EXPERIMENTAL STUDIES ON THE PATHOLOGY OF RANIKHET DISEASE IN RELATION TO AGE IN POULTRY

C. D. N. SINGH

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I certify that this Thesis has been prepared under my supervision by C. D. N. Singh, a candidate for the M. Sc. (Vet.) with Pathology as major subject, 1962, and that independent incorporates the results of his study.

Dated the 15th October, 1962.

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EXPERIMENTAL STUDIES ON THE PATHOLOGY OF

" RANIKHET DISEASE "

IN RELATION TO AGE IN POULTRY

A Thesis

submitted to the Magadh University, Gaya, in partial fulfilment of the requirements for the Degree of M.Sc., (Vet.) in the Faculty of Veterinary Science.

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C.D.N.Singh
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CHAPTER I

INTRODUCTION

Ranikhet Disease (RD) may be defined as an acute, febrile, contagious and infectious disease of fowls caused by a filterable virus and characterised by difficulty in respiration with high mortality. It is quite probable that Ranikhet Disease has been existing for centuries in this country and destroying the poultry in villages and towns with enormous losses. It was first identified in this part of South East Asia by Edwards (1927). Ranikhet Disease is the most serious and outstanding poultry disease that requires great effort to be combated effectively.

The host range of Ranikhet Disease virus appears to be an expanding one and is capable of attacking pigeons, guineafowls, chicks, geese, pheasants, partridges, crows, sparrows, grouse, doves, ducks, mayas and several other species of birds while ducks and geese are relatively resistant. Artificially mice, hamsters, monkeys, dogs, guinea-pigs, rabbits, ferrets, swine, calves, cats, bats, hedge-hogs and chinchillas have been infected with this virus. A few cases of laboratory infections in man have been recorded.

In nature, fowls contract the infection by ingestion of contaminated food and water and cohabitation of healthy and disease birds. The alimentary and respiratory channels are the main avenues of natural

infection. Air borne spread of Ranikhet Disease virus has been shown by several workers.

According to Kaschula (1961), two main types of Ranikhet Disease, namely Mild American form and Virulent Asiatic form of the disease are recognised. The mild form of American NewCastle Disease is usually air borne and appears to infect mainly through respiratory tracts whereas the virulent Asiatic form of Ranikhet Disease appears to spread through the infected drinking water and food. The principal lesion in the American form is an aerocystitis and no significant lesions in the alimentary canal are noticed on autopsy. In per acute or acute virulent Asiatic type which is found in most tropical and subtropical countries including India, the mortality rate is very high and even it may be upto cent per cent. Characteristic haemorrhagic or ulcerative lesions occur in the alimentary tract. The lesions such as extensive hyperaemia and proliferation of the endothelial cells in the central nervous system have also been recorded by Sullivan (1958). Respiratory difficulty appears to be due to nervous failure rather than due to lesions in respiratory system. The incoordination, tremor and the resulting torticollis and paralysis of the limbs or viscera are also said to be due to involvement of the central nervous system. In this connection, it will be interesting to note that in pegeons, the virulent experimental form produces nervous symptoms as the main feature of the infection (Doyle, 1927) and in

mild American form, a reaction is noticed when injected intracerebrally.

The Ranikhet Disease virus is very labile and undergoes variation and mutation. The variability in pathogenicity has been shown by Hanson (1949). Jungherr et al (1946) showed that changes in tissue tropism can account for variations in such properties as invasiveness of the virus and in the symptomatology, course and pathology of NewCastle Disease infection. It is also well known that the tropisms of Ranikhet Disease virus may vary i.e., from low to high degree with neural, visceral and pneumal affinity. In short, the survivality, adaptibility, long range of infectivity for avian and mammalian species and variability for different tissues among different species as well as among strains of Ranikhet Disease virus from place to place are surprisingly peculiar to this disease.

According to Sahai (1937), it breaks out usually in June and July after the rains and out-breaks may continue upto the December or January and occasionally it may occur at other times of the year also. In many areas in this State the disease is enzootic subsiding during the extreme hot weather and breaking out again after the rains.

It affects chicks of both sexes and of all ages with a mortality of about 95 % - 100 % and so upsets the economics of the poultry industry. In U. S. A. and Canada, younger chicks of brooder age are killed by this disease while in

the layers not only the egg yield is reduced but owing to the dysfunction of the oviduct, the eggs are invariably thin shelled and deformed.

From the foregoing it would be seen that there are considerable variation in symptoms and in clinical and hist o-pathology probably due to the variation in the strains of the virus. But unfortunately the literature available on the haematological aspect of the disease is meagre and very few workers particularly in India have made histo-pathological studies, though as already mentioned, the disease is a great menace to poultry industry. Hence it is essential to find out the exact position with reference to symptoms and pathology of the disease in relation to a particular strain of the virus associated with the disease to facilitate correct diagnosis of the disease for effective control. To achieve this, experimental studies shall have to be undertaken with a given strain of the virus. It is with this object in view the following experiments were carried out with the local strain of the virus to get a true pathological picture of the disease as it occurs in this State of Bihar in particular and probably in India in general so that the results obtained would serve as a valuable guide in the diagnosis of the disease by pathological procedures. In this connection it may not be irrelevant to quote Smith and Jones (1958) who said," Even some of the first of these diseases to be recognised, anthrax, for example, could be restudied with profit in the light of present day knowledge and with modern techniques".

CHAPTER II

REVIEW OF LITERATURE

A historical account of Ranikhet Disease

Edwards (1927) identified this disease for the first time in this country and gave the earliest description of the disease under the title of " A New Fowl Disease". It occurred in the form of a serious outbreak with considerable loss at Ranikhet, a town in Almora district in the Kumaon foot hills of the Himalayas. The disease was later recognised in the Garhwal district and at Lucknow. This was followed by its recognition in several other parts of the country within a couple of years.

Doyle (1926) had discovered a similar disease in a poultry farm near New Castle on Tyne in U.K. which he had described under the title of "New Castle Disease" after the name of the place and also demonstrated the filterability of the causative organism.

What is now generally designated as New Castle Disease was first reported in 1926 by Kraneveld to be prevalent in the Dutch East Indies as a highly diffusible and fatal infection of the poultry.

In India, Cooper (1931) and Sahai (1937) had observed the disease in crows in the vicinity of the poultry farms where the disease was raging but now it is uncommon to find crows dying in the vicinity of the infected farm.

Following the above discoveries, similar if not

identical diseases were recorded by investigators in various parts of the world, including India and under the same or other names. Wave (1930) and Cooper (1931) reported that the Indian virus was immunologically identical with the strains of the virus from Great Britain, Java and Philippines, i.e. these were one and the same virus.

Literature on the symptoms and pathology of Ranikhet Disease-

Doyle (1926) reported NewCastle disease as an acute febrile contagious infectious disease resembling fowl plague. There was rise of temperature from 2nd day which fell abruptly to below normal just before death, a gasping inhalation through half opened beak, a thick mucous discharge from the nostrils and a varying amount of frothy exudate hanging occasionally in threads, from the end of the beak and a general cyanosis of the comb and wattles and a watery yellowish diarrhoea with a characteristic nauseating odour. There was no characteristic postmortem lesion. Haemorrhages were found on the gizzard fat, pericardial sac, heart muscle and lining membrane of the proventriculus. There was generally a catarrhal enteritis in the duodenum. The lungs were generally normal in appearance.

Since then, several workers gave description of the symptoms of Ranikhet Disease which differed in respect of some minor points.

Edwards (1927-28) laid stress upon the almost entire absence of lesions in Ranikhet Disease.

Doyle (1926), Farinas (1930), Cooper (1931) and Orr and John (1946) found the incubation period to be 5 days, 2 to 4 days, 3½ days and 2 to 4 days generally in acute cases.

Farinas (1930) noted the NewCastle Disease to be a febrile disease which was followed by an irregular or sub normal temperature. Cooper (1931) saw a characteristic drop in temperature to a marked degree in artificially infected birds. Doyle (1938) found his majority of artificially infected birds show febrile reaction. Orr and John (1946) found that the average maximum temperature reached was 111°F in artificially affected cases and the temperature fell rapidly from the peak to a normal or sub normal temperature. It was later on shown by workers in this country that a febrile reaction is fairly common symptom of the disease caused by the Indian strain of the virus. Kaschula and Canham (1946) noted that in the birds affected with NewCastle Disease, temperature seldom exceeded 109° F. Cooper (1931), Kylassamaier (1931), Iyer and Dobson (1940), Albiston and Gorrie (1942), Iyer (1943), Orr and John (1946), Kaschula and Canham (1946), Gordon, Reid and Asplin (1948) and Kaschula (1961) reported discrete petechiae or ecchymoses in the proventiculus of the birds affected with Ranikhet Disease. Cooper (1931) found that except the proventiculur lesions, post mortem lesions of diagnostic value were almost entirely absent. He saw completely no recognisable lesion in the lungs of the birds showing extreme respiratory distress during life. Kylassamaier (1931) found the histopathological lesions in the proventriculus in

the form of distortions of the gastric follicles and in the intestines as distortions of the intestinal villi with super imposed bran like deposits. Kuppuswamy (1934) concluded that in fowls, the lesions are more marked in the alimentary canal than any where else and the nervous symptoms were much more marked in pigeons affected with Ranikhet Disease Virus than the lesions found in the alimentary canals. Shirlaw (1937) noted that neuroparalytic form of the disease is on the increase and recent work has shown that the causal virus is probably neurotropic in character. Haddow (1937-38) found that the occurence of the neurotropic form of the Doyle's disease in fowls was again observed in several experimentally infected fowls. Iyer and Dobson (1940) noted that the trachea and lungs were usually unaffected.

Albiston and Gorrie (1942) found the usual symptom of Newcastle disease. They saw that the manifestation of nervous abberrations characterised the chronic case. Paralysis of one or both limbs, torticollis accompanied by a circling movement of the head and a general disturbance of equilibrium were noticed. Iyer (1943) noted that there was a considerably prolonged course of the disease in birds showing involvement of the nerves and paralysis. In chronic cases, as a result of injury to nerves, twitching of the head and neck and lameness and paralysis of the legs and wings may occur (Kaura and Iyer 1937). He found haemorragic lesions in the duodenum and rectum and congestion in the liver, spleen, kidneys and strangely little change in the lungs.Orr and John (1946) reported

characteristic localised lesion resembling diphtheritic pseudomembranes on the buccal and pharyngeal mucosa.They noted enteritis in duodenum, bran like diphtheritic lesions and ulcerations in the large intestines and caeca, congestion of liver, spleen and kidneys and slight tumour splenis. Kaschula and Canham (1946) found diphtheritic laryngitis and small cheesy deposits on the congested mucosa of the pharynx. They found leg weakness and the fowl frequently lying on their sides. Different forms of paralysis of the wings, legs and neck were seen in some of the recovered birds. Kaschula (1961) found necrotic patches in the proventriculus and caeca and bluish red lesions in the wall of the intestines and also pointed out that the pattern of distribution of these lesions in any one site is very similar in birds dying at the same stage of the acute disease. He saw swelling of the spleen in per acute and acute cases, congestion and acute inflammation of the kidneys in acute cases and not highly characteristic pin point haemorrhages and catarrhal inflamma tion in the tracheal mucosa.

Jungherr and Minard (1943), Jungherr et al (1946) Beach (1948) and Schoening and Osteen (1948) reported that in the Avian Pneumoencephalitis i.e. the American equivalent of NewCastle Disease symptoms such as involvement of the respiratory and central nervous system are usually seen. Jungherr and Minard (1943) studied NewCastle Disease from the gross and histological stand point and found grayish or occasionally yellowish discolorations of the pulmonary air

sacs in 80 % of cases. They recorded splenic and nervous lesions but no constant lesion in heart, liver, pancreas, gizzard, intestine and bursa of Fabricious. Beach (1948) found that in laying flocks, the disease manifests itself solely by coughing, decline in egg production and imperfectly formed eggs. Jungherr et al (1946) found that the basic microscopic pathology of NewCastle Disease was found to be necrotizing in character in the abdominal viscera and proliferative in the lung and central nervous system. Biswal and Morril (1954) found that imperfect and soft shelled eggs were laid by some of the infected birds and degeneration of the Graafian Follicles resulted in the arrest of ovulation and subsequent oviposition.

Haematology in Ranikhet Disease

The studies on haematology in Ranikhet Disease have been undertaken by very few workers in India.

Sharma and Seetharaman (1950) examined heart blood of fifteen adult Rhode Island Red male fowls during health and after the onset of Ranikhet Disease induced artificially. During the disease they found a marked leucopenia associated with a distinct rise and fall in the percentage of heterophiles and lymphocytes respectively, and the number of erythrocytes and haemoglobin concentration dropped markedly and the corpuscular volume was reduced. There was a significant reduction in the time taken for the complete sedimentation of cells. Percentages of essinophils, monocytes and basophils did not undergo any appreciable variation during the disease.

Chandra Sekharan and Krishnan (1958) made a study in respect of an outbreak of Ranikhet Disease and using standard methods examined blood picture in 10 spontaneous cases. They found that during the course of the disease, there was slight rise in total RBC count, haemoglobin value and packed cell volume and reduction in total white cell count and sedimentation rate. Differential count of leucocytes showed marked reduction in lymphocytes and a significant increase in heterophiles. But the basophiles, monocytes and eosinophils remained almost unchanged.

No literature on chloride value of blood in Ranikhet Disease was available. The chloride value of blood in normal chicken as quoted by Sturkie (1954) varies from 376.0 to 563.0 milligrams per 100 c.c. of blood as recorded by different workers.

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CHAPTER III

MATERIALS AND METHODS

Management

Day-old-chicks of both sexes were obtained from one source i.e., Central Poultry Farm, Patna. They were kept in Electric Foster mother in an insect proof room and were fed on the same standard chick mash as used in the said poultry farm during the period of experiment. There was constant availability of water in the room. The same feeder and water-pans were utilised for a particular group of experimental chicks. The droppings of the chicks were removed two times a day (morning and evening). All equipments taken into this room were thoroughly cleaned. Hands and legs were washed in a disinfectant (2.5 percent lysol) before entering and leaving the room.

Blood collection and examination

When the chicks became 56 days old, they were divided into three groups, each group consisting of 12 birds and a control group of 18 birds to provide 6 controls for each experimental group by random assortment on the basis of their weights. The numbered birds were kept in three big separate cages to avoid any mixing. Blood was collected from the hearts of the chicks before the birds were fed. 2.5 cc of blood were immediately placed into a test tube containing a suitable quantity of Wintrobes isotomic ammonium and potassium oxalate mixture. Blood fils for differential counts were generally made from a separate drop of blood direct from the syringe. The blood was collected from the heart by Hofstad's technique. The tubes were shaken for about 2 minutes to ensure a thorough

mixing of the anticoagulant and blood. The blood was examined for haemoglobin percentage, sedimentation rate, corpuscular volume, total erythrocyte and leucocyte counts, fragility of the red cells, differential count and blood chloride value.

In the present investigation 36 experimental birds were subjected to different haematological tests during the pre and post infection period. The age difference among the birds of three batches was of two weeks duration. The same haematological procedures were adopted in the three batches of the experimental birds. After the blood from each of the 3 batches of fowls was examined, all the birds were subjected to H.I.test for the presence of immune body present either by congenital immunity or acquired immunity. Their excreta was examined for protozoal or other parasitic infections. Only those birds found free from any disease and carrying no antibodies for Ranikhet Disease and showing normal cloacal temperature were infected with a local strain of virulent Ranikhet Disease virus intramuscularly into their thigh muscles with 0.5 cc of 1/100 dilution of virulent material. The route of infection and dose of the virulent Ranikhet Disease virus were kept constant in all the three batches during the course of experiment. Haematological values including blood chloride value of all the birds were determined during the post-infection period prior to their killing. Two birds were usually killed at the interval of 24 hours. Two healthy

birds (controls) of the same age group of each batch were killed for histological studies. The temperatures of the birds were recorded every day after infection. Some of the birds died due to acute infection of Ranikhet Disease before any haematological test could be performed. Although two birds per unit of observation would not be expected t o give satistically reliable data, main pathologic trends over the observational period seemed to be highly significant. The data obtained includes total red cell count, total and differential white cell counts, haemoglobin percentage sedimentation rate, packed-cell volume, cell-fragility and chloride in milligrams of Nacl per 100 cc of whole blood.

