

STUDIES ON THE HISTO-PATHOLOGY AND PATHOGENESIS OF NEW-  
CASTLE DISEASE OF FOWLS IN SOUTH AFRICA, WITH SPECIAL  
REFERENCE TO THE LYMPHOID TISSUE.  
(A PRELIMINARY REPORT).

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Before considering the pathological studies, it may be of interest to briefly refer to the incidence of Newcastle disease in South Africa.

Newcastle disease was for the first time diagnosed in fowls in South Africa in May 1945, although it was subsequently believed that it was present in Durban, Natal, in September 1944 (Kaschula *et al.*, 1946). Everything pointed to the possible introduction of the disease through the Port of Durban, from where a large part of the Coastal belt of Natal became infected. The disease apparently died out in 1946. Serious outbreaks again occurred in South Africa in 1949 and 1950 in all the Provinces of the Union. It is estimated that more than 300,000 fowls died, and that more than a million were vaccinated with an attenuated live virus. In the Cape West, the last outbreak of Newcastle disease was recorded early in 1951.

According to Kaschula and others (1946), it would appear that the dyspnoea recorded in about half of the number of cases in the Natal outbreaks, might partly have been due to the changes in the upper part of the respiratory tract. Apparently the *lungs*, except for slight reddening, were not affected. In the upper part of the respiratory tract the following lesions were recorded by them: catarrhal rhinitis, diphtheritic laryngitis and haemorrhages in the trachea. Small haemorrhages in the proventriculus were encountered, as well as a certain degree of enteritis, typhlitis, hyperaemia of the rectum sometimes with diphtheritic lesions, and a low grade fibrinous peritonitis (and sacculitis?) was also seen in some of the cases. Most of the fowls revealed nervous symptoms, and it was stated that a combination of respiratory, intestinal and nervous symptoms at once suggested Newcastle disease.

A BRIEF REVIEW OF THE LITERATURE.

Jungherr and Minard (1945) indicated that "Pneumoencephalitis" was characterised by catarrhal tracheitis, bronchitis, *infiltrative sacculitis* of the pulmonary air sacs, haemorrhages, and/or hyaline necrosis in the adenoid sheaths of the spleen. There was no evidence of pulmonary consolidation, but the large bronchi at times revealed marked increase and dilatation of the mucosal goblet cells, indicating a catarrhal bronchitis. Pneumotropism was therefore slightly developed, and it found its principal expression in a sacculitis. The *spleen* was *outstanding* amongst the visceral organs as *exhibiting significant lesions* in over

half of the experimental cases. In their 1946 publication they referred to the necrotic foci of the spleen, and indicated, (in their experimental cases with various strains) that the haemorrhagic necrotic foci of the Peyer's patches, caecal tonsils and lymph follicles, an outstanding feature of the European strains, were but rarely seen in the American strains. The necrotic foci in the intestinal tract usually occurred near or in the normal lymphoid aggregates, and affected tissue cells underwent cytological changes. "Necrotizing" and haemorrhagic lesions were occasionally seen in the liver, gall-bladder, heart and proventriculus. Of interest are their remarks about an increase of non-circumscribed lymphocytic aggregates particularly in the liver and spleen of *immune* birds. They referred to characteristic lesions in the *central nervous system*. These consisted of capillary haemorrhages, myelin degeneration, localised meningitis, *endotheliosis*, neuronal changes, and numerous scattered *foci of gliosis*. The latter were at times associated with perivascular infiltrates. The central nervous lesions occurred particularly in the cord, the brain stem, and adjacent structures. The endotheliosis as an expression of vascular damage was an important feature of the neuro-pathology, particularly vessels of the white matter showed hyperplasia of the endothelial lining. They were of opinion that the *European strains* were endowed with remarkable *enterotropic potentialities* as expressed by necrosis of the spleen and intestine, but with limited affinity for the respiratory tract, and virtually none for the central nervous system. By contrast the *American strains* showed predominance of *neurotropism* or *pneumotropism*, depending on whether the virus was injected parenterally or intratracheally.

Karzon and Bang (1951) in studying the pathogenesis of infection with virulent and avirulent strains of Newcastle disease virus in the chicken, referred to wide variations noted among different strains. After the intramuscular injection of the virus there was a general dissemination of the lesions throughout the brain, characterised by primary neuronal injury, inflammatory infiltrates, either diffuse or clustered around a neurone, and *perivascular "cuffing"*. These three types seemed to appear almost simultaneously. The *lymphocyte* was the cell-type predominantly involved during the acute phase of the infection. *Endothelial proliferation* as a prominent feature of this disease was not noted by them, but they recorded *perivascular* infiltration of large numbers of lymphocytes. Birds which died on the 3rd or 4th day after virulent infection with typical neurological symptoms and with high titre in the brain, showed very few lesions. They indicated that the nervous system was the primary focus of damage after parenteral administration.

De Moulin (1951) referred to the following characteristic histological changes in the central nervous system: a few cases with *endothelial hyperplasia*; absence of the so-called "cuffing"; sometimes slight diffuse infiltration of glia cells; chromatolysis of ganglion cells; etc. He believed that the function of the *spleen* was markedly interfered with (atrophy; loss of lymphocytes) and that this lymphogenous organ had lost its function. He described "*reaction products*" in the spleen in the chronic form of Newcastle disease. Usually they resembled "round bodies" surrounded by a narrow connective tissue capsule, and consisting of large basophilic cells, resembling reticulum cells—probably associated with *antibody formation*. Besides various degrees of degeneration of the liver, there were small infiltrations of lymphocytes, and probably an increase of adventitia cells around small interlobular arteries. He did not see an inflammation of the intestines; and the respiratory symptoms, in the absence of a pneumonia, were probably due to a disturbance in the respiratory mechanism, the amount of blood being reduced as a result of damage to the nervous centre (medulla oblongata). Of great interest were the changes encountered by De Moulin in the pituitary,

thyroid, and adrenals. The degeneration of the basophiles of the pituitary (gonadotropins), according to him probably explained the marked reduction in egg production. There was an increase of monocytes with a reduction of polymorphs and lymphocytes. He explained that marked thyroid deficiency might produce such changes in the blood, which were apparently not due to a direct effect of the virus on the haemopoietic system. De Moulin regarded the disease of the nature of an encephalomyelitis, and a subsequent polyneuritis, the virus having a neurotropic action, not only on the central, but also on the autonomic system, and through the latter it secondarily affected the vegetative nerves. According to him only neurotropism occurred in the case of the Newcastle disease virus.

There appeared to be no finality about the nature ("nodules" or "aggregate"), distribution, and amount of lymphoid tissue in the *normal* chicken and adult fowl. The *spleen* was regarded by Kaupp (1918) as essentially a lymphatic organ, and compact lymphatic tissue occurred in the spleen as spherical, oval or cylindrical masses, known as Malpighian bodies, or splenic corpuscles or follicles. Each corpuscle contained one or more small arteries, rarely situated in the centre. According to Biester and Schwarte (1948) lymphoid tissue was also present between the superficial glands of the *proventriculus*, and the *intestine* also contained much lymphoid tissue.

Bradley (1950) referred to the occurrence of lymphoid tissue in the mucous membrane of the hard palate, pharynx and tongue, and at the junction of the oesophagus and the glandular stomach there was a mass of lymphoid tissue. In the intestine, according to him, the mucous membrane was rich in lymphoid tissue, which was especially abundant in the two caecae, and the bursa cloacae (Fabricius). He maintained that this bursa, like the thymus was prominent in the young fowl, and then underwent involution.

In respect of the *spleen* Bradley described one or two blood vessels in the white pulp, surrounded by concentrically arranged oval nucleated reticular cells. Apparently the tunica media of these vessels was missing, and the tunica adventitia was replaced by lymphoblasts and lymphocytes. The endothelium of the artery was thus placed directly in contact with the sheath of reticular cells and was called the sheath of the artery. By some it has been named the adenoid sheath. The interstices of the red pulp were filled with *lymphocytes*, monocytes, *plasma* cells, macrophages containing iron pigment (with Prussian blue), and all the formed elements of the blood. Clumps of red cells indicated the existence of blood sinuses, which were absent in the area between the red and the white pulp.

According to Lucas (1949) lymphoid tissue in parenchymatous organs of chickens, developing in response to external agents (even in defence of the host), must be considered abnormal. Oakberg and Lucas (1949), however, stated that no finality could be reached concerning "normality" or "abnormality" of lymphoid areas occurring in various tissues in birds, until it was possible to obtain individuals free of lymphomatosis.

