# A STUDY ON THE CYTOLOGY OF THE NERVE LESIONS IN NEUROLYMPHOMATOSIS GALLINARUM

by

FRANZ LEIDLER

M. D., University of Vienna, Austria, 1938

---

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Bacteriology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1941

# TABLE OF CONTENTS

# INTRODUCTION

This investigation was conducted in the hope of making some small contribution to the knowledge of abnormal reactions of certain cells which have been observed in diseased nerves. The disease known as "neurolymphomatosis" of fowls appeared to be particularly suited for such an investigation. Furth (17) well expressed the reason for the numerous investigations which have been conducted in the last thirty years with the purpose of elucidating this condition when he said, ".....lymphomatosis of numerous nerves and ganglions with slight or no alteration of the blood-forming tissues is a unique phenomenon observed only in chickens."

Originally the process was considered to be a "neuritis" or inflammatory reaction as in the work of Marek (29), Van der Walle and Winkler-Junius (40) and others. With the investigations of Pappenheimer and co-workers (34), the relationship of this condition to other lymphomatous cell aggregations became obvious and since that time the disease has been considered as a "lymphomatosis." This opinion was accepted by Bayon (4) who placed the disease in the "diffuse lymphoma", thus indicating the possibility of its leukemic nature.

From then on, it became the prevailing tendency to classify this condition somewhere within the group of disorders characterised by a more or less generalized involvement of the lymphatic system with a possible neoplastic tendency. No consensus

has been reached, however, in regard to the exact nature of neurolymphomatosis or its relationship to other alterations of the lymphoid elements.

In view of the complexity of the concepts dealing with leukemic and allied reactions, an investigation such as the one presented, cannot hope to solve the problem. Yet the importance of the disease calls for unceasing attempts to add to our limited knowledge of the subject.

## REVIEW OF LITERATURE DEALING WITH NERVE LESIONS AND BLOOD PICTURE IN NEUROLYMPHOMATOSIS GALLINARUM

In view of the large number of publications dealing with "fowl paralysis" as a disease-entity, without going into any detail regarding the histology of the nerve lesions, it seemed to be best to limit this review to papers relating directly to this investigation and omitting all others.

The first report on this disease was given by Marek (29) in 1907. In his specimens the nerve fibers in the lumbar plexus and sciatic nerves were almost completely destroyed. A dense and uniform infiltration of mononuclears, in places aggregated to clumps, replaced the normal structures. The perineurium showed but slight thickening and contained only a few scattered cells. Marek regarded the process as inflammatory in nature and termed it "polyneuritis." The nerve degeneration was, in his opinion, secondary to the infiltration.

In the United States, Kaupp (23) in 1921 first recorded observations on this disease. His attention was mainly directed

towards the lesions of the central nervous system which he classified as "transverse myelitis."

An apparently identical disease, reported in the Netherlands by Van der Walle and Winkler-Junius (40) in 1924 showed infiltrations in the peripheral nerves followed by degeneration of the nerve fibers. These authors being also under the impression of dealing with an inflammatory process, introduced the term "neuromyelitis gallinarum."

Doyle (13) in 1928 described the nerve lesions as either patchy infiltrations, perhaps determined by the location of blood vessels; or, in a great many cases, as a diffuse cellular invasion occupying a major portion of the nerve trunk.

The first comprehensive study on the pathology of "fowl paralysis," by Pappenheimer, Dunn and Cone (34) appeared in 1928. The section of this publication dealing with pathology was based on the examination of sixty spontaneous cases obtained from California, Massachusetts, Maine, New Hampshire, New Mexico, Ontario and Pennsylvania. The following breeds were represented: White Leghorns, Silkies, Silver-Spangled Hamburgs, Plymouth Rocks, Buff and White Wyandottes, and Rhode Island Reds. The lesions observed in the nerves were the most severe alterations found and were identical regardless of breed.

In regard to their cytological nature, the authors made the following statement:

> The smaller forms of infiltrating cells appear to be morphologically identical with lymphocytes. In the nodular accumulations, which crowd aside the adjacent nerve fibers, the center of the mass is composed of somewhat larger elements, with more vesicular nuclei, and here mitoses are frequent. In addition to

cells of the lymphocytic type, which predominate, there are numerous cells with more abundant basophilic cytoplasm, peripherally disposed chromatin and a juxtanuclear clear space, which closely resemble mammalian plasma cells.....There are also large mononuclear cells with vacuolar cytoplasm corresponding to the fat laden phagocytes derived from the cells of Schwann's sheath.

Regarding the structure of the visceral lymphomata, which were sometimes concurrent with nerve lesions, it was stated:

> Histologically, these were all identical in structure. They were composed of closely packed, small round cells with deeply stained nucleus and relatively scant cytoplasm....In the more actively proliferating areas where mitotic figures were extremely numerous, the cells were larger in size, the nuclei more vesicular, with distinct chromatin structure, and the cytoplasm more abundant. Such cells are perhaps comparable to the larger lymphoid elements present in the centers of the germinal follicles.

In a discussion on the proposed nomenclature for the disease under observation, Pappenheimer and co-workers did not consider it possible to find an altogether satisfactory name as long as the cause of the disease was not definitely established. For this reason they suggested the term "neurolymphomatosis," thus indicating the most outstanding pathological lesions, namely the lymphoid infiltrations in peripheral nerves and the lymphomatous growths in other localizations.

Bayon (4), 1930, tried to classify neurolymphomatosis within a system of avian hemopathias. He placed it in the group of "diffuse lymphoma" which was characterized as a simple multiplication and dissemination of lymphoid elements, the blood lymphocytes being increased only slightly and irregularly.

In 1931, Bayon published two papers on this disease. The first of these (5) dealt with an acute outbreak in a strain of Rhode Island Red fowls. The author studied four birds with

severe symptoms. An examination of the blood showed average figures, except for a slight increase in the leucocytes (average 31,000). This increase was due to lymphocytosis. It should be mentioned in regard to this interpretation of the white cell count, that many authors would consider 31,000 leucocytes to be an average figure. On the other hand, Bayon mentions an increase in "various immature cells" from normally 500 to 2000 (actual numbers) in the paralytic birds. This observation may have been of more bearing for a characterization of the blood picture, than the total white count as figured above.

In the examination of the sciatic nerve, the author found slender, fusiform accumulations of lymphoid cells. These infiltrations differed in degree only from the ones observed by Pappenheimer and co-workers (34) who apparently described a more chronic type of the same condition.

A second paper published by Bayon in 1931 (6) dealt with observations in eight outbreaks of neurolymphomatosis. The lesions differed in acute and chronic cases. Such differences could, however, be understood as the intensification and after-effects of the same morbid process. In accord with his idea of a progressive pathological development, the author suggested that the terms "acute" and "chronic" be dropped and such statements as "early" and "late" stages be substituted.

Warrack and Dalling (41) in 1932 discussed the conclusions they had obtained from the observation of seventy-six outbreaks. No detailed report on histological findings was given in this paper. The authors mentioned, however, that accumulations of

cells of lymphocyte, mononuclear, and plasma cell type, as well as "cuffing" in the central nervous system, were accepted as diagnostic for this disease.

Patterson, Wilcke, Murray and Henderson (35) reported in 1932 on histological studies in 267 positive cases. The authors included leukemic and erythroleukosis-like conditions, which have not been referred to in this place.

The histological studies revealed edema and an infiltration of "rather large, undifferentiated, mononuclear, basic-staining, non-granular cells." They regarded these elements as undifferentiated embryonic blood-cell progenitors, apparently identical with the so-called lymphoid cells. It was impossible, however, to determine to what type these "blast" cells were differentiating. In the opinion of these authors similar elements were found in aleukemic and leukemic lymphocytoma, erythroleukosis, large round-cell sarcoma and lymphosarcoma. Mitotic figures were noted in some of the lesions.

Replacement of the primary invading tissue by fibrous proliferation took place in some of the chronic cases. It even appeared probable that such fibrous formations were occasionally established in locations which had undergone little or no previous lymphoid infiltration.

In classifying the pathological manifestations taking place in the peripheral nerves, the authors pointed to the resemblance with a malignant neoplastic process as well as with inflammatory alterations. They also mentioned that intermediate stages between the tumor-like and the inflammation-like types were ob-

served. An alteration in the blood picture was observed only in those few nerve cases which showed concurrent lesions in the bone-marrow.

Johnson (21), in 1932, stated in a study on lymphomatosis that the cells observed in nerve, brain and visceral lesions could not be differentiated from small lymphocytes. He furthermore denied the presence of a leukemic blood picture.

Johnson and Conner (22) in 1933 made a study of the blood in lymphomatosis. In Table 1 some of the average values obtained by these authors are reproduced together with values for normal chicken blood.