1. Preparation of stained blood smears

Properly spread blood films were made on flammed and polished absolutely clean slides. Uniform smears thus prepared were stained by modified Wright's staining technique. 200 leucocytes were counted in each slide following the "battlement" system (1 mm.down, 1 mm across and 1 mm up)

2.Haemoglobin determination

Haemoglobin percentage was estimated by Sahli-Haden Haemoglobinometr following the directions for its use.

3. Total erythrocyte and leucocyte counts

Mammalian type blood techniques are unsuitable for avian blood. Disrupted nucleated erythrocytes leave nuclei that may be confused with lymphocytes. The methods tried to date fall into three main categories - the indirect, semi direct and the direct methods of counting leucocytes.

A. Indirect - this method consists of blocking out an area of a blood smear and counting all the cells in the area.

B.Semi direct

Wiseman's method ; makes use of special staining wbc diluent that intensified the eosinophilic cells and stains also the erythrocytes a distinct pink colour.

C. Direct

A stained wbc diluent is used that stains the wbc allowing them to be differentiated from the rbc in the counting chamber, this allows the direct counting of wbc.

In the present work, semi direct diluent was used.

Wiseman's

Phloxine - 50 mgm

Formalin - 5 cc

Ringer's solution - 95 cc

The erythrocyte pipette was filled to 0.5 mark with blood and rest of pipette was filled to 101 marks with Wiseman's diluent. The pipette were kept in a refrigerator over night to obtain maximum staining of the cells and on the following morning, the red cells were counted in 80 of the smallest squares and as the blood was diluted 200 times, the number of red cells counted in the 80 squares was multiplied by 10,000 to give the number of erythrocytes per cubic millimeter of blood.

The number of acidophilic granulocytes was counted in the entire ruled area of the haemocytometer. A differential count on a properly stained blood smear was performed to obtain the proportion of heterophilis, eosinophils, lymphocytes, monocytes and basophils in

percentage. The total number of leucocytes was calculated according to the following formula -

$$\text{Total leucocytes count} = \frac{\text{No. of acidophilic cell count} \times 10 \times \text{dilution} \times 100}{\text{Percentage of acidophilic cells in the blood smear} \times \text{Number of square mms counted in the haemocytometer}}$$

4. Corpuscular volume and sedimentation rate

Scrupulously clean Wintrobes haematocrit tubes (length 11 c.m. ; bore 2.5 mm. graduated with centimeter and millimeter scale 10 cm. in lenth) were used. Two such tubes were filled with oxalated blood from the two birds upto the mark '10' and were allowed to stand vertically. Simply one reading was taken at the end of one hour. The Wintrobes tubes were afterwards centrifugalized at the speed of 3000 r.p.m. for 50 minutes to determine the corpuscular volume which was recorded as the number of ml of cells per 100 ml of blood.

5. Fragility of the red corpuscles

Fragility or resistance of the erythrocytes were determined following the method based on Creed's Technique (1938). The blood collected previously into Wintrobe's isotonic ammonium and potassium oxalte mixture was used in this test. The point at which haemolysis began i.e. minimum resistance and the point at which all cells were haemolyzed i.e. maximum resistance were recorded.

6. Blood chloride value estimation

It was estimated by adopting the technique given in the manual of colorimetric clinical analysis with the Fisher Clinical Electrophotometer.

Preparation of tissue and staining

The different organs of the killed birds and the birds died due to infection were preserved at the earliest opportunity in 10% formol-saline solution for histopathological examinations. Tissue sections were stained with haematoxylin-eosin and sections from brain materials were stained by Macchiavello's technique (modified) to demonstrate the presence of inclusion bodies.

CHAPTER IV

RESULTS

The artificial infection experiments in the chicks were performed during the months of May and June, 1962. The disease (RD) appeared in its acute form in the infected chicks. No subacute, sub-clinical, asymptomatic or chronic case could be seen. The disease was recognisable 48 hours after infection and none of the birds survived beyond the fourth or fifth day after infection. The birds were destroyed during the fourth and fifth day after infection when the symptoms were most severe. The haematological studies in some of the birds could not be made because they were found dead before collection of blood could be made. The birds killed at intervals of 24 hours upto third day did not show well developed lesions of Ranikhet Disease but the birds dying or killed three to four days after artificial exposure showed typical symptoms and lesions. The naked eye lesions were prominently haemorrhagic and necrotic in nature and confined to the alimentary tract. The haemorrhagic and necrotic lesions of the intestine and exudative changes in the lungs appeared to be the outstanding feature with this local RDV. This strain of the virus showed viscerotropism and pneumotropism as evidenced by lesions in the alimentary tract and lung.

The autopsy of the controls did not reveal any lesions in their body.

Symptoms

The birds appeared reasonably normal for the first 48 hours. There was a clear rise in temperature on the 3rd day in all batches. Febrile reaction was of uniform type in all the birds but it was seen to fall to normal before death in a few cases as is shown in the table 1, 2 and 3. The dullness shown by the birds in the beginning developed rapidly into a marked depression and weakness (Fig.2). The bird no.2633 of the first diseased batch (62 days old) showed a body temperature of 107° F after 24 hours of infection while the bird no.2628 of the same diseased batch showed 110 ° F after 96 hours of inoculation.

Copious greenish white or yellowish white watery diarrhoea resulting in a rapid dehydration was noticed. The body feathers were ruffled while the ventral feathers were soiled with faeces.

The birds were gasping for breath with extreme dyspnoea,with half opened beak (Fig.3.). Variable amount of frothy or tenacious exudate in the mouth and pharynx and dribbling of stringy saliva were seen.

The birds showed complete debility and prostration with paralysis of the wings and legs in most of them. They were lying on their sides and unable to stand in the cages and pick up their feed. The main striking symptoms were the signs of incoordination which developed into twitching of one or more extremities of of head and neck. The birds were found crouched in the cage. They were dozing,closing and opening partly their eyes. There was drooping of the

wings leading to their paralysis (Figs. 4 & 5). Some of the birds were in a somnolent stage and these were invariably paralysed.

Thus digestive, respiratory and nervous disturbances were found to be associated with this local strain of the virus (RD).

Gross-pathology

The necropsied birds presented mainly haemorrhagic inflammatory and necrotic lesions of the disease. The appreciable lesions could not be noticed in the birds killed during the first 72 hours but the lesions appeared generally in their fully developed forms in the birds killed on the 4th or 5th day. The alimentary tract showed variable degree of congestion in its different portions. The proventriculus revealed ecchymoses in its mucosa only on the 4th or 5th day of infection in the chicks in 1st, 2nd and 3rd batch respectively. Petechiae and haemorrhages were seen in the proventricular lining membrane in most of the cases. Catarrhal enteritis was usually present in the duodenum and also other portions of the intestine. Oblong and bluish red or grayish lesions were also noticed in certain sites in the small and large intestinesof the infected chicks of 1st, 2nd and 3rd batches quite constantly irrespective of age (Fig.6,7,8 & 9). The pattern of distribution of the lesions has been shown diagrammatically in Fig.A. Caecal tonsils and caeca

revealed haemorrhagic necrotic lesions in all cases. The cloaca showed several localised haemorrhages and also a few ulcers in some cases. Petechiation of the proventricular mucosa, duodenum, caecal tonsil and cloaca were constant postmortem findings. The gross-pathological changes were more or less same in all cases killed during acute stage of the disease. Bird No.317 showed oedema on left auricle and also haemorrhages on the heart. Bird No.84 also showed pinpoint haemorrhages on the heart. The lungs were congested and consolidated. In spite of careful observations, the trachea and air sacs were almost normal. In 'chronic respiratory disease,' caseous exudate in the air sacs and beaded appearance of these sacs are usually found, in 'infectious bronchitis 'catarrhal or fibrinous inflammation of the air sacs and presence of yellowish and caseous plugs in the lower trachea and bronchi are found, while in 'infectious laryngotracheitis,' haemorrhagic tracheitis is found ; none of these lesions were found in cases of Ranikhet disease.The liver, spleen, kidneys and pancreas were congested and in a few cases, the liver was pale, soft and friable. The spleen was either normal or slightly enlarged in most of the cases, whereas Todd (1954), Beaudette (1956) and Kaschula (1961) had found that the spleen was considerably enlarged. In the present series of experiment, only in one or two cases greatly enlarged spleen was noticed. The brain did not reveal any visible change in several cases but in a few cases, it was also slightly congested or abnormally pale. In bird no.86

and bird no.79 the breast muscle was highly gelatinised.

Histopathology

The tissues of the affected organs presented haemorrhagic and necrotic changes and necrosis was seen particularly in and around the lymphoid patches. The lesions were mainly necrotizing in the intestine, proventriculus, spleen, kidney and liver, exudative and some what proliferative in the lung and degenerative in the heart and brain.

Proventriculus

Parietal glands and proventricular mucosa especially the epithelial lining of the villi showed necrosis. There was haemorrhagic exudate and necrosed tissue fallen from the parietal glands in the ductal openings of these glands. Haemorrhages could be noticed between the parietal glands There was necrosis, shedding of the surface epithelium particularly near the areas where the lymphocytes are located in the propria.(Figs. 10 & 11).

Intestine

Haemorrhagic and necrotic areas were noticed in the lymphfollicles and caecal tonsils. In the intestinal tract, congestion and necrosis were seen in the villi which also presented a distorted appearance in some cases. Necrosis was generally found near or in the lymphoid plaques (Fig. 12). Complete necrosis of the lymphoid plaques and desquamation of the villi were also seen (Figs. 13, 14, 15 & 16).

Liver

The cells of the hepatic laminae were necrosed. Central veins were found to be dilated more or less filled with blood. Individualization and disorganisation of the hepatic architecture were also seen. There was vacuolation of the cytoplasm, and hyperchromatic staining of nuclei. Areas of necrosis and haemorrhages and bile in some places were occasionally seen.(Figs.17,18 & 19).

Spleen

In some cases necrosis and in a few others hyalinisation in the spleen were noticed.(Fig. 20).

Pancreas

Necrosis of the acini and hyaline changes in the necrosed areas were observed. In some cases, there was lymphocytic infiltration. The islets of Langerhans were necrosed and showed eosinophilic staining.(Figs.21,22 & 23).

The observations in the proventriculus, intestine, spleen, liver and pancreas mostly confirmed the findings of Jungherr et al (1946).

Kidney

There were clear areas of hyalinisation. The tubular epithelial cells showed degenerative changes leading to necrosis and desquamation (Fig. 24.). Glomeruli also showed degenerative and necrotic changes (Figs 25 & 26).

Lungs

Alveoli were distended mostly with red cells and

bronchioles were plugged with bloody exudate. Large areas of haemorrhages and consolidation could be seen in many cases. In some cases, there was thickening of the alveolar epithelial cells and interstitial tissue as will be seen in the Fig.27. Haemorrhagic exudate was present in the parabronchi (Fig.28).

Heart

There was oedema of pericardium and fragmentation of the cardiac muscle fibres in some cases. Degenerative changes and round cell infiltration were noticed in the myocardial fibres (Fig. 29).

Brain

Neuronal degeneration was clearly marked and neuronophagia and satellitosis were also seen. Haemorrhages and liquefaction were noticed (Figs. 30 & 31).

Haematology

It is clear from the comparison of the average blood values of the chicks during the pre and post infection periods that the birds infected with Ranikhet Disease showed a marked fall in leucocyte count [Vide Table 1 and 1 A 12163 (Disease)-19666 (Normal)], an increase in the percentage of heterophiles [Vide Table 1 and 1A 62.9% (D)-35.3 %(N)], a clear rise in the red cell count [Vide Table 1 and 1A 3.27 millions (D)-2.77 millions (N)], a slight rise in haemoglobin percentage [Vide Table 2 and 2A 10.6% (D)-9.5%(N)] and a decrease in sedimentation rate [Vide Table 1 and 1A 1.24 mm per hour (D)-2.5 mm per hour (N)]. These findings corrobrate the findings of Chandrasekharan and Krishnan(1957).

1 1 1 1 1 1 3

TABLE I A

Figures in respect of the same chicks (Batch No.1) during the pre infection period.

Bird No.	Age when examined (days)	Body temperature (°F)	R. B. C. per c.mm. (millions)	W. B. C. per c. mm.	Haemoglobin gms. %	Sedimentation rate mm.	Packed cell volume c. cm. per 100 c. cm. blood	Red cell fragility Nacl %	Mean corpuscular volume c	Mean corpuscular haemoglobin r.r.	Mean corpuscular haemoglobin concentration %	Chloride value Nacl milligrams per 100 c.cm. blood	Differential count				
													Hetero-philes	Lympho-cytes	Mono-cytes	Baso-philes	Baso-philes
2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
673	54	107.2	2.6	13000	10.4	2	28.5	0.44-0.32	109.61	40	36.49	360	45	50	3	1	2
628	54	106.8	3.87	16000	10.1	2	27	0.44-0.32	69.76	26.09	37.40	340	42	47	6	3	2
722	54	105.8	2.50	33000	11.0	2.5	25	0.44-0.28	100.00	44.00	44.00	385	27	60	9	3	1
623	54	105.8	2.56	17000	9.3	2.00	30.5	0.48-0.32	119.14	36.32	30.49	345	27	63	3	1	2
86	54	106.0	2.48	15000	11.2	3.00	32	0.44-0.32	129.00	45.16	35.00	495	39	52	8	-	-
647	54	106.5	3.10	22000	8.8	2.5	32	0.44-0.32	103.22	28.39	27.5	260	32	64	2	-	-
84	54	106.8	2.83	20000	11.1	3.0	28	0.44-0.32	98.94	39.22	39.64	490	23	70	4	1	3
79	54	107.2	2.37	18000	10.1	3.5	27.5	0.48-0.28	116.03	42.61	36.73	415	33	56	3	1	3
273	54	106.5	3.44	17000	11.2	2.5	34.1	0.44-0.28	99.12	32.56	32.84	395	36	62	3	-	-
654	54	106.4	2.42	25000	8.7	2.0	24	0.44-0.32	99.17	35.95	36.25	345	40	57	3	1	1
766	54	106.8	2.72	18000	9.0	3	30	0.44-0.32	110.29	33.08	30.00	460	56	42	5	1	-
17	54	106.0	2.80	22000	9.1	2	27	0.48-0.32	96.43	32.50	33.70	320	24	70	4	2	-
G E S		106.5	2.77	19666	10.0	2.5	27.96	0.45-0.31	104.23	36.32	35.00	392.5	35.3	57.72	4.33	1.22	1.1

The chloride values in two chicks per unit of observation were determined at the intervals of 24 hours for 6 days continuously.