In the literature differences of opinion are continually being ventilated as regards the terminology, derivation, morphology, function, and species differences, in respect of lymphocytes, plasmacytes, monocytes and macrophages. Many terms have been applied to the intermediate developing stages of lymphocytes and plasmacytes, i.e. between the undifferentiated stem cell, the reticulum cell, and the mature cell. In the present study it will, however, only be possible to refer to a few aspects of these many contentious and involved problems.

## THE PRESENT INVESTIGATION.

Unfortunately Newcastle disease in Cape Town was virtually under control when these pathological-histological studies, the basis of this paper, were undertaken. No suitable series of specimens were collected from natural cases, and practically all material for these investigations emanated from: (a) clinically healthy fowls infected intramuscularly with virulent virus and (b) immune fowls challenged with virulent strains. The Roakin strain was used for immunization, whereas for the susceptible fowls, and for the challenge of immunity, the Punt and Sweet virulent strains were used, respectively Cape and Natal strains isolated in egg embryos.

Besides the collection of specimens from various organs for sections, *blood smears* as well as *imprint smears* were made from the spleen, liver and bone marrow in the majority of cases. They were stained with Giemsa, and the sections, fixed in formalin, and embedded in paraffin, were stained with Haemalum-eosin (H.E.). The following stains were applied in some of the cases: Giemsa; Phosphotungstic acid—Haematoxylin; Berliner-Blue (B.B.); Mann's stain, Haemalum-Phloxine, and Periodic-Acid-Schiff (P.A.S.).

In some cases blood smears were made prior to destruction, whereas in others at the time of post-mortem, the time after death varying. It was realised that smears from the blood should be made immediately after death, which was not always possible, to avoid coagulation, precipitation, or even disintegration. (which seemed to affect the leucocytes more than the erythrocytes).

In Annexure I dealing with the pathological anatomy, the changes varied from those in which no *significant lesions* were seen (apart from the reddening of the lungs, liver, kidneys, mucous membrane of the intestines and slight degenerative changes in the liver and kidneys), to those recorded in the literature as *significant for Newcastle disease*, namely: localised necrosis of the pharynx, (one case): hyperaemia or/and haemorrhages proventriculus, air sacs, peritoneum, brain, meninges; muco-catarrhal enteritis; haemorrhages and/or localised necrosis, intestines and caecal tonsils; slight atrophy of the spleen; localised sacculitis. A number of cases revealed cyanosis of the comb, and soiling of the ventral feathers with faeces. In *most* of the cases *nervous* symptoms were seen, such as signs of inco-ordination, tremor, torticollis, twitching, paralysis, affecting either a leg or a wing, or both. Progressive weakness led to prostration, and eventually to death, or the fowl was killed *in extremis* for post mortem and the collection of suitable smears and specimens.

## MICROSCOPICAL STUDIES.

(See Annexures 2-4)

*Blood.*

Some difficulty was experienced in differentiating between certain monocytes and large lymphocytes of the fowl's blood. The following criteria were taken into consideration in the examination of the Giemsa stained smears:— (a) shape and character of the nucleus, (b) blue or greyish blue colour of the cytoplasm of the cell, and (c) the presence of phagocytosed debris in the cytoplasm of the cell. In this paper the term *immature* has been adopted for the developing stage of lymphocytes and plasmacytes, and the term *heterophile* has been applied to the so-called polymorph pseudo-eosinophiles with rod-shaped granules. In view of the limited number of cases studied, it is appreciated that a guarded interpretation of the findings, recorded in the annexures, has to be submitted.



In a few cases "immature" erythrocytes were identified, but an oligocythaemia could not be substantiated morphologically in the majority of cases. There appeared to be a *monocytosis* in a number of cases, with a reduction in the number of small lymphocytes. In some of the cases the monocytes revealed phagocytosed debris. In some the remains of erythrocytes could be identified. In some of the blood smears, as well as in the imprint spleen smears, stained with Giemsa, monocytes or macrophages with phagocytosed red cells could be identified (see plate No. 1). These broken down remains varied from light yellowish brown granules to discs, slightly more intensely stained. In some cells vacuoles of various sizes were present between these remains. Some of the monocytes and macrophages also showed the presence of "blue-blotches" between the phagocytosed remains. They were homogeneous in nature, and with the Giemsa stain assumed a dark greenish blue colour. In some cells these blotches almost completely filled the cell, and partly obscured the nucleus.

#### *Imprint Spleen Smears.*

Imprint smears made at post mortem from cases of Newcastle disease, were compared with smears from clinically healthy fowls. The following characteristics of plasmacytes as compared with lymphocytes were taken into consideration: the character and size of the nucleus which was in most cases eccentrically situated; the greater amount of cytoplasm as compared with that of the lymphocyte; the deeper blue colour of the cytoplasm and clear zone, the so-called halo next to the nucleus. In the interpretation of the frequency of the lymphocytes and plasmacytes, it is essential that a large number of impressions be examined, because these cells are sometimes not evenly distributed throughout the smears. The plasmacytes varied in the early deaths from more frequent, to those cases in which they seemed to dominate the picture. In some cases the immature plasmacyte seemed to be more frequent. Of interest is the spleen smear of immune fowl No. 41054 killed *in extremis* 20 days after it was challenged with virulent virus. It showed the lymphocytes not depleted, whereas the plasmacytes were rare. Control Fowl No. 41167 killed on account of paralysis 36 days after the injection, showed clusters of mature lymphocytes, while plasmacytes were only infrequently present.

In the *spleen smears* examined great variation occurred in the numbers and nature of the *macrophages*. In some smears they were infrequent, whereas in others they were frequent. The phagocytosed material varied from ingested erythrocytes, in which the outlines of their nuclei could be faintly identified, to pigment granules or discs of various sizes and shapes. In some cases blue blotches, were also seen in some of the macrophages, almost filling the cytoplasm of the cell, and partly obscuring the nucleus.

#### *Imprint Liver Smears.*

In Fowl No. 43 macrophages with phagocytosed erythrocytes were identified. In some of the Newcastle disease cases macrophages with blue blotches were observed. In No. 1209 heterophiles were not infrequent. In the majority of the Newcastle disease cases vacuoles in the cytoplasm of liver cells stained with Giemsa varied in size and frequency.

#### *Imprint Bone-marrow Smears.*

Apparently the bone marrow was not affected. Active regeneration of erythrocytes and granulocytes was identified (43, 50, 60, 61, 80, 1934). In 43 and 80 a few macrophages with "blue blotches" were seen. No proliferation of plasma cells was observed.

*Myocardium.*

No significant features were identified, except hyperaemia, which in some of the cases was fairly well marked. The presence of lymphocytic "aggregates", associated with a few blood vessels, although very rare, should be recorded.

*Lungs.*

The outstanding feature in some of these cases was the hyperaemia. There was no evidence of any exudation or induration except in cases Nos. 39805 and 39220; excess of mucus was identified within the lumen of one of the bronchi.

*Liver.*

Hyperaemia and/or degeneration of the liver varied from slight to fairly well marked. In some cases macrophages with haemosiderin granules were identified in the sinusoids (39805 and 40953). With the Giemsa stain blue blotches were revealed in some of the macrophages (40956). The presence of mononuclears, mainly lymphocytes, was encountered in the adventitia of some of the blood vessels in some cases (39219, 40955, 41167, 30 and 39914). There was no evidence of necrosis, and in a few cases there was a fair number of heterophiles. In some sections it was difficult to differentiate between heterophiles and eosinophiles, but in this respect the imprint smears were most helpful.

*Pancreas.*

Specimen No. 40957 showed several clusters of cells of the acini in which the eosin stain was lighter in colour, the cells appeared swollen, and some showed the presence of a few vacuoles. In specimen No. 39222 there were a few small areas which appeared to be of the nature of an early necrosis. Circumscribed lymphocytic "aggregates", associated with blood vessels were encountered in a few cases (40957, 39222 and 41167).

*Kidneys.*

In the majority of cases examined an hyperaemia was present which varied from slight to fairly well marked. Degenerative changes of the tubular epithelium was also seen in a number of cases. The presence of circumscribed lymphocytic aggregates should be recorded in the following cases Nos. 40957, 41054 and 41167.

From a survey of the results of the investigations as reflected above, as well as those recorded in the earlier cases in Natal, no pathognomonic Newcastle disease changes were identified in *the lungs, the liver, the pancreas, the kidneys and the myocardium*. The circumscribed lymphocytic aggregates noted in the liver, kidneys and myocardium, will be referred to later on, as well as the observations made in respect of the frequency of plasmacytes in the imprint spleen smears. Pathognomonic lesions, as recorded in the literature, were encountered in the *spleen, the gastro-intestinal tract and the central nervous system*.