Table 1. Showing average blood counts of normal and paralytic chickens.

| | Normal birds | Paralyzed birds | Paralyzed birds with tumors |
|---|---|---|---|
| | Total count | | |
| Erythrocytes | 3,054,168 | 3,387,173 | 3,249,900 |
| Leukocytes | 27,845 | 36,316 | 36,508 |
| | Differential count (Per cent) | | |
| Polymorphonuclears | 30.30 | 32.28 | 36.34 |
| Large mononuclears | 12.8 | 18.71 | 18.58 |
| Lymphocytes | 46.26 | 42.06 | 37.24 |
| Eosinophiles | 6.0 | 3.37 | 4.18 |
| Mast cells | 2.7 | 3.44 | 3.63 |

Unfortunately no definition of the nomenclature was included. It can, however, be assumed that the term polymorphonuclears has been applied to elements which in this paper will

be described as rod-bearing eosinophile granulocytes, while the eosinophiles of Johnson and Conner most probably correspond to the granule bearing eosinophile granulocytes.

In the discussion, these authors pointed to the increase in large mononuclears, and in the total leukocyte count, associated with "budding" of numerous lymphocytes. It was contended that an increase in leukocytes with "budding" of numerous lymphocytes would indicate the onset of lymphatic leukemia. The significance of high mononuclear counts in fowl was, according to this paper, not known at the time of its publication.

Seagar (37) in 1933 studied the blood of neurolymphomatosis birds. In order to establish normal standards, he examined a number of healthy chickens. The average values obtained by him were 2,900,000 erythrocytes, 27,000 leukocytes and a differential count of 39 per cent polymorphonuclears, 5 per cent eosinophiles, 54 per cent lymphocytes and 5 per cent monocytes. He mentioned the difficulty in differentiating between large lymphocytes and monocytes and ascribed the wide range of monocyte values in counts performed by different investigators to this fact.

In neurolymphomatosis he found an increase in polymorphonuclear leukocytes during what he considered to have been the incubation and acute stage, while a lymphocytosis appeared to have been characteristic for the advanced stage.

Furth and Breedis (17) in 1935 made a study of lymphomatosis in relation to fowl paralysis on the basis of transmission experiments. Their strains (five and six) produced mainly lymphomatosis of the nerves characterized by predominance of usually

the small, rarely the medium sized lymphocytes. It was seldom accompanied by blood changes. Strain two caused the formation of lymphomatous tumors composed mostly of large lymphocytes. This condition was almost invariably associated with lymphatic leukemia.

These authors came to the conclusion that -

> neurolymphomatosis is a neoplastic disease allied to leukosis and sarcoma, but it is not produced by the agent that causes erythroleukosis and myeloid leukosis; neither does the agent of neurolymphomatosis produce erythroleukosis and myeloid leukosis.

Gibbs and Johnson (18) in 1936 reported on the use of a modified Unna eosin-methylene blue stain by means of which it was found that the nuclei of the pathological cell in neurolymphomatosis were more vesicular than the nuclei of the lymphocytes of the blood studied under identical conditions. There were, furthermore, numerous mitoses among the former cell type.[1]

Barber (3) in 1937 classified the cell types found in peripheral nerve lesions as large and small lymphocytes, plasma cells and Schwann's sheath cells.

In a paper by Lee and co-workers (24) in 1937, cells found in nerves were described as small, intermediate or large, mononuclear, basic staining, non-granular. Mitotic figures were often observed especially in the intermediate group. The authors characterized these elements as hemocytoblasts.

In Bushnell's poultry practice (9) 1940, Stubbs outlined the position of neurolymphomatosis at that recent point of time.

---

[1] These authors did not in that paper characterize the mentioned "pathological" cell.

This author stated that -

> Lymphomatosis is a term commonly used to indicate a hyperplasia or proliferation of lymphoid tissue...Neurolymphomatosis (fowl paralysis) is one of the most prevalent forms..The alteration first described, was a cellular infiltration of the nerves .....It is generally stated that these cellular infiltrations of the nerves belong to the lymphoid series...The original work of Pappenheimer in neurolymphomatosis indicated that it is infectious in nature, yet most experiments recorded leave doubts in this regard. Particularly when fowl paralysis is accompanied by localized cellular infiltrations in the nature of tumors, there is an apparent analogy to neoplasms.

## REVIEW OF THE LITERATURE ON THE CYTOLOGY OF THE NERVE

In order to create a basis for the interpretation of the pathological cytology, a short discussion of the pre-existing normal cells and their potentialities, is called for. A detailed account of the cellular morphology characteristic of neurolymphomatosis lesions will be given in a separate chapter.

### Cytology of Normal Nerve Tissue

The peripheral nerve consists of ectodermal and mesodermal elements. Of the ectodermal tissue, it is only the Schwann cells which are of importance in connection with this problem. According to Hassin (20), a Schwann cell has a cytoplasm which extends from its nucleus to the nodes of Ranvier, and a number of processes of which the Schwann membrane is the most conspicuous. The cytoplasm has a condensed perinuclear area and contains vacuoles with various inclusions, products of nerve metabolism.

The most important tissue elements involved in neurolymphomatous lesions are of mesodermal origin. A structure of connective tissue, conventionally divided into three sheaths, surrounds

tracts of peripheral nerve, the single nerve fiber possessing an endoneural sheath, bundles of several nerve fibers being enveloped by the perineurium, and the entire nerve being held together by the epineural sheath, which merges into the surrounding connective tissue. Actually these "sheaths" represent a continuous network protecting the nervous matter embedded in it.

The cells involved in the formation of those structures in the normal state are primarily connective tissue cells, fibroblasts, resting-wandering cells, mast cells, and the wandering-lymphoid cells. In addition to these, the blood vessels contribute a variety of elements, such as endothelial, adventitial cells and the elements of the circulating blood.

The cellular basis of the connective tissue is the fibroblast (also spoken of as fibrocyte, desmocyte). In the opinion of Maximow-Bloom (31), Cowdry (12) and a great number of other histologists, this cell is highly differentiated, and does not easily change into another cell type, although it is admitted that under intense stimulation such cells occasionally became free and phagocytic. Within this concept, the fibroblast assumed a very minor role in the pathological tissue reactions.

It appears to be of interest to contrast the standpoint of v. Moellendorf (32) against the above mentioned definition. Applying an especially balanced iron-hematoxylin stain, this author was able to obtain a picture in which the cytoplasm of fibroblasts formed a continuous network. The fibroblast nuclei lay within this cytoplasmic syncitium. In the normal state, single fibroblasts were never completely isolated. Furthermore, Moel-

lendorf found that there were other than fibroblast nuclei in the plasmatic net. Specific mention was made of a small dark nucleus surrounded by intensively staining cytoplasm. The author identified this structure with the resting-wandering cell which will be mentioned below.

The second important cell of the connective tissue is the resting-wandering cell, characterized by its phagocytic properties. No consensus in regard to its derivation and its potencies has been reached up till now. The disagreement is best reflected by the widely varied nomenclature which has been applied to this element. Maximow-Bloom (31) gave it the following morphological description:

Shape. Rounded or oval, spindle-shaped; sometimes, with branched processes.

Nucleus. Irregular, oval or kidney-shaped. Smaller than the average fibroblast nucleus. The chromatin particles coarser and darker than in the latter. There are no large nucleoli. The membrane is thick and slightly folded.

Cytoplasm. Stains eosinophilic with a distinct cytocentrum. The outline is ragged but well defined.

Maximow-Bloom (31) termed this cell as "resting-wandering cell," also as "fixed macrophages," and considered them as part of the reticulo-endothelium (in which they did not include the lining of blood-vessels proper). In Moellendorf's opinion, these elements were directly derived from the fibrocyte-net. Aschoff and Kiyono (2) called them "histiocytes" and considered them as representatives of the reticulo-endothelial system within the

connective tissue. Sabin, Doan and Cunningham (36) distinguished between an "anchored endothelial phagocyte," e.g., the Kupffer cells, etc., and a "free endothelial phagocyte" which they also spoke of as "plasmatocyte." By adopting this nomenclature, these authors wished to imply that the respective cells originated from the endothelium.

According to Marchand (28), adventitial cells of primitive mesenchymal potencies gave rise to phagocytosing tissue mononeuclears.

This enumeration, which is by no means complete[2], would have demonstrated sufficiently how divergently various histologists have placed this cell. For this reason, no mention of that element, as such, will be made in the chapter on morphology. Its possible place in the nerve lesions will be a matter of discussion in the final part of this paper.

Other, less numerous, elements of the normal connective tissue are the wandering lymphoid cells and the mast-cells.

The first group can be divided into large forms which closely resemble the blood-monocytes, and small forms which are morphologically identical with lymphocytes. The mast-cells (in mammals) were given the following description by Maximow-Bloom (31).

Shape. Irregular oval, in some animals (rat and mouse) very large, spherical or polyhedral.

Nucleus. Spherical shape and small size. It stains very inconspicuously, appearing lighter than the cytoplasm.

---

[2]It must be said that a majority of the listed authors are rather cautious in their statements regarding the origin of the phagocytes in normal connective tissue, since most of the investigations have been based on reactions to abnormal stimuli.