TABLE 2

Figures in respect of the chicks (Batch No.2) infected with Ranikhet Disease Virus

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
305	76	24	107.5	2.58	15000	9.4	2	26	0.44-0.32	100.8	36.15	36.15	475	57	37	4	-	2
321	76	24	108.1	2.90	25000	8.4	2.5	29	0.44-0.28	100	28.96	28.96	555	48	39	8	2	3
325	76	48	110	2.59	8400	8.5	3	27.5	0.40-0.28	105.77	32.69	30.90	640	54	41	2	1	2
310	76	48	108.3	2.8	23000	11	3	31.2	0.44-0.32	111.43	39.5	35.25	600	54	43	3	-	-
324	76	72	110.2	2.6	14000	8.4	1.5	28	0.44-0.32	107.54	32.30	30.0	640	45	50	3	2	-
326	76	72	109.4	2.58	26000	8.3	2.5	24	0.36-0.28	92.30	30.2	34.58	640	45	46	6	2	1
316	76	96	109	5.20	16000	13.8	0.5	40	0.32-0.24	76.98	26.53	34.5	800	71	22	5	-	2
308	76	96	109.5	3.4	18000	12.2	0.5	37.5	0.32-0.24	110.29	35.88	32.53	900	63	24	9	2	2
314	76	120	110.2	3.6	15000	12.1	1	34.5	0.36-0.28	95.83	33.61	35.07	860	65	32	2	1	-
318	76	120	110.6	3.4	11000	11	1.5	32.6	0.36-0.28	95.88	32.35	33.74	980	78	11	10	-	1
307	76	120	107	3.9	8900	12	0.5	32	0.36-0.24	82.05	30.77	37.5	800	76	17	6	-	1
303	76	120	112	3.98	9600	12.2	0.75	35	0.32-0.24	87.5	30.5	34.85	810	72	22	3	2	1
E R A G E S --	-		109.3	3.29	15825	10.6	1.6	31.44	0.38-0.27	97.2	32.7	33.67	724.9	60.33	32	5.08	1	1.25

TABLE 2 A

Figures in respect of the same chicks (Batch No.2) during the pre infection period.

3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
66	107.5	3.24	22000	9.0	2.0	28 .0	0.44-0.32	86.42	27.77	32.14	322	35	58	6	2	3
66	106.4	2.7	18000	11.5	2.0	28.5	0.44-0.32	105.55	42.59	40.35	340	49	42	5	2	2
66	106.2	3.10	29000	10.5	3.0	26	0.48-0.32	83.87	33.87	40.38	380	30	62	7	1	-
66	107.2	2.42	33000	10	3.5	24	0.48-0.32	99.17	41.32	41.66	540	22	68	8	2	-
66	107.0	2.64	18000	8.2	3	28	0.44-0.32	106.06	31.06	29.28	420	52	40	6	1	1
66	107.5	2.62	21000	8 .0	2.5	31	0.40-0.28	118.32	30.53	25.81	340	25	66	4	3	2
66	107.0	3.14	24000	11.0	2.2	27	0.42-0.28	85.98	35.93	40.74	500	41	56	2	-	1
66	107.0	3.20	27000	8.5	1	28	0.48-0.36	87.50	26.56	30.36	400	23	68	4	3	2
66	106.5	3.04	19000	8.3	2.4	30	0.44-0.32	98.68	27.30	27.66	300	65	27	7	2	1
66	107.0	2.50	21000	12.3	3	27	0.44-0.32	108.00	50.00	46.29	410	42	55	2	1	1
66	107.5	3.00	21000	9.2	1	28.5	0.44-0.32	96.00	30.66	32.28	460	35	55	8	-	2
66	106.5	2.58	15000	7	1.5	27.2	0.36-0.28	105.42	28.00	25.73	440	24	67	8	3	1
s -	107.0	3.01	22300	9.5	2.26	27.76	0.44-0.32	98.33	33.72	34.38	404.3	37	55.33	5.5	1.66	1.33

loride value in two chicks per unit of observation were determined at the intervals of 24 hours for 6 days continuously.

TABLE 3

Figures in respect of the chicks (Batch No.3) infected with Ranikhet Disease Virus

2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2633	90	24	108	2.2	27000	8.5	3	24	0.40-0.32	109.09	38.63	35.42	320	32	63	2	-	3
311	90	24	107.2	3	25000	10.2	2	28	0.48-0.32	93.33	34.0	36.43	380	40	49	8	2	1
309	90	48	110.0	2.47	29000	10.8	2	25	0.44-0.36	101.21	43.72	43.2	420	48	50	2	-	-
302	90	48	109	2.96	22000	11.8	2	27	0.40-0.32	90.00	39.34	43.70	500	68	30	2	-	-
313	90	72	110	3.04	21000	11	2.5	28	0.44-0.32	93.32	36.65	39.28	640	59	33	3	3	2
322	90	72	110.2	3.07	14000	10.6	1	30	0.48-0.32	100.00	34.52	35.33	420	70	27	2	1	-
320	90	96	111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
312	90	96	110	2.9	10000	11.5	-	-	-	-	-	-	640	84	13	2	1	-
330	90	96	110	3.1	11000	-	0.5	29.5	-	98.33	-	-	560	88	9	2	-	1
304	90	120	109	3.5	14000	12	1.5	34	0.48-0.32	97.14	34.28	35.29	640	74	19	4	3	-
301	90	120	108.4	3.6	11000	10	1	36	0.36-0.28	100.00	27.77	27.77	620	70	20	3	2	2
306	90	120	106.3	3.4	12000	11.0	0.5	34	0.36-0.24	100.00	32.35	32.35	680	77	16	5	2	-
ERAGES	-	-	109.1	3.02	17817	10.74	1.6	29.55	0.42-0.31	98.24	35.69	36.5	529.03	64.5	29.9	3.2	1.3	0.8

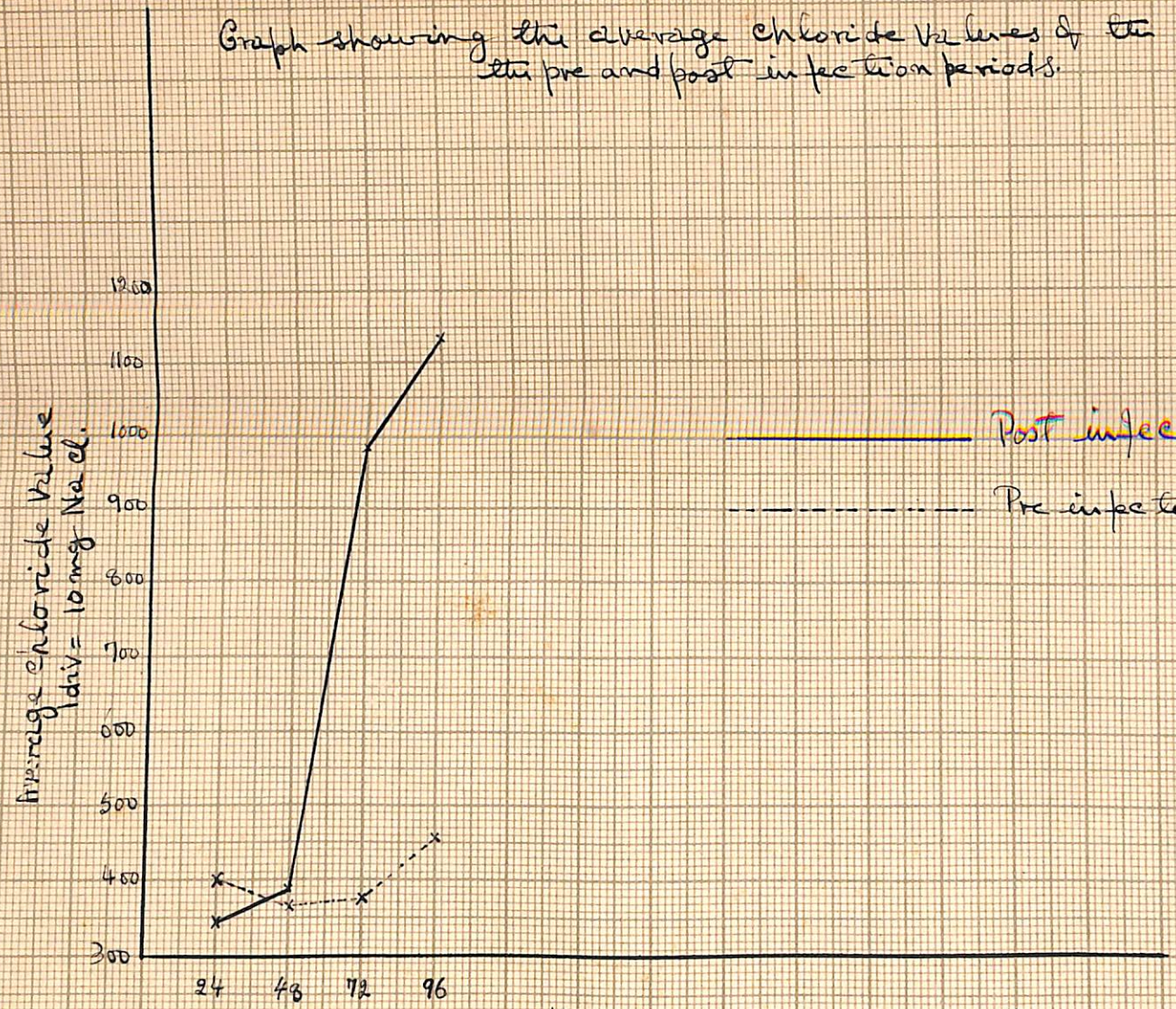
TABLE 3 A

Figures in respect of the same chicks (Batch No.3) during the pre infection period.

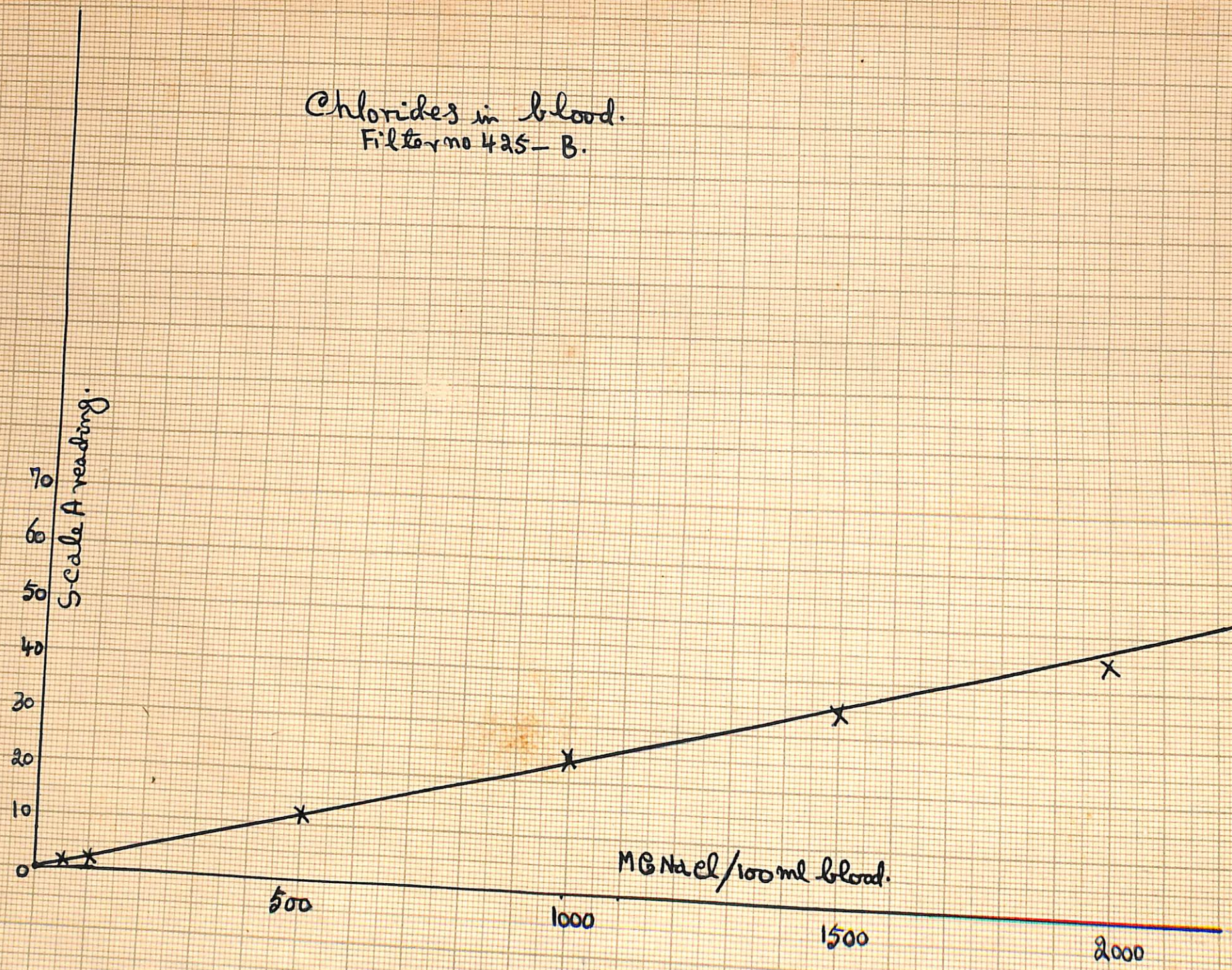
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0	82	107.0	2.89	24000	8.2	3	25	0.44-0.32	86.50	28.37	32.80	450	24	70
33	82	108.0	2.58	20000	9.7	2.5	29	0.48-0.32	112.40	37.59	33.45	390	52	42
0	82	107	2.80	18000	9.2	1.5	24	0.48-0.28	85.71	32.85	38.33	468	27	69
2	82	108	2.2	21000	10.5	1.5	26	0.44-0.32	118.18	47.73	40.38	510	30	65
1	82	106.2	2.62	15000	8.7	2	29	0.44-0.32	110.68	33.20	30.00	430	42	54
2	82	107.3	3.1	12000	9	2.2	30	0.44-0.32	96.77	29.03	30.00	320	58	32
9	82	107.2	3.0	12000	11.0	2.4	30	0.48-0.28	100.00	36.66	36.66	425	22	71
2	82	108	2.5	22000	7.5	2.0	24	0.48-0.28	96.0	30.0	31.25	468	35	61
	82	107.4	2.3	19000	11.0	3.0	25	0.44-0.28	108.61	47.82	40.00	320	28	66
	82	107.3	2.4	17000	10.5	2	22.5	0.46-0.32	93.75	43.75	46.66	315	26	69
	82	106.2	2.66	22000	10.2	3.2	27	0.44-0.32	101.50	38.34	37.77	335	30	65
	82	107.2	2.75	25000	11.2	1.5	25	0.44-0.32	90.91	40.73	44.80	440	25	69
G E S -		107.4	2.82	18917	9.72	2.3	26.37	0.45-0.30	100.07	38.00	37.7	406	33.2	61

chloride values in two chicks per unit of observation were determined at the intervals of 24 hours for 6 days continuously.

Graph showing the average chloride values of the birds in the 1st batch during the pre and post infection periods.