*The Spleen.*

In practically all the "early" deaths, there was a certain degree of degeneration and loss of cells around the artery of the follicle (see plate No. II). In one or two cases this was associated with a fairly extensive hyalinisation, as an irregular homogeneous pink (with H. E.) network around the follicular artery (see plate No. III). There was a depletion of lymphocytic cells. In some of the cases small irregular haemorrhages were encountered in the periphery, as

well as a varying number of heterophiles and macrophages. With the H.E. stain the latter revealed the remains of phagocytosed red cells, which with the B.B. stain appeared to be haemosiderin. In some cases (e.g. sections No. 39915, 39805, 42072, 42073), the degenerated cells around the follicular artery of some of the follicles of the spleen, showed the presence of flake-like granules.

The nature of the flakes is well illustrated in the sections stained with H.E., Giemsa and P.A.S. (see plates No. IV and V). It would appear that cells of the adenoid sheath were involved in this change. Between these degenerated cells and the periphery of the follicle there was a fair number of large spherical or polyhedral-shaped cells with round nuclei with sparse chromatin, the so-called reticulum cells. These cells, as well as the plasmacytes, seemed to be increased at the expense of the lymphocytes (see references above in respect of the imprint spleen smears). Intra-nuclear inclusions were identified in some of these reticulum cells. They were polyhedral in shape, slightly larger than a nucleolus and reddish pink, with H.E. and Mann's stains. These so-called inclusions are from time to time met with in reticulum cells.

#### *The Gastro-intestinal Tract.*

The main changes in the gastro-intestinal tract were observed in the *proventriculus, small intestine and caecal tonsils*. They appeared to be of the nature of a localised necrosis with haemorrhages, rather than of an inflammation. The examination of portions of the intestine was, however, limited to those cases which showed naked-eye lesions. It is quite possible that there might have been disturbances, especially in regard to the subepithelial lymphoid tissue, in other portions of the intestine, or in other earlier cases, which did not reveal any evident naked-eye lesions of the mucous membrane. In a number of cases the mucous membrane of the intestine revealed small haemorrhages, and these might have been associated with earliest evidence of disturbance in the lymphoid tissue. The degree of the breaking down of the lymphoid tissue varied from slight to almost complete depletion. In the propria clusters of "flakes" of a light brown (tan) colour with the H.E. stain occurred in the interstices, (see Plate No. VI). It was apparently of the same nature as those described in the spleen above. There was also a depletion or loss of the intestinal glands, and the goblet cells, and in some cases there was an infiltration of heterophiles.

#### *The Central Nervous System.*

In view of the fact that nearly all the cases revealed symptoms associated with the central nervous system, specimens were only collected from the brain, and in some cases from the medulla. In cases of paralysis, important peripheral nerves were examined for neuro-lymphomatosis, however with negative results. It is appreciated that specimens from the spinal cord should have been collected at various levels for histological examination.

The changes encountered in the sections of the central nervous system are tabulated in Annexure IV. Unfortunately the specimens were selected at random at varying levels of the brain, but an attempt has been made to study the changes in: (a) cerebellar cortex and white matter, (b) optic lobes and (c) cerebrum.

The majority of the cases revealed a hyperaemia, which varied from slight to fairly well marked. Small haemorrhages were recorded in some of the cases, (see plate No. VII), and in one they were prominent around the blood vessels of the white matter. In nearly all the sections changes were recorded in the cells of the walls of the small blood vessels. Some of the endothelials appeared to



be swollen, and assumed a polyhedral shape. A few of these cells were desquamated, and collected with mononuclears, red cells, and plasma within the lumen of the blood vessels. The walls of the blood vessels were also infiltrated with lymphocytes, monocytes and other cells of the blood, and in a few cases formed several layers of cells (see plate No. VIII). In a few cases a few of these cells were found in the peri-vascular lymph spaces. (Probably the earliest evidence of perivascular infiltration).

Many ganglion cells in the brain showed a chromatolysis of varying degree. It usually commenced as a swelling of the cell, dissolution of Nissl's substance and vacuolation of the cytoplasm of the cell. Gradual loss of the staining properties of the nuclei followed, with their ultimate disappearance. In practically all cases, a number of the Purkinje cells were affected to a varying degree.

The neuroglia was involved to a less extent, and here and there foci of gliosis were encountered. In the cortex of the cerebellum a few areas of slight gliosis were seen to extend from the granular layer into the molecular layer. In several cases the meninges showed hyperaemia, and in some a localised leptomeningitis could be identified.

#### *Observations on Fowls Immunized against Newcastle Disease.*

Fowl No. 52 (post mortem 1609, specimen No. 41224) and Fowl No. 33 (p.m. 1610, specimen No. 41224A) were immunized in May 1950, and on the 29/5/51 challenged with virulent virus. They showed no clinical symptoms, and were destroyed on 9/7/51, fowl No. 33 by chloroform, and fowl No. 52 by wringing the neck. This was undertaken to ascertain whether the hyperaemia, and/or the haemorrhages noted in the cerebellum in cases of Newcastle disease, could be attributed to the latter method of destruction. The haemorrhages observed in the spinal cord, medulla and base of the cerebellum of fowl No. 52 were present as irregular lagoons, and were not in any way associated with the blood vessels, nor were these dilated. The blood had apparently filtered in from the severed arteries as a result of the traumatic influences.

The spleen of these immune birds revealed "aggregates" of lymphocytes, like nodules, in juxta-position to certain arteries (not the arteries of the follicle). Some of these nodules were encircled by a thin layer of tissue associated with the adventitia of the artery (see plate No. IX). The lymphoid tissue in *caecal tonsils* were also hyperplastic, and in these, nodules with secondary nodules, could be identified (see plate No. X). In the *lungs* (see plate No. XI) a similar lymphoid hyperplasia was encountered, and "aggregates" of lymphocytes were also seen in the myocardium, liver, kidneys, and even in the medulla (specimen No. 41224). In fowl specimen No. 41167 (a chronic case) lymphoid tissue was also extravagantly present in the caecal tonsils, and as nodules in the spleen, and as lymphocytic aggregates in the myocardium, pancreas, liver and kidneys.

#### DISCUSSION.

This preliminary investigation and the brief review of the literature revealed disagreement as regards the *pathogenesis* of Newcastle disease. One or more of the following might probably be responsible for this: strain of virus; age of the fowl (baby chickens as compared with adults); the possibility of the presence of an immunity; port of entry of the virus (per os, intranasal, parenteral); or whether death occurred during the earliest stages, or during a more protracted course of the disease. According to Jungherr and others, *pneumotropism* was



slightly developed, and found its principal expression in a sacculitis. In the absence of pneumonia, could the respiratory symptoms (Beach, 1948), especially in young chickens, be associated with slight lesions in the air sacs, or were they due to damage of the nerve centre, as stated by De Moulin?

There are differences of opinion as regards the changes observed in the nervous system. Biggart (1949) indicated that one should be guarded about the interpretation of degenerative changes in the ganglion cells, depending on how soon after death specimens were collected, and the kind of fixative used. He referred to the shrinkage and more intense staining of the nerve cells produced occasionally as an "artefact", for the outer layers of ganglion cells of the cortex of quite normal animals, after formalin fixation. This was observed in some of the cases, and referred to in the Annexure. The presence of "satellites" in connection with ganglion cells in the grey matter should also be very carefully considered, in view of their not infrequent presence near healthy ganglion cells at various levels. Biggart indicated that many *changes of function* of the nerve cells in their earlier stages, represented merely some alterations in cell metabolism, which could not be demonstrated by histological methods. Karson and Bang (1951) referred to birds, which died on the 3rd or 4th day after virulent infection with typical neurological symptoms, and, with high virus titre in the brain, had very few lesions. In a number of the Newcastle Disease cases studied here, showing definite nervous symptoms during the earliest stages, lesions in the brain and medulla were remarkably slight.

Apart from regressive changes observed in the ganglion cells and circulatory disturbances (hyperaemia, haemorrhages) Jungherr is of opinion that "endo-heliosis" of the blood vessels is an important feature of the neuropathology, and that *neurotropism* is predominant in the American strains. De Moulin also referred to an endothelial hyperplasia in the smallest blood vessels. According to him no perivascular infiltration of lymphocytes, the so-called "cuffing" was seen. Karson and Bang on the other hand stressed the presence of perivascular cuffing, in which the lymphocyte was apparently the dominant cell-type, and in which endothelial proliferation, as a prominent feature of this disease, was not found. Apparently the nervous system was the site of damage after parenteral administration, and caused a general dissemination of lesions throughout the brain.

The cases investigated here revealed, apart from hyperaemia and/or haemorrhages, swelling and degeneration of the *endothelials*, and changes within the lumen of some of the small blood vessels of the brain. There was also an infiltration (probably from the blood) of lymphocytes, monocytes and red cells, into the wall of these vessels. Peri-vascular infiltration of lymphocytes was represented in a few cases by the presence of a few mononuclears. Is the damage to the cells of the walls of the blood vessels due to the direct action of the virus? Chromatolysis of ganglion cells and areas of gliosis were also identified in a number of these cases. Satellitosis was only seen to a slight extent in a few cases.

