EXPERIMENTAL STUDIES ON THE PATHOLOGY OF FOWL POX IN CHICKENS

Thesis

SUBMITTED TO THE RAJENDRA AGRICULTURAL UNIVERSITY BIHAR IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (VET.) IN THE FACULTY OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

1971

By

Hemendra Nath Varma

B. V. Sc. & A. H.

Junior Research Fellow (I. C. A. R.)

Post-Graduate Department of Pathology and Bacteriology

BIHAR VETERINARY COLLEGE, PATNA

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This is to certify that the thesis entitled "EXPERIMENTAL STUDIES ON THE PATHOLOGY OF FOWL POX IN CHICKENS" submitted in partial fulfilment for the award of his degree of Master of Science (Vet.) in Pathology to the Rajendra Agricultural University, Bihar, Patna by Dr. Hemendra Nath Varma incorporates the results of his independent study which he has carried out under my guidance.

T. S. Sharma.
25-3-72

March, 1972. (T.S. Sharma)

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C H A P T E R - I

INTRODUCTION.

I N T R O D U C T I O N

Poultry diseases have proved to be a serious problem for the poultry industry all over the world since it has been taken on a commercial scale. There are several diseases which affect fowls. Fowl pox is the viral disease which brings considerable loss to the poultry industry. Inspite of high morbidity, this disease has a low mortality. However, in 1936 Vittoz, reported that as high as 35 percent of death in poultry was due to fowl pox. Although recovery takes place, the recovered birds are uneconomical.

Fowl pox, which has several synonyms, is an acute, febrile, infectious disease. The disease is characterised by cutaneous eruptions, wart-like nodules on the unfeathered parts of the body and diphtheritic lesions or barn-like deposits in the mouth. Occasionally, watery or muco-purulent discharge from eyes and nose is observed.

Goodpasture (1928) and Beaudette (1949, 1953) have written excellent reviews of this disease as reported by Biester and Schwarte (1965). Bollinger bodies, which are observed as intracytoplasmic inclusions, are specific for the fowl pox disease.

The disease occurs naturally in fowls, turkeys, pheasants, geese, ducks, guinea fowls and other birds.

Transmission of the virus generally takes place by

'droplet infection', by bites of intermediary carriers like Culex pipiens and Aedes aegypti groups of mosquitoes or by mechanical inoculation.

The etiological aspect of the virus has been well covered by several workers. It has been also well established that vaccination or recovery results in immunity.

Detailed work has been carried out by several workers to explain the pathology of the disease, but the haematological aspect of the pathology of affected birds has been meagrely worked out. This study was therefore undertaken to examine the haematological pictures of the birds which were experimentally inoculated with the virus and efforts were made to substantiate the existing knowledge on the problem.

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C H A P T E R - II.

REVIEW OF LITERATURE.

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(1) HISTORY :

Rivolta (1869) was the first to describe the presence of inclusion bodies in the cutaneous lesions of fowl pox. Later on, Bollinger in 1873 described similar inclusions in fowl pox (F.P.) cases and through histological studies differentiated the lesions of fowl pox from variola and erroneously classified fowl pox among the tumours as epithelioma contagiosum.

Marx and Sticker (1902) were first to demonstrate that fowl pox is caused by a filterable virus. Carnwarth (1908) and others showed that the virus was responsible for both the cutaneous and diphtheritic forms of the disease. Gallagher (1917) described an outbreak of pox in quail which was transmissible to chickens. Ward and Gallagher (1920) stated that pox occurs naturally among geese, ducks, guinea fowl, pheasants and various wild birds.

Te Hennepe (1926) reported that of 268 ducks examined by him during 1924, 17 were affected with mouth lesions and 14 with cutaneous lesions of pox.

Brandly and Dunlap (1938) inoculated chickens with a strain of pox virus obtained from a natural case of the disease in turkeys and observed only a mild cutaneous reaction. No infection was produced at the fifth passage of the virus in chickens.

Beaudette and Hudson (1941) reported that a strain of turkey pox virus produced more severe, local and systemic reactions and a higher percentage of secondary head lesions in chickens than the fowl pox virus.

Rooyen (1954) described four different strains of virus causing pox among birds : - fowl pox virus, pigeon pox virus, canary pox virus and turkey pox virus. The basis of his classification was host susceptibility. Each virus is infective for its homologous host and in some instances for heterologous hosts.

(2) SYMPTOMS :

Buddingh (1938) demonstrated that if fowl pox virus suspension was inoculated intracranially it results into meningoencephalitis within 4 - 5 days, followed by spastic paralysis and convulsions on the 5th/7th day, and death on 7th/8th day.

Barger and Card (1949) recorded that the symptoms produced by the fowl pox depend on the form of the disease occurring. In severe cases listlessness, inappetance, loss of weight were more marked. Interference with breathing, accompanied by rattling noises were caused by the presence of exudates or, patches near the larynx. Affected birds were off-feed due to severe mouth lesions.

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Govind Rao (1965) found an initial rise of body

temperature by 1-2°F. and the birds were dull and had an irregular appetite. The egg production was stopped completely in the affected layer hens.

As reported by Biester and Schwarte (1965) the mortality rate is variable. When layers are affected the egg production temporarily gets retarded and the mortality may assume serious proportions. Chickens of all ages, sexes and breeds unless previously exposed are susceptible to the virus.

(3) PATHOLOGY :

Good pasture (1928), Matheson, Brunett and Brody independently described in 1931 the appearance of cutaneous eruptions or wart-like nodules on the unfeathered parts of fowl and diphtheritic membranes in the mouth. The cutaneous lesions were observed over the head region, but they might appear on legs, feet, around the cloacal aperture, and under the wings.

Hutyra et. al. (1938) described the cutaneous lesions in the fowl pox disease, as a local epithelial hyperplasia, involving both epidermis and underlying feather follicles with the formation of nodules which might be few to numerous in number and might not necessarily erupt at the same time.

They, described the cutaneous nodules as first appearing small, whitish foci which grow rapidly in size and become yellowish in colour. In some instances, the

closely adjoining lesions coalesce and develop into larger lesions which are rough and grey to dark brown in colour. After about two weeks, sometimes earlier, the lesions may become haemorrhagic. Then they undergo a process of desiccation and results in scab formation. The scab lasts for one to two weeks. In uncomplicated cases the process ends into desquamation of the degenerated epithelial cells leaving behind a scar tissue.

Manninger's (1938) observations are like those of Marek (1938). However, when he removed the undried scabs, observed a moist, seropurulent exudate underneath with a granulating surface. The scar tissue was observed to be negligible in milder cases. In case of secondary bacterial invasion, the course of the disease and the pathology were slightly modified by setting up suppurative or fibrinous inflammation of the deeper epithelial layer.

Goodpasture (1928), as quoted by Biester and Schwarte (1965), found that the eruptions on the mucous membranes (m.m.) were white, opaque and looked like slightly elevated nodules. They rapidly increased in size, often coalescing to become a yellowish, cheesy, necrotic mass giving the appearance of a pseudomembrane. Beneath the pseudomembrane, a zone of reaction was observed and the removal of this membrane resulted in a granulomatous bleeding surface. The invasion by contaminating bacteria aggravated the diphtheritic form of the disease. The classical description is quoted as follows :

"The inflammatory process may extend from the mouth region into the sinuses, particularly the infra orbital sinuses, resulting in a tumor like swelling, and may extend into the pharynx, resulting in respiratory disturbances".

Hutyra et. al. (1938), Bigland (1962), Kirmse (1969) and Löftin (1969) reported that the pox lesions develop chiefly on the borders of the eyelids, at the angles of the mouth and on the feet. When Bigland (1962) administered the virus by intramuscular (i/m) injection it produced an inflammatory necrotic lesion at the inoculated site and a generalised infection. The postmortem appearance resembled that of a subacute bacterial cellulities. Haemorrhages under the serous membranes, oedema of the lungs and pericarditis were observed.

Durant (1938) and Mc Dougle (1938) observed cheesy exudates in the commissures of the mouth and at the anterior larynx. In common with many virus diseases, the significant feature of the fowl pox is the presence of intracytoplasmic inclusion bodies in the affected epithelial cells.

Goodpasture (1928) & Prasad, et. al. (1967) Gupta (1967) observed only the squamous epithelium of the skin and mucous membranes appeared to have been affected in the viral infection and the basal layer of the epithelium showed little change from the normal. They observed that the inclusion bodies appeared peripheral to the basal layer in the early stages of the infection. The cells in which the

inclusions were present, were first enlarged, and the cytoplasm appeared oedematous. The inclusion material first appeared near the periphery of one or more vacuoles situated at the proximal pole of the cell. Then it increased in size rapidly, in some cases becoming as large as the original cell. But when degenerative changes appeared in the cell, the inclusion bodies became more defined in the cytoplasm.

Borrel (1904) discovered in smear preparations from the cutaneous lesions of fowl pox myriads of minute coccus-like structures apparently small enough to pass through the pores of a Berkefeld filter and described these bodies as the specific etiological agent for fowl pox.

Burnet (1906) in a critical pathological study of fowl pox confirmed Borrel's observations of the coccus-like structures in fresh preparations and showed that these bodies were derived from the same cell in which inclusion bodies were demonstrated.

Goodpasture (1928) reported that these bodies were about 0.25 microns in diameter and due to the large size, were seen easily under the microscope. Woodruff and Goodpasture (1930) reported that an inclusion body may contain as many as 20,000 elementary bodies each of which was capable of inciting the disease in the susceptible host.

In 1931, Ledingham described the borrel bodies of fowl pox disease as infectious and suggested them as aggre-

-gation of virus particles. Danks in 1932 made chemical analysis of these inclusions, and found them inorganic in nature. Goodpasture's (1933) observations were similar to those of Danks (1932). But he further observed that the crushed granules were agglutinated by specific immune sera and were also able to stain these bodies by silver method of Morosov staining.

The disease is manifested in three different forms. It was reported that cutaneous and diphtheritic forms of the disease were caused by different types of virus (Jowelt, 1902; Fally, 1908; Bordet and Fally, 1910). However, Carnwarth (1908), Uhlenhuth and Mantenfel (1910 and 1913), Betegh (1913) and Bayen (1933) reported that cutaneous and diphtheritic forms were the manifestations of the same virus. Based on the experimental trials Pannisset and Verge (1923) reported that all the three classical forms of the disease were caused by the same virus.