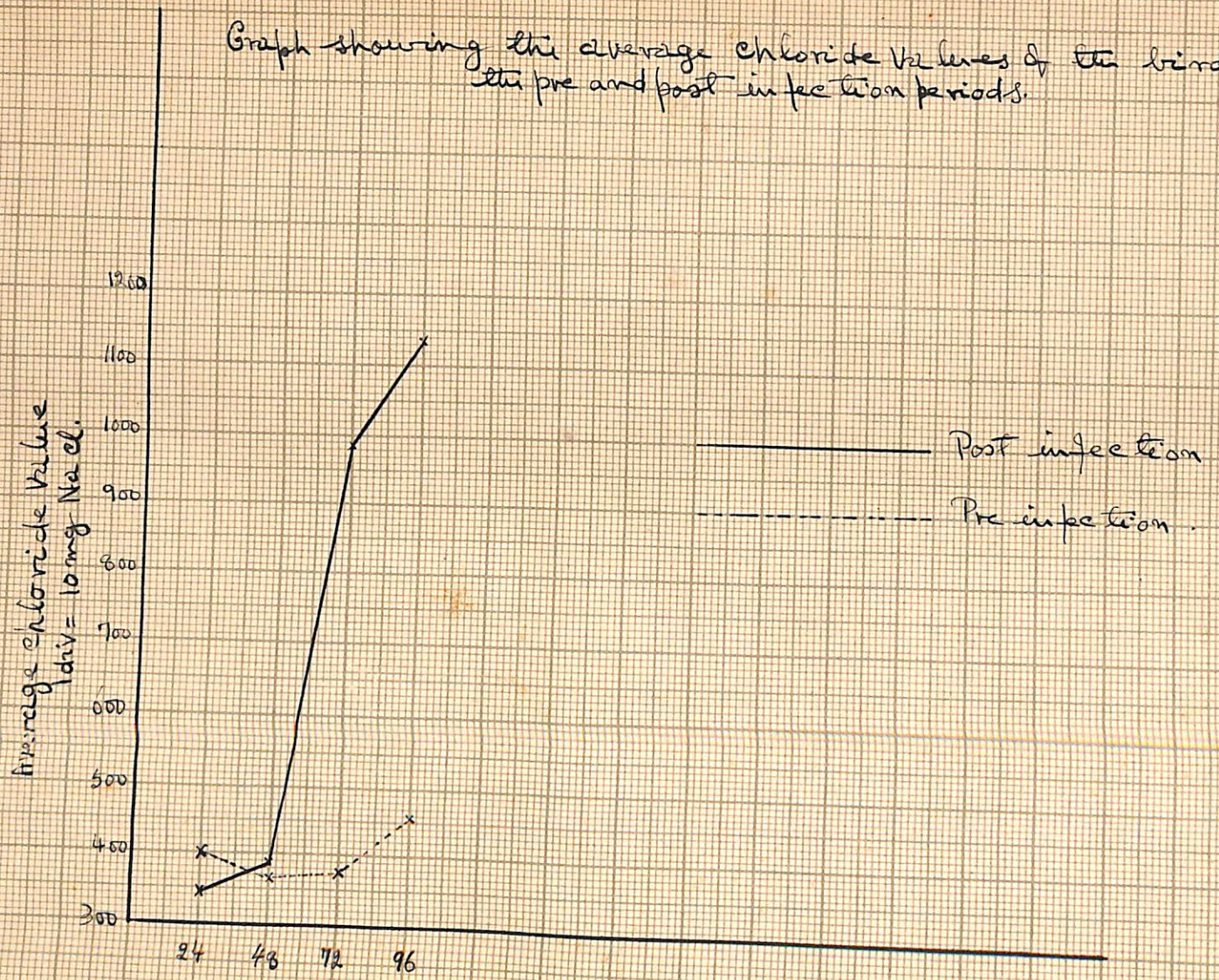


Chlorides in blood.
Filter no 425 – B.

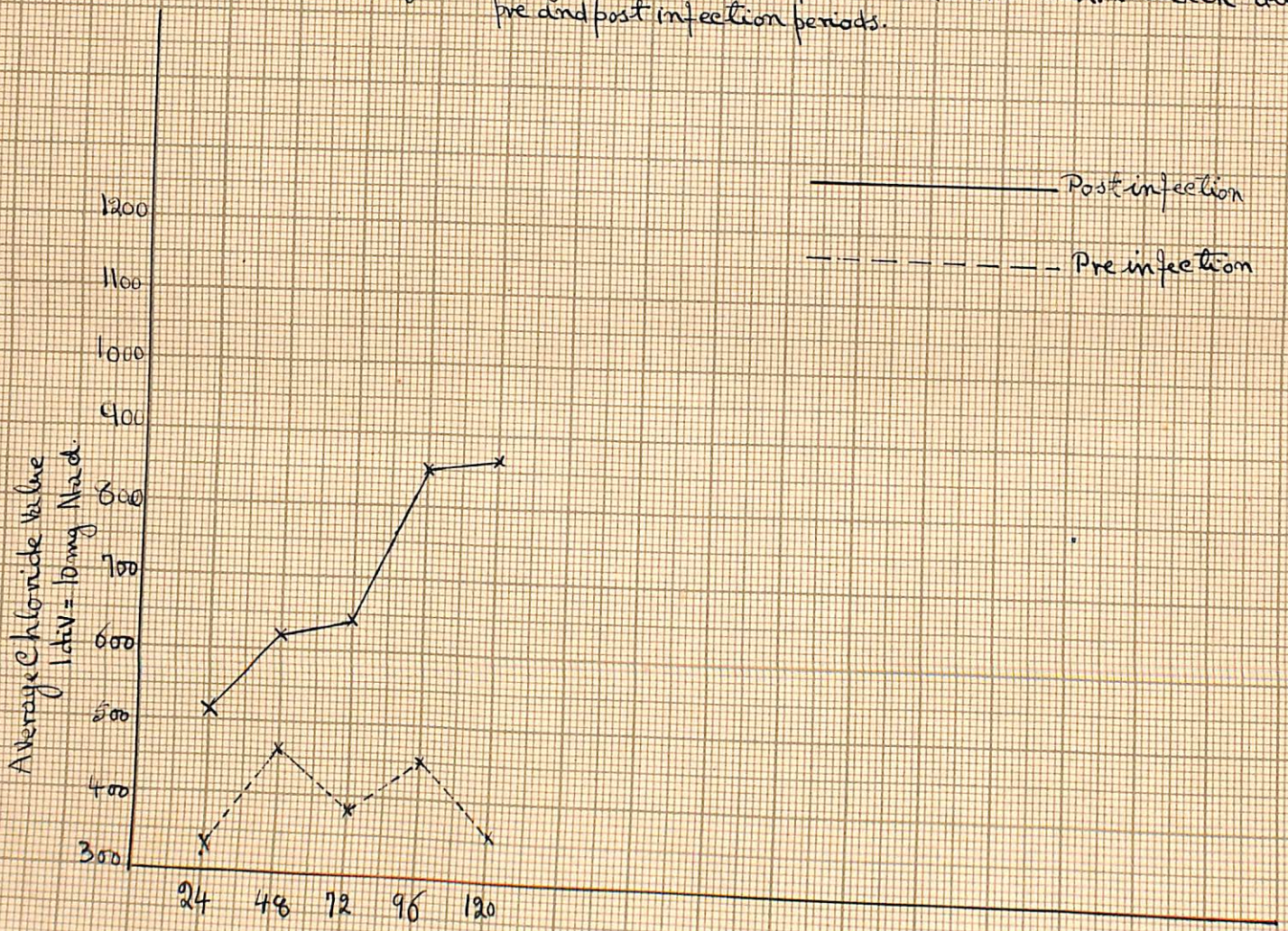


Calibration curve.

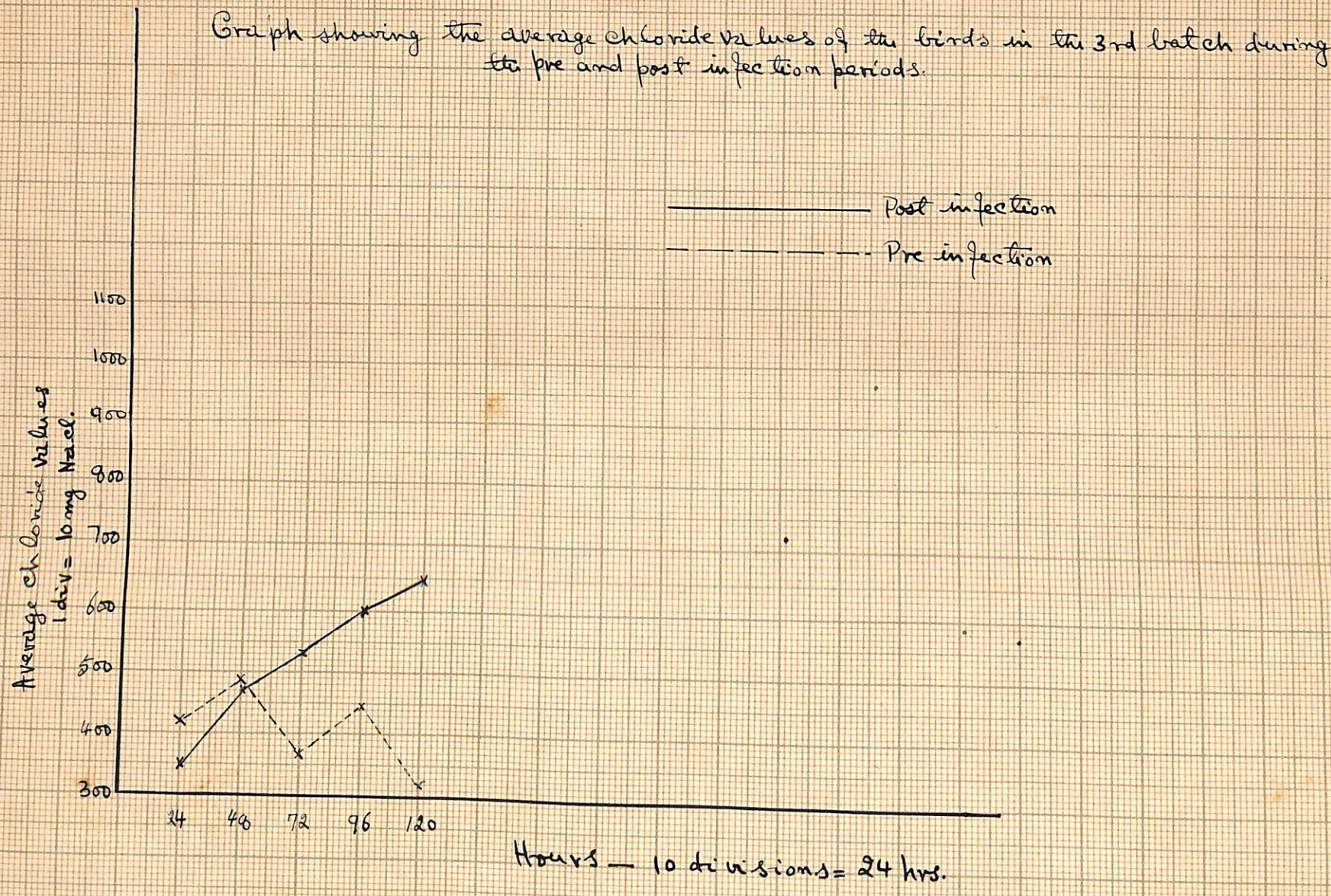
Graph showing the average chloride values of the birds in the 1st batch during the pre and post infection periods.



Graph showing the average chloride values of the birds in the 2nd batch during the pre and post infection periods.



Graph showing the average chloride values of the birds in the 3rd batch during the pre and post infection periods.



The percentage of monocytes, eosinophiles and basophiles did not undergo any appreciable alteration. There was generally a visible decrease of mean corpuscular volume mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration in the birds of all the three batches. More or less similar is the case in the chicks of other batches.

The increase in body temperature, RBC count and chloride content of the blood and fall in the total WBC count were found to be significant ones after 72 hours and onwards of infection.

The tables 1, 2 and 3 reveal that the onset of Ranikhet Disease is marked by leucopenia and a higher rise in the percentage of heterophiles and no appreciable variation in eosinophiles, monocytes and basophiles which confirm the findings of Sharma and Seetharaman (1950) and Chandrasekharan and Krishnan (1958). Sharma and Seetharaman (1950) also pointed out that the results embodied in their article are in conformity with those obtained by Lewis and Shope (1929) and Kern Kamp (1939) in hog cholera, Hammon and Enders (1939) in a virus disease of cats, Kirk and Collin (1945) in ferret distemper and Verma (1947) in rinder pest in goats. They therefore remarked that leucopenia can not be taken as specific for any particular viral disease and in majority of the virus diseases haemopoietic tissues are known to be affected in such a manner as to produce leucopenia. In the present experiments since Ranikhet Disease appeared in its acute diarrhoeic form with severe catarrhal enteritis, it is but natural that when there is fluid loss in the early stage, the haemoconcentration should occur as explained by Chandrasekharan and Krishnan(1958). They also observed that the mean erythrocyte sedimentation rate in Ranikhet Disease was found to be lower than in normal,probably due to resultant erythrocyte concentration.

It is well known that the chloride value goes up in nephritis cases. Since the Ranikhet Disease infected birds showed inflammatory changes in the kidney, the rise in chloride value may therefore be due to inflammatory changes in the kidney as well as haemoconcentration.

The red cells from diseased birds were found to be fragile in lower concentration of Nacl when compared to the healthy group. Cell fragility was observed to occur in 0.43 to 0.27 per cent Nacl in the diseased batch No.1 (62 days old) while it was seen in saline of 0.45 to 0.31 percent Nacl in the corresponding healthy group (vide table 1 and 1 A). Similarly in the 2nd diseased batch (76 days old) cell fragility occurred in 0.38 to 0.27 per cent Nacl while it occurred in saline of 0.44 to 0.32 per cent Nacl in the corresponding healthy batch (vide Table 2 and 2 A). Thus it is clear that the cell fragility occurred in respect of the infected chicks (76 days old) at lower concentration when compared to the corresponding healthy controls.It is interesting to note that in the 3rd diseased batch the cell fragility occurred in saline of 0.42 to 0.31 per cent while it was observed in 0.45 to 0.30 percent Nacl in the corresponding healthy group (vide Table 3 and 3 A). In this batch there was no appreciable change in cell fragility between the healthy and diseased birds so far as the lower limits of salines are concerned. A possible explanation may be that in the lower age groups, the erythrocytes are more liable to be fragile whereas in higher age groups the red blood corpuscles are not so much affected by the virus probably due to some increased resistance which requires further investigation. It is likely that the cells of the diseased birds might become haemolysed in lesser time than the cells of the healthy birds of the corresponding age group. And this also merits further investigation. This fact may due to

the weakening effect of the cell membrane due to the action of the haemolytic and necrotising effects of RDV and to some extent this is explained by the fact that cell fragility in the infected birds gradually increased in the lower concentrations of Nacl from 24 hours after infection to 120 hours after infection e.g. in Bird No.305 of batch No.II, the cell haemolysis was complete in 0.32 percent Nacl whereas in Bird No.303 of the same age group but after 120 hours the cell haemolysis occurred in 0.24 percent Nacl (vide Table 2). Similar is the case in respect of Bird No.2633 and Bird No.306 in the batch No.3.

Fukushima and his coworkers (1932,1933) were probably the first to describe histopathological lesions in the chicken naturally infected with Newcastle Disease. The avian pneumoencephalitis was studied by Beach (1942) and Jungherr and Minard (1944) from the gross and pathological points. Jungherr et al (1946) made an exhaustive pathological study of different organs of chicks artificially infected with Newcastle Disease. They noted,"Most striking and prominent however, were the haemorrhages and early secondary necrosis of the relatively large lymphoid patches". The basic histopathological changes of Ranikhet Disease were found to be haemorrhagic and necrotising in character in the present study. It appears quite likely that distribution of RD lesions corresponds with the distribution of lymphoid patches.

Jungherr et al (1946) found that the necrotic foci

occurred near or in the normal lymphoid aggregates in the intestinal tract. deKock (1954) also considered the lesions to be present in the lymphoid tissue. In the present studies the intestinal villi and glands showed varying degree of degeneration leading to necrosis. The intestinal lesions appeared at certain constant sites in the affected chicken. The lymphoid patches of small and large intestines were invariably necrosed in most of the acute cases of RD.

Fukushima et al (1933) described lymphocytic hyperplasia in the splenic follicles of birds naturally infected with Newcastle Disease. They also described hyaline degeneration of the spleen and intima of the vessel walls. Zangerle (1952) described lymphocytic hyperplasia in spleen, liver and intestines of birds infected with New castle Disease. In the present artificial infection experiments with RD in the chicks, the spleens were either normal or enlarged slightly in most of the cases. There was frank necrosis in the lymphoid follicles of the spleen and lymphocytic hyperplasia.