The *spleen* as well as *Peyer's patches*, and *caecal tonsils* were, according to Jungherr and Minard, outstanding amongst visceral organs in exhibiting significant lesions of an haemorrhagic-necrotic nature, i.e. in important lymphoid tissue depots. De Moulin indicated that the function of the spleen was markedly interfered with, and that this lymphogenous organ had lost its service. According to Wolfe *et al.* (1950) splenectomy of chickens between the ages of 6 and 12 weeks reduced their ability to produce precipitating antibodies.

## HISTO-PATHOLOGY AND PATHOGENESIS OF NEWCASTLE DISEASE.

In these preliminary studies of Newcastle disease, changes have been described in the main lymphoid depots of the fowl, particularly in the early cases that died of this disease, or were destroyed *in extremis*. These changes were of the nature of a degeneration and a depletion of the cells of the lymphocytic series, whereas there was an apparent proliferation of reticulum cells, and an increase in the number of plasmacytes. Reference was made to the presence of *flake-like granules* in some of the degenerating cells of the lymphoid tissue, particularly in the spleen, and at certain levels of the alimentary tract.

In their experiments on the functional alterations of lymphoid tissue, induced by adrenal or cortical secretions in mice and rabbits, Dougherty and White (1945) referred to a flaky-cytoplasmic material, and they associated it with the cytoplasm of degenerating lymphocytes. Flewett in dealing with the growth of viruses of the influenza group as revealed by the electron microscope of infected cells, wished to know whether influenza virus elementary bodies break up within the cell into large numbers of smaller units, which subsequently combined to form fresh elementary bodies. Sommers *et al.* (1951) referred to the lymphoid tissue in poliomyelitis, and stated that, aside from the central nervous system, the lymphoid tissue exhibited the most frequent and marked changes. Around the edges of the affected follicles of the lymphoid tissue active regeneration of reticulum cells was indicated. Baner in discussing aspects of virus multiplication, postulated that virus particles after entering the cell break up into a large number of sub-units which diffuse through the cell. It seemed quite probable that the virus particles of influenza were mere droplets of diseased cytoplasm.

Are the flakes in the cells observed in these studies on Newcastle disease in any way related to a development, and possible multiplication of the virus units? Are the reticulum cells and plasmacytes in any way associated with antibody formation in the lymphoid depots?

On the one hand there appears to be a *preponderance of the plasmacytic series* with a certain degree of depletion of the *lymphocytes*, whereas in the later phases there is an apparent hyperplasia of the lymphoid tissue in various organs. Does this hyperplasia (also commented on by De Moulin), observed in these immune fowls, and chronic case, stand in relation to an "immunity reaction", or is it of the nature of an early *lymphomatosis*, a disease apparently fairly prevalent in the Union? It may, however, be pointed out that in none of the batch of Cape Town fowls used for these experiments, were any significant lesions of lymphomatosis seen.

Apart from the considerable significance of the lymphocyte to the general body economy, Craddock, *et al.*, (1947) stated that in spite of the diversity of opinion as to the relative value of various tissues (lymphocyte, plasmacyte, and reticulo-endothelial cell) in antibody mechanism, recent findings revived the concept that lymphoid tissue and its product the lymphocyte were fundamental in this process. Fagraeus (1948) was of opinion that the differentiation of the transitional cells of the spleen into immature plasma cells implied a considerable rise in the capacity of these tissues to form antibodies. This took place side by side with the development of the reticulum into plasma cells, and the conclusion is drawn that antibodies were formed in the R.E. cells. Burnet and Fenner (1949) maintained that the *plasma cells response* in the spleens of fowls, *immunized against Newcastle disease*, was completely in line with the findings of Fagraeus, and that the reticulum cell, while producing antibody, took on a plasma-cell "character". The lymphocytes according to them were probably responsible for the maintenance of *low levels of antibody, long after the antigenic stimulus*. Ehrlich (1946) referred to the close co-existence of lymphocytes and

plasmacytes, and suggested that both were engaged in antibody production, either in different phases of the same problem, or in the production of different antibodies. Some regarded the lymphocyte as a multipotent cell that might develop into monocyte and a macrophage; while others, e.g. Ehrich (1946), denied the possibility of further development of the lymphocyte and considered it to be a differentiated mature cell. According to Parsons (1943) lymphocytes might be entirely absent from glands, when plasma cells were abundantly active, and it was suggested that reticulum cells might be the direct precursors of the plasma cell. Fadem and McBirnie (1950) lent support to the suggested reticulum origin of the plasmacyte series. In the above preliminary investigations one is inclined to agree with Ehrich and others as regards the independent nature of the lymphocyte, from which plasmacytes are not derived.

Does a reaction between the virus of Newcastle disease and antibody formation in the lymphoid tissue result in one of the following:—

1. The setting free of sufficient virus in the circulation to produce the characteristic lesions in the nervous system, resulting either in early death, or in a more protracted course of the disease?
2. Destruction of some of the ganglion cells in one or more centres of the nervous system, resulting in paralysis of the wings and/or legs; in the interim the virus is brought under control, as revealed by a lymphoid hyperplasia in several organs?
3. Sufficient antibody is produced in the lymphoid tissue in the early stages of the disease to control the virus leading to recovery and immunity?

The significance of the blue blotches is at present not understood. Are they in any way associated with a breaking-down process of nuclei of nucleated cells?

De Moulin referred to the changes encountered in the *pituitary*, *thyroids*, and *adrenals*. This disintegration of the basophiles (gonadotropins) of the pituitary may, according to him, explain the marked reduction of egg production. Apparently Newcastle disease is not the only one in fowls in which this occurs. According to Beaudette (1951) the effect of Newcastle disease and Infectious Bronchitis on laying birds are quite alike; production is markedly reduced but more likely to reach zero in Newcastle disease infection. Kaschula mentioned the severe drop in egg production in some cases for as long as nine weeks, in fowls immunised with (Fort Dodge) *killed Newcastle disease vaccine*. Unfortunately specimens were not collected from the endocrines in these studies. Their importance was appreciated in the recent investigations of White and Dougherty (1944), Craddock *et al.* (1949), Feldman (1951) and others, in which the endocrine influence on the lymphoid tissues was considered. Apparently the pituitary and the adrenal cortex control the structure of lymphoid tissue; but according to Craddock and others, there appears to be no unanimity as to what influence they exert on antibody formation.

Further investigations seem to be indicated to clarify some of the issues raised above. Susceptible, immune, and even splenectomised chickens and adult fowls, infected with Newcastle disease virulent or attenuated strains, should be destroyed at various stages of the disease for the collection of suitable smears and specimens, in order to study:—

- (a) the concentration of the virus, especially in the lymphoid tissue and the brain, and at the same time to ascertain the nature of the morphological changes in earliest cases, and compare these with the findings in more protracted cases;



## HISTO-PATHOLOGY AND PATHOGENESIS OF NEWCASTLE DISEASE.

- (b) the pathogenesis of the disease in baby chickens compared with adult fowls;
- (c) electron microscopic studies of the possible existence and nature of virus particles in the lymphoid tissue during certain phases of the disease;
- (d) haematological studies, and changes in the endocrines, and their possible influence on the lymphoid tissue, and indirectly on the virus;
- (e) the possible co-existence of lymphomatosis, and whether it has any influence on the course of Newcastle disease.

### SUMMARY.

1. Newcastle Disease, after intramuscular inoculation of virulent virus, was characterised by pathognomonic lesions in the spleen, the gastro-intestinal tract, and the nervous system.

2. In the *Spleen* degenerative changes in the cells of the lymphoid sheath were noted in early deaths. In some of them flake-like granules were observed. There were depletion of lymphocytic cells in the follicle, increase in the number of plasmacytes, and proliferation of reticulum cells. In cases with a more protracted course, hyperplasia of the lymphoid tissue was apparent.

3. In the gastro-intestinal tract the chief changes were observed in the *Proventriculus*, the *Small Intestine*, and *Caecal Tonsils*. In early cases, this was of the nature of a localised necrosis with haemorrhage. The changes in the lymphoid tissue were of a similar nature as those observed in the spleen.

4. The *Nervous system* in the majority of cases revealed a hyperaemia, and in some, small haemorrhages. The changes in the small bloodvessels were of the nature of a so-called "endotheliosis" with an infiltration of the walls with lymphocytes, and other cells of the blood. In some of these vessels the presence of plasma, mononuclears and red cells was noted within the lumen. In some cases chromatolysis and slight gliosis were seen.