Cytoplasm. It is rather abundant and filled with granules staining metachromatically with basic anilin dyes (purple with methylene blue). According to the authors quoted above, the function of this cell is unknown. It tends to be located near blood vessels, without being identical with the basophilic granulocyte of the circulating blood.

The elements which form the wall of the small vessels present in the nerve, as endothelial and advantitial cells, do not seem to have a direct function in pathological changes. Indirectly they may be active by giving rise to the tissue phagocytes.

## Cytology of Nerve Tissue Under the Stimulation of an Irritating Agent

After having discussed the normal aspect of cells which may participate in the pathological process under observation, it is desirable to report on some of the concepts dealing with their potencies under abnormal conditions.

In regard to the role of Schwann cells in nerve degeneration, Hassin (20) made the following statement:

Within twenty-four hours after section of a peripheral nerve[3] the cells show an increase in size; their nuclei become

---

[3]It has to be kept in mind that the process described by Hassin refers to secondary degeneration of the peripheral segment after division of a nerve. In neurolymphomatosis, the infiltration disrupts the nerve. While this may cause changes, identical to the ones described, at a more peripheral point, it is obvious that the various influences active in the area of destruction by invading cells may greatly obscure the cytological picture.

rich in chromatin and mitotic figures occur within the first four days. The cytoplasm harbors inclusions in an increasing number of vacuoles. The cytoplasmic processes gradually disappear and the cell body assumes the shape and appearance of a honey-comb or lattice. Finally the plasma is transformed into minute vacuoles, each of which harbors a drop of lipoid, while the nucleus, rich in chromatin, is displaced to the periphery. In Hassin's opinion this process constitutes the transformation of a Schwann cell to a fat granule body, or "Gitter cell." The further fate of these cells appears to be uncertain. They are either taken up by mesodermal elements or removed into the general circulation.

It is important to note that neither Cajal (10) nor Nageotte (33) shared this interpretation. These authors were inclined to consider the "Gitter cells" as derivatives of immigrated cells (possibly leucocytes) or fibroblasts. They did not bring them in connection with Schwann cells.

As has been mentioned before, a majority of histologists doubted the capability of fibroblasts for further development. Contrary to this attitude, Moellendorf (32) and his school assumed that the main cellular reactions taking place in an irritated connective tissue originated from fibroblasts.

This author described the tissue reaction to the injection of trypan blue as an irritating stimulus. The first cell to separate from the continuous network of the mouse connective tissue was the "Gewebsleukocyt." This cell had a "Lochkern" (ring-like nucleus) and eosinophilic or pseudoeosinophilic granules.

Next to this the number of "resting-wandering cells" (see

page 12) increased. In the further development these more intensely stained cytoplasmic areas with their dark nuclei lost the normally present connection with the plasmatic syncitium, rounded off and formed free cells. The latter were identical with "active macrophages" or "polyblasts" (Maximow, 30).

Under further disintegration of the original fibroblast network and constant transformation of the evolving structures, a great variety of cells developed. The shape of the nucleus ranged from round to polymorphous, the outline of the cell from lobate to round. Elements with round nuclei tended to stain darkly, while others with polymorphous nuclei were often nearly colorless. In this phase, the fibrous network of the affected area was practically completely split into individual cells. The typical fibroblast disappeared to be replaced by ameboid forms with elongated pale nuclei and all transitions to a polynuclear aspect.

A stage of cell scarcity followed and from it the connective tissue was regenerated in its normal structure.

Although Moellendorf admitted that the origin of his "polynuclear" cells remained doubtful, his report seemed to imply that the histological changes following a minor irritation took place among connective tissue elements exclusively.

While there is little belief in the multipotentiality of the fibroblast, considerable evidence has been produced indicating the possibility of other elements being transformed into that cell.

Research leading to this result was carried out by Carrel

and Ebeling (11), Fischer (15), Timofejewski and Benewolenskaja (39), Maximow (30), and Bloom (8) to mention only a few of the most important investigators. Carrel and Ebeling (11) in observing tissue cultures found a transformation of chicken monocytes into fibroblast-like forms. Fischer (15) repeated essentially the same experiment. Timofejewski and Benewolenskajs (39) in culturing myeloblasts from a case of acute human myeloid leukemia described a transition into fibroblast-like forms, either directly or through a stage of polyblast and clasmatocyte.

Considering their applicability to the problem under investigation, the investigations of Maximow and Bloom will be discussed more in detail.

Maximow (30) divided the cells found in an area of inflammation into: (a) fibroblasts, (b) polymorphonuclear special leucocytes, and (c) mononuclear exudate cells or polyblasts.

The first cell type was denied any active participation in the tissue reaction. The blood leucocytes were not considered of much importance from the histological picture obtained in later stages; they had a relatively short life-time and were highly specialized forms with little or no ability of transformation.

The most potent cell in Maximow's opinion was the "polyblast." It could transform into any of a variety of types such as epitheloid cells, giant cells, etc. This multipotent cell arose from two sources, the relative number originating from one or the other source varying with the nature of both the inflamed tissue and the noxious stimulant. The first source was the local fixed histiocytes; in connective tissue this would be the "rest-

ing-wandering cell." The second source, which probably was more important, was the immigrated lymphocytes and monocytes. The lymphocyte in its development to a polyblast first passed through a monocytoid stage, from there on it developed into a polyblast. The latter changed into a fibroblast or a "resting-wandering cell," thus restoring the normal composition of the connective tissue.

Bloom (8), in an impressive study on the culture of lymphocytes, extended Maximow's contention of the role of this cell in tissue reactions. His material was obtained from the thoracic duct lymph of rabbits and stained with hematoxylin-eosinazur II. After three hours culturing, nucleus and cytoplasm increased in size. The nucleus of the small lymphocytes lost its round shape, and became angular. Many transitions between small and large cells were observed, the development being directed toward larger sized elements. A great number of these cells began to lose their lymphoid characteristics. The nucleus assumed an eccentric position, became somewhat elongated and bent. The chromatin particles were more finely divided and the nucleoli less prominent.

The histological picture at four to eight hours of culturing was described in the following manner:

> The great number of small and larger lymphocytes...have begun to disappear. In their place one finds a new type of cell which is patently quite different from the original lymphocytes but which is nevertheless connected with the lymphocytes by a very intimate series of transition cells. These new cells show great variation in size. Some of them are hardly larger than an erythrocyte, while others are much larger than the largest lymphocytes. They possess an abundant cytoplasm, when compared with the lymphocytes, which is purple at the periphery of the cell and they usually have a large, acidophilic area opposite the indentation of the nucleus.

The nuclei were transformed from those of typical small and medium sized lymphocytes, into forms which were round or kidney-shaped, with chromatin scattered in finer particles or arranged in comparatively heavy clumps, and with a thick nuclear membrane staining fairly dark.

After twenty-four hours, large numbers of these cells - the so-called "polyblasts" - became vacuolated, their nuclei being eccentric and dark. Such cells were highly ameboid and had occasionally several thin pseudopodia.

Between the second and sixth day, the polyblasts increased in size, the fine psuedopodia transforming into extensions of the cytoplasm as tapering non-motile processes. The cytoplasm stained a varying shade of blue with eosin-azure II; it was finely reticulated and sometimes vacuolated; the large vacuoles, as seen before, disappeared, except in dying cultures where they coalesced, thus filling the cells almost completely. The nucleus increased in size and remained somewhat angular. The nucleoli were much larger and more prominent, the chromatin usually became more finely divided.

During the following days, the cells gained in size. They lost their roughly round shape and began to extend into very long processes. The nucleus assumed an oval shape and the chromatin became dust-like. In this stage, all phases from polyblast to typical fibroblast could be observed.

Summarizing Maximow's and Bloom's scheme, it could be said that the blood lymphocyte had been declared the stem-cell for a large fraction of phagocytic cells, the "polyblasts," which commonly occur under abnormal conditions, such as an inflammation.

The monocyte was given a secondary position, since the latter cell was believed to originate from lymphocytes, under certain conditions. The tissue "resting-wandering cell" (the fixed macrophage, histiocyte, etc.) admittedly shared in the production of polyblasts, but, in Maximow's opinion, to a lesser degree than the blood cells. In regard to the plasma cell, these authors mentioned that cells morphologically identical with that form occurred among the "polyblasts." It was their opinion that this cell as such did not take an active part in the transformations as described above. It may, however, have changed into a polyblast occasionally.

The active participation of fibroblast and endothelial cell in inflammatory cell transformations was denied by these authors.

To complete this enumeration of concepts by means of which it has been attempted to elucidate the origin of the macrophages in irritated tissue, the names of Lewis (25), Aschoff and Kiyono (2), Aschoff (1), Marchand (28), Mallory (26), Evans (14), Foot (16), Sabin, Doan and Cunningham (36) have to be included.