Iyer (1943) and Orr and John (1946) described congestion in the kidney. Kaschula (1961) stated that congestion and acute inflammation of kidneys were usually present in acute cases. In the present studies of the experimental cases of RD, there was vivid tubular degenration leading to desquamation, necrosis and hyalinisation of the tubular epithelial cells.

Jungherr et all (1946) noticed that there was often interstitial pancreatitis and in the affected areas, the acinar lobules appeared reduced in size and separated by

oedema and proliferated interstitial tissue. In the present RD infected chicken, there was necrosis of the acini and islets of Langerhans and lymphocytic infiltration in a few cases.

Fukushima et al (1933) observed cellular infiltration in the myocardium of the chicks naturally infected with Newcastle Disease. Jungherr et al (1946) stated that necrotizing and haemorrhagic lesions were observed in the heart. In the present RD cases, the myocardium revealed round cell infiltration in some cases and fragmentation of the muscle fibres with onset of degenerative changes.

Jungherr et al (1946) stated that the lesions in the lungs were primarily proliferative and secondarily exudative in character. In the chicks affected with the local strain of RDV, areas of congestion, consolidation and haemorrhages were present in the lung. There were some hyperplasia of alveolar epithelium and thickening of interstitial tissue as revealed in the American form.

The appearance of hyperaemia, haemorrhages and so called endotheliosis with an infiltration of the capillary walls with lymphocytes and other blood cells have been noted in the central nervous system by various workers (Jungherr et al, 1946 ; Karzon and Bang, 1951 ; deKock 1954). Sullivan (1958) observed that in the cases of Newcastle Disease the predominant tissue damage was an extensive hyperaemia of central nervous system accompanied by proliferation of the endothelial cells. In the present studies

the degenerative changes in the neurons accompanied with satellitosis and neuronophagia, areas of liquefaction and proliferation of the endothelial cells of the blood vessels even to the point of occluding the lumen were clearly seen.

As the gross lesions were in the most developed forms on the 4th and 5th day, the histopathological findings of the chicken died or killed on the said days in the first, second and third batches were compared to each other and it was found that these were more or less the same in the acute cases and as such it is considered that the influence of age does not markedly alter the nature and pattern of the lesions within the age group of 62 days to 90 days. It is also interesting to note that the duration of the disease in the infected birds was slightly longer in the birds of the higher age group, namely, in the 2nd and 3rd batches than in the lower age group i.e. the first batch. This may be due to developing age resistance in the birds as remarked by Jungherr et al (1946) who observed greater resistance with increasing age.

CHAPTER VI

SUMMARY

Heart blood of 36 Rhode Island Red Chicks of both sexes of different ages was examined during the pre and post infection periods. The chicks were artificially infected with a local Ranikhet Disease virus strain by intramuscular route in all batches. The dose of Ranikhet Disease Virus and its route of administration were not changed in any batch of the chicks.

During the course of the disease there was a rise in total red cell count, haemoglobin value, and packed cell volume and fall in total white cell count and erythrocyte sedimentation rate. The cell fragility occurred in lower percentages of Nacl when compared to the corresponding healthy control birds. Possible correlation with cell fragility and age has been indicated with suggestions for further line of investigation. The blood chloride value significantly increased in the artificially infected chicks. Differential counts of leucocytes revealed a clear decrease in lymphocytes and a marked increase in heterophiles and there was almost no change in the percentages of basophiles, monocytes and eosinophiles during the disease.

Symptoms, lesions duration of the disease and histopathological changes in tissues noticed in the acute cases of Ranikhet disease have been fully described and discussed.

In the abdominal viscera, the Ranikhet Disease virus showed clear affinity for the lymphoid tissues of the alimentary tract with haemorrhagic and necrotising effects in the liver, pancreas, spleen and kidneys. Petechiae and necrosis were invariably found in the proventriculus and caeca. Haemorrhagic and necrotic lesions were constant lesions in certain sites in the small and large intestines of the chicks of all the three batches irrespective of age which may thus be taken as the diagnostic pathological lesions. These lesions were found not only in those cases that died of disease but also in those that were killed on the 4th and 5th day of infection.

In the lung, the lesions were in the form of congestion, haemorrhages and proliferation of alveolar epithelium and to some extent in the interstitial tissue. In the brain, the changes were mainly of degenerative type. This local strain though viscerotropic it tended to be also pneumotropic in character. Its neuroparalytic effect has been emphasised. No significant influence of age could be seen in the histopathological lesions in the acute cases of RD.

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APPENDIX

Fig. 1
Chicken (Batch no.2)
Showing normal
posture.

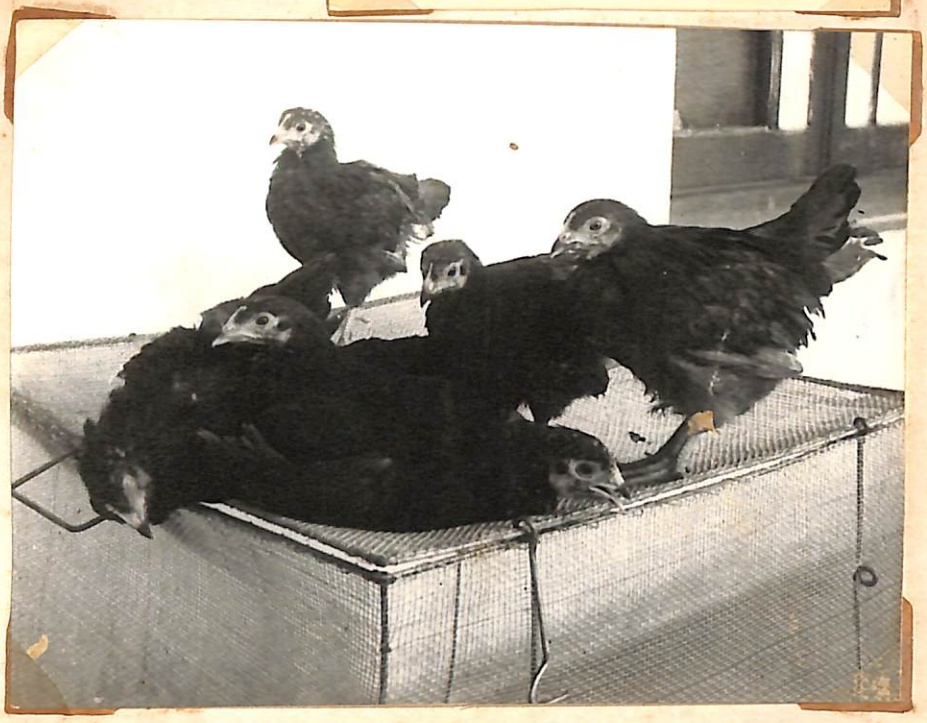

Fig.2
Chicken of the Batch
No.2 (120th hour post
inoculation) Showing
ruffled feathers,
drowsiness, weakness,
drooping of the wings,
somnolence and dyspnoea.

Fig. 3
Chick No.307
(Batch No.2)
Gasping for air
with partly opened
beak.

Fig. 4
Chick No.2628
(Batch No.1)
Showing crouching position and paralysis of the wings and legs.

Fig. 5
Chick No.84(Batch no.1) showing paralysis of the wings and legs, closed eyes and complete prostration.

Fig.6
Chick No.314
(Batch No.2)
Duodenum showing an oblong lesion.

Fig.7
Chick No.306
(Batch no.3)
Duodenum showing
an elongated lesion.

Fig. 8
Chick No.84
(Batch No.1)
Haemorrhagic and
necrotic lesions
shown in duodenum ,
caecal tonsils and
cloaca.

Fig.9.
Chick No.307
(Batch No.2)
Haemorrhagic and
necrotic lesions
in the ileum and
caecal tonsils.

Fig.10.
Proventriculus
H.& E.x 100.
Haemorrhage between
two parietal glands.

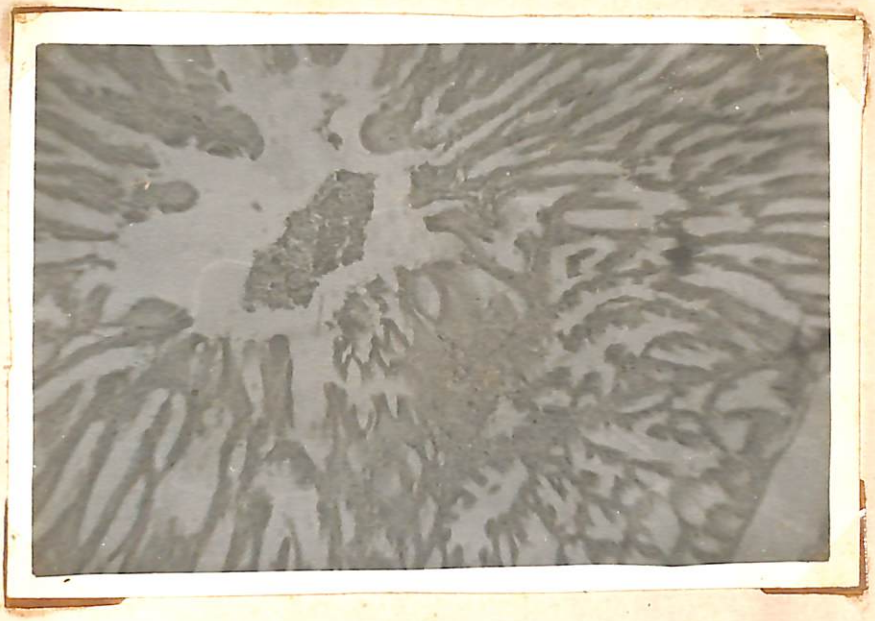

Fig.11
Proventriculus
H.& E. x 100
Necrosis of the
glandular epithelium
and tissue debris
in the ductal opening
of the parietal glands.

Fig.12
Small intestine
H.& E. x 400.
Degenerative changes
and necrosis in the
intestinal gland and
lymphoid patch.

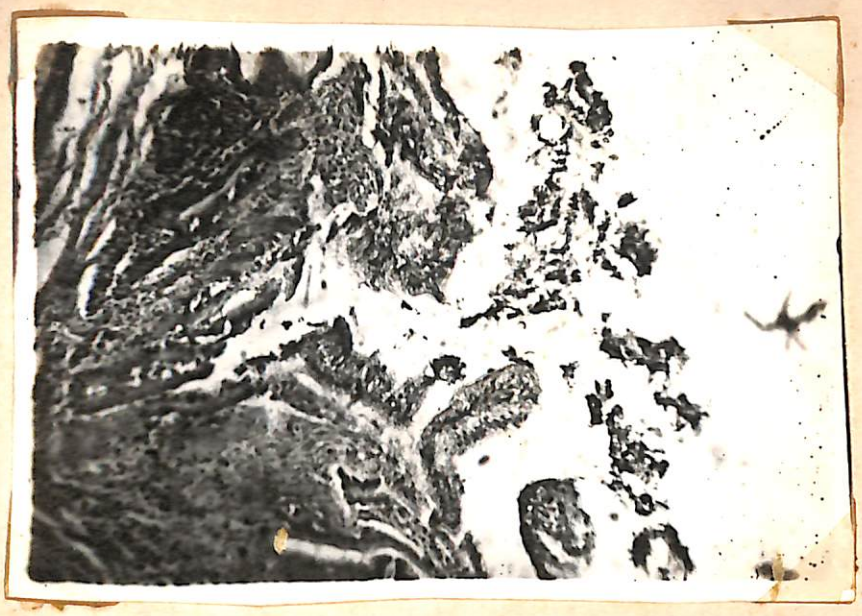

Fig.13
Small intestine.
H.& E. x 100.
Necrosis, distortion and desquamation of the intestinal villi.

Fig. 14
Small intestine
H. & E. x 100
Necrosis, and desquamation of the intestinal villi and glands.

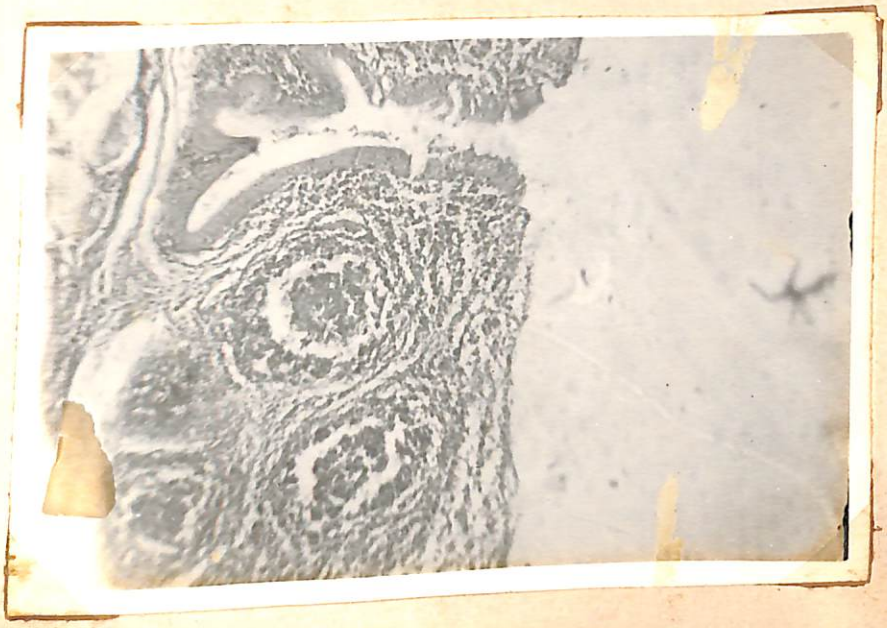

Fig.15.
Cloaca. H. & E.x 100
Hyperplasia and necrosis of the lymphoid patches.

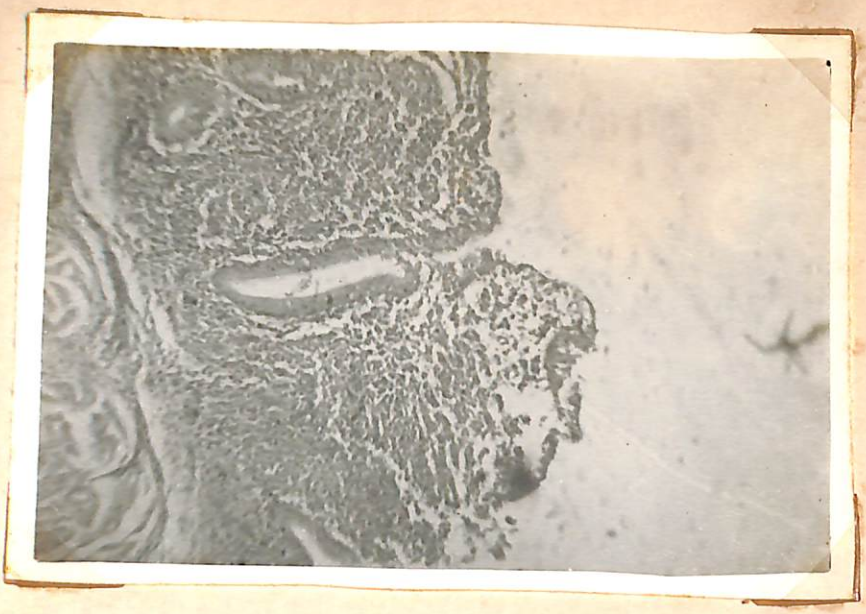

Fig. 16
Large intestine.
H. & E. x 100.
Necrosis of the lymphoid tissue and desquamation of the epithelial lining of the villi.