5. More information is desired about the depots, morphology, and function of the lymphoid tissue of the fowl. It would, however, appear that lymphocytes and plasmacytes have an independent origin, and that they are probably implicated in the propagation of the virus and antibody mechanism. At this stage it is not possible to indicate what the nature is of the flake-like granules in some of the cells of the lymphoid tissue. Further investigations are indicated to clarify some of the problems raised in this preliminary study.

### ACKNOWLEDGMENT.

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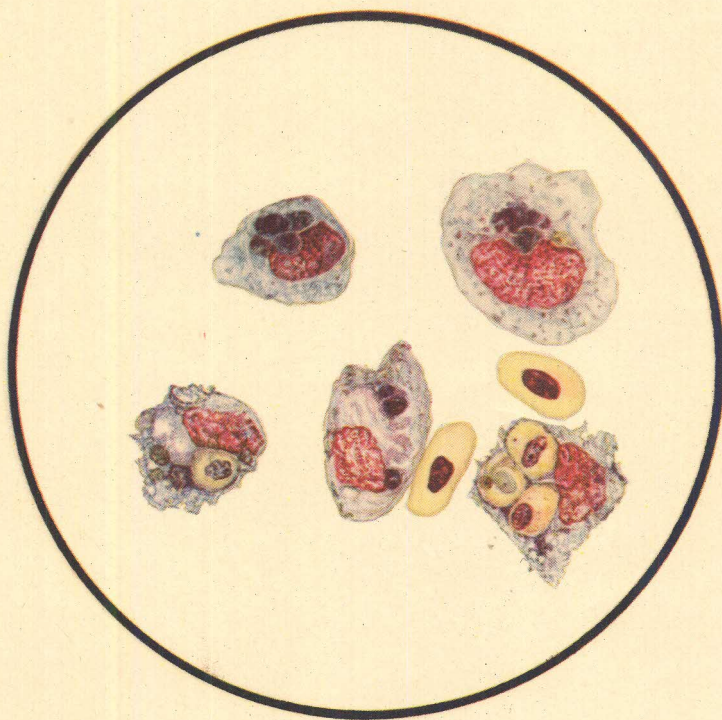
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PLATE No. I.



FOWL No. 1212: *Peripheral blood*: Stained with Giemsa; note monocytes with phagocytosed erythrocytes, and "Blue-blotches". (Specimen No. 40956.)

PLATE II.



FOWL No. 60.—*Spleen*: Note disintegrating follicle of the spleen with loss of lymphocytes, and haemorrhages in the periphery. Susceptible Fowl: Died 5 days after inoculation with virulent virus. (Specimen No. 42072.)



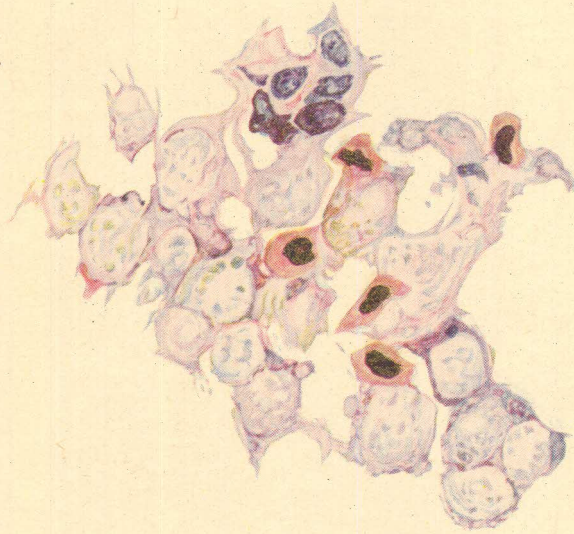
PLATE III.



FOWL No. 61.—*Spleen*: advanced disintegration of the follicle of the spleen, with loss of lymphocytes, and areas of hyalinisation. Susceptible Fowl: Killed *in extremis* 5 days after inoculation with virulent virus. (Specimen No. 42073.)

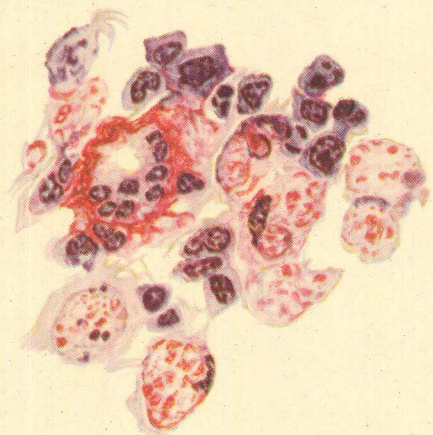


PLATE No. IV.



FOWL No. 150: *Spleen*: Note the presence of flake-like granules in some of the cells around the follicular artery. (Specimen No. 39915.) Objective 2m.m., Ocular K7X.

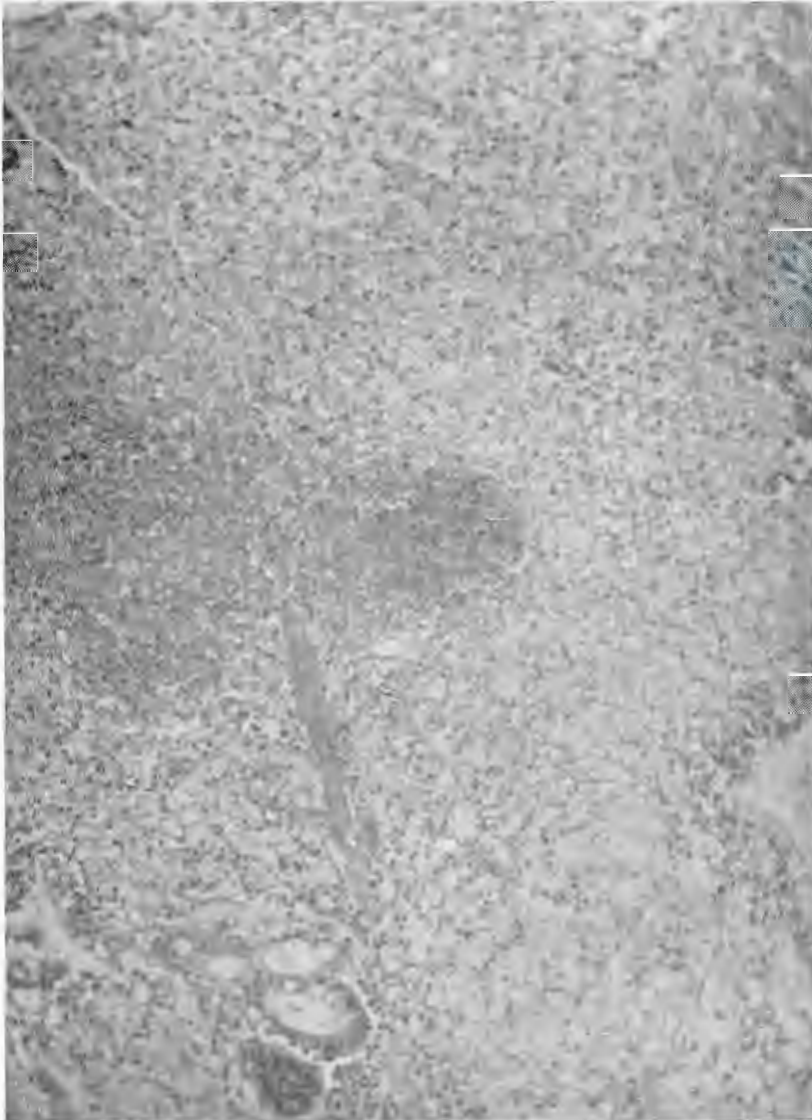
PLATE No. V.



FOWL No. 60: *Spleen*: Note the presence of the flake-like granules in some of the cells around the follicular artery. (Specimen No. 42072.) Stain: Periodic-acid, Schiff.



PLATE VI.



FOWL No. 29.—*Caecal Tonsil*: Note extensive disintegration of lymphoid tissue and the tubular glands. In the network are the so-called flakes which stain a tan with H.E. Susceptible Fowl: killed *in extremis* 6 days after inoculation with virulent virus. (Specimen No. 39805.)