James (1931) observed that diphteritic form (mouth form) of fowl pox disease was prevalent in Illinois but the mortality rate was low. Labage and Golub (1934) isolated the virus from cases of pigeon pox.

Wiltoz (1936) according to the severity of the disease classified fowl pox disease as per acute, acute, subacute and chronic forms.

Brandly and Dunlop (1938) described the feet lesions

in fowl pox as infectious.

Mathey (1953) and Hill (1953) isolated together three types of virus on the basis of lesions produced. One of them produced a small discrete lesion with inclusion bodies, appapently limited to the cells infected at the time of inoculation. The second variety produced a confluent lesions lasting about two weeks. The inclusion bodies were seen in all the infected cells. The third type produced a heavy confluent lesion, lasting for a month or more. Inclusions were similar to those of the second type.

Inclusion bodies were observed as early as on the following day of post inoculation and were found in salivary glands from a bird experimentally infected by i/v injection with fowl pox virus.

CHORIO ALLANTOIC MEMBRANE:

Woodruff and Goodpasture (1931) reported that the chorio allantoic membranes of chicken eggs infected with fowl pox virus showed a marked susceptibility for ectodermal cells to infection, although entodormal cells could also be infected. Inclusion bodies were usually less numerous and hyperplasia was less marked in entodermal cells than in ectodermal cells.

Brandly (1941) concluded from studies of infected chorio allantoic membranes that "both fowl and pigeon pox" viruses produced microscopic retrograde changes in all three

germ layers of the chorio allantois. Fowl pox strains produced more severe alterations in the ectodermal and entodermal layers, while with the pigeon pox virus the reactions were more marked in the mesoderm (cellular infiltration and edema). He found necrosis of the limiting cellular layers.

Mayr (1957) and Wittmann (1957) reported independently that the histological examination of embryonated eggs inoculated on the chorio allantoic membrane with fowl pox virus revealed that the virus spread outwardly from its foci in alternate phases of high and low activity and at a certain point stopped. The process was characterized by the formation of ring zones, undulation of the entodermal proliferations, and the appearance at regular intervals of shaggy ectodermal proliferations. The entodermal proliferation at the periphery extended far beyond the last alteration in the ectoderm.

Wolff (1960) and de Laforest (1960) found that the fowl pox virus cultivated in explants of skin from chick embryos 8-12 days old caused changes similar to those seen in birds infected cutaneously; - hyperplastic thickening of the epidermis, the cells of which contained Bollinger bodies. The virus underwent 15 passages in skin explants in 5 months.

(4) <u>INCIDENCE AND DISTRIBUTION</u> :

Although the disease is known since time immemorial, it was only in the beginning of the twentieth century that

Mark et al. (1902), Glover (1931) and a number of other workers established a viral theory for the disease.

In India, the disease has been reported from many states such as, Uttar Pradesh, Maharashtra, West Bengal, Madras, Andhra Pradesh, Bihar, Assam, Gujrat and Kerala (Uppal, 1967). The disease mostly occurs during the hot and rainy season. Kaura and Iyer (1938) were the first to report a natural outbreak of pigeon pox in India.

Tietz (1932) reported that all the grain eating birds were susceptible to the disease. Burnet (1933) isolated virus from canary, Kaura and Iyer (1938) from pigeons, Dobson (1937) from pheasants, Syvertan and Cowan (1944) from sooty grouse, Mcgaughey and Burnet (1945) from wild sparrows, De Blieck and Jansen (1946) from turkeys. Recently, Hroiuchi et al. (1965) isolated the virus from ptarimigan and found it be be identical to fowl pox virus.

Doyle (1930) recorded that amongst fowls 2-3 weeks old chicks were most susceptible. The mortality in these chicks might reach as high as 60 to 90 percent, whereas in adult birds; the mortality might be upto 5 to 10 percent with marked decrease in egg production.

(5) HAEMATOLOGY IN FOWL POX DISEASE :

Very little information is available about the

haematological studies of fowl pox disease.

Blount (1947) examined the blood of Leghorn cockerels infected with fowl pox and found that the blood picture in the disease was quite different from that seen in experimentally produced infection. In cases of natural outbreak, the percentage of neutrophils and eosinophils was reduced.

In experimental cases, however, there was a well marked neutrophilia with a reduction in both eosinophils and basophils.

Schalm (1961) said that leucopenia was common to most diseases caused by viruses.

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CHAPTER - III

MATERIALS AND METHODS.

MATERIALS AND METHODS

VIRUS :

Infective fowl pox scabs received from Indian Veterinary Research Institute, Mukteshwar - Kumaon by the Livestock Research Station, Bihar, Patna, described as vaccine-cum-challenge strain of F.P. virus was used as the virus material for this study.

0.25 gm. of scab were triturated into 10 ml. of sterile normal saline solution (N.S.S.). Antibiotics were added to the final concentration of 40,000 units of Penicillin and 50 mgm./ml. of Streptomycin.

For producing experimental infection of F.P. in chicks, the above material was used.

MANAGEMENT :

Day old unvaccinated chicks of both sexes were obtained from the Central Poultry Farm, Patna. These chicks were maintained in electric foster mother in an insect proof animal shed and were fed on standard chick mash, prepared by the aforesaid farm authority during the period of experiment. Tap water was supplied to chicks for drinking purposes. Feeders and water fountain pots were used for maintaining the chicks. Fresh water was provided to them every 12 hours. The droppings

of the chicks were taken out twice daily. All utensils, equipments etc. used in the poultry shed were properly washed with disinfectant (2.5% lysol). Hands and legs were washed with germicidal soap and dettol (dilute solution) before starting and finishing the work.

BLOOD COLLECTION AND EXAMINATION :

The chicks were maintained as above, for 85 days when they were divided into three groups. The first group consisted of 9 birds and two controls, the second group consisted of 12 birds and two controls and the third group also consisted of 12 birds and two controls.

These birds in each of the above 3 groups were numbered and were kept in three separate animal sheds to avoid any mixing. The blood was collected from wing veins of the chickensbefore feeding. Two ml. of the collected blood were immediately placed into a test tube containing a suitable quantity of Wintrobes isotonic ammonium and potassium oxalate mixture. The tubes were shaken for about 2 minutes to ensure a thorough mixing of the anticoagulant and blood. The blood was then examined for haemoglobin percentage, erythrocyte sedimentation rate, packed cell volume, total erythrocyte and leucocyte counts, buffy layer, clotting time and differential count. Blood for differential count were generally prepared from a drop of blood taken directly from the wing vein.

In the present investigation, 33 experimental birds were subjected to different haematological tests during pre- and post-infection period.

The experimental birds were regularly examined for protozoan and other parasitic infestations. The birds which were found free from any disease and having normal cloacal temperature, were infected with the vaccine-cum-challenge strain of fowl pox disease virus obtained from the Livestock Research Station, Bihar, Patna. Inoculations were done subcutaneously into thigh, comb and wattles with a total of 0.5 c.c. of 0.25 gram of virus material dissolved in 10 cc. of sterile N.S.S. The route of infection and dose of the virulent fowl pox disease virus were kept constant in all the three batches of chicks during the course of experiment. Temperature of the inoculated birds and the control birds were taken twice daily.

Two inoculated birds were slaughtered every 24 hours for making observations. Necessary controls were incprporated at every step of the experimentation.

METHOD OF COLLECTION OF BLOOD :

(1) ANTICOAGULANT :

Small sterile empty penicillin vials and test tubes were filled with 0.25 ml. of the anticoagulant mixture cons-

-isting of the following components :-

Potassium Oxalate -	0.8 gm.
Ammonium Oxalate -	1.2 gm.
Neutral Distilled water -	100 ml.

The 0.25 ml. mixture contained in vials was evaporated to dryness by placing them in hot air oven at 60°C, so that they did not alter the volume of blood collected for the test. In each of the tubes/vials containing dried anticoagulant mixture, blood was collected in amount of not more than 2.5 ml.

(2) COLLECTION OF BLOOD :

The blood required throughout this study was taken from the wing vein. The feathers covering the wing vein region were plucked and the skin was sterilised with rectified spirit. The bird was then put on its side on a stool and was secured by an attendant by holding the legs and wings with one hand and the neck with the other. A sterile hypodermic needle was used to puncture the wing vein and blood was collected directly into the penicillin vials/tubes containing the dried anticoagulant. Approximately 2 ml. of blood was collected from each bird and mixed properly with the anticoagulant.

All haematological tests were followed immediately after the collected blood was brought to the laboratory.

(3) CLOTTING TIME :

The technique described by Varma (1960) was used for this study. Pasteur's capillary pipette was used for studying the clotting time. The skin of the wing vein was sterilised with rectified spirit. After the spirit evaporated completely, the vein was pricked with a pin and the blood was allowed to flow out. The tip of the pipette was then placed over the blood. With the capillary action, a blood column of 3 to 4 inches was taken, then the capillary portion from the pasteur's pipette was broken for observing the clotting time.

Observations were made at every 10-15 seconds interval, by breaking a small portion of the capillary tube for the presence of blood clot. This procedure was continued till a thin thread of clotted blood was observed between the two broken ends. The time when this clotted blood was observed first, and the difference from the actual filling of the capillary tube gave the clotting time.

(4) HAEMOGLOBIN :

The haemoglobin estimation was carried out by Sahli-Haden haemoglobinometer.

The empty graduated tube was filled with N/10 hydrochloric acid upto the mark 10. Blood was drawn into the

measuring pipette upto the 20 c.m.m. mark and was then carefully added into the tube containing N/10 hydrochloric acid. The mixture was then thoroughly shaken, care being taken not to form air bubbles. The resulting dark brown fluid was then diluted with neutral distilled water until it matched with the brown glass standard provided in the haemoglobinometer. The haemoglobin value was read directly from the scale in percentage and in gms./100 c.c. of blood.

(5) DIFFERENTIAL LEUCOCYTE COUNT :

Clean, grease free microscopic slides were used for making uniform blood film, for the differential count. The smear was stained with the Wright's staining technique before the count was made.

(6) STAINING TECHNIQUE :

The modified Wright's technique was used for staining blood films for the differential count.

The air dried blood film was fixed in methyl alcohol for three minutes and then flooded with the stain. After one minute, the stain was diluted two folds with neutral distilled water and was allowed to stand for 30 minutes.