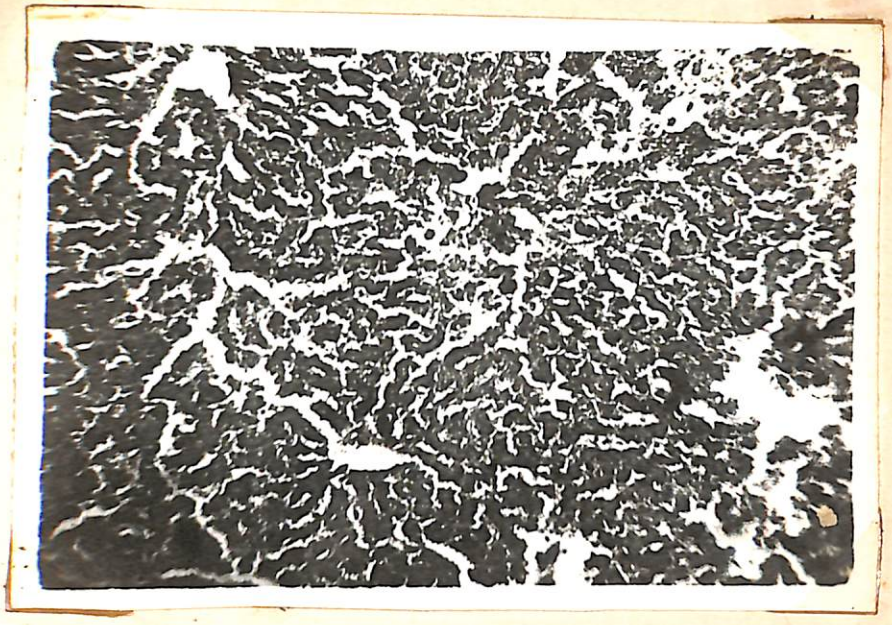

Fig. 17
Liver. H.&E. x 100.
Necrosis and disorganisation of the hepatic laminae.

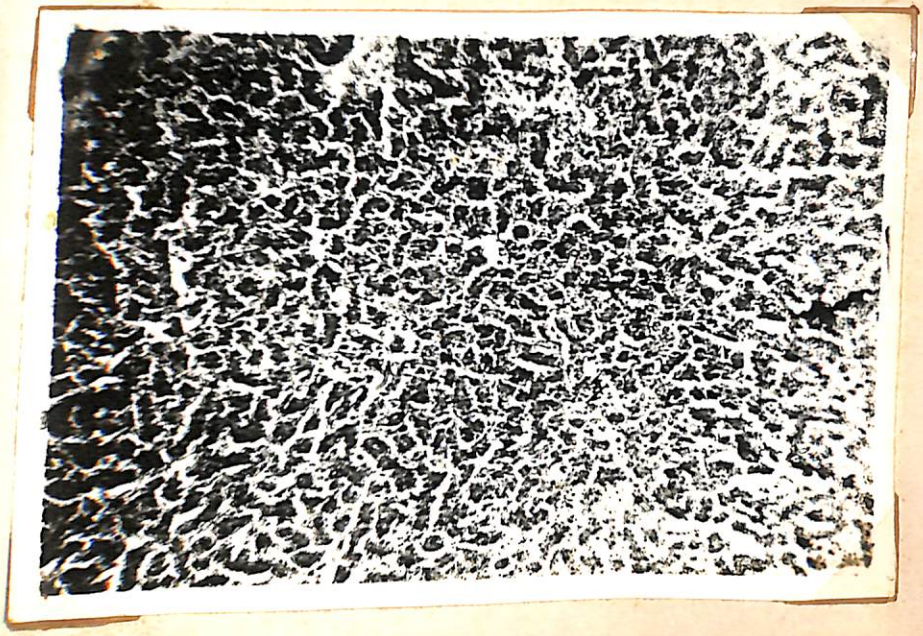

Fig. 18
Liver.H.& E. x 100.
Necrosis of the hepatic laminae

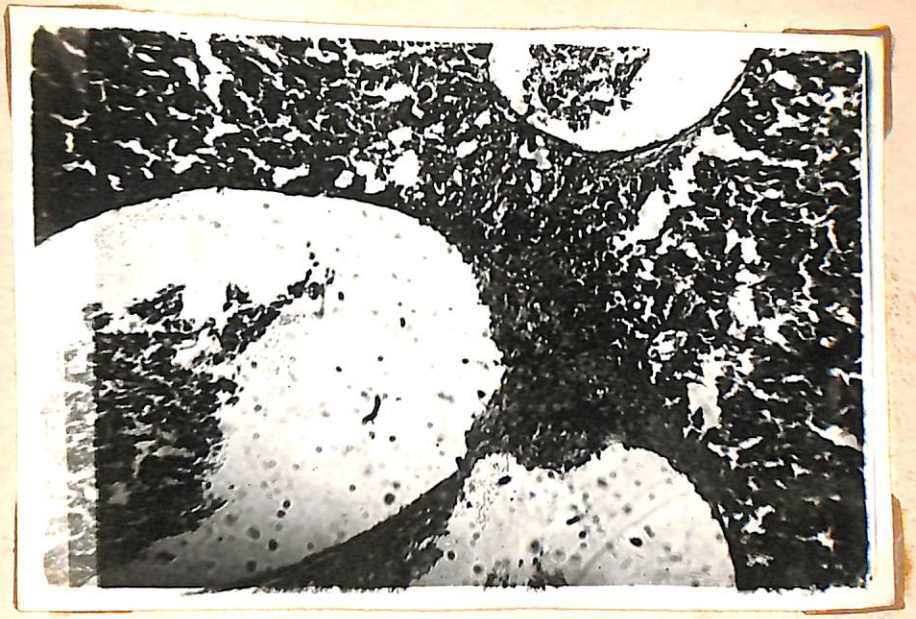

Fig.19
Liver.H.& E. x 100.
Areas of haemorrhages
in the liver parenchyma.

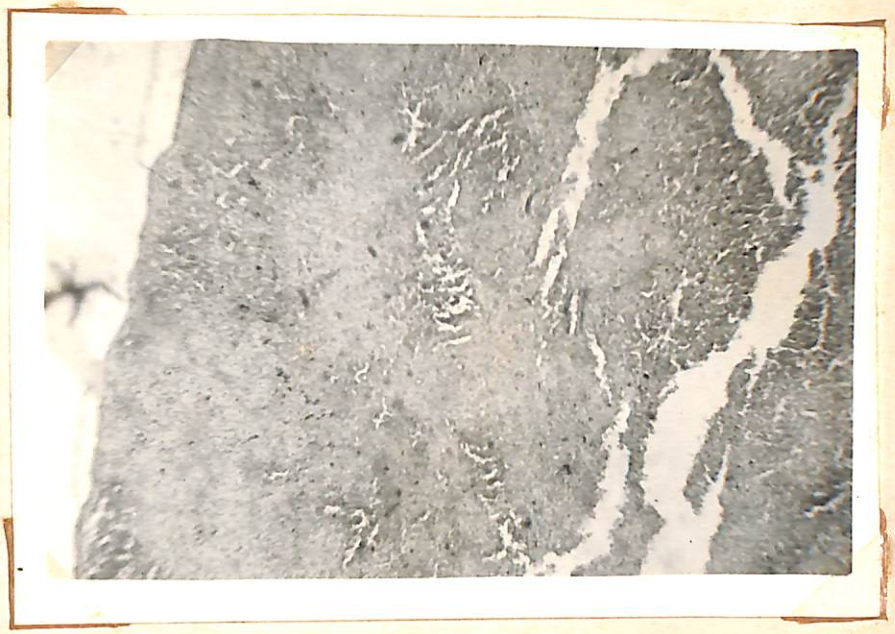

Fig. 20
Spleen.H.& E. x 100.
Areas of Necrosis.

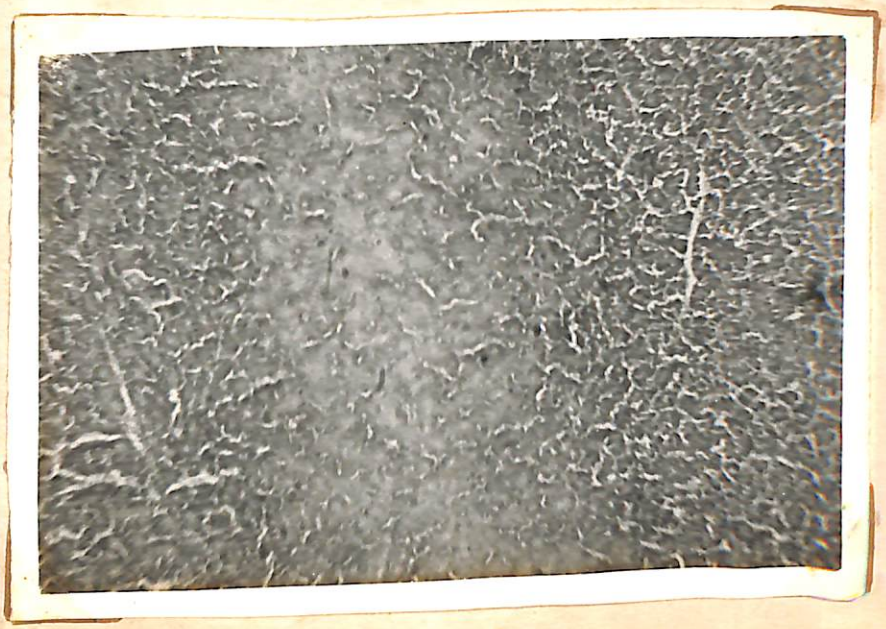

Fig.21
Pancreas H.& E.x 100.
Areas of Necrosis

Fig. 4
Chick No.2628
(Batch No.1)
Showing crouching position and paralysis of the wings and legs.

Fig. 5
Chick No.84(Batch no.1) showing paralysis of the wings and legs, closed eyes and complete prostration.

Fig.6
Chick No.314
(Batch No.2)
Duodenum showing an oblong lesion.

Fig.7
Chick No.304
(Batch No.3)
Duodenum showing
an elongated lesion.

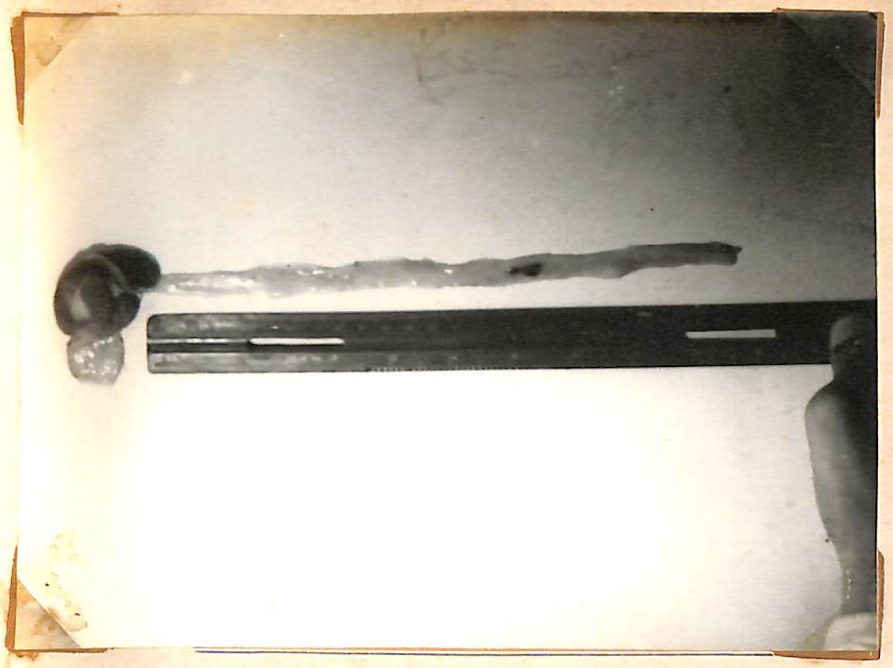

Fig.7
Chick No.306
(Batch no.3)
Duodenum showing
an elongated lesion.

Fig. 8
Chick No.84
(Batch No.1)
Haemorrhagic and
necrotic lesions
shown in duodenum ,
caecal tonsils and
cloaca.

Fig.9.
Chick No.307
(Batch No.2)
Haemorrhagic and
necrotic lesions
in the ileum and
caecal tonsils.

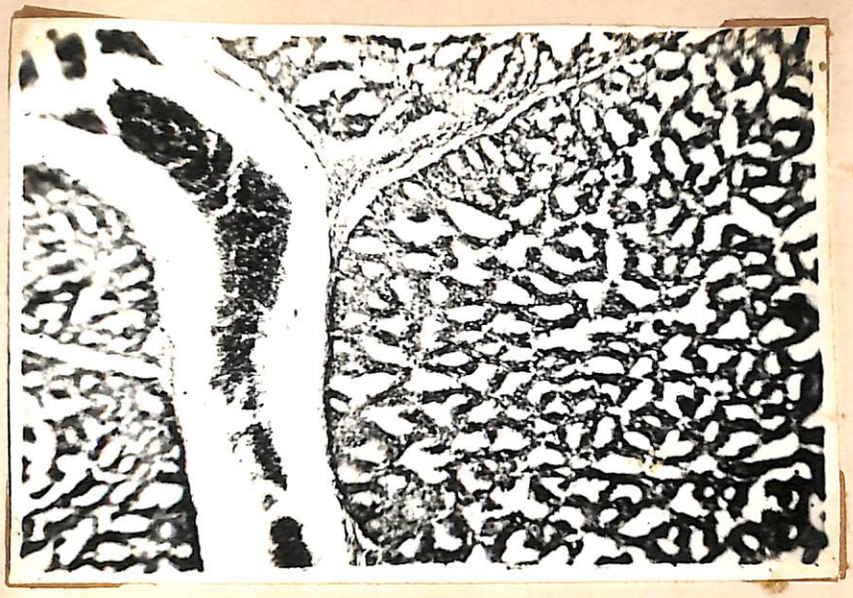

Fig.10.
Proventriculus
H.& E.x 100.
Haemorrhage between
two parietal glands.

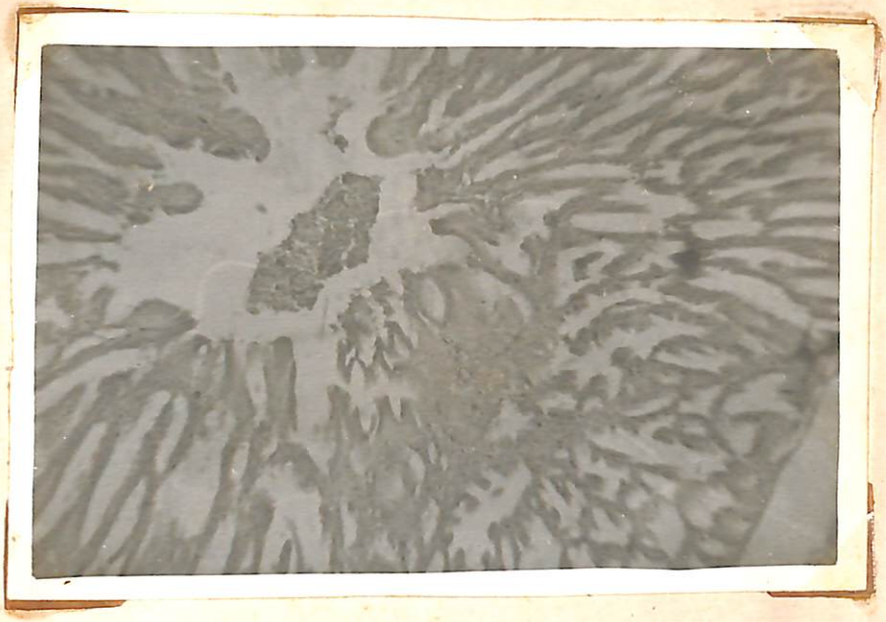

Fig.11
Proventriculus
H.& E. x 100
Necrosis of the
glandular epithelium
and tissue debris
in the ductal opening
of the parietal glands.