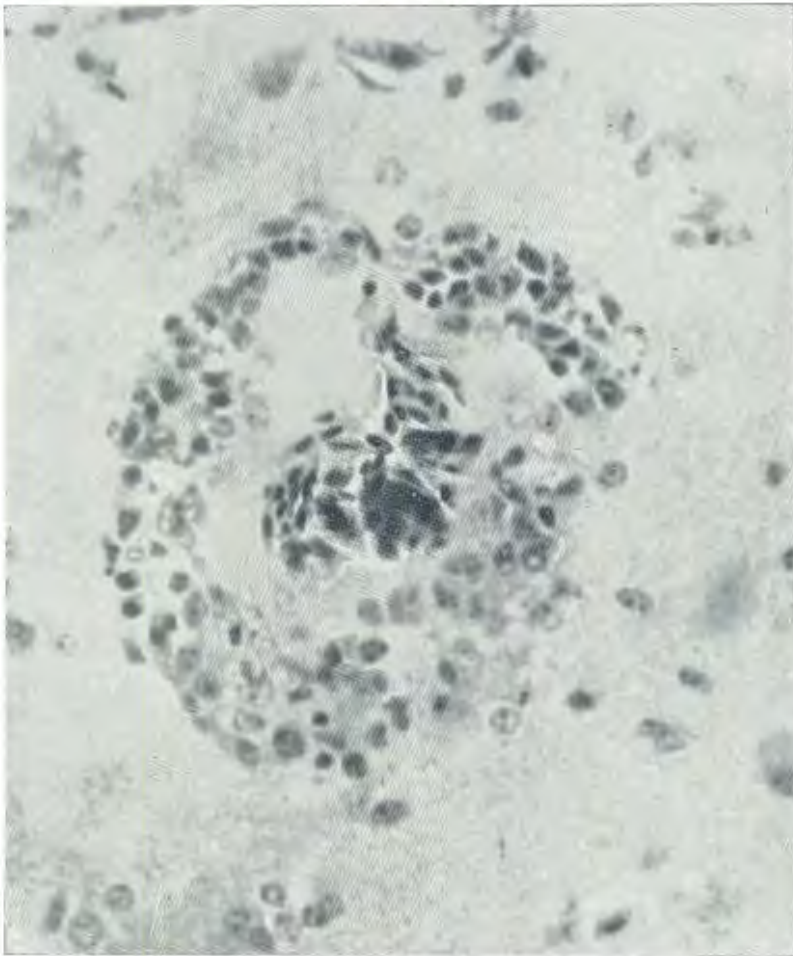


PLATE VII.



FOWL No. 30.—*Cerebellum*: note the cell changes in the wall of a bloodvessel, and a small perivascular haemorrhage. Susceptible Fowl: *Killed in extremis* 6 days after inoculation with virulent virus.

PLATE VIII.



FOWL No. 1212.—*Brain*: Note the cell changes in the wall of a small blood vessel, and within the lumen the presence of plasma, and the “clumping” of the red cells. Susceptible Fowl: Died 5 days after inoculation with virulent virus. (Specimen No. 40956.)

PLATE IX.



FOWL No. 1609.—*Spleen*: Note lymphoid nodule associated with the wall of an artery. Immunised May 1950; challenged with virulent virus 29/5/51; no clinical symptoms; destroyed 9/7/51 for post mortem. (Specimen No. 41224.)



PLATE X.



FOWL No. 1609.—*Caecal Tonsil*: Note lymphoid hyperplasia and formation of a nodule. Immunised May 1950; challenged with virulent virus 29/5/51; no clinical symptoms; destroyed 9/7/51 for post mortem. (Specimen No. 41224.)



PLATE XI.



FOWL No. 1610.—*Lung*: Note the lymphoid hyperplasia in the mucous membrane of a bronchus. Immunised May 1950; challenged with virulent virus 29/5/51; no clinical symptoms; destroyed 9/7/51 for post mortem. (Specimen No. 41224A.)

## ANNEXURE I.

## PATHOLOGICAL ANATOMY.

P.M. No.	Specimen No.	N. Natural, C. Virus Control, I. Immunity Test.	Date of Death.	Died. or killed.	No. of Days after Inoculation.	H.I. Test.	Breed.	Significant Changes.
29	39805	Control R.I.....	26/5/50	Killed in <i>extremis</i>	6 days	Negative before injection with Punt strain	W.L.	Liver lighter in colour and more friable; hyperaemia proventriculus; reddish areas intestine.
30	—	Control R.I.....	26/5/50	Killed in <i>extremis</i>	6 days	Negative before injection with Punt strain	W.L.	Liver lighter in colour and more friable; hyperaemia proventriculus; reddish areas intestine.
31	—	Control R.I.....	26/5/50	Died....	6 days	Negative before injection with Punt strain	W.L.	Petechiae mucous membrane intestines; blood vessels of the brain injected.
41	39219	N. (N.D. virus isolated in eggs)	7/6/50	Died....	—	—	B.A.	Liver light brown; hyperaemia of intestine; symptoms of paralysis.
41A	39220	N. (N.D. virus isolated in eggs)	7/6/50	Killed....	—	—	B.A.	Liver light brown; hyperaemia of intestine; symptoms of paralysis.
43	39221	C.....	13/6/50	Died....	6 days	Negative before injection with Punt strain	W.L.	Liver lighter in colour with darker patches; erosions proventriculus; localised necrosis intestines.
50	39222	C.....	16/6/50	Killed in <i>extremis</i>	9 days	Negative before injection with Punt strain	W.L.	Negative P.M., but nervous symptoms. It was sick since 11/6/50.
60	—	C.....	30/6/50	Died....	5 days	Injected with Sweet strain	W.L.	Blood vessels of the brain and dura mater injected; petechiae dura mater; dark reddish areas raised above the mucosa small intestine and caecal tonsils. Spleen smaller and lighter colour.
61	—	C.....	30/6/50	Killed in <i>extremis</i>	5 days	Injected with Sweet strain	W.L.	Blood vessels of the brain and dura mater injected; petechiae dura mater; dark reddish areas raised above the mucosa small intestine and caecal tonsils. Spleen smaller and lighter colour.
149	39914	C. (3842).....	28/10/50	Died....	5 days	Negative before injection with Sweet strain	B.A.	Spleen lighter in colour and smaller; multiple haemorrhages mesentery and mucous membrane of intestines; few localised necroses pharyngeal mucous membrane; hyperaemia of kidneys.

ANNEXURE I—(Continued).

P.M. No.	Specimen No.	N. Natural. C. Virus Control. I. Immunity Test.	Date of Death.	Died or killed.	No. of Days after In-oculation.	H.I. Test.	Breed.	Significant Changes.
150	39915	C. (3850).....	28/10/50	Killed...	5 days	Negative before injection with Sweet strain	B.A.	Spleen lighter in colour and smaller; multiple haemorrhages mesentery and mucous membrane of intestines; few localised necroses pharyngeal mucous membrane; hyperaemia of kidneys.
1209	40953	C. (4123).....	3/6/51	Died....	5 days	—	W.L.	Slight icterus; slight degeneration of the liver; spleen smaller in size; hyperaemia of the kidney; slight muco-catarrhal enteritis; few haemorrhages caecal tonsils.
1210	40954	C. (4146).....	3/6/51	Died....	5 days	—	W.L.	Hyperaemia liver and kidneys; general cyanosis; small haemorrhages caecal tonsils.
1211	40955	I. (35).....	3/6/51	Died....	5 days	—	W.L.	P.M. changes; hyperaemia kidneys; general cyanosis.
1212	40956	C. (4173).....	4/6/51	Died....	6 days	—	W.L.	Slight localised acute sacculitis; haemorrhages proventriculus; few haemorrhages in gizzard, and in caecal tonsils; slight hyperaemia of kidneys; general cyanosis.
1244	40957	C. (4168).....	5/6/51	Killed in <i>extremis</i>	7 days	—	W.L.	Slight localised acute sacculitis; few haemorrhages caecal tonsils; slight hyperaemia kidneys.
1394	41054	I. (30).....	18/6/51	Killed in <i>extremis</i>	20 days	—	W.L.	Nervous symptoms clinically; no specific changes at post mortem, except slight muco-catarrhal enteritis; hyperaemia liver and kidneys.
—	41167	C. (4131).....	4/7/51	Killed in <i>extremis</i>	36 days	—	W.L.	Paralysed; negative post mortem.

The Roakin strain is the Beaudette vaccine strain.  
 The Punt strain is a virulent Cape strain.  
 The Sweet strain is a virulent Natal strain.  
 They were all egg embryo material.

R.I. Robben Island.  
 W.L.: White leghorn.  
 B.A.: Black Australorps.

ANNEXURE II.  
BLOOD SMEARS.