Counting of W.B.C. was done according to the "battlement" system (1 m.m. down, 1 m.m. across and 1 m.m.up).

A total of 200 leucocytes were counted in each case.

The different leucocytes were characterized according to the description given in Veterinary Pathology by Smith and Jones (1970).

(7) TOTAL RED AND WHITE CELL COUNT :

Throughout this study, the method described by Natt and Herrick (1952) was followed. The diluent was prepared as follows :-

Sodium chloride (NaCl) -	3.88 gms.
Sodium Sulphate (Na_2SO_4) -	2.50 gms.
Disodium hydrogen phosphate ($Na_2HPO_4.12\ H_2O$) -	2.91 gms.
Potassium hydrogen phosphate -	0.25 gms.
Formalin (formaldehyde 37%) -	7.50 ml.
Methyl Violet 2B -	0.10 gm.

The above chemicals were dissolved in distilled water in the order prescribed as above and diluted to a total volume of 1,000 ml. in a volumetric flask. After standing overnight, the solution was filtered through Whatman No.2 filter paper and the pH was adjusted to 7.3 with alkali (NaOH) and acid (HCl) solution.

The Thoma red blood cell (R.B.C.) counting pipette was filled with the blood to the 1.0 mark and the diluting

fluid was sucked to the 101 mark. The mixture was allowed to stay for 5 minutes. The blood sample which was thus diluted 1 : 100, was then shaken in order to obtain an equal cell distribution. All the counts were made by means of improved Neubauer haemocytometer. Clean, grease free counting chamber and the cover slip were used. The cover slip was placed on the counting chamber so that it covered the entire ruled area. It was ensured that the contact between the counting chamber and the cover slip was well established. The first few drops of the diluted blood were discarded by blowing into the pipette. The pipette was then held in an inclined position and small drop of diluted blood was placed at the junction of the cover slip and counting chamber. The drop immediately passes under the cover slip by capillary attraction. Every care was taken to ensure that no air bubble was included in the chamber. The cells were then allowed to settle for about 2 minutes before they were counted under 40X magnification.

The R.B.Cs. were counted in 5 large squares, four at the corners and one in the centre. Each of the large square contained 16 small squares, thus making the area of 80 (16x5) small squares.

The leucocytes were counted in all the 400 (16x25) small squares. The number of erythrocytes counted was multiplied by 5,000 which the number of leucocytes in the 400 small squares were multiplied by 1,000 to give the total red and white cell count respectively. All the cells touching the

upper and the left hand line of each square were included for counting while those touching the lower line and the right hand line of each square were not counted.

The nucleus of the oval R.B.C. stained violet and the cytoplasm stained the same colour but to a much less intensity. Since the shape of the R.B.C. is so characteristic that no difficulty could be experimnced in differentiating the same from the leucocytes. The other white blood cells stood distinctly with their distinctly violet stained nuclei while the cytoplasm took up very light stain.

(8) ERYTHROCYTE SEDIMENTATION RATE (E.S.R.) AND PACKED CELL VOLUME (P.C.V.) :

Wintrobe and Landsberg's method was used for E.S.R. and P.C.V. estimation (Wintrobe, 1956).

The haematocrit tube was filled up to the 10 mark at the right side of the scale, with the oxalated blood; by means of a pipette drawn from a glass tubing to such a length that it can be passed to the bottom of the tube. The pipette was passed to the bottom of the haematocrit tube and an uniform pressure was applied to the bulb of the rubber teat. Thus the blood was forced out as the pipette was withdrawn. This was done at such a rate that no bubbles of air could form in the tube. The tube was then closed by means of a rubber stopper to check evaporation of blood.

The haematocrit tube was then allowed to stand vertically and the readings were noted at the end of three hours.

After the erythrocyte sedimentation rate was noted, the haematocrit tube was centrifuged for 30 minutes at 3,000 r.p.m. The tubes were taken out of the buckets and the readings were noted. The P.C.V. was expressed in percentage. The reading of "Buffy layer" was also taken and expressed in m.m./ 100 ml. of blood.

(9) HISTOPATHOLOGY :

Necropsy procedure recommended by Carter (1962) was followed for the routine postmortem examination of the chickens.

The general condition of the body and other abnormalities, if any, were noted. The carcasses were laid on the tray with their ventral side up. A longitudinal incision was given on the skin ventrally, and the skin was stripped digitally from the break to the vent. The abdomen was opened by removing the ventral wall and the breast was removed by cutting it forward on both the sides through the ribs and humeroscapular articulations with the help of a bone cutter. The organs were first observed in situ and then examined systematically by removing from the neck to the cloaca. Gross lesions observed in the organs were noted. Suspected materials from the different organs were collected for histopatho-

-logical examination.

The techniques recommended in the Laboratory Mannual of special staining Techniques (Coffin , 1953) for histopathological examination were followed. The tissues were cut in small pieces measuring about one c.cm. taking half portion of healthy and half portion of diseased tissue together for comparative study. The tissues were first fixed in 10% formal-saline solution and then washed in running tap water for 24 hours to remove theexcess of fixative. The tissue pieces were then dehydrated in ascending grades of alcohol, cleared in xylol and embadded in paraffin wax. Sections were cut at 5 microns.

For staining Harris's haematoxylin and alcoholic solution of Eosin Y were used as a routine in all the sections unless otherwise stated. The sections were deparaffinised in xylol, carried through descending grades of alcohol to water. The sections after being stained with haematoxylin, excess of the stain was removed by 1% acid alcohol; were dehydrated through ascending grades of alcohol, and counterstained with alcoholic solution of eosin. The stained sections were then cleared in two changes of xylol and mounted in D.P.X. before examined microscopically.

(10) <u>METHODS OF CHORIO ALLANTOIC MEMBRANE (C.A.M.) INOCULATION</u> :

Dropped C.A.M. of 12 day old hen's embryo as per the

technique described by Merchant and Packer (1967) was used for tais inoculation. Each of the embryonated eggs were inoculated with 0.1 ml. of virus dilution ranging from 10^{-3} to 10^{-9}. After inoculation, the eggs were shaken to disperse the virus uniformly, and were then; incubated at 37°C with the inoculated area facing upwards, for 4 days.

After the incubation period, the eggs were chilled overnight at 4°C. The following morning, the infected C.A.M. from each of the chilled eggs were harvested and examined for the lesion.

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CHAPTER - IV

RESULTS

R E S U L T S

The transmission experiments in the chickens were performed during the period between August, 1971 to September, 1971.

The infected birds suffered from the acute form of the disease. No per acute, sub-acute, or chronic form of the disease, however, was observed. The disease became obvious in 6 days post-infection with characteristic lesions. The birds which were sacrificed in batches each day starting with 24 hours post-infection upto the fifth day did not show characteristic lesions of fowl pox, but the birds which died or were killed after six to seven days of the artificial exposure, showed typical symptoms and characteristic lesions.

SYMPTOMS :

The infected birds appeared normal upto the second day after infection. Pyrexia was noticed in all the birds excluding controls on the third day. The body temperature ranged from 106°F to 110°F. The febrile reaction was of uniform nature in all the inoculated birds but became normal 11-12 days post-infection.

The other symptoms were observed characteristic of the inherent form of the disease. In severe cases, restlessness, inappetance and dullness were marked. The affected

birds were showing marked depression, debility and weakness. Respiratory distress accompanied by rattling noise was noticed in some of the affected birds (Fig. 7 & 8). The "Ocular form" of the disease was characterised initially by conjunctivitis along with watery discharge from the eye. It was observed in three birds of batch number 3. The watery discharge became purulent with the advancement of the disease. The eyelids were swollen and closed due to a thick yellow viscid discharge. Some of the birds having mouth lesions were off-feed. Purulent discharge occluded the nasal passage and the birds were noticed labouring for respiration with half-opened beak.

HAEMATOLOGY :

Haematological pictures are presented in Table I - IX. The average blood values of the chickens during pre and post-infection period showed that the birds infected with fowl pox disease showed a marked fall in total leucocyte count but there was an increase in the percentage of neutrophils, increase in red cell count in few cases and decrease in red cell count in others. A slight decrease in haemoglobin percentage, erythrocyte sedimentation rate in some cases was accelerated whereas in few cases it slowed down.

The percentage of eosinophils and basophils decreased in the affected birds with slight increase in the

percentage of monocytes. There was a general decrease in the percentage of packed cell volume in the affected birds, except in a few cases. Lymphopenia was recorded in almost all the infected birds.

Slight increase in monocytes and body temperature were found to be significant after fifth day and onwards. Clotting time revealed no appreciable changes.

TABLE I.

Haematological Picture of Chickens (Batch-I) During the Pre-Infection Period.

Bird No.	Age when examined (days)	Body temp. (^{o}F)	R.B.C. per c.m.m. (millions)	W.B.C.per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (½ hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils.	Lymphocytes	Basophils	Eosinophils.	Monocytes
51	70	106.6	2.58	32,000	10.6	7	25	190	1.2	30	58	0	10	2
40	70	106.8	2.92	28,000	10.0	9	30	235	1.2	25	68	0	5	2
67	70	107.0	2.62	35,000	13.6	10	30	225	1.0	28	59	2	9	2
41	70	107.2	2.75	29,000	9.8	10	33	253	1.2	31	59	1	6	3
42	71	106.8	2.95	30,000	11.0	9	32	238	1.4	28	65	1	4	2
43	71	107.4	3.02	27,000	9.8	9	31	253	1.2	26	65	1	5	3
44	71	107.0	3.18	29,000	11.2	9	32	255	1.0	30	60	1	7	2
45	71	107.2	2.89	27,000	8.8	9	31	243	1.2	29	62	1	5	3
46	71	107.0	3.15	32,000	11.0	10	32	235	1.2	31	60	1	5	3

TABLE II.

Haematological Picture of Chickens (Batch-I) Infected with Fowl Pox Disease Virus.