Lewis and Lewis (25) concluded from blood-culture experiments that monocytes, macrophages and epitheloid cells represented merely different phases of the same cell; according to their investigations, the morphological differentiation was greatly dependent on the type and size of ingested material.

Aschoff (1) stressed the importance of the reticulo-endothelium in general, while Marchand (28) introduced "Gefaesswandzellen"[4] of two kinds, endothelial-adventitial and adventitial-

---

[4]Vessel wall-cells.

mesenchymal cells into the process of macrophage formation.

Mallory, (26) Evans, (14) and Foot (16) believed that the vascular endothelium, in any location, might give rise to the respective phagocytes, while Sabin, Doan and Cunningham (36) found definite proof for the endothelial function in the spleen only.

According to the outlined theories, it could be assumed that "resting-wandering cells" (fixed macrophages, histiocytes) possibly fibroblasts and Schwann cells of the tissue, endothelial, adventitial cells, lymphocytes and monocytes of vascular system and blood may have participated in such a pathological process as the one under discussion.

## REMARKS ON BLOOD CYTOLOGY

In view of the generally accepted morphological classification of the cells of normal blood, it will suffice to point out a few details concerning the characteristics of chicken blood.

The average numeric values as given by Kaupp (23) are the following:

Total erythrocyte count: 2,600,000 - 4,000,000

Total leukocyte count: 28,000 - 35,000

Differential count (in per cent):

| | | |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 28 - 32 |
| | granules | 4 - 6 |
| basophiles | | 2 - 4 |
| Lymphocytes: | | 40 - 44 |
| Monocytes: | | 18 - 20 |

The designation "eosinophile granulocytes," as it has been used in this paper, refers merely to the staining qualities of the granula or rodlike inclusions. It does not imply any functional identification with the "eosinophiles" of mammal blood. On the contrary, it is believed that the polymorphonuclear cells with eosinophilic rods in chicken blood correspond to the neutrophilic granulocytes in the mammals.

Another point to be stressed is the extreme difficulty in the differentiation between large lymphocytes and monocytes. This obstacle in classification has been observed by a number of investigators and cannot be solved except by application of special staining methods. In view of the minor importance of this question in connection with the problem presented, no special attempt for increased accuracy was made.

The term "relative lymphocytosis" applies to an increase of the lymphocyte count within a normal total leukocyte count (in contrast to "absolute lymphocytosis" signifying a high total count due to mounting lymphocyte values).

"Irritation forms" among the lymphocytes were designated in accordance with Gradwohl's (19) definition and illustrations. He stated, (p. 345), that -

> These cells are very large, although small forms have been observed. The cytoplasm is intensely blue usually showing vacuoles. There is a fine reticular structure visible. The nucleus is eccentric and stains a deep purple with dark spots, usually with a distinct, clear, central "sphere." The nucleus gives the appearance of being divided into zones. There are no granules.

In a number of large and medium sized lymphocytes, heavy azurgranula, surrounded by a clear halo, possibly a vacuole, can

be seen. No information on their significance could be obtained.

In regard to the cell measurements, it must be pointed out that similar elements in blood smear and tissue section always have different size, the cell in the smear being larger. The considerable difference in the method of preparation may well afford an explanation for this observation.

## SOURCE OF MATERIAL AND METHODS OF STUDY

The material for this investigation was obtained from chickens, the majority of which were furnished by the Poultry Farm of Kansas State College. A smaller number was collected among cases sent in to the poultry diagnostic laboratory of the Bacteriology Department. Twenty-eight clinically suspect birds were examined. Eight of these showed nerve lesions. One healthy hen served as a histological control.

### Preparation of Histological Specimens

Specimens of the sciatic nerve were removed from the thigh (in some cases wing nerves were included.) In two cases specimens of tumor tissue were taken.

In the embedding process, the following outline was followed:

| | |
|---|---|
| 1. Zenker's fluid | 12 - 24 hours. |
| 2. Running water | 24 hours. |
| 3. 80 per cent alcohol | 24 hours or more. |
| 4. 95 per cent alcohol | 24 hours. |
| 5. Absolute alcohol | 4 - 12 hours. |

6. Cedar oil until cleared (12 hours usually sufficed).
7. Tissue mat, (Fisher, melting point, 54° - 56°C.) twenty-four hours, with one change.
8. Embedded in tissue mat.

The specimens were sectioned at a thickness of seven micra. In order to allow direct comparison between the elements of blood smears and of the tissue sections, the blood was stained by Wright's method and the tissue by a modification adapted after Bensley (7):

1. Xylol until all paraffin is dissolved.
2. 100 per cent alcohol 3 - 5 minutes.
3. Alcoholic iodine solution 5 - 10 minutes.
4. 95 per cent alcohol 3 minutes.
5. 5 per cent sodium thiosulfate a few seconds (until bleached.)
6. Rinsed in tap water.
7. Tap water 5 - 10 minutes.
8. a. The slide was stained on staining-rack in the manner of a blood smear for 1 - 2 hours.
   b. A dilution of Wright's stain was prepared by adding approximately twenty drops of concentrated dye solution to a Copeland jar of neutralized or buffered water. The slide was kept in this solution for approximately twenty-four hours. (The exact time of staining and degree of dilution had to be determined with each batch of stain.)
9. Differentiation in absolute methyl alcohol, less than one second to two or more seconds, depending upon the

intensity of the stain.

10. Dipped into second absolute methyl alcohol.
11. Acetone 1 - 5 seconds.
12. Acetone-toluol (equal parts) 2 - 5 minutes.
13. Toluol 2 minutes.
14. Toluol 2 minutes.
15. Mounted in neutral balsalm.

### Preparation of Blood Smear

The usual Wright technique was employed.

### Examination of Tissues

In order to establish a basis for comparing and analyzing the cellular composition of the lesions under observation, differential cell counts were performed. It must be said here that such counts did not compare in their significance with the differential leukocyte counts in blood. It became obvious. that the accuracy of counting greatly suffered in cell accumulations. Values obtained by this method could not be designated as "per cent." It was felt, however, that the figures were roughly representative of true conditions. In their evaluation, care was taken of this limitation.

Blood vessels and their vicinity were examined separately, and significant findings recorded. In two cases which had lymphoid tumors besides nerve lesions, mitotic figures and dying cells were counted in both tissues in order to compare the re-

spective cellular activity. The examination of lymph vessels, which could not be shown by the applied technique, had to be omitted.

## Examination of Blood

The red blood cells were counted in the same manner as in human samples.

White blood cells were counted indirectly.[5]

Smears were examined by the usual technique.

---

[5] In this method, the white blood cells occurring with 1000 red blood cells were counted. The number of leukocytes was then expressed as per cent of the erythrocytes. By taking the same percentage of the total number of erythrocytes, the actual leukocyte value was obtained.

Although this method seemed to be one of the most reliable wherever nucleated red blood cells were present, it apparently did not yield results as accurate as the count in a chamber. The low counts which were obtained in some of the cases to be reported, may have been partly due to a deficiency of this technique.

## TISSUE CYTOLOGY

The purpose of this chapter is to give the morphology of elements commonly found in the nerve lesions and to arrange them in a scheme adapted for differential counts.

As it was intended to use the method of cell-counting in the analysis of the nerve alteration, it appeared to be necessary to establish a definite order in which the cellular elements present could be enumerated.

In the first place three size standards were set-up, the large, medium and small cells. This division was adopted in consideration of the important position taken by the lymphoid series; as will be seen in the case reports, the latter group constituted a nearly constant majority among the cells present. Since lymphoid cells can readily be divided into large, medium, and small cells, their respective average measurements were made a basis for the three size standards.

The next questions arising in connection with this scheme concerned the nomenclature to be adopted for the variety of cells which were to be listed in the above mentioned divisions. Such elements as the lymphoid series,[6] the fibroblasts and the

---

[6]The term "lymphoid series" was introduced for two reasons: (a) The cells so designated were, morphologically, related to lymphocytes more closely than to any other known cell group; (b) at the beginning of the investigation, it was not known for certain whether the lymphoid cell and the lymphocyte were identical. Until a proof for such identity had been established (which will be attempted in the last chapter), it was, therefore, considered a matter of accuracy to apply the descriptive term "lymphoid series" instead of classifying the cells of this type as "lymphocytes."

Schwann cells could be enumerated under their conventional name.

A certain difficulty was encountered in finding adequate designations for cells which did not belong to any of the groups named in the preceding paragraph. Such elements were apparently either pre-existing or immigrated forms which had undergone changes in their morphology under the influence of the noxious stimulus. No consensus has ever been reached by different pathologists in regard to the exact position (considering origin as well as role) of such cells in a pathological process. Consequently, there is no general agreement as to the terms which should be applied to these elements.