P.M. No.	Specimen No.	Significant Changes.
29	39805	Increased number of leucocytes, particularly monocytes, some of which are vacuolated.
30	—	Increase in the number of the monocytes; note the occurrence of these cells in clusters with heterophiles and thrombocytes.
43	39221	Smear unsatisfactory, but an increase in the number of monocytes, some with large vacuoles, irregular in size.
50	39222	Increase in the number of the monocytes; heterophiles also frequent.
60	—	Monocytosis, some with phagocytosis.
61	—	Monocytosis, some with phagocytosis.. Some monocytes with phagocytosed erythrocytes in all stages of disintegration.
149	39914	Monocytosis.
150	39915	Monocytosis.
1209	40953	Heterophiles prominent; increased number of monocytes some with blue blotches.
1210	40954	Monocytosis, some with phagocytosis.
1211	—	Smear unsatisfactory, disintegrated.
1212	40956	Few "immature" erythrocytes; large numbers of monocytes, some with phagocytosed erythrocytes in various stages of disintegration; some with blue blotches.
1244	40957	Monocytes prominent, some with blue blotches of various sizes.
1394	41054	Fair distribution of leucocytes.
—	41167	Increased number of monocytes in places appearing in clusters; heterophiles not infrequent.

ANNEXURE III.  
IMPRINT SPLEEN SMEARS.

P.M. No.	Specimen No.	Significant Changes.
29	39805	Plasma cells more frequent than lymphocytes, especially in certain places. A number of macrophages with "blue blotches".
30	—	Plasma cells more frequent; macrophages not infrequent.
31	—	Plasma cells more frequent; a number of "immature cells"; macrophages not infrequent, some with blue blotches, some with granular debris
43	39221	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent.
50	39222	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent. Some macrophages with blue blotches.
60	—	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent.
61	—	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent. Large number of immature cells.
149	39914	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent. Large number of macrophages, either with ingested erythrocytes, or granular debris.
150	39915	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent. Cells with blue blotches frequent.
1209	40953	Plasma cells much more frequent than lymphocytes, a number of immature cells; macrophages not infrequent. "Mature" lymphocytes rare.
1210	40954	Plasma cells more frequent; macrophages with debris not frequent.
1211	40955	Decomposed, but a preponderance of plasma cells. Also cells with blue blotches. N.B.—Number of granules-discs lying free.
1212	40956	Plasma cells frequent; macrophages with blue blotches. Possible difference between "flakes" and broken down phagocytosed red cells.
1244	40957	Diffuse distribution of plasma cells, more frequent than lymphocytes of which mature types infrequent; fair number of heterophiles.
1394	41054	Lymphocytes not depleted; plasma cells rare; macrophages with blue blotches not infrequent.
—	41167	Clusters of mature lymphocytes; plasma cells infrequent; macrophages frequent, some with blue blotches.



ANNEXURE IV.

HISTOLOGY—SPLEEN.

*P.M. No. 29: Specimen No. 39805.*

The cells around the artery of the "follicle" (also called the artery of the "adenoid" sheath) are degenerated or lost. In some the presence of flakelike granules is seen. A narrow irregular zone of mononuclears forms the periphery of the nodule. Dispersed through the latter are macrophages, some of which with the H.E. stain resemble phagocytosed erythrocytes, and with the B.B. stain show up as haemosiderin. Heterophiles are not infrequent, as well as large spherical cells and nuclei with less dense chromatin, probably "reticulum cells", the precursors of plasmacytes, of which there are a good few present. There is a difference between the flakes in cells around the follicular artery, and the remains of phagocytosed red cells in the macrophages of the red pulpa. These flakes stain characteristically with Giemsa, MPAH P.A.S. and AFMG stains.

*P.M. No. 30.*

Degeneration of the cells around the follicular artery, with a reduction of the number of lymphocytes. "Reticulum cells" and plasmacytes are not infrequent and irregularly distributed around the above. Macrophages with haemosiderin are not infrequent with a fair number of heterophiles.

*P.M. No. 50: Specimen No. 39222.*

The loss of cells around the artery of the follicle is minimal. Macrophages with phagocytosed erythrocytes are not infrequent; fair numbers of heterophiles, and blood in the red pulpa. Small circumscribed nodules of lymphocytes surrounded by a thin capsule are closely associated with the arteries, which are larger and better defined than the artery of the "follicle".

*P.M. No. 60.*

Lymphoid tissue is depleted; remains of it are identified as a narrow irregular zone of mononuclears (reticulum cells etc.) with slight haemorrhage. Macrophages rare.

*P.M. No. 61.*

Similar loss of lymphoid tissue as described above, but in the centre of the nodule there is fairly extensive hyalinisation forming irregular patches, around the follicular artery. They stain pink with H.E. and are homogeneous. Reticulum cells, and plasmacytes are not infrequent around these areas.

*P.M. No. 149: Specimen No. 39914.*

Hyperaemia; degeneration of cells in and around the adenoid sheath. There is evidence of haemorrhage, which seems to be associated with the extensive erythrophagocytosis and haemosiderosis. This latter is not uniformly distributed, but occurs in irregular clusters in the cell. Slight lymphoid hyperplasia associated with the walls of the arteries (not follicular arteries). No blue blotches seen in the Giemsa stained section, although they are frequent in the imprint smear.

*P.M. No. 150: Specimen No. 39915.*

Hyperaemia; lymphoid tissue reduced and irregularly distributed along the periphery of the follicle. Macrophages with haemosiderin not infrequent; around the arteries of some of the follicles there appears to be flakelike granules in some of the degenerated cells. On the periphery of these, reticulum cells and plasmacytes are not infrequent.

*P.M. No. 1209: Specimen No. 40953.*

Loss of the cells associated with the adenoid sheath, resulting in an open-network. The lymphocytic cells are reduced in number and present as an irregular zone in the periphery of the follicle. Numerous macrophages are distributed through the peripheral zone, and when stained with B.B. they reveal greenish blue pigment (haemosiderin). Some heterophiles also present. Some of the degenerating cells show the presence of flake-like granules which in places assume a homogeneous pink appearance. Note on the periphery of this, reticulum cells and plasmacytes identified. These reticulum cells are large, almost polyhedral in shape with round nuclei.

*P.M. No. 1211: Specimen No. 40955.*

A few haemorrhages; degeneration of the cells of the adenoid sheath with slight hyalinisation in places around the follicular artery; slight erythrophagocytosis.

*P.M. No. 1212: Specimen No. 40956.*

Fairly well marked degeneration of cells around the artery of the follicle, depletion of lymphoid tissue, and hyalinisation; erythrophagocytosis frequent in the periphery, and when stained with B.B. shows extensive haemosiderosis. Along the periphery the frequency of reticulum cells and plasmacytes noted.

*P.M. No. 1244: Specimen No. 40957.*

Loss of lymphoid tissue; cells with flakes noted around follicular artery; erythrophagocytosis in the periphery. Note the presence of lymphoid nodules in juxtaposition to some of the other arteries of the spleen, and the frequency of "reticulum cells". There is a good deal of blood in the red pulp.

*P.M. No. 1394: Specimen No. 41054.*

Hyperaemia and irregular appearance of the lymphoid follicles, probably the result of regeneration following on the loss of some of the cells of the adenoid sheath. Many lymphoid nodules in juxtaposition to some of the other arteries of the spleen are noted. Only occasional cells with haemosiderin seen. There is a good deal of blood in the red pulp, and a number of heterophiles.

*P.M. No.: Specimen No. 41167.*

Lymphoid nodules mainly in juxtaposition to the arteries of the spleen not infrequent.

*P.M. No. 41: Specimen No. 39219.*

An unusual spleen with loss of lymphoid tissue around the follicular artery. It is difficult to map out the majority of the follicles. In the periphery macrophages and a number of heterophiles identified.

*P.M. No. 41A: Specimen No. 39220.*

Open network around the follicular artery of the follicle, which is not well defined. In the periphery note the frequency of heterophiles and the presence of haemorrhages.

#### HISTOLOGY: PROVENTRICULUS AND INTESTINES.

*P.M. No. 29: Specimen No. 39805.*

##### *Proventriculus or Glandular Stomach.*

At the junction of the stratified epithelial layer of the oesophagus, and the glandular stomach, the epithelial covering in places is lost with a certain amount of disintegration of the tissue in the propria. Irregular flake-like granules, staining a light yellowish brown colour with H.E. are identified in the interstices. This is not haemosiderin. In places there is an hyperaemia, and here and there a few heterophiles. There is an indication of haemorrhage and loss of the goblet cells. No lymphoid tissue identified. The flakes stain characteristically with H.P. and P.A.S. stains.

##### *Caecal Tonsils.*

Caecal tonsils show enlargement of some of the villi, some of which are without epithelial covering. In places the goblet cells are swollen, and in the propria necrotic areas can be defined with complete depletion of lymphoid tissue and disintegration of the intestinal glands. Here and there isolated lymphocytes are identified. In places in the interstitial spaces of the propria light brownish flake-like granules are identified, but no defined cell wall can be made out. Haemorrhages are associated with the necrotic areas. In places macrophages with haemosiderin (B.B. stain) and heterophiles are seen. With H.P. stain the flakes stand out prominently and are characterised by their uniform size. Sections stained with M.P.A.H. give a more or less similar impression.