Fig.12
Small intestine
H.& E. x 400.
Degenerative changes
and necrosis in the
intestinal gland and
lymphoid patch.

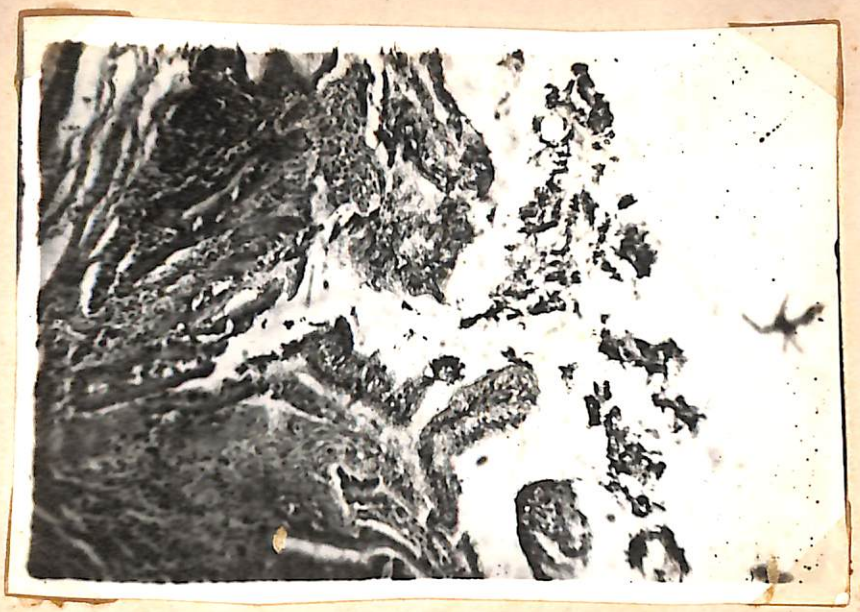

Fig.13
Small intestine.
H.& E. x 100.
Necrosis,distortion
and desquamation of
the intestinal villi.

Fig. 14
Small intestine
H. & E. x 100
Necrosis,and desqua-
mation of the
intestinal villi
and glands.

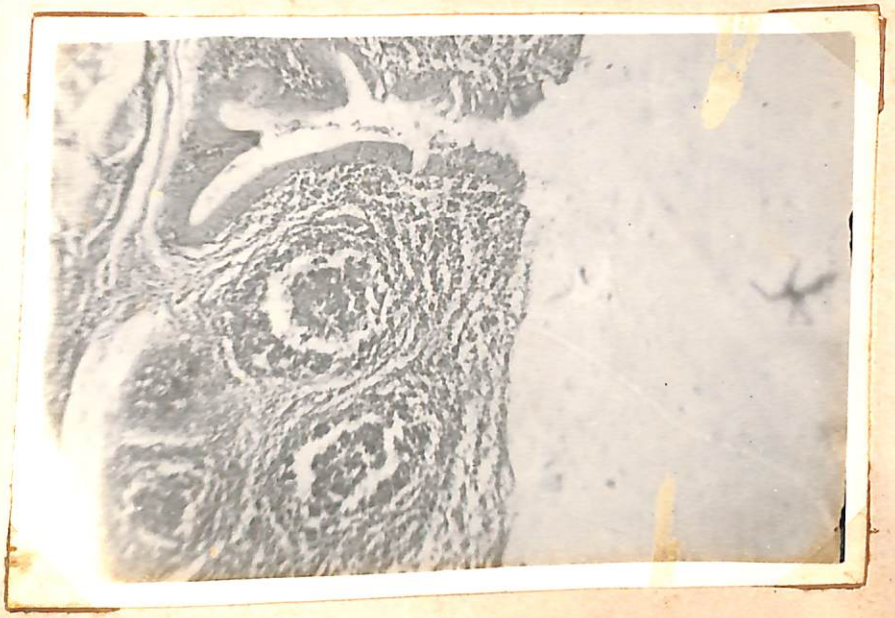

Fig.15.
Cloaca. H. & E.x 100
Hyperplasia and
necrosis of the
lymphoid patches.

Fig. 16
Large intestine.
H. & E. x 100.
Necrosis of the
lymphoid tissue
and desquamation
of the epithelial
lining of the villi.

Fig. 17
Liver. H.&E. x
Necrosis and
disorganisation
the hepatic la

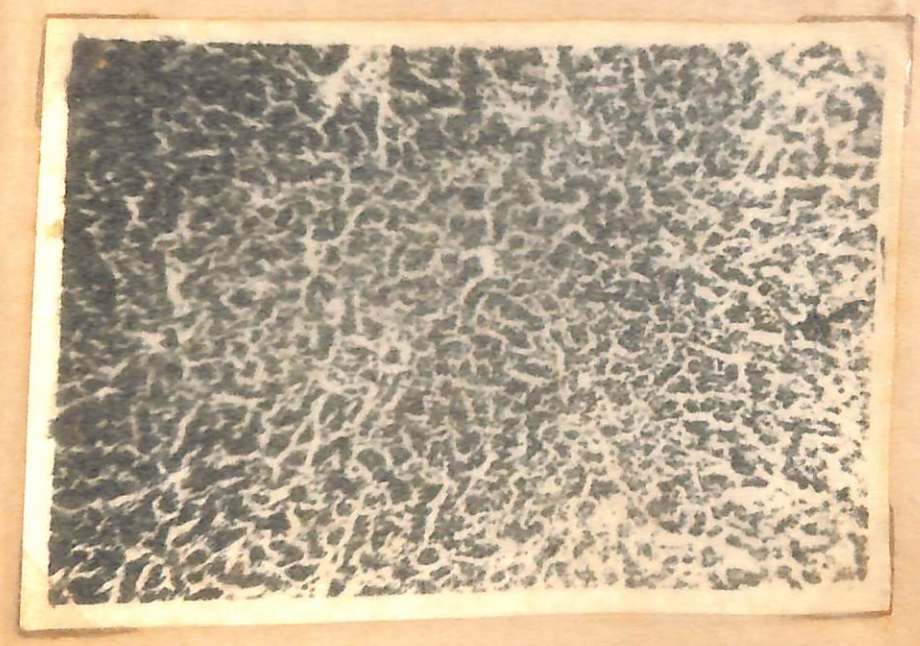

Fig. 18
Liver.H.& E. x 100.
Necrosis of the
hepatic laminae

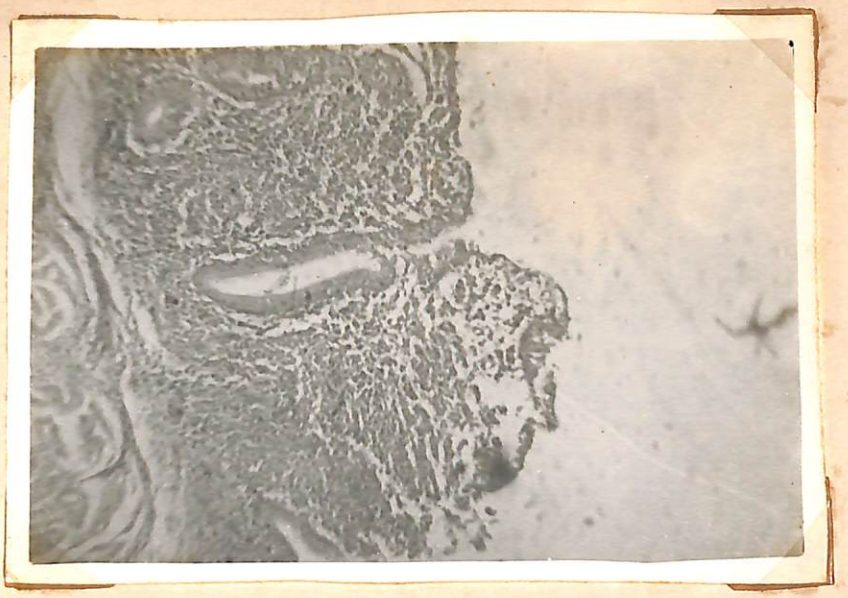

Fig. 16
Large intestine.
H. & E. x 100.
Necrosis of the
lymphoid tissue
and desquamation
of the epithelial
lining of the villi.

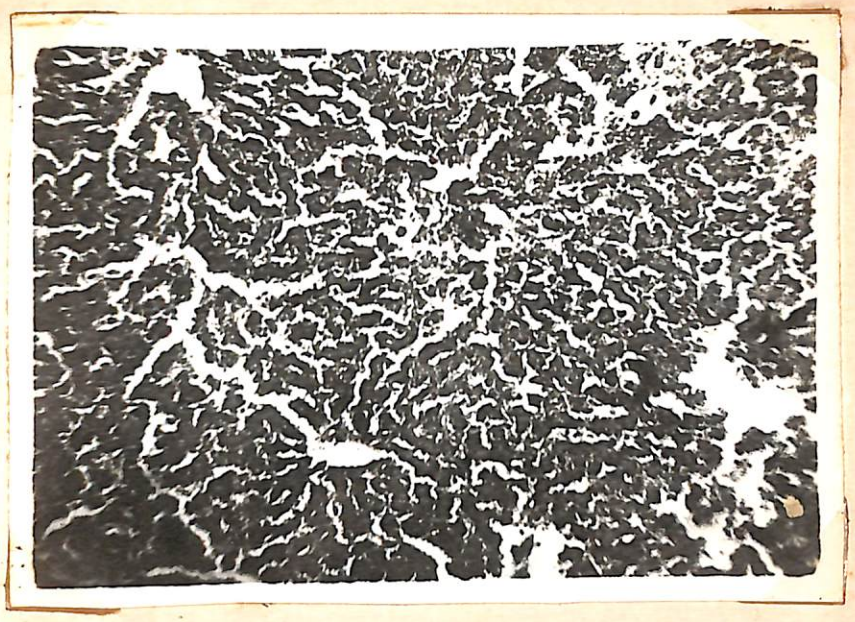

Fig. 17
Liver. H.&E. x 100.
Necrosis and
disorganisation of
the hepatic laminae.

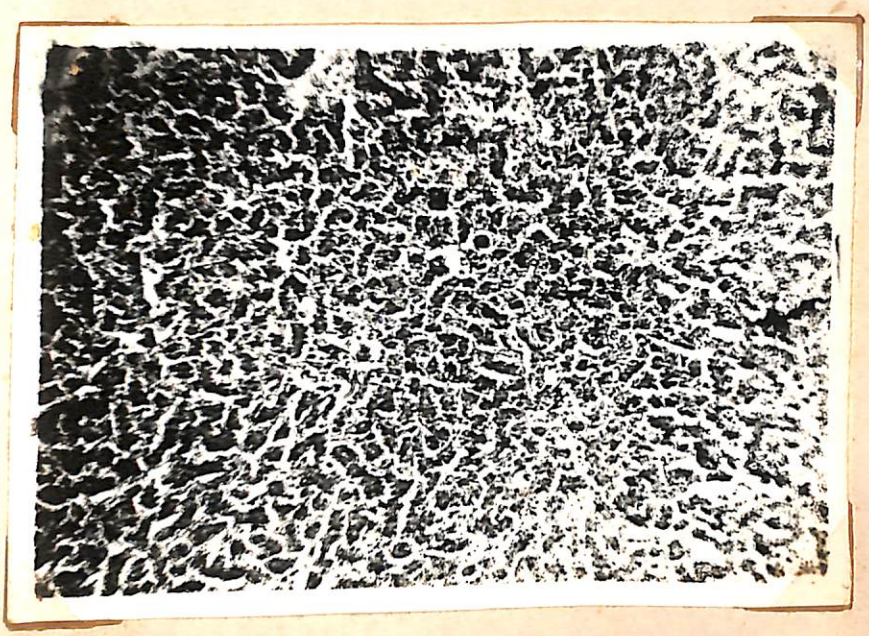

Fig. 18
Liver.H.& E. x 100.
Necrosis of the
hepatic laminae

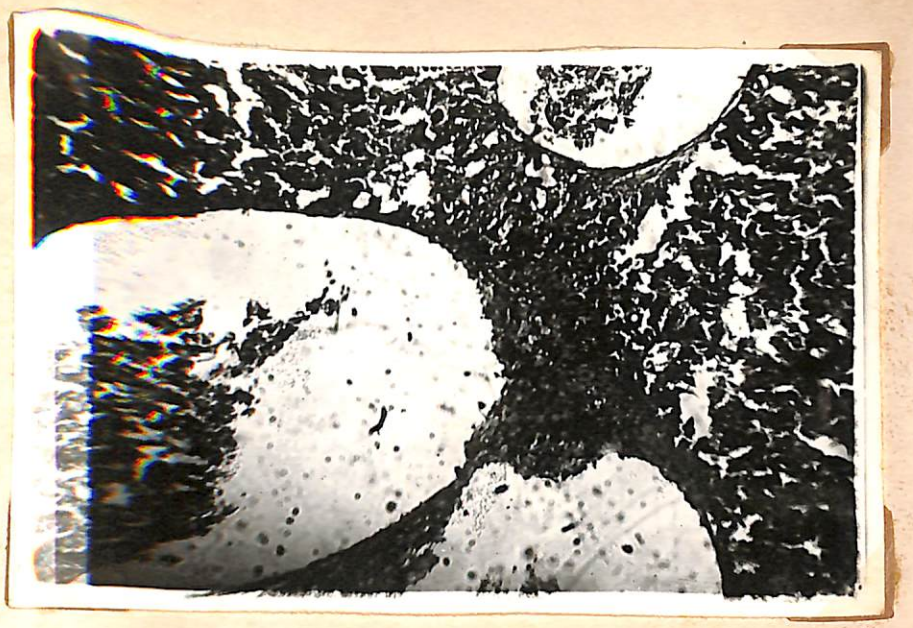

Fig.19
Liver.H.& E. x 100.
Areas of haemorrhages
in the liver parenchyma.

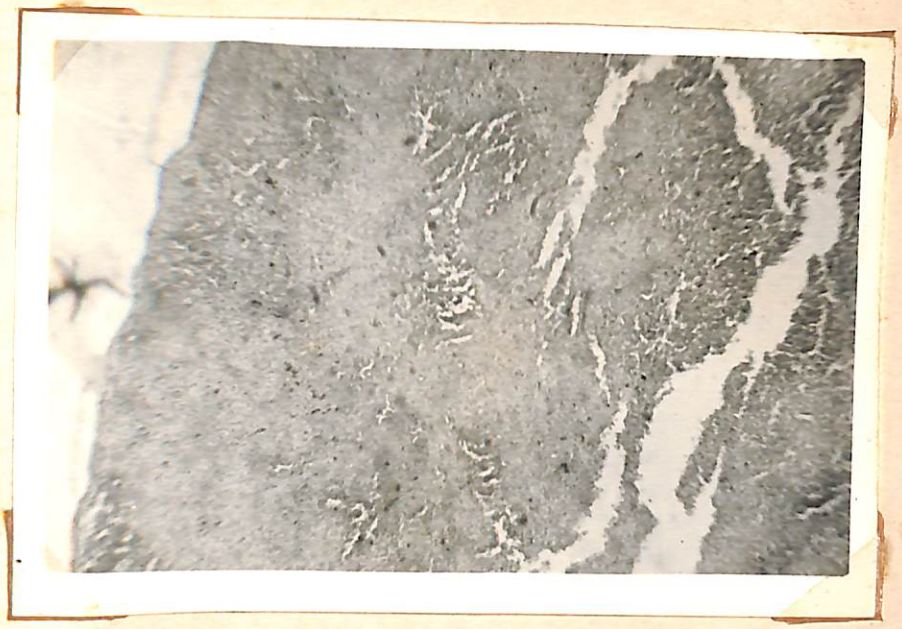

Fig. 20
Spleen.H.& E. x 100.
Areas of Necrosis.