It was the aim of this investigation to arrive at a basis for a "classification" (as far as it could be considered permissible to "classify" where only one staining technique was used) by the process of impartial observation rather than by uncritical adoption of one of many pre-conceived cytological systems. The use of terms found in the literature to describe various pathological tissue cells was purposely omitted in this chapter. It seemed to be the method of choice, in the examination of the cells under discussion, first to assemble them under definite headings, using their morphology and their obvious relation to the tissue alteration as a basis for their characterization; secondly to outline their position in the lesion on the basis of the cell counts and other observations made in the case reports. In a final discussion, an attempt will be made, under consideration of the recorded findings, to classify these elements within the cytological systems outlined in the section on the review of the

literature on the cytology of the nerve.

According to that method, the purpose of this section, in respect to the cells under discussion, was to assemble them under definite headings, using their relation to the tissue reaction and their morphology as a basis for their characterisation. The heading "transition forms" was chosen as a group title. This term was selected in the belief that such atypical cells had originated from pre-existing or immigrated elements, but had undergone a process of transformation due to the influence of a noxious agent. Among the subgroups listed under the general heading "transition forms" were certain forms, the morphological characteristics of which pointed toward a lymphoid, fibroblast and endothelial origin. Such cells were labelled as "cells related to the lymphoid series," "cells related to the fibroblasts," and "cells related to the endothelial series." Another type was distinguished by its intensely basophilic cytoplasm, and for it the name "basophilic cells" was adopted. Finally there were forms characterized by unusually large nuclei. They were designated as "cells with hypertrophied nuclei."

After having outlined the considerations which lead to the adoption of a certain nomenclature and arrangement of cells, it is proposed to proceed by inserting an enumeration of the divisions, groups, and subgroups in the order in which they will be listed in the cell counts. Following this, detailed morphological descriptions of all cell types will be concluded in this section.

## ORDER OF CELLS

### Large Cells

The Lymphoid Series

The Fibroblasts

The Schwann Cells

Transition Forms

- Cells related to lymphoid series
- Basophilic cells
- Cells with hypertrophied nuclei
- Cells related to the fibroblasts
- Cells related to the endothelial series

### Medium Cells

The Lymphoid Series

Transition Forms

- Cells related to the lymphoid series
- Basophilic cells
- Cells related to the endothelial series

### Small Cells

The Lymphoid Series

Transition Forms

- Basophilic cells

Dying Lymphoid Cells

## MORPHOLOGICAL DESCRIPTION OF CELL TYPES

### Large Cells

(nuclear diameter more than 4.6 micra, cellular diameter up to 28 micra).

### Lymphoid Cells

Shape and size. Smoothly round, or polygonal with a tendency to round off. Diameter 7 - 8 micra.

Nucleus. Round. Diameter 5 - 6 micra. Central position. The coarse and dense chromatin structure seen in the lymphocyte nucleus as observed in blood-smears was rare. In most cases, the chromatin was broken up into finer granulations along the thick nuclear membrane with a few coarse granules near or in the center. A well defined linin network stood out against a more or less homogeneous, dark-blue background. False nucleoli were present in a number of cells.

Cytoplasm. From a narrow, hardly discernible margin to a rim occupying not more than one-third of the cell volume. Wherever there was a narrow zone only, no structures could be distinguished. In the case of more abundant cytoplasm a foamy or fibrillar character came to observation in a number of cells. A perinuclear halo was present

in a majority of these elements. The color varied from blue to purplish (in the foamy and fibrillar types.) The outline was smooth.

## The Fibroblasts

Shape and size. Could not be determined with the applied method.

Nucleus. Slendar spindle. Diameter 8 - 13 micra. Finely distributed dust-like chromatin; often one or more false nucleoli. A fine linin network and a light staining background. Thin membrane.

In a majority of cells seen in "proliferations" (see case reports) and in rare, scattered elements, the nucleus presented itself as a homogeneously dark-blue body without differentiation of internal structures.

Cytoplasm. Was not stainable by the applied technique.

## The Schwann Cells

The stains employed in this investigation did not seem to permit a structural differentiation of this element from the fibroblasts of the endoneural sheath. Only by very careful observation of the localization in relation to the nerve fiber could the Schwann cell be identified as such. Since the regular structure was severely interfered with in the affected portions of the nerve, it was not attempted to differentiate Schwann cells in the counts.

## Transition Forms

Cells related to the lymphoid series.

Shape and size. Oval, elongated, polygonal. Diameter 7 - 12 micra.

Nucleus. Round, oval, angular. Diameter 4 - 6.5 micra. Position central and eccentric. The nuclei showed varying characteristics, which could be summarized under the following points:

(a) Lymphoid nuclei.

(b) Angular or round. Diameter 4 - 4.5 micra. The chromatin was distributed in the manner of small lymphoid cells, or concentrated in one heavy clump, lying in an undifferentiated dark blue background. The membrane was thick.

(c) Round. Diameter 6 - 7 micra. Chromatin bars, sometimes reaching a length of four micra and a width of 1.5 micra, were present in some nuclei. In others, one or more heavy chromatin particles came to observation. The latter sometimes assumed the shape of false nucleoli. A linin network was visible in a number of these elements, while in another group a light, structureless background contrasted with

the heavy chromatin. The nuclear membrane was thick.

(d) Oval, irregularly elongated. 5 - 9 micra. A variety of forms has been grouped under this heading. Heavy chromatin, as described in the preceding paragraph, as well as light scarce particles were observed. Linin structures could be seen in some cases, but were absent in others. The background stained varying dark or light. The nuclear membrane was thick in most of these cells, thin in a few. Some of the nuclei were indented and some divided, touching each other at the surface of division.

Cytoplasm. Variable in relative quantity. While it was abundant in many of the cells with smaller nuclei, it formed a narrow margin only in other cells with very large nuclei. It showed four types of structure:

(1) Fibrillar, staining blue or violet with smooth outline and, sometimes, process-like extensions.

(2) Foamy, or reticulated, staining blue or violet, with smooth outline.

(3) Folded, staining blue. Linear intensifications of the otherwise light blue

color-base, created the impression of "folds." Smooth outline.

(4) Homogeneously blue. This cytoplasm had the appearance of blue-tinted glass. The color-shade was usually rather light but often did increase in density toward the smooth cell membrane. It did not appear possible to correlate any specific plasma structure to any of the afore mentioned nuclear types. All combinations could be observed.

Basophilic Cells[7]

Shape and size. Oval, rectangular, polygonal and entirely irregular. Total diameter up to 8.5 micra.

Nucleus. Round and oval. Diameter 4 to 5 micra. Eccentric. Often the entire cell was so darkly stained that nuclear structures could not be distinguished. Those nuclei which showed details had either the structure of small lymphocyte nuclei, or showed scanty chromatin in a densely

---

[7] A number of these cells resembled the plasma cells which have been described by Maximow-Bloom (31), in the following manner:

Shape and size. Spherical, often flattened. From small lymphocyte size to twice or three times the size of that cell.

Nucleus. Round or oval. Small lymphocyte size. Eccentric position. Coarse, dark, regularly distributed chromatin particles.

Cytoplasm. Homogeneous, strongly basophilic, abundant.

staining, homogeneous background. Sometimes false nucleoli were seen. The nuclear membrane, wherever observed, was thick.

Cytoplasm. Very abundant. In a majority of cells, it was more or less homogeneous, in a smaller number the cytoplasm seemed to be broken up into irregular particles. Such elements often showed vacuoles, and signs of cytoplasmic decay. No perivascular halo was present. The predominant coloration was a deep blue, often with a purplish tinge. In a number of cells, the area of the attraction sphere stained distinctly acidophilic. The outline in homogeneously blue elements was nearly smooth. In a few cases, the cytoplasm was drawn out into well-sized pseudopodium-like protrusions. The forms with coarsely granular cytoplasm had an irregular surface, giving the appearance of a cellular membrane ruptured in many locations.

Cells with Hypertrophied Nuclei

Shape and size. Oval, elongated, undetermined. Total recognizable diameter up to 15 micra.

Nucleus. Oval. Diameter 5 to 10 micra. Where cytoplasm was visible, the nucleus had a slightly eccentric position. In the smaller forms the chro-

matin distribution resembled that of fibroblasts (see under fibroblast).[8] With increasing size of the nucleus, the basophilic substance was lost step by step. In the larger elements, chromatin could be observed in the form of small basophilic knots in the linin network. Some of the largest nuclei were completely bleached, being recognizable only by their distinct one to two false nucleoli and the relatively thick nuclear membrane.

Cytoplasm. Here again changes took place apparently correleted to the above described transformation of the nuclei. Those smaller forms which closely resembled fibroblasts, did not show any cytoplasm with the applied staining method.[9] As the nucleus lost in staining intensity and increased in size, the cytoplasm became more clearly visible. At some point of the nuclear changes, which could not be schematically fixed except for the fact that it did not usually involve the largest nuclei, a well stained cytoplasm came to observation. This was homogeneous to finely

---

[8]Such cells presumably were active fibroblasts, but were listed here on account of their atypical size and continuous transformations to the other types of hypertrophied cells.