HISTO-PATHOLOGY AND PATHOGENESIS OF NEWCASTLE DISEASE.

*Small Intestine.*

No significant changes.

*P.M. No. 43: Specimen No. 39921.*

*Small Intestine.*

Fairly extensive necrosis affecting the propria up to the muscularis mucosae, with loss of the covering epithelium and disintegration of the intestinal glands and lymphoid tissue. A number of lymphocytes lie free. Note the infiltration of heterophiles and the presence of haemorrhages. Flake-like granules are identified in the interstices of the propria. In places it seems to assume an homogeneous pink-staining character.

*P.M. No. 61.*

*Caecal Tonsils.*

Necrosis extending in places to the muscular layer; the haemorrhages are more extensive than in 29. In the propria note again the light brown flake-like granules in the interstitial spaces, not of the nature of haemosiderin, nor of necrotic debris. The disintegration of the lymphoid tissue is fairly advanced. There is a diffuse distribution of lymphocytes and other mononuclears.

*P.M. No. 149: Specimen No. 39914.*

*Small Intestine.*

Fairly extensive necrosis with loss of the covering epithelium and disintegration of the intestinal glands. Skeleton remains of lymphoid tissue can be identified, with a good deal of haemorrhage and fairly extensive infiltration of heterophiles. Note the presence of so-called Raneth cells and Russell bodies. Note also the presence of flakes.

*Caecal Tonsils.*

Extensive hyperaemia, necrosis and haemorrhage extending to the muscular layer. No lymphoid tissue as such can be identified. Marked disintegration of the intestinal glands. Note here and there in the necrotic material, clusters of bacteria. Flakes also identified.

*P.M. No. 1209: Specimen No. 40953.*

*Proventriculus.*

The main changes seem to be present in the superficial glandular portion which seem to be of the nature of a disintegration of the lymphoid tissue in the propria mucosae.

*Caecal Tonsils.*

Early disintegration of the lymphoid tissue with small haemorrhages, and in places with the loss of the covering epithelium. Note the presence of light brownish flakes in places in the interstices of the propria mucosae. This case is complicated by the presence of cysts in the mucous membrane.

*P.M. No. 1210: Specimen No. 40954.*

Ditto.

*P.M. No. 1212: Specimen No. 40956.*

*Proventriculus.*

The superficial glandular portion shows extensive disintegration of the propria mucosa, and lymphoid tissue, with loss of the covering epithelium, hyperaemia, and small haemorrhages. Note the presence of the flake-like granules.

*Caecal Tonsils.*

Advanced necrosis and haemorrhage.

*No P.M. No.: Specimen No. 41167.*

*Caecal Tonsils.*

Note the prominence of the lymphoid tissue. This case showed no clinical symptoms except paralysis. It was destroyed 36 days after the challenge with virulent virus.

HISTOLOGY: CENTRAL NERVOUS SYSTEM.

*P.M. No. 29: Specimen No. 39805.*

*Medulla.*

Hyperaemia; a few areas of gliosis, and in one area slight chromatolysis.

*Cerebellum.*

*White matter:* Changes in the cells of small blood vessels; foci of gliosis. *Cortex* in places shows chromatolysis in a number of Purkinje cells; extension of cell activity into medullary layer.

*Cerebrum.*

Slight chromatolysis. Note changes in the cells of some of the blood vessels.

*P.M. No. 30.*

*Cerebellum.*

*White matter:* Hyperaemia; changes in the cells of the walls of a few of the small blood vessels, in some with a few small haemorrhages, and the presence of plasma. Are there flake-like granules in some of these cells?

*Cortex.*

A large number of the Purkinje cells shows chromatolysis, and in a few places with slight cellular activity extending into the molecular layer.

*Cerebrum.*

Slight chromatolysis; some of the small blood vessels show changes of endothelials, some with swelling and vacuolation. These cells with lymphocytes, other mononuclears, and red cells form cell-layers two to five deep. The lumen of some of these vessels show desquamated endothelial cells, red cells, and plasma. Some of the larger ganglion cells show chromatolysis.

*Optic Lobes.*

Ditto, but changes in the blood vessels less extensive.

*P.M. No. 43: Specimen No. 39221.*

*Cerebellum.*

*White matter:* an area of gliosis, and chromatolysis of some of the ganglion cells. Slight changes in the cells of the walls of small blood vessels. Some of the Purkinje cells in the cortex show chromatolysis with very slight cellular activity extending into the molecular layer, i.e. a slight gliosis.

*Optic Lobes and Peduncles.*

Slight changes in the cells of the walls of some of the small blood vessels and infiltration of lymphocytes, monocytes, etc. In places chromatolysis of some of the ganglion cells; slight gliosis; hyperaemia.

*Cerebrum.*

Affected to less extent.

*P.M. No. 50: Specimen No. 39222.*

*Cerebellum.*

*White matter:* Hyperaemia and in places slight changes in the cells of the walls of a few of the blood vessels. Marked haemorrhages associated with some of the blood vessels, infiltrating irregularly into the surrounding tissues. In the cortex some of the Purkinje cells show chromatolysis, and in a few places cellular activity extend into the molecular layer; i.e. a slight gliosis.

*Optic Lobes.*

Changes in the cells of the walls of a few blood vessels; in one vessel with haemorrhage. In some of the vessels mononuclears predominate in the wall. Fairly well marked chromatolysis, seen in respect of the ganglion cells. Slight gliosis.



HISTO-PATHOLOGY AND PATHOGENESIS OF NEWCASTLE DISEASE.

*Cerebrum.*

Ditto. Note the large numbers of mononuclears in the walls of some of the walls of the blood vessels, extending into the lymph spaces.

*P.M. No. 60.*

*Cerebellum.*

Few ganglion cells show chromatolysis. Cerebellum and rest of the brain show hyperaemia.

*P.M. No. 61.*

Ditto.

*P.M. No. 149: Specimen No. 39914.*

*Cerebellum.*

Ditto.

*Cerebrum.*

Slight chromatolysis of some of the ganglion cells. Hyperaemia.

*Peripheral Nerve.*

Nothing unusual.

*P.M. No. 150: Specimen No. 39915.*

*Cerebellum.*

Hyperaemia; a few of the Purkinje cells show early chromatolysis.

*Peripheral Nerve and Medulla.*

Nothing unusual.

*P.M. No. 1209: Specimen No. 40953.*

*Cerebellum.*

Slight changes in the cells of the walls of small blood vessels; chromatolysis of some of the Purkinje cells.

*Optic Lobes.*

Slight hyperaemia; changes in the cells of walls of small blood vessels. Besides the endothelials, note the presence of several lymphocytes, red cells, and probably monocytes and plasma within the lumen.

*P.M. No. 1210: Specimen No. 40954.*

*Cerebellum.*

Hyperaemia; some of the Purkinje cells show swelling, loss of Nissl substance, and vacuolation, whereas others appear shrunken and more intensely stained (probably as a result of fixation). Portion of the medulla shows hyperaemia, and change in the walls of some of the small blood vessels, and infiltration with mononuclears.

*Optic Lobes.*

Hyperaemia and slight changes in the cells and oedema of the walls of a few of the blood vessels. Some ganglion cells show chromatolysis.

*P.M. No. 1211: Specimen No. 40955.*

*Cerebellum.*

Hyperaemia; some of the Purkinje cells show chromatolysis.

*Cerebrum and Optic Lobes.*

Hyperaemia; slight chromatolysis.

*P.M. No. 1212: Specimen No. 40956.*

*Cerebellum.*

Hyperaemia; chromatolysis; some of the vessels show changes in the cells of the walls.

*Cerebrum.*

Chromatolysis of a number of ganglion cells; hyperaemia; changes in some of the cells of the blood vessels. Some of the walls of the affected blood vessels show an infiltration of lymphocytes, monocytes and red cells. Some of these cells lie free within the lumen, together with clumps of red cells and plasma.

*Optic Lobes.*

Ditto.

*P.M. No. 1244: Specimen No. 40957.*

*Brain.*

Hyperaemia and slight chromatolysis. Dilatation of the blood vessels without changes in the walls of the blood vessels.

*P.M. No. 1394: Specimen No. 41054.*

*Cerebellum.*

Hyperaemia; here and there chromatolysis of Purkinje cells with slight cellular activity into the molecular layer.

*Cerebrum.*

Hyperaemia and slight chromatolysis, with slight satellitosis. A few of the blood vessels show changes in the cells of the walls.

*Optic Lobes.*

Hyperaemia.

*No P.M. No.: Specimen No. 41167.*

Nothing unusual noted in any of the brain sections which were utilised as a control for comparison with the above sections. Probably the paralysis was due to changes in the spinal cord (sections not available). Note the distribution of Nissl's granules in the large, and smaller ganglion cells.