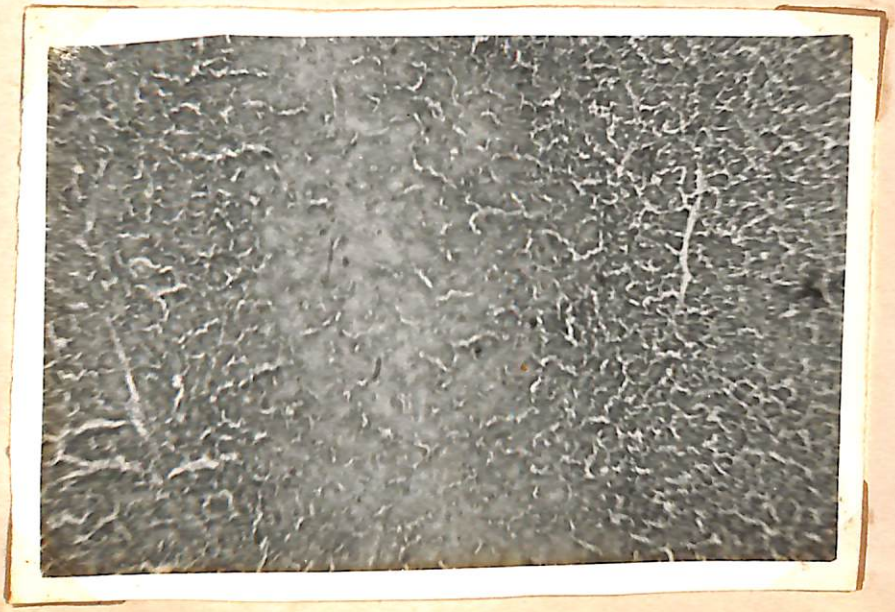

Fig.21
Pancreas H.& E.x 100.
Areas of Necrosis

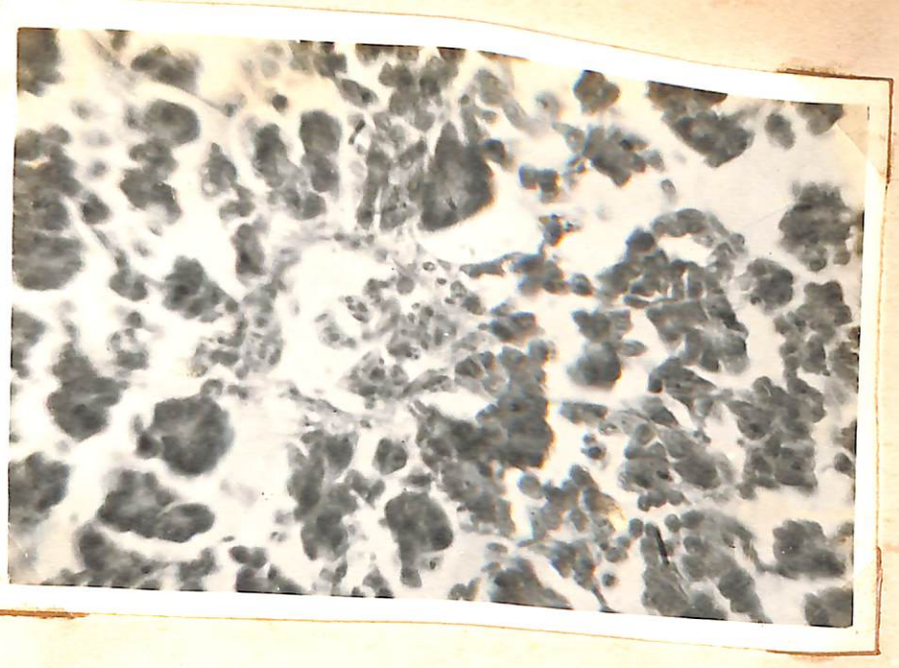

Fig. 22.
Pancreas H.& E.x 400.
Necrosis and
disorganisation of
the acini.

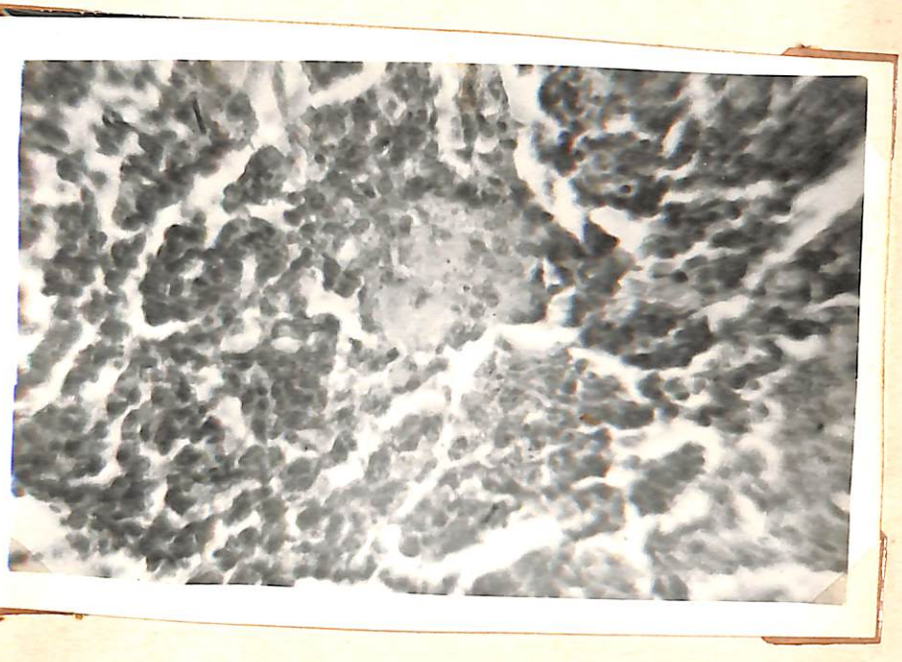

Fig.23.
Pancreas H.& E.x 400
Necrosis of the
acini and islets of
langerhans.

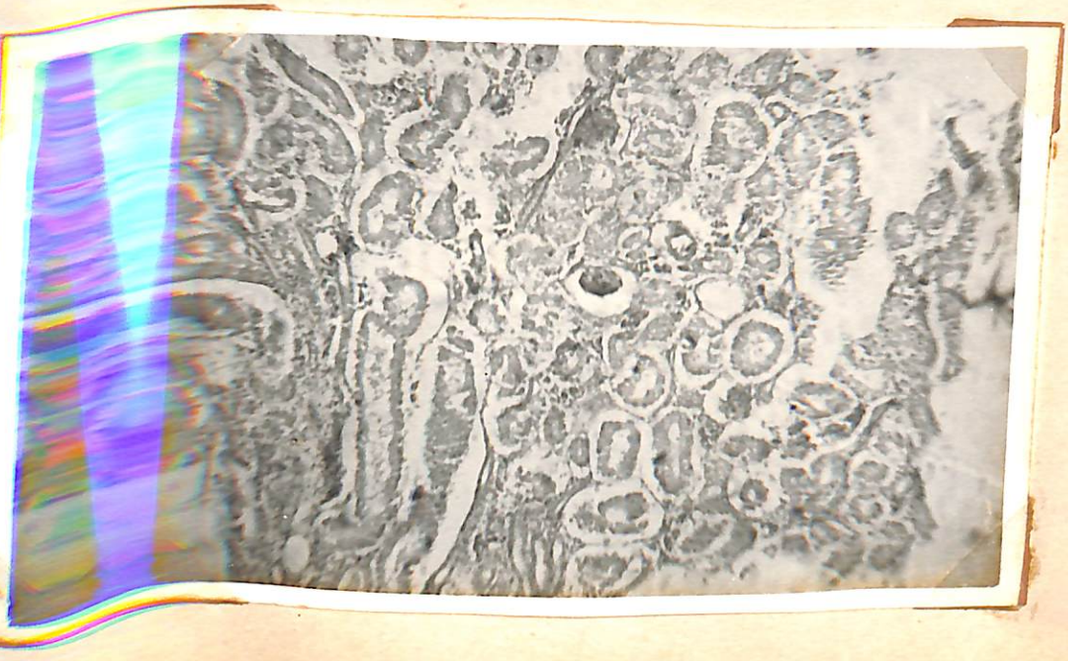

Fig.24.
Kidney. H.& E.x 100.
Degeneration, necrosis
and desquamation of
the tubular epithelial
cells.

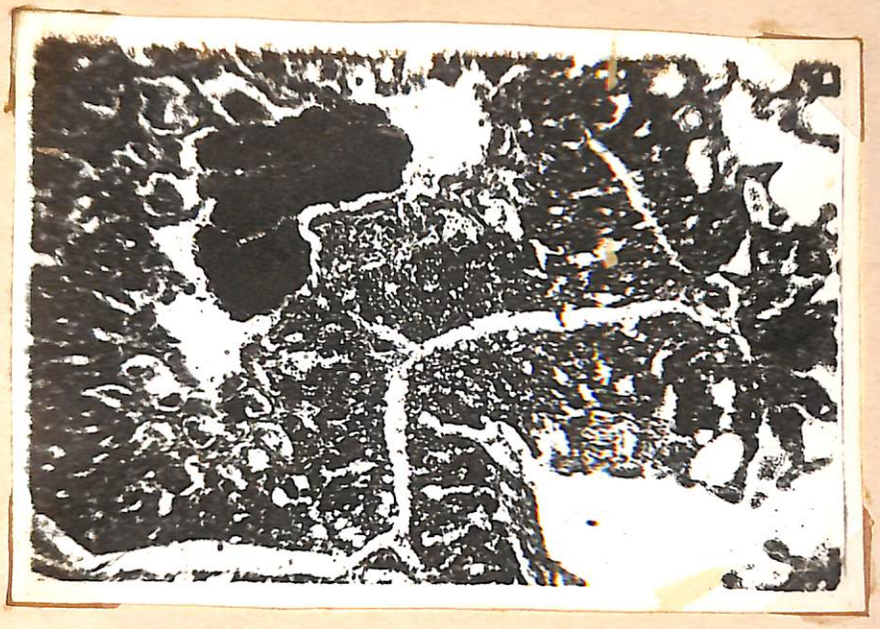

Fig. 28
Lung.H.& E.x 100
Showing an area of haemorrhage and exudate in the alveoli.

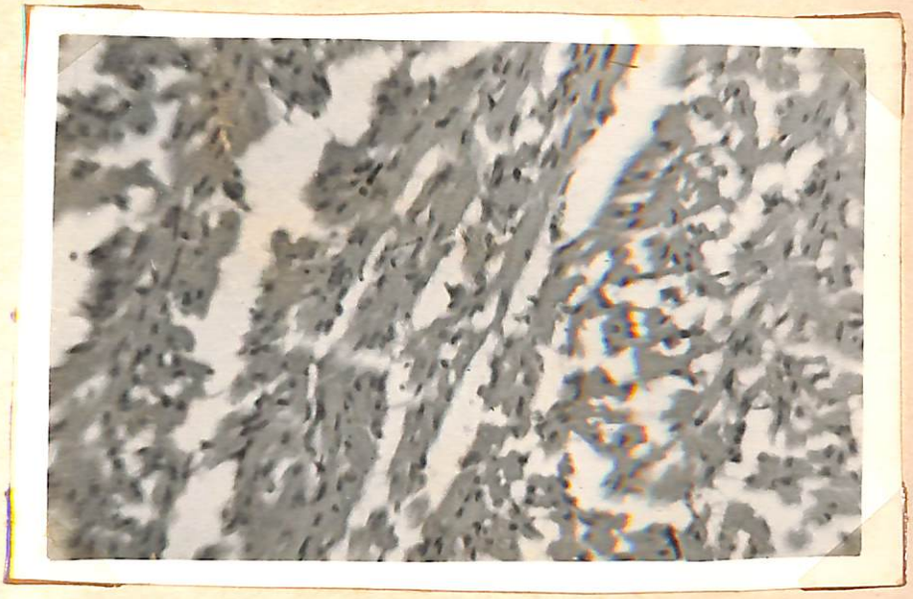

Fig. 29
Heart. H.& E.x 400
Degenerative changes in the myocardial fibres.

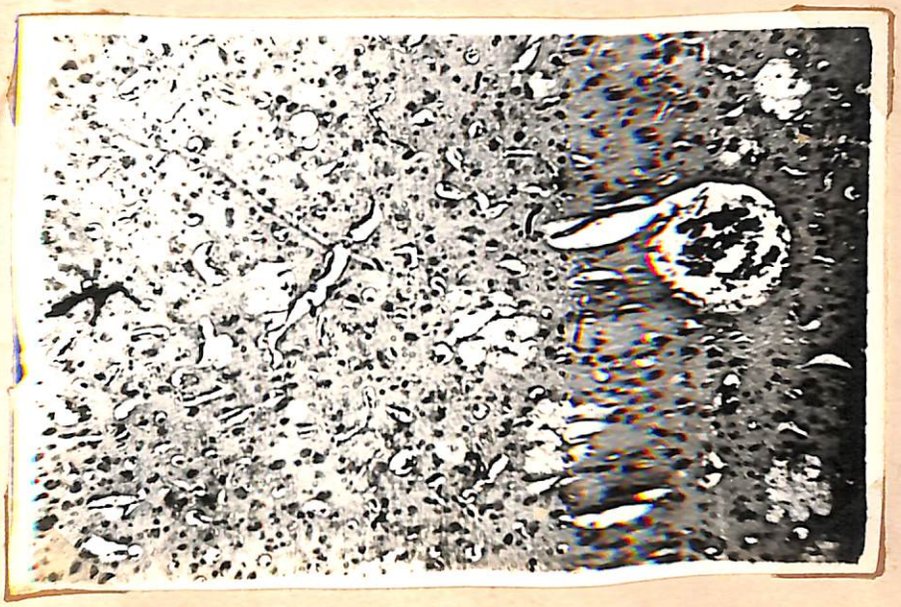

Fig.30
Brain(Cerebrum)
H.& E. x 100
Showing an area of haemorrhage.

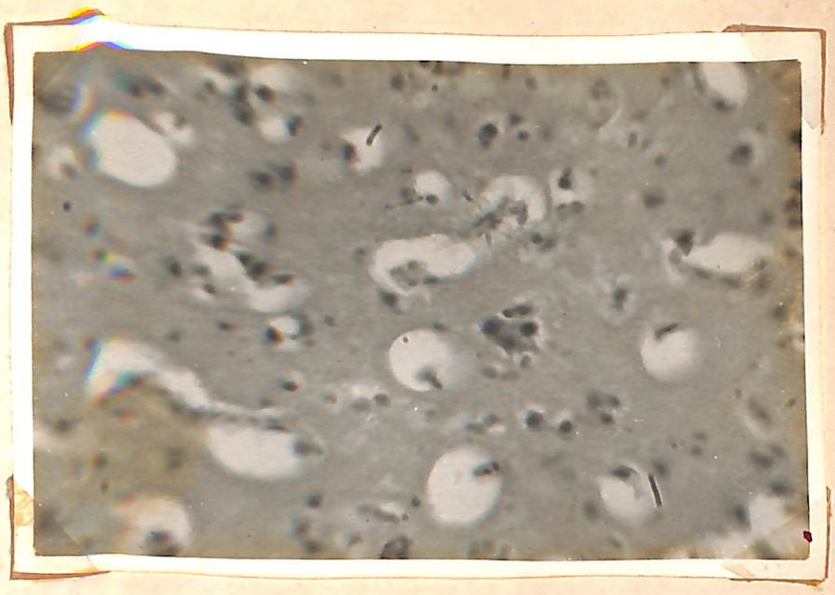

Fig.31
Brain.H.& E.x 400.
Showing neuronal degeneration and neuronophagia in cerebrum.