[9]The fiber-like processes which could frequently be seen to project from the poles of such nuclei, resembled collagenous material rather than cytoplasmic extensions.

reticular, violet in color, with smooth outline, and tapering, splitting processes. Such processes most frequently extended in the longitudinal axis of the cell; sometimes, however, they pointed in all directions of the space, thus producing the well-known "star-shape" of the fibroblast.

At the same stages of the nuclear development, the cytoplasm afforded an entirely different aspect in some other cells. Scanty in stainable substance, it showed wide, irregular meshes of a blue staining net. Only a minority of these cells was limited by a fairly distinct and closed cellular membrane, from which, in most cases, extensions went to either side of the field. In a majority of these elements, the nucleus was embedded in honey-combed cytoplasm, extending indefinitely into the surroundings, until it was lost, either by interference of other structures, or by decrease in stainability.

The cytoplasm correlated to the largest nuclei under observation seemed to have the same general characteristics as that of the last group described. It was, however, very poorly stained and could be observed only in the close neighborhood of the nucleus. In rare cases, a

dense, violet staining cytoplasm was present with a finely reticular or fibrillar structure and indefinite limits.

Cells Related to the Fibroblasts

Shape and size. Oval. Diameter could not be determined.

Nucleus. Oval. Diameter 5 - 7 micra. The chromatin was either finely granular and well distributed, or, more often coarse, being concentrated into a few irregularly located particles. Only rare structures of the linin-net were visible. The background stained pale blue, frequently more intensive toward the thick nuclear membrane. Thus the nucleus often obtained a distinctly vesicular aspect.

Cytoplasm. In most cells not visible. In a few, it formed a narrow margin of non-homogeneous, pale-violet, material of indistinct outline.

Cells Related to the Endothelial Series

Shape and size. Oval. Size was not determined because the cytoplasm remained nearly unstained.

Nucleus. Oval, irregularly elongated. 5 - 9 micra. Few, dust-like chromatin particles could be observed, but were not present regularly. The linin network was indistinct. The background stained a fairly homogeneous light blue. The membrane

was thin.

Cytoplasm. Remained nearly unstained with the applied method.

Medium Cells

(nuclear diameter 3 - 4.5 micra; cellular diameter up to 9 micra)

The Lymphoid Series

Shape and size. Polygonal to round. Diameter from slightly more than 3 to 4.5 micra.

Nucleus. Round. Central position. The chromatin arrangement, as well as the other nuclear structures, resembled the lymphocytes in blood smears rather closely, much more so than in the large cell.

Cytoplasm. Formed a more or less narrow margin. It was homogeneous and a perivascular halo could be seen, wherever the plasma-rim was wide enough to permit detailed inspection. The color was blue with, sometimes, a violet tinge. The cell outline was smooth.

## Transition Forms

### Cells Related to the Lymphoid Series

Shape and size. Oval, elongated, irregular. Size, in the elongated forms, near to 9 micra.

Nucleus. Oval, round, sometimes irregularly deformed. Except for this alteration in shape, the nuclei retained all lymphoid characteristics.

Cytoplasm. Usually more abundant than in the lymphoid cell, except in case of simply deformed nuclei. The structure was fibrillar, foamy or like blue-tinted glass. With the deformed nuclei, the original characteristics of the lymphoid cytoplasm were most often maintained. The color was violet or blue and the outline smooth.

### Basophilic Cells

Except in regard to their size, these cells had all the characteristics of the large basophilic cells.

### Cells Related to the Endothelial Series

Here again a structure similar to that described under the large cells was found. It could, perhaps, be said that the medium sized nuclei of this type were still more void of structural details than those of the larger type.

Small Cells
(nuclear diameter less than 3 micra, very small amount of cytoplasm)
The Lymphoid Series

These elements resembled the small lymphocytes of blood smears so closely that it seemed unnecessary to give a special description. Here again, deformation of the nucleus, same as with the medium-sized cells, could be observed.[10]

Dying Lymphoid Cells[11]

Shape and size. Round or oval. Total diameter 2.5 - 4.5 micra.

Nucleus. Sickle or half-moon shaped, irregular. It was often broken into pieces (two, rarely three); its diameter varying from 2.0 to 2.5 micra. The nucleus stained homogeneous, strongly baso-

---

[10] Transverse sections: More or less frequently, two types of irregularly oval nuclear structures could be seen. They were not surrounded by cytoplasm. The appearance of the intranuclear substance, permitted to classify these elements under cells related to the fibroblasts and cells related to the endothelial series respectively. Their measurements, however, were deceiving; no elements of the types mentioned above fell into the size limits of the small cells. It must be assumed, therefore, that these oval structures represented transverse sections of normal sized nuclei belonging to the two quoted groups.

[11] The classification of this element as "dying lymphoid cell" had been adopted for the following reasons. The size and shape of the cell strongly indicated its derivation from a small element of the lymphoid series. Signs for nuclear disintegration were presented by the high basophilia of this structure, the complete absence of internal differentiation, the frequent lack of a distinct outline, and the final disruption of the nucleus. The structureless muddy aspect of the cytoplasm strongly supported the assumption of cell degeneration.

philic, void of any internal differentiation. A membrane could not be distinguished. In a number of cells, the basophilic substance diffusely extended into the cytoplasm.

Cytoplasm. Scarce to abundant. Structureless, staining a muddy violet. The outline was most often smooth, although not always very distinct.

CASE REPORTS

Case 5: White Leghorn Hen, Adult.

Clinical symptoms. Paralysis of the left leg.

Blood examination:

Total erythrocyte count: 2,670,000

Total leukocyte count: 21,400

Differential leukocyte count: (100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 9 |
| | granules | 6 |
| basophiles | | 2 |
| Monocytes | | 1 |
| Lymphocytes | | |
| large | | 7 |
| medium and small | | 75 |

"Irritation forms" were present among the large lymphocytes.

Pathology.

Gross findings. Not recorded.

Microscopic findings. The left sciatic nerve was diffusely infiltrated, one division distinctly more so than the other. Only small areas of the nervous substance were completely destroyed.

The cellular proliferation was made up predominantly of round-cells and connective tissue elements. There was no region

of complete absence of fibroblasts, and in a circumscribed zone dark fibrous elements had proliferated intensively; in this location no round cells were seen. This area was not included in the count.

Cell count: (100 cells).

Large cells

| | |
|---|---|
| The lymphoid series | 4 |
| The fibroblasts | 42 |
| Transition forms | |
| basophilic cells | 2 |
| cells with hypertrophied nuclei | 28 |

Medium cells

| | |
|---|---|
| The lymphoid series | 5 |
| Transition forms | |
| cells related to the lymphoid series | 9 |
| basophilic cells | 4 |

Small cells

| | |
|---|---|
| The lymphoid series | 6 |

Observations on blood vessels: Three significant vessels were selected for description. In one, no cells were present in the lumen.

Outside of the endothelial layer, a number of nuclei were scattered as classified under cells related to the endothelial series. At a little distance from the vessel wall were a few basophilic cells. They all showed a non-homogeneous cytoplasm with distinct vacuole formation.

The lumen of another blood vessel contained a red blood

cell, one eosinophile granulocyte and one large lymphocyte, apparently just engaged in the act of migration. A few basophilic cells, cells related to the endothelial series, cells with hypertrophied nuclei, and a considerable number of cells related to the fibroblasts were located in the surroundings. Lymphocytes were astoundingly rare.

A count in the vicinity of a capillary, including cells which apparently had originated in the blood vessel lumen or wall, gave the following figures:

| | |
|---|---|
| Large cells | |
| The lymphoid series | 0 |
| Transition forms basophilic cells | 13 |
| Medium cells | |
| The lymphoid series | 2 |
| Transition forms basophilic cells | 10 |
| cells related to the endothelial series | 1 |
| Small cells | |
| The lymphoid series | 0 |
| Transition forms basophilic cells | 1 |

Summary: The blood picture showed normal total erythrocyte and leukocyte counts. There was, however, a considerable relative lymphocytosis with the appearance of atypical large and medium sized lymphocytes.

The nerve lesion showed an area of fibrous cell proliferation. The count presented signs of a predominance of fibrous

elements, and of such with hypertrophied nuclei.

The observations on blood vessels showed basophilic cells and cells related to the fibroblasts to be numerous in the surrounding of occasional vessels, while lymphoid cells in this location were scarce.

Case 15: White Rock Rooster, Adult.

Clinical symptoms. Paralysis of both legs.

Blood examination

Total erythrocyte count: 4,000,000

Total leukocyte count: 44,000

Differential leukocyte count: (100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 48 |
| | granules | 2 |
| basophiles | | 4 |
| Monocytes | | 7 |
| Lymphocytes | | |
| large | | 7 |
| medium | | 29 |
| small | | 3 |

Pathology.

Gross findings. The sciatic nerves were slightly enlarged.

Microscopic findings. In the section of the right nerve, no pathological changes were encountered; this may have

been due to the location of the plane of sectioning. In the left nerve, one limited area of dense infiltration with complete destruction of the nervous substance could be seen. Besides it, scattered round cells occupied most of the endoneural spaces. No fibrous cell proliferation was observed.

Cell count: (200 cells)[12]

| | count 1 | count 2 |
|---|---|---|
| Large cells | | |
| The lymphoid series | 4 | 0 |
| The fibroblasts | 4 | 41 |
| Transition forms | | |
| cells related to the lymphoid series | 8 | 0 |
| basophilic cells | 0 | 1 |
| cells with hypertrophied nuclei | 3 | 3 |
| cells related to the endothelial series | 4 | 0 |
| Mitotic figures | 1 | 0 |
| Medium cells | | |
| The lymphoid series | 10 | 4 |
| Transition forms | | |
| cells related to the lymphoid series | 2 | 2 |
| basophilic cells | 1 | 5 |
| cells related to the endothelial series | 10 | 2 |
| Small cells | | |
| The lymphoid series | 53 | 42 |

Observations on blood vessels: In the lumen of a longitudinally sectioned capillary extending through more than

---

[12]Count 1 in heavily infiltrated area. Count 2 comprising scattered elements.

one field of vision, three small lymphocytes and one red blood cell were observed. Elements related to the endothelial series, basophilic cells of the vacuolarized type, a few cells with hypertrophied nuclei and a considerable number of typical small lymphoid cells were located outside the endothelial layer.

Another vessel of the same order was bordered by the striking number of nine basophilic cells. The lumen contained only one small lymphocyte and there were no typical lymphoid cells in the near neighborhood.

Summary. The erythrocyte and leukocyte count lay at the upper limit of the normal. The differential count presented average values, atypical cells were absent.

In both counts of tissue cells, the strongest fraction belonged to the lymphoid series; within this series, the smaller outnumbered the larger elements.

The values obtained for the dense infiltration indicated a dominating role of the lymphoid cells. In the count of scattered elements, the numerical superiority of that cell over the fibroblast was only slight.

Observations in the vicinity of blood vessels showed both the occurence of lymphoid and of basophilic cells.

Case 16: White Leghorn Hen, Adult.

Clinical symptoms. Paralysis of both legs.

Blood examination:

Total erythrocyte count: 3,000,000

Total leukocyte count: 39,000

Differential leukocyte count: (200 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 44 |
| | granules | 1 |
| basophiles | | 5 |
| Monocytes | | 6 |
| Lymphocytes | | |
| large | | 10 |
| medium | | 21 |
| small | | 13 |

Among the large lymphocytes, 4 - 6 percent were "irritation forms."

Pathology.

Gross findings. The sciatic nerves were considerably enlarged.

Microscopic findings. In both sciatic nerves, one division was but slightly infiltrated by mononuclear cells. The other division was heavily infiltrated, although only relatively small areas appeared to be completely destroyed. There were no zones of fibrous cell proliferation.

Cell count: (200 cells).[13]

| Large cells | count 1 | count 2 |
|---|---|---|
| The lymphoid series | 11 | 9 |

[13]Count 1 was taken from a longitudinal section, count 2 from a transverse section. 1 from the right, 2 from the left nerve.

| | | |
|---|---|---|
| The fibroblasts | 5 | 22 |
| Transition forms | | |
| cells related to the lymphoid series | 5 | 16 |
| basophilic cells | 1 | 1 |
| cells related to the endothelial series | 5 | 6 |
| Medium cells | | |
| The lymphoid series | 26 | 10 |
| Transition forms | | |
| cells related to the lymphoid series | 3 | 4 |
| cells related to the endothelial series | 6 | 10 |
| Mitotic figures | 1 | 1 |
| Small cells | | |
| The lymphoid series | 37 | 21 |

Observations on blood vessels: A transverse section of a pre-capillary contained three erythrocytes and two small lymphocytes in its lumen. Elements related to the endothelial series, cells related to the fibroblasts, and cells with hypertrophied nuclei were in the neighborhood. Small and medium sized lymphocytes were rather numerous, while not a single basophilic cell came to observation.

The examination of nine pre-capillaries and capillaries showed only one basophilic cell close to a blood vessel. In every instance, however, numerous typical lymphoid cells of medium and small size, fewer of large size were present.

A pre-capillary situated in a densely infiltrated area contained numerous lymphocytes of all sizes, in their morphology very similar to a majority of cells in the vicinity

of this vessel. The endothelial cells had apparently undergone severe changes. Very few of the latter had retained a normal appearance. Some had hypertrophied nuclei, embedded in a dense violet homogeneous cytoplasm extending processes in its longitudinal axis. One endothelial cell showed a mitotic figure. Great parts of the endothelium seemed to be broken up.

Summary: The total erythrocyte and leukocyte count was within normal limits. The differential count represented average values. The only suggestion of a blood affection was given by the presence of 4 - 6 percent "irritation forms" among the large lymphocytes.

The tissue-cell counts showed a predominance of lymphoid cells, again with smaller elements in the majority.

The observations on blood vessels demonstrated the probable derivation of the lymphoid cells from the blood. A break up of vessel walls in heavily infiltrated areas could be seen. Hardly any basophilic cells were present in these sections.

Case 17: White Leghorn Hen, Adult.

Clinical symptoms. Paralysis of the left leg.

Blood examination:

Total erythrocyte count: 2,600,000

Total leukocyte count: 10,400

Differential leukocyte count:
(100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 23 |
| | granules | 5 |
| basophiles | | 1 |
| Monocytes | | 2 |
| Lymphocytes | | |
| large | | 24 |
| medium | | 25 |
| small | | 20 |

Fifteen percent of the large lymphocytes were "irritation forms." A number of medium sized lymphocytes had pseudopodia.

Pathology.

Gross findings. Typical gross lesions caused by Histomonas meleagridis. The sciatic nerve did not show any gross changes.

Microscopic findings. One division of the left sciatic nerve was diffusely infiltrated by round cells. Only in small areas was the nervous substance completely replaced; no foci of fibrous cell proliferation could be observed.

Cell count: (100 cells)

| | |
|---|---|
| Large cells | |
| The lymphoid series | 20 |
| The fibroblasts | 12 |
| Transition forms cells related to the lymphoid series | 17 |

cells with hypertrophied nuclei 3
cells related to the endothelial series 2

Medium cells

The lymphoid series 22

Transition forms
cells related to the lymphoid series 7
basophilic cells 1
cells related to the endothelial series 2

Mitotic figures 1

Small cells

The lymphoid series 13

Observations on blood vessels: A capillary surrounded by considerable infiltration showed the following count (45 cells counted).

Large cells[14]

The lymphoid series (1) 6

Transition forms
cells related to the lymphoid series 10
cells with hypertrophied nuclei 3
cells related to the endothelial series 2

Medium cells

The lymphoid series (6) 6

Transition forms
cells related to the lymphoid series 2

---

[14]Figures in brackets indicate cells in the vessel lumen. They were all lymphocytes.

Small cells

The lymphoid series (6) 3

In the surroundings of another blood vessel, essentially the same picture was seen, except for the presence of one, disintegrating basophilic cell.

It was noticeable that in a transversally sectioned capillary the lumen of which was filled by ten lymphocytes of all sizes, the largest lymphocytes showed a very pale nucleus, void of the common chromatin pattern.

Summary: The presence of Histomonas meleagridis introduced a noxious agent which commonly is not brought in connection with a neurolymphomatosis. It has to be mentioned, furthermore, that an egg-sized mass of dried necrotic material was located in the left lower half of the abdomen. This tumor might well have caused the paralysis by pressure on the lumbar plexus. Since the histological picture of the nerve, however, had the appearance of neurolymphomatosis lesions, this case was included.

In view of the parasitic infection, the blood counts could hardly be considered significant.

In the sciatic nerve, the lymphoid cells constituted a majority. Large lymphocytic nuclei in one of the vessels had the same pale staining properties as lymphoid cells in the tissue; this observation offered evidence for the similarity of the tissue cells and the blood lymphocytes. Basophilic cells were rare.

Case 18: White Leghorn Hen, Adult.

Clinical symptoms. Paralysis of the left leg.

Blood examination:

Total erythrocyte count: 3,000,000

Total leukocyte count: 30,000

Differential leukocyte count: (100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 7 |
| | granules | 8 |
| basophiles | | 0 |
| Monocytes | | 1 |
| Lymphocytes | | |
| large | | 14 |
| medium | | 46 |
| small | | 29 |

Pathology.

Gross findings. The left sciatic nerve showed a slight increase in size.

Microscopic findings. A considerable infiltration with little destruction of nervous substance was present. No fibrous cell proliferation could be seen.

Cell count: (200 cells)[15]

| Large cells | count 1 | count 2 |
|---|---|---|
| The lymphoid series | 2 | 8 |

[15]Count 1 referred to scattered elements, count 2 to a more densely infiltrated area.

| | | |
|---|---|---|
| The fibroblasts | 24 | 3 |
| Transition forms | | |
| cells related to the lymphoid series | 7 | 9 |
| basophilic cells | 2 | 0 |
| cells related to the endothelial series | 5 | 0 |
| Medium cells | | |
| The lymphoid series | 33 | 27 |
| Transition forms | | |
| cells related to the lymphoid series | 3 | 2 |
| basophilic cells | 13 | 3 |
| cells related to the endothelial series | 0 | 1 |
| Small cells | | |
| The lymphoid series | 11 | 47 |

In this section a number of cells were seen, the nuclei of which were "hypertrophied" and located at the surface of a nerve-fiber. The cytoplasm was finely fibrillar and blue. It seemed to envelop a portion of the nerve.

Observations on blood vessels: A capillary situated in a heavily infiltrated area had a few medium sized lymphocytes in its lumen and was surrounded by a large number of mostly medium sized lymphoid cells, which were somewhat deformed but had not otherwise changed their aspect.

A transversally sectioned capillary offered the picture of perivascular "cuffing." The lumen contained one small, one medium sized, and one large lymphocyte. In the immediate surroundings, 39 cells were counted. Twenty-five of these were small and medium sized lymphocytes. Five cells resembled

endothelium, being definitely outside the endothelial layer. Five others were of the size and shape of the small lymphoid series, their nuclei staining deeply basophilic, void of any internal structures. The remaining four cells belonged to the large lymphoid series. Hardly any basophilic cells were present in "cuffs." In areas surrounding blood vessels without cuffs, they were more or less numerous. In view of the lymphoid character of the perivascular infiltration, this specimen was a good proof of the migration of lymphoid elements from the circulation. In addition to the above reported instances, a number of other vessels were seen, surrounded by cuffs or heavier infiltrations. The latter, like the former, were composed mainly of small and medium sized lymphocytes.

Summary: The total erythrocyte and leukocyte counts were within normal limits. The differential count showed a high relative lymphocytosis (89 percent).

Two cell counts in different areas of the section showed a majority of lymphoid cells; in both instances the medium sized and small cells predominated. Fifteen basophilic cells were present in one of the counts.

The observation of blood vessels offered a good proof for the migration of lymphocytes (mainly small and medium sized) from the blood.

There were cells with hypertrophied nuclei which possibly originated from Schwann's cells.

Case 19: White Leghorn Hen, Adult.

Clinical symptoms. Paralysis of the left leg.

Blood examination:

Total erythrocyte count: 2,680,000

Total leukocyte count: 8,400

Differential leukocyte count: (100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 58 |
| | granules | 4 |
| basophiles | | 1 |
| Monocytes | | 4 |
| Lymphocytes | | |
| large | | 18 |
| medium | | 11 |
| small | | 4 |

Pathology - Nerve.

Gross findings. No distinct increase in the size of the left sciatic nerve could be observed.

Microscopic findings. The nerve fibers of the left sciatic nerve were nearly completely replaced by invading cells; the remainder of the original tissue consisted of scattered fibers and a few marginal foci of dense fibrous cell proliferation lacking any intercellular fibrillar substance.

Cell count: (200 cells).[16]

| | count 1 | count 2 |
|---|---|---|
| Large cells | | |
| The lymphoid series | 14 | 24 |
| The fibroblasts | 13 | 13 |
| Transition forms | | |
| cells related to the lymphoid series | 4 | 7 |
| basophilic cells | 0 | 1 |
| cells related to the endothelial series | 0 | 1 |
| Granulocytes | | |
| eosinophiles | 0 | 2 |
| Medium cells | | |
| The lymphoid series | 44 | 17 |
| Transition forms | | |
| cells related to the lymphoid series | 1 | 7 |
| basophilic cells | 1 | 1 |
| cells related to the endothelial series | 1 | 12 |
| Mitotic figures | 0 | 2 |
| Small cells | | |
| The lymphoid series | 22 | 13 |

Observations on blood vessels: A group of five small arteries and three capillaries located in the perineurium was examined. The arteries were empty and did not show any changes in their muscular layer. The adventitia of two of the vessels was embedded in an accumulation of the dark, homogeneous fibroblast nuclei.

[16]Count 1 referred to an area in which a few nervous fibers were left. Count 2 was taken in a portion in which no normal tissue was retained.

In the lumen of a capillary which was surrounded by a dense layer of fibrous cells, three large, two medium sized and one dying lymphocyte[17] were present. The cytoplasm of one of the large lymphocytes was elongated into a tapered process, longer than the diameter of the main cell body, possibly indicating the first step toward migration of the cell. The vessel-wall was continuous but wavy, apparently yielding to the pressure of the surrounding fibrous tissue.

A small vessel, probably a capillary, located in an area of the nerve which had been completely replaced by partly lymphoid and partly fibrous cells, seemed to be in a state of decomposition. The clear outline of the endothelial layer was retained in only one sector of the vessel-wall. In all other portions an irregular arrangement of cells, bordered the lumen. The elements in the immediate vicinity were endothelial, fibroblasts, and lymphoid cells. The first group constituted a majority.

In an area of predominantly lymphoid infiltration, a capillary was examined which contained two large and two medium sized lymphocytes and two eosinophilic granulocytes. The vessel-wall was intact and the surrounding cells were predominantly of lymphoid character.

Pathology - Tumor.

Gross findings. A round, flat, tumor, two inches in diameter was embedded in the muscles of the left thigh. It

---

[17]The description given under "dying lymphoid cells" applies to this cell also.

apparently had a well developed connective tissue capsule and a slightly higher consistency than the surrounding muscle. On the cut surface, the tissue was pink and protruding. A few foci of softening were present.

Microscopic findings. This growth consisted of lymphoid tissue, embedded in which were strands of connective tissue and very few fat cells.

Cell count: (100 cells).

| | |
|---|---|
| Large cells | |
| The lymphoid series | 21 |
| The fibroblasts | 6 |
| Transition forms | |
| related to the lymphoid series | 26 |
| cells related to the fibroblasts | 3 |
| cells related to the endothelial series | 10 |
| Medium cells | |
| The lymphoid series | 8 |
| Transition forms | |
| cells related to the lymphoid series | 17 |
| cells related to the endothelial series | 2 |
| Mitotic figures | 1 |
| Small cells | |
| The lymphoid series | 6 |

Count for the comparison of cellular activity in nerve and tumor: (200 cells).

| | Nerve | Tumor |
|---|---|---|
| Mitotic figures | 4 | 2 |
| Dying cells | 5 | 2 |

Observations on blood vessels: The contents of the vessel lumina was partly insignificant, but consisted in some instances of rows of lymphocytes.

The vicinity of several capillaries did not show any elements other than those most commonly found throughout the growth. However, near one branching capillary or pre-capillary, seven mitotic figures and five dying cells were seen. This may have been due to the higher vascularization of the respective part of the tumor.

Summary: The erythrocyte count was normal, while there was a rather low total leukocyte count. The differential count presented average values.

Among the cells counted in the nerve, the lymphoid element was predominant. Unlike the distribution in the tumor, however, the smaller type of cell prevailed. Foci of proliferated fibroblasts could be observed; the cell counts were nowhere free of fibrous elements. Basophilic cells were rare.

In the examination of blood vessels, the lumina were found to harbor lymphocytes besides small numbers of other blood cells. In the vicinity of the vessel wall, lymphoid elements and fibroblasts dominated the picture. No basophilic cells were seen.

Lymphoid cells mainly of the larger type dominated the cellular make-up of the tumor. No cells with hypertrophied nuclei and no basophilic cells could be seen. In general, there was relatively low cellular activity. In a well vascularized area, however, numerous mitotic figures could be

noticed.

For the purpose of comparing the cellular activity in nerve and tumor the number of dying and dividing cells in a total of 200 was determined. This count showed that mitotic figures and dying cells were more numerous in the nerve lesions than in the examined area of the tumor.

Case 22: Barred Rock Hen, Adult.

Clinical symptoms. General droopiness, weakness in both legs, no paralysis. Blood smears could not be obtained.

Pathology.

Gross findings. Histomonas meleagridis lesions. No apparent enlargment of the sciatic nerves or wing nerves was observed.

Microscopic findings. Both divisions of the sciatic as well as of the wing nerves were invaded by scattered round cells; there was also a distinct increase in connective tissue elements. In one division of one of the sciatic nerves, a localized accumulation of round cells as well as a focus of fibrous cell proliferation was present.

Cell count: (100 cells).

Large cells

| | |
|---|---|
| The lymphoid series | 6 |
| The fibroblasts | 32 |
| Transition forms cells related to the lymphoid series | 12 |

| | |
|---|---|
| cells with hypertrophied nuclei | 6 |
| cells related to the endothelial series | 1 |
| **Medium cells** | |
| The lymphoid series | 19 |
| Transition forms | |
| cells related to the lymphoid series | 4 |
| basophilic cells | 2 |
| cells related to the endothelial series | 6 |
| **Small cells** | |
| The lymphoid series | 13 |

Observations on blood vessels: A number of capillaries and small arterioles did not show any significant changes in lumen, wