Fig. 16**
Lungs.
Showing hyperplasia of the peribronchial lymphatic follicles.
2 hours post infection.
Group IV animals.
H.E. (x 30)

**Fig. 17**
Trachea.
Showing succoid degeneration of the epithelial cells.
2 hours post infection.
Group II animals.
H.E. (x 440)
Fig. 18
Trachea.
Showing mononuclear infiltration in submucosal connective tissue, congestion of blood vessels and swelling of lining endothelial cells. 13 hours post infection.
Group III animals.
H.E. (x 320)

Fig. 19
Trachea.
Showing desquamation of lining epithelial cells and intense congestion of blood vessels in submucosa. 18 hours post infection.
Group IV animals.
H.E. (x 80)
**Fig. 20**

Kidney.

Showing granular degeneration in lining epithelial cells of convoluted tubules.

5 hours post infection.

Group I animals.

H.E. (x 440)

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**Fig. 21**

Kidney.

Showing hydropic degeneration in cells lining the collecting tubules and hyaline casts in the lumina of tubules.

2 hours post infection.

Group I animals.

H.E. (x 560)
**Fig. 22**

Kidney.

Showing small eosinophilic masses in the intercapillary spaces of glomerular tufts.

18 hours post infection.

Group I animals.

H.E. (x 550)

**Fig. 23**

Kidney.

Showing congestion of capillaries and general disorganisation in medulla.

18 hours post infection.

Group II animals.

H.E. (x 40)
Fig. 24  Kidney.

Showing reduction of capsular space due to engorgement of glomerular capillaries, congestion and haemorrhage in interlobular blood vessels.

5 hours post infection.

Group III animals.

H.E. (× 440)
Fig. 25  Adrenal.
Showing general disorganisation of zona glomerulosa and retrogressive changes in cytoplasm and the nuclei of component cells.
10 hours post infection.
Group I animals.
H.E. (x 560)

Fig. 26  Adrenal.
Showing marked nuclear changes in the cells of zona fasciculata and slight congestion of the sinuses.
18 hours post infection.
Group I animals.
H.E. (x 140)
Fig. 27  
Adrenal.  
Showing hydropic degeneration in the 
cells of zona fasciculata.  
5 hours post infection.  
Group II animals.  
H.E. (x 560)

Fig. 28  
Adrenal.  
Showing granular degeneration in 
the cells of zona fasciculata.  
16 hours post infection.  
Group II animals.  
H.E. (x 360)