Bird No.	Age when exposed (days)	Hours of ex-am. post-inoculation	Body temp. (^{o}F)	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
											Neutrophils	Lymphocytes	Basophils	Eosinophils.	Monocytes.
51	85	48	109.0	2.55	20,000	10.0	8	24	180	1.0	50	44	0	3	3
40	85	48	110.0	2.85	14,000	9.6	10	30	240	1.2	45	49	1	2	3
67	85	48	109.0	2.74	15,000	12.8	9	31	220	1.2	53	40	1	2	4
41	85	96	108.6	3.07	13,000	10.4	9	34	248	1.2	55	40	0	3	2
42	85	96	108.8	2.81	11,000	10.6	10	31	232	1.2	40	56	0	2	2
43	85	96	109.2	3.12	9,000	10.0	9	32	260	1.2	57	36	1	2	4
44	85	144	109.0	3.09	10,000	11.0	10	32	252	1.2	45	49	0	3	3
45	85	144	109.4	2.76	12,000	8.6	9	31	249	1.0	49	45	0	3	3
46	85	144	108.4	2.72	16,000	10.4	11	30	239	1.0	68	25	1	2	4

TABLE III.

Haematological Picture of Chickens (Batch-II) During the Pre-Infection Period.

Bird No.	Age when examined (days)	Body temp. (°F)	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils.	Lymphocytes	Basophils	Eosinophis.	Monocytes.
79	88	107.0	1.87	28,000	6.8	10	28	245	1.2	25	65	0	8	2
53	88	106.8	2.65	33,000	11.0	10	30	238	1.0	20	69	0	7	4
54	88	107.0	2.55	25,000	11.8	10	30	254	1.2	30	62	0	6	2
55	89	107.2	1.35	18,000	9.2	10	28	248	1.2	20	74	0	5	1
56	89	107.0	2.99	18,000	12.0	8	27	254	1.2	18	69	0	10	3
57	89	106.8	3.07	28,000	13.8	15	26	254	1.0	15	71	0	11	3
58	90	107.2	2.93	14,000	13.2	10	28	245	1.0	19	64	0	15	2
59	90	106.6	3.07	25,000	11.2	10	27	240	1.2	18	72	1	7	2
60	90	107.0	2.51	25,000	11.6	12	26	270	1.0	20	70	0	8	2
61	91	107.2	2.56	25,000	10.6	12	26	245	1.2	22	68	1	7	2
63	91	107.0	2.96	30,000	9.0	10	25	255	1.2	20	71	1	7	1
65	91	107.2	2.76	24,000	10.0	11	27	260	1.2	20	72	0	5	3

TABLE IV.

Haematological Picture of Chickens (Batch-II) Infected with Fowl Pox Disease Virus.

Bird No.	Age when exposed (days)	Hours of ex-am. post-inoculation.	Body temp. (°F)	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
											Neutrophils	Lymphocytes	Basophils	Eosinophils.	Monocytes.
79	95	96	108.6	2.10	14,000	7.0	9	30	240	1.2	40	54	0	2	4
53	95	96	109.0	1.98	10,000	11.4	12	28	235	1.2	45	50	0	2	3
54	95	120	108.2	1.58	7,000	12.2	12	28	255	1.0	57	36	0	3	4
55	95	120	108.4	1.25	6,000	10.0	11	26	250	1.2	51	44	0	2	3
56	95	144	109.2	2.13	14,000	10.2	12	25	252	1.2	45	51	0	2	2
57	95	144	108.8	3.24	8,000	11.2	12	32	256	1.2	40	53	0	3	4
58	95	168	109.2	3.48	18,000	12.4	9	33	242	1.2	46	46	0	4	4
59	95	168	108.6	2.62	12,000	10.5	12	25	236	1.0	39	55	0	2	4
60	95	192	109.0	2.60	11,000	11.0	11	27	280	1.2	57	36	0	2	5
61	95	192	108.6	2.40	9,000	10.0	13	25	250	1.2	43	51	0	2	4
63	95	216	109.4	2.85	8,000	8.6	12	24	252	1.2	54	40	0	3	3
65	95	216	110.0	2.99	19,000	11.0	10	29	262	1.2	59	36	1	2	2

TABLE V.

Haematological Picture of Chickens (Batch-III) During the Pre-Infection Period.

Bird No.	Age when examined (days)	Body temp. (^{o}F)	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils	Lymphocytes	Basophils	Eosinophils.	Monocytes.
62	100	107.0	3.44	26,000	8.2	10	28	220	1.2	27	65	1	5	2
78	100	107.2	1.84	27,000	6.4	10	27	240	1.2	26	64	0	8	2
77	100	107.0	2.87	25,000	9.0	9	30	250	1.2	23	67	1	7	2
76	102	106.8	2.79	24,000	9.0	10	29	266	1.0	29	63	0	6	2
76	102	106.4	3.25	20,000	10.2	10	31	263	1.2	25	62	1	10	2
74	102	107.2	3.15	18,000	11.0	9	33	245	1.0	26	65	1	7	1
73	102	106.6	3.35	15,000	10.5	8	31	225	1.2	21	70	1	6	2
72	103	106.8	3.10	24,000	13.0	10	29	238	1.0	30	62	2	5	1
71	103	107.0	3.05	25,000	10.5	11	29	282	1.0	28	66	0	4	2
70	103	107.2	2.63	27,000	10.0	10	31	260	1.2	30	61	0	7	2
69	103	107.0	3.47	29,000	10.8	9	33	256	1.4	18	70	1	8	3
68	103	106.4	2.22	32,000	8.2	11	30	249	1.2	27	66	0	6	1

TABLE VI

Haematological Picture of Chickens (Batch-III) Infected with Fowl Pox Disease Virus.

Bird No.	Age when exposed (days)	Hours of ex-am. post-inoculation.	Body temp. (°F)	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
											Neutrophils.	Lymphocytes	Basophils	Eosinophils.	Monocytes.
62	110	240	109.0	3.34	12,000	8.5	10	27	225	1.2	41	53	0	3	3
78	110	240	108.6	1.92	14,000	6.8	9	28	243	1.2	55	39	0	2	4
77	110	264	108.8	2.72	12,000	8.8	10	30	248	1.2	56	39	0	2	3
76	110	600	109.4	2.88	9,000	9.4	9	30	262	1.2	45	50	1	2	2
75	110	288	109.2	3.21	10,000	10.0	10	30	267	1.2	44	50	1	2	3
74	110	288	108.4	3.02	11,000	10.6	10	32	250	1.2	60	34	1	2	3
73	110	312	107.4	3.05	8,000	10.7	8	32	222	1.2	62	31	0	2	5
72	110	312	107.0	2.98	11,000	12.8	10	30	235	1.0	50	43	1	2	4
71	110	336	107.2	3.10	15,000	11.0	10	33	276	1.2	44	50	1	2	3
70	110	336	107.8	2.52	13,000	9.6	11	30	257	1.0	44	48	1	2	5
69	110	360	107.0	3.41	12,000	10.9	9	32	260	1.2	52	42	0	3	3
68	110	360	107.4	2.42	16,000	9.0	10	32	254	1.4	53	41	1	2	3

TABLE VII.

Haematological Picture of Chickens (Batch-I) Infected with F.P.Virus with Control.

Bird's age (days) at the time of final observation.	Condition of birds	Period of blood collection.	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms./100 ml	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils.	Lymphocytes	Basophils	Eosinophils.	Monocytes.
70	-	Pre-infection	2.58	32,000	10.6	7	25	190	1.2	30	58	0	10	2
71	-	"	2.95	30,000	11.0	9	32	238	1.4	28	65	1	4	2
72	-	"	3.82	22,000	11.6	12	27	252	1.2	26	63	1	8	2
87	I*	Post-infection.	2.55	20,000	10.0	8	24	180	1.0	50	44	0	3	3
87	C**	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
89	I	"	3.07	13,000	10.4	9	34	248	1.2	55	40	0	3	2
89	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
91	I	"	3.09	10,000	11.0	10	32	252	1.2	45	49	0	3	3
92	C	"	3.75	24,000	11.4	12	28	256	1.2	30	62	1	4	3

* denotes infected. ** denotes control. N.D. denotes not done.

Remarks :- Birds were inoculated with the F.P.V. at the age of 85 days. Haematological observation was made every 48 hours beginning 2 days post-infection.

TABLE VIII.

Haematological Picture of Chickens (Batch-II) Infected with P.P.Virus with Control.

Bird's age (days) at the time of final observation.	Condition of birds	Period of blood collection.	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms/100 ml.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils.	Lymphocytes	Basophils	Eosinophils.	Monocytes.
88	-	Pre-infection.	1.87	28,000	6.8	10	28	245	1.2	25	65	0	8	2
92	-	"	2.97	26,000	9.0	9	31	253	1.2	31	63	2	3	1
99	I*	Post-infection.	2.10	14,000	7.0	9	30	240	1.2	40	54	0	2	4
99	C**	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
100	I	"	1.58	7,000	12.2	12	28	255	1.0	57	36	0	3	4
100	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
101	I	"	2.13	14,000	10.2	12	25	252	1.2	45	51	0	2	2
101	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
102	I	"	3.48	18,000	12.4	9	33	242	1.2	46	46	0	4	4
102	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
103	I	"	2.60	11,000	11.0	11	27	280	1.2	57	36	0	2	5
103	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
104	I	"	2.85	8,000	8.6	12	24	252	1.2	54	40	0	3	3
105	C	"	3.04	28,000	9.2	9	30	255	1.0	29	65	0	4	2

* denotes infect. ** denotes control. N.D. denotes not done.

Remarks :- Birds were inoculated with the P.P.V. at the age of 95 days. Haematological observation was made every 24 hours beginning 4 days post-infection.

TABLE IX.

Haematological Picture of Chickens (Batch-III) Infected with F.P.Virus with Control.

Bird's age (days) at the time of final observation.	Condition of birds	Period of blood collection.	R.B.C. per c.m.m. (millions)	W.B.C. per c.m.m.	Haemoglobin in gms /100 ml.	E.S.R. m.m. (3 hours).	Packed cell volume in %	Clotting time in seconds.	Buffy layer in %	Differential count				
										Neutrophils.	Lymphocytes	Basophils	Eosinophils.	Monocytes.
100	-	Pre-infection.	3.44	26,000	8.2	10	28	220	1.2	27	65	1	5	2
104	-	"	3.03	28,000	9.8	10	31	242	1.0	31	61	2	5	1
120	I*	Post-infection.	3.34	12,000	8.5	10	27	225	1.2	41	53	0	3	3
120	C**	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
121	I	"	2.72	12,000	8.8	10	30	248	1.2	56	39	0	2	3
121	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
122	I	"	3.21	10,000	10.0	10	30	267	1.2	44	50	1	2	3
122	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
123	I	"	3.05	8,000	10.7	8	32	222	1.2	62	31	0	8	5
123	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
124	I	"	3.10	15,000	11.0	10	33	276	1.2	44	50	1	2	3
124	C	"	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
125	I	"	3.41	12,000	10.9	9	32	260	1.2	52	42	0	3	3
126	C	"	3.00	24,000	9.6	10	30	246	1.0	25	68	1	4	2

* denotes infect. ** denotes control. N.D. denotes not done.

Remarks : - Birds were inoculated with the F.P.V. at the age of 110 days. Haematological observation was made every 24 hours beginning 10 days post-infection.

GROSS LESIONS IN LIVE AND KILLED CHICKENS:

Gross pictures are presented in Fig. 1 to 8. On autopsy, the birds presented chiefly inflammatory and necrotic lesions when examined six days post-infection.

The gross lesions were nodular and necrotic in nature. The lesions were predominantly confined to the cutaneous regions. The nodular lesions and exudative changes in the lungs appeared to be outstanding feature with the local (F.P. Vaccine-cum-challenge strain) virus. The virus showed dermotropism as evidenced by lesions in the skin, comb and wattles.

Control birds were negative for any lesions of the disease.

No characteristic lesions were observed in birds sacrificed in the initial stage of the infection but the lesions developed fully in the birds sacrificed on the sixth day and subsequently thereafter.

(1) Cutaneous form :

In the "Cutaneous form", wart-like nodular eruptions were observed on the unfeathered parts of chickens, and diphtheritic membranes appeared in the mouth. The lesions practically covered the entire unfeathered skin of the head

including the comb, wattles, face, eyelids and corners of the mouth (Fig. 1 to 3). In one case (3%) similar lesions but minute in size were detected around the feet, on the digits, webs, near vent, and under the wing. The eruptions were numerous in number and measured approximately 2 m.m. to 3 m.m. in diameter and few were as large as 10 m.m. The eruptions were gradual in appearance and increased in size with the advancement of the disease. At first, the nodular eruptions were small and white in colour; they rapidly increased in size and became yellowish in colour. In few cases, closely adjoining lesions coalesced together and the larger developing lesions were found to be rough, and grey or dark brown in colour.

After about two weeks, the lesions became slightly haemorrhagic which later on underwent a process of desiccation and resulted in scab formation. The scabs dropped off in 20 to 26 days. In some of the uncomplicated cases this process ended with the desquamation of the degenerated epithelial layer leaving behind a scar tissue. When the immature scabs were removed, a moist, seropurulent exudate was observed underneath covering a bleeding, granulating surface. Different forms of the disease produced are presented in Table X - XII.

(ii) Mouth form :

The "Mouth forms" showed whitish, opaque, slightly

elevated nodules over the mucosa of the mouth, oesophagus and crop. It rapidly increased in size, coalescing to become a yellowish, cheesy and necrotic material which later on became ulcerated (Fig. 4).

(iii) Ocular form :

This was characterised initially by a watery discharge from the eye, due to conjunctivitis which in course of time became purulent (Fig. 6).

The cutaneous form was commonest, and accounted for 85 percent of the cases. The ocular form of the disease was observed in approximately 9 percent of the birds, and the rest of six percent of birds suffered from the mouth form.

(iv) Gross-pathology :

On autopsy, liver was slightly enlarged in most of the cases. Greyish discoloration and fragility were noted in some of the cases. In some necrotic areas were also seen.

The involvement of spleen was variable. In few cases, spleen was enlarged about one and one-half times from the normal. Minute greyish spots were seen all over the enlarged surface of spleen.

In most cases kidneys showed slight enlargement

with little fragility. Pathological changes in the digestive tract were noticed in only few cases. The lesions were mostly confined to the upper digestive tract.

Oesophagus and crop showed numerous minute nodular eruptions in the mucosa. Deeper layer seemed to be undamaged.

Lungs were congested and consolidated in most of the cases. Few slight haemorrhagic areas were seen in some of the lungs. Trachea contained little frothy mucoid exudates in its lumen in few cases.

Heart showed slight enlargement and congestion in few birds(Table XIII to XV).

TABLE X.

Chickens (Batch-I) showing Different Forms of F.P. after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection.	Cutaneous form			Mouth form	Ocular form	Mixed form
			Skin	Beak	Comb			
B-51	F	2	-	-	-	-	-	-
B-40	F	2	-	-	-	-	-	-
B-67	M	2	-	-	-	-	-	-
B-41	F	4	-	-	-	-	-	-
B-42	M	4	-	-	-	-	-	-
B-43	F	4	-	-	-	-	-	-
B-44	M	6	+	-	+	-	-	-
B-45	F	6	+	+	+	-	-	-
B-46	M	6	+	-	+	-	-	-

- denotes No lesions.
+ denotes Mild.
F denotes Female.
M denotes Male.

TABLE XI.

Chickens (Batch-II) showing Different Forms of F.P. after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection.	Cutaneous form			Mouth form	Ocular form	Mixed form
			Skin	Beak	Comb			
B-79	F	4	-	-	-	-	-	-
B-53	F	4	-	-	-	-	-	-
B-54	F	5	-	-	-	-	-	-
B-55	F	5	-	-	-	-	-	-
B-56	F	6	+	-	+	-	-	-
B-57	F	6	+	-	+	-	-	-
B-58	F	7	+	-	+	-	-	-
B-59	M	7	++	-	++	-	-	-
B-60	F	8	++	-	++	++	-	-
B-61	F	8	++	-	++	-	-	-
B-63	F	9	++	-	++	-	-	-
B-65	M	9	+++	-	+++	++	-	-

- denotes no lesions.
+ denotes mild.
++ denotes severe.
+++ denotes more severe.

TABLE XII.

Chickens (Batch-III) showing Different Forms of P.P.after Artificial Insemination.

Bird No.	Sex	Time of sacrifice (no.of days post-infection.	Cutaneous form			Mouth form	Ocular form	Mixed form.
			Skin	Beak	Comb			
B-62	M	10	+++	-	+++	-	-	-
B-78	F	10	++	-	++	-	+++	-
B-77	F	11	++	-	++	-	-	-
B-76	M	25	++	-	++	-	-	-
B-75	M	12	++	-	++	-	+++	-
B-74	M	12	++	-	++	-	-	-
B-73	F	13	++	+	++	-	+++	-
B-72	F	13	++	-	++	-	-	-
B-71	F	14	-	-	-	-	-	-
B-70	M	14	+++	-	+++	-	-	-
B-69	F	15	++	-	++	-	-	-
B-68	M	15	++	-	++	-	-	-

Page No. 44.

\- denotes no lesions
\+ denotes mild.
++ denotes severe.
+++ denotes more severe.

TABLE XIII.

Chickens (Batch-I) showing Gross Lesions in Different Organs of the Body after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection.	Liver	Kidney	Heart	Lungs	Spleen	Digestive tract.
B-51	F	2	-	-	-	-	-	-
B-40	F	2	-	-	-	-	-	-
B-67	M	2	-	-	-	-	-	-
B-41	F	4	-	-	-	-	-	-
B-42	M	4	-	-	-	-	-	-
B-43	F	4	-	-	-	-	-	-
B-44	M	6	-	-	-	-	-	-
B-45	F	6	-	-	-	-	-	-
B-46	M	6	-	-	-	-	-	-

- denotes no lesions.

TABLE XIV.

Chickens (Batch-II) showing Gross Lesions in Different Organs of the Body after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection.	Liver	Kidney	Heart	Spleen	Digestive tract.	Lungs.
B-79	F	4	-	-	-	-	-	-
B-53	F	4	-	-	-	-	-	-
B-54	F	5	-	-	-	-	-	-
B-55	F	5	-	-	-	-	-	-
B-56	F	6	-	-	-	-	-	+
B-57	F	6	-	-	-	-	-	+
B-58	F	7	-	-	-	-	-	+
B-59	M	7	-	-	-	-	-	+++
B-60	F	8	++	+	-	+	+	++
B-61	F	8	++++	+	-	-	+	++
B-63	F	9	++	+	-	+	+	++
B-65	M	9	++	+	-	+	+	++

- denotes no lesions.
+ denotes mild.
++ denotes severe.
+++ denotes more severe.
++++ denotes very severe.

Page No.46.

TABLE XV.

Chickens (Batch-III) showing Gross Lesions in Different Organs of the Body after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection)	Liver	Kidney	Heart	Lungs	Spleen	Digestive tract.
B-62	M	10	++	+	-	++	+	+
B-78	F	10	-	+	-	+	+	-
B-77	F	11	++	-	-	++	-	-
B-76	M	25	-	-	-	++	+	-
B-75	M	12	++	+	-	+	-	-
B-74	M	12	-	+	+	++	+	+
B-73	F	13	++	+	+	++	+	+
B-72	F	13	++	+	+	++	+	+
B-71	F	14	-	+	+	++	-	+
B-70	M	14	++	+	+	++	+	+
B-69	F	15	-	+	+	+	-	+
B-68	M	15	-	++	-	+++	+	+

- denotes no lesions.
+ denotes mild.
++ denotes severe.
+++ denotes more severe.

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HISTOPATHOLOGY :

Histopathological pictures are presented in Fig. 9 to 23.

(i) Skin :

The skin nodules revealed necrotic changes in superficial epithelial layers, resulting in complete loss of cellular details. The underlying epithelial cells showed proliferative changes with marked distension, "ballooning" of individual cells. Some of these cells, revealed the presence of globular, refractile inclusion bodies. Pyknosis and eccentrical situation of nucleus was seen in many of the epithelial cells. The dermal blood vessels showed congestion along with lymphocytic infiltration, and were severely engorged. Keratinisation, distortion and disintegration of the dermal tissue with diffuse lymphocytic infiltration were observed in most of the advanced cases. Vacuolation of the epidermal epithelium was also well marked in the affected skin (Fig. 9 to 11).

(ii) Liver :

The hepatic cords appeared distorted and disorganised. Degenerative changes were marked in the phepatic lobules. Focal and diffuse necrotic areas were seen surrounded

by reactionary zones. In some cases, lobular architect was lost and perivascular areas were occupied by lymphocytic infiltration (Fig. 15 & 16).

(iii) Lungs :

The architecture of alveolar walls were broken at several places. Alveoli were distended mostly with red blood cells. The walls of alveoli had become thinner at some places. Severe congestion along with pneumonic changes was noticed in several cases (Fig. 12 to 14).

(iv) Kidney :

Peritubular and periglomerular cellular (lymphocytic) infiltration was seen in some of the cases. Affected kidney showed focal necrosis in several areas (Fig. 19 & 20).

(v) Spleen :

Slight degenerative changes along with congestion were noticed in spleen (Fig. 17).

(vi) Testis :

Slight inflammation was observed (Fig. 18).

Microscopical lesions are presented in Table XVI to XVIII.

TABEL XVI.

Chickens (Batch-I) showing Microscopical Lesions in Different Organs of the Body after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection).	Liver	Kidney	Heart	Lungs	Spleen	Digestive tract.	Skin(Comb & wattles).
B-51	F	2	-	-	-	-	-	-	-
B-40	F	2	-	-	-	-	-	-	-
B-67	M	2	-	-	-	-	-	-	-
B-41	F	4	-	-	-	-	-	-	-
B-42	M	4	-	-	-	-	-	-	-
B-43	F	4	-	-	-	-	-	-	-
B-44	M	6	-	-	-	-	-	-	+
B-45	F	6	-	-	-	-	-	-	+
B-46	M	6	-	-	-	+	-	-	+

- denotes no lesions.
+ denotes mild.

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TABLE XVII.

Chickens (Batch-II) showing Microscopical lesions in Different Organs of the Body after Artificial Infection.

Bird No.	Sex	Time of sacrifice (no.of days post-infection).	Liver	Kidney	Heart	Lungs	Spleen	Digestive tract.	Skin (Comb & wattles).
B-79	F	4	-	-	-	-	-	-	-
B-53	F	4	-	-	-	-	-	-	-
B-54	F	5	-	-	-	-	-	-	-
B-55	F	5	-	-	-	-	-	-	-
B-56	F	6	-	-	-	+	+	-	+++
B-57	F	6	-	-	-	+	-	-	++
B-58	F	7	-	-	-	+	-	-	++
B-59	M	7	-	-	-	+++	++	-	+++
B-60	F	8	+++	-	-	++	-	-	++++
B-61	F	8	+++	-	-	++	-	-	+++
B-63	F	9	-	-	-	++	-	-	+++
B-65	M	9	+++	-	-	++	-	-	+++

- denotes no lesions
+ denotes mild.
++ denotes severe.
+++ denotes more severe.
++++ denotes very severe.

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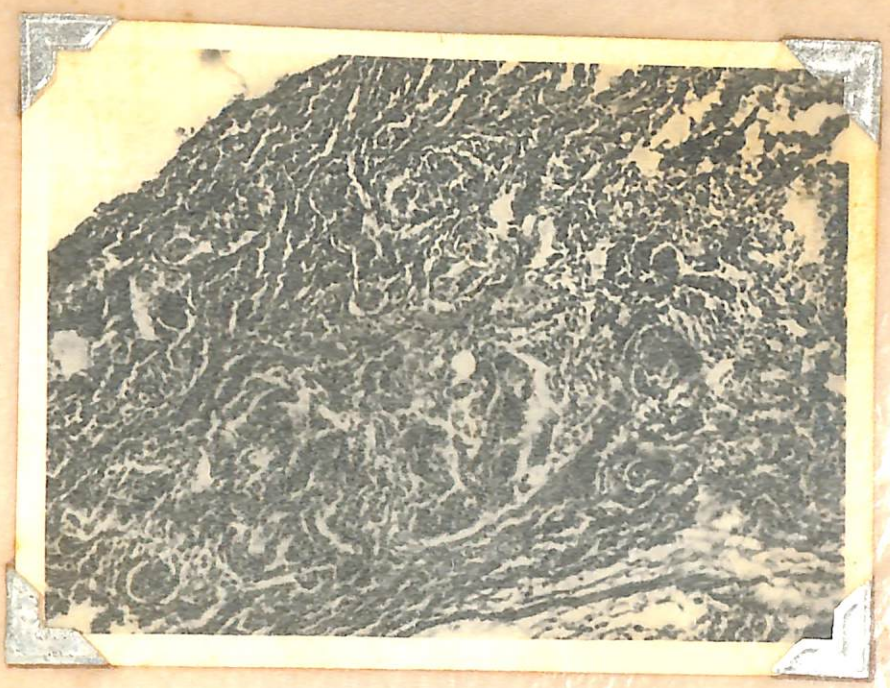
TABLE XVIII.

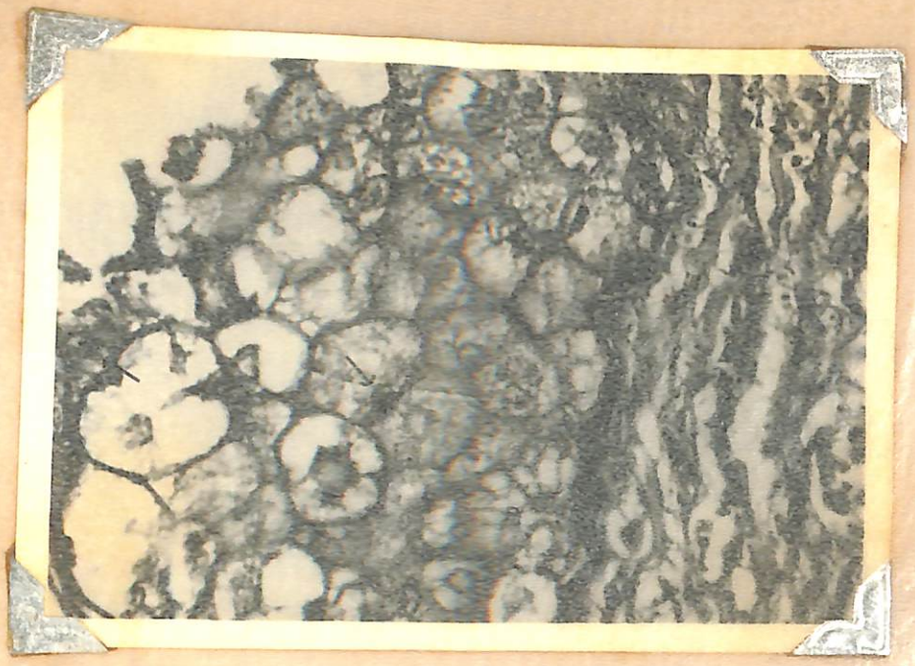
Chickens (Batch-III) showing Microscopical Lesions in Different Organs of the Body after Artificial Infection.

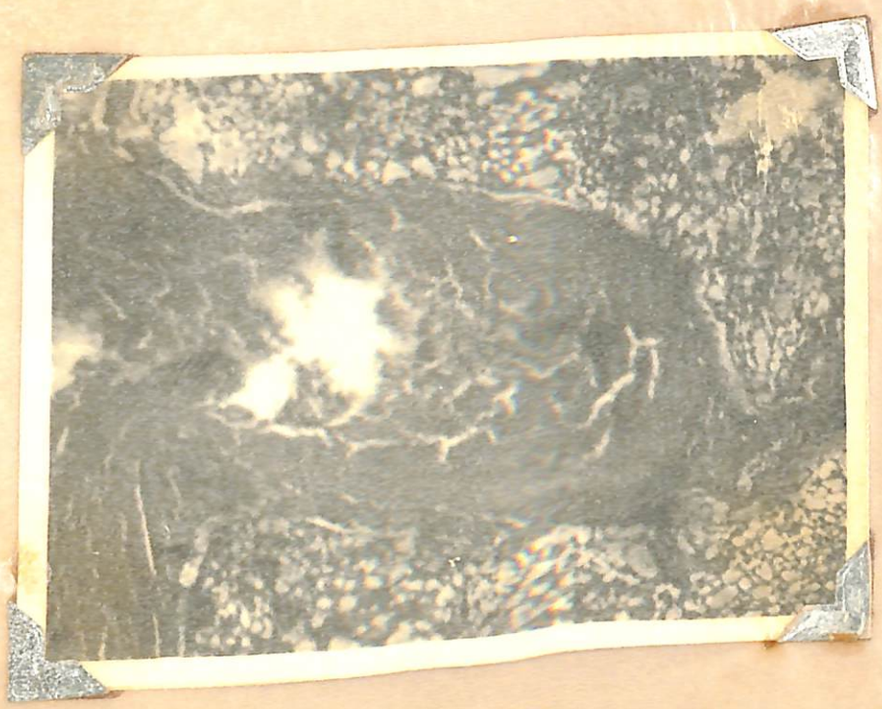
Bird No.	Sex	Time of sacrifice (no.of days post-infection).	Liver	Kidney	Heart	Lungs	Spleen	Digestive tract.	Skin (Comb & wattles).
B-62	M	10	+++	-	-	++	+	-	++
B-78	F	10	-	-	-	+	+	-	++
B-77	F	11	-	-	-	++	-	-	++
B-76	M	25	-	-	-	++	-	-	+++
B-75	M	12	-	-	-	+	-	- -	+++
B-74	M	12	-	-	-	++	-	-	++
B-73	F	13	-	-	-	++	-	-	+++
B-72	F	13	-	-	-	++	-	-	++
B-71	F	14	-	-	-	++	-	- -	++
B-70	M	14	-	-	-	++	-	-	++
B-69	F	15	-	+++	-	+	-	-	++
B-68	M	15	+++	+	-	+++	-	-	++++

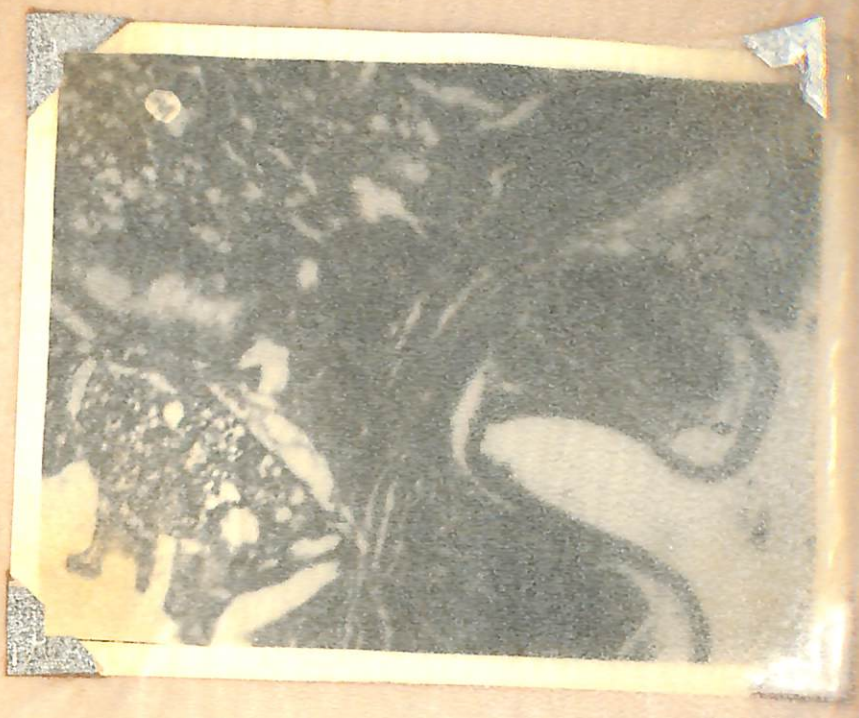
Page No. 52.

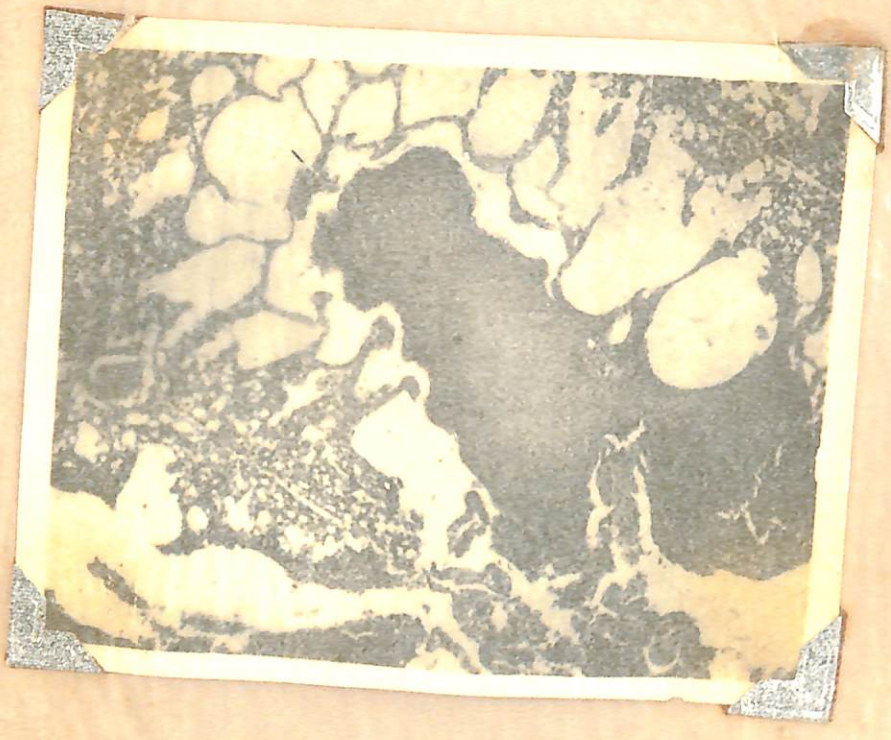
- denotes no lesions.
+ denotes mild.
++ denotes severe.
+++ denotes more severe.
++++ denotes very severe.

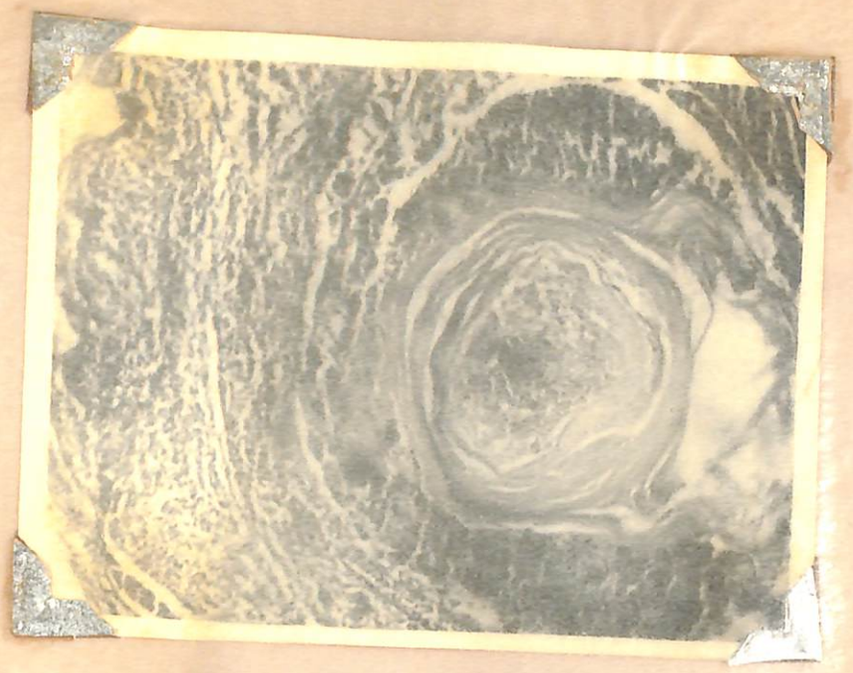


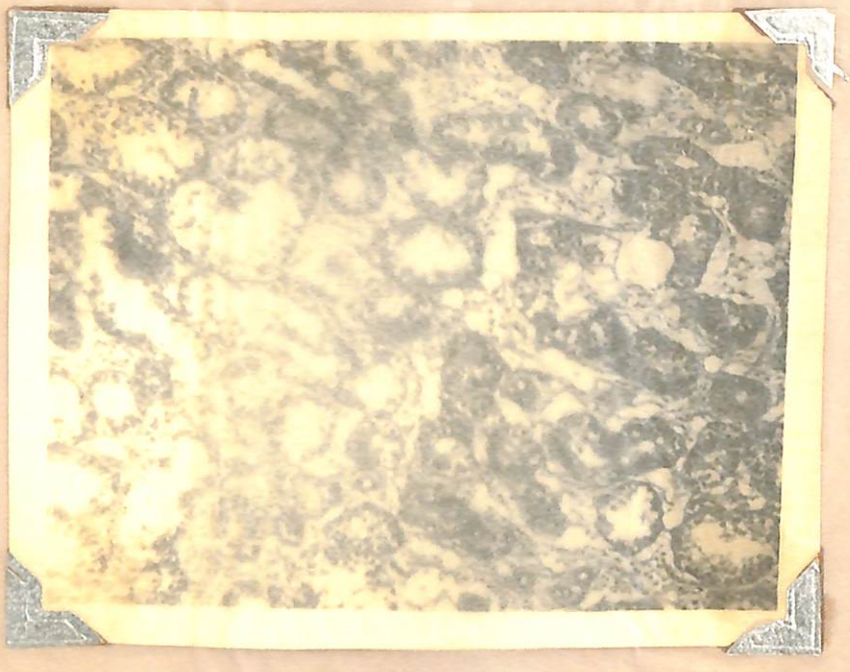


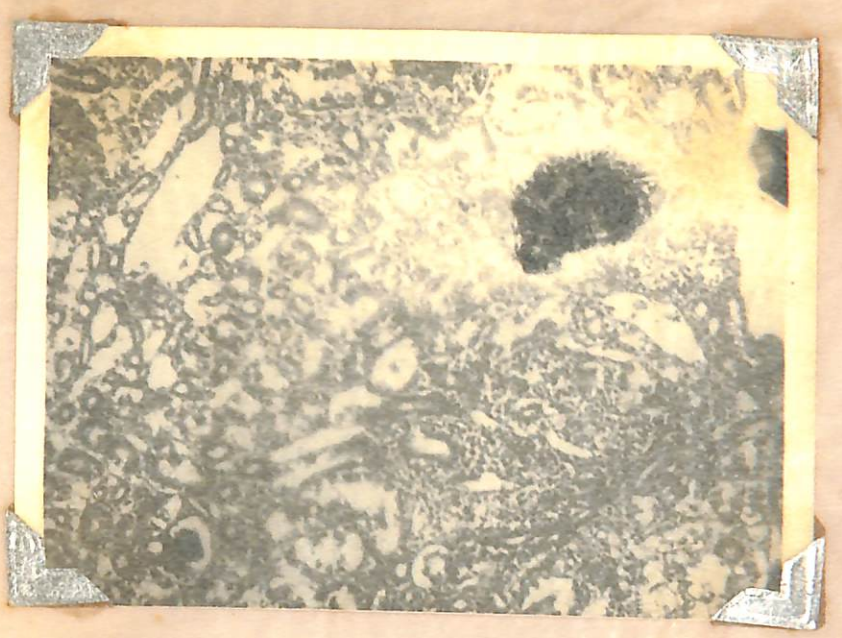












CHORIO ALLANTOIC MEMBRANE (C.A.M.) :

(i) Gross-pathology :

The chorio allantoic membranes infected with F.P. virus suspension ranging from 10^{-3} to 10^{-6} dilutions showed congestion and slight oedema. The C.A.M. infected with 10^{-7}, 10^{-8} and 10^{-9} dilutions revealed no appreciable gross lesions. Controls were negative for any gross lesions.

(ii) Histopathology :

The cellular lymphocytic infiltration along with oedematous changes were well marked throughout the zone of ectoderm, mesoderm and entoderm. At some places, areas of congestion along with perivascular lymphocytic infiltration were observed. Many of the ectodermal and entodermal cells were vacuolated, resulting in loss of cellular details at some places. Exudative changes were also seen. C.A.M. from controls and C.A.M. infected with 10^{-7}, 10^{-8} and 10^{-9} dilutions showed no remarkable pathological changes (Fig. 21 to 23).

MORTALITY RATE :

Mortality rate is presented in Table XIX. 16.6 percent of infected birds died in batch-II and in batch-III

due to F. P. disease. There was no mortality recorded in batch-I. The general mortality in male affected chickens was 8.33 percent whereas in female birds it was approximately 14.28 percent.

TABLE XIX.

Mortality Rate in Different Batches of the Chickens after Artifical Infection.

Batch No.	Bird No.	Sex	Time of sacrifice (no.of days post-infection)	Mortality.
I	Nil	Nil	Nil	Nil
II	B-56	F	6	+
II	B-60	F	8	+
III	B-78	F	10	+
III	B-75	M	12	+

+ denotes mortality recorded.

Percentage of Mortality.		General Mortality in Male birds(%)	General Mortality in Female birds(%)
Batch- I	0.0	8.33	14.28.
Batch- II	16.6		
Batch- III	16.6		

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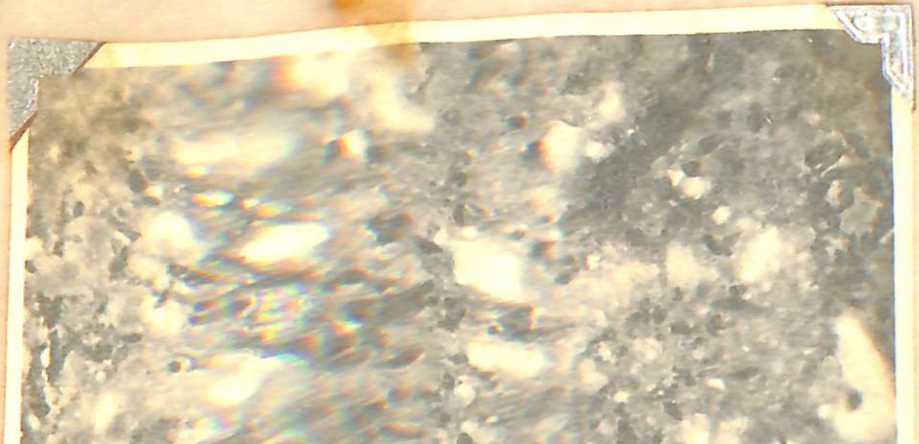

DISCUSSION AND CONCLUSIONS.

A considerable difference in the nature of the disease occurring naturally and the disease produced experimentally has been observed, probably due to the change brought in the virus during the laboratory adaptation. Goodpasture (1959) reported that lesions in birds experimentally infected with a mutant strain of fowl pox virus, differ considerably from those caused by classical field strain.

The results obtained in this study are due to infection of W.L.H. chickens of 2-4 months age with the local virus strain (Livestock Research Station, Bihar, Patna).

The symptoms observed were dullness, drowsiness, rise of temperature, and restlessness in few. In general, the birds were off-feed. The temperature of all the inoculated birds showed a rise of 2-3°F on the 3rd day and continued almost upto 11th day. The characteristic cutaneous form of lesion appeared on the 6th day after the birds were experimentally infected. The macules turned quickly to papular form and by the 8th to 10th day post-infection vesicles were formed. Some of the vesicles were observed to coalesce with the adjacent vesicles resulting into large diffused lesions. The birds were observed to be very restless and showed signs of respiratory distress. Three birds which were observed to show the "Ocular form" of the disease, exhibited watery

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discharge from the swollen eyes. The discharge turned purulent, thick and viscid with the advancement of the disease which resulted in closure of the eyelids. By the 15th to 16th day from the onset of the disease, the vesicles progressed to scab formation. Inoculated birds showed a tendency to return to feeds after the pyrexia stage was over.

Reduction in egg production could not be recorded as the inoculated chickens were not layers.

In the present study 85 percent of the inoculated birds showed cutaneous form of the lesions; 9 percent of the birds showed ocular form of the lesions and the rest accounted for the mouth form of the lesions. The different forms of the disease observed were probably due to (1) the change in the viral infectivity because of maintaining the virus in the laboratory conditions for a long period of time and due to (2) the individual response of the inoculated chickens.

The fowl pox inoculated birds revealed marked leucopenia with a high degree of neutrophilia. As a general rule, lymphocytes were reduced in all the infected birds. The infected birds which showed increase in the R.B.C. count and Hb. percentage also showed a higher erythrocytic sedimentation rate.

The percentage of eosinophils and basophils decreased in the affected birds whereas monocytes were slightly

increased. A general decrease in the percentage of the packed cell volume was observed in the affected birds but it was not constant. Clotting time of blood remained unchanged.

The increased percentage of neutrophils could have been due to reaction to the infection by the host. Lymphopenia might have been simply because of neutrophilia and leucopenia was due to the result of inappetance, and debility. Further, the virus might have a depressant action on the haemopoietic system. Slight reduction in haemoglobin content may be due to disturbed appetite during the course of the disease.

Findings of this study are similar to those recorded by Blount (1947) so far the leucocytic picture in experimental cases is concerned.

Schalm (1961) said that leucopenia was common to most diseases caused by viruses, which have been also confirmed by this study.

The affected birds on the postmortem examination presented mainly the inflammatory and necrotic changes. In the present experiment, no characteristic lesions were observed till five days post-infection. The lesions appeared from the sixth day onwards.

The "Cutaneous form" presented wart-like nodular

eruptions on the unfeathered parts of the body and diphtheritic membranes in the mouth in the oral form was seen. The lesions almost covered the entire unfeathered skin over head including comb, wattles, face, eyelids, earlobes, and corners of the mouth. Characteristic cutaneous lesions as described in text books were observed.

In the "Mouth form", whitish, opaque slightly elevated nodules were observed over the mucosa of the mouth, oesophagus and crop which contained yellowish, cheesy and necrotic material.

The "Ocular form" was characterised by a watery discharge in the eye due to conjunctivitis which later on turned to become purulent.

Macroscopic examination of liver showed slight enlargement, with greyish discoloration and paleness in some of the cases. Necrotic areas were also seen in few. Spleen was enlarged in majority of the cases possessing minute greyish spots. Kidney was enlarged in most of the cases with slight fragility associated with areas of discoloration.

Lungs grossly showed areas of congestion and consolidation in 60 percent of the cases, associated with slight haemorrhagic patches over the air sacs. In case of, birds showing respiratory distress, the trachea was filled with mucus exudates. Heart was enlarged with prominent coronary

blood vessels on its surface.

The skin nodules revealed necrotic changes in the superficial epithelial layer, resulting in complete loss of cellular details. The underlying epithelial cells showed proliferative changes with marked distension or "ballooning" of the individual cells. Few of these cells revealed the presence of globular, refractile inclusion bodies due to which the nuclei were pyknotic and eccentric. The underlying stromal elements were oedematous and there were presence of keratinization, distortion and disintegration of dermal connective tissue with diffuse lymphocytic infiltration. Vacuolation was well marked in epidermal epithelium accompanied by engorgement of dermal blood vessels.

In liver, disintegration of hepatic lobules, diffuse necrotic areas surrounded by reactionary zones were seen in some of the cases. Loss of lobular architecture, focal necrosed areas with disintegration of hepatic cords associated with lymphocytic infiltration in perivascular areas were significant features in some of the cases.

Parenchymatous focal degenerative and disintegrative changes of the kidney associated with peritubular and periglomerular cellular (lymphocytic) infiltration were the significant lesions which might be of some diagnostic value. In few cases, spleen showed severe congestion.

In lungs, the architecture of alveolar walls were

broken at several places and thinness of alveolar walls were marked at several places. Severe congestion along with pneumonic changes ware the most significant feature noticed in about 60 percent of the affected birds. It could be deduced that the birds after losing resistance due to the disease might have got secondary bacterial invasion leading to the respiratory trouble. Access to other bacterial infections may be due to debility, weakness, loss of body resistance owing to viral invasion. Congestion along with slight degenerative changes in spleen might be due to acute viraemia.

Mortality rate was 8.33 percent in male birds and 14.28 percent in females. It shows that female birds had less resistance than the males due to which the former has recorded higher mortality.

The chorio allantoic membranes infected with F.P. virus suspension ranging from 10^{-3} to 10^{-6} dilutions grossly showed congestion and slight oedema but higher dilutions than this had no effect on the C.A.M.

Microscopically, the C.A.M. revealed cellular lymphocytic infiltration, oedema, congestion, perivascular lymphocytic infiltration, vacuolation in ectodermal and entodermal cells and exudative changes when virus dilutions of 10^{-3} to 10^{-6} were used. Similar observations about the lesions produced on the C.A.M., have been made by several workers as recorded earlier.

CHAPTER - VI.

SUMMARY.

S U M M A R Y.

1. Blood of 33 unvaccinated W.L.H. chickens of both sexes of different ages was examined both before and after experimental infection.

2. The chickens were experimentally infected with a local strain of fowl pox virus (Livestock Research Station, Bihar vaccine-cum-challenge strain) by the skin scarification method in different batches.

3. Three groups each of 9-12 chickens were experimentally inoculated with two control birds in each group.

4. Inoculated birds began to show clinical symptoms viz. rise in temperature on the third day post-infection, by 2-3°F., lachrymation, discharge from nostrils, inappetance, depression and weakness.

5. The cutaneous form was observed to be the commonest (85 percent), with 9 percent ocular and 6 percent mouth form.

6. Haematological studies were carried out in all the groups right from the commencement of the experiment.

(a) Decrease in haemoglobin, variable red cell count, E.S.R. and packed cell volume were recorded.

(b) Leucopenia was the significant feature found in all the affected birds.

(c) There was slight increase in the percentage of monocyte with decrease in eosinophils and basophils.

(d) The differential count showed marked neutrophilia and lymphopenia.

7. (a) Lungs were severely congested along with other pneumonic changes.

(b) The skin nodules revealed degenerative and necrotic changes in the superficial epithelial layers resulting in the complete loss of cellular details. Epithelial cells showed proliferative changes with marked distension of cells revealing the presence of globular, refractile inclusion bodies. Keratinisation and vacuolation in epidermal cells were clearly marked.

(c) Liver showed necrotising and degenerative changes along with lymphocytic infiltration around blood vessels.

(d) Kidney showed degenerative changes and periglomerular and peritubular lymphocytic infiltration.

(e) Spleen was congested.

(f) Testis was inflammd.

(g) This strain of virus though dermotropic has also shown pneumotropic characteristics.

(h) The age did not have any significant effect on

histopathological or haematological changes.

8. The mortality rate was observed to be higher in female birds.

9. The chorio allantoic membranes infected with this virus revealed vascular, oedematous, exudative and degenerative changes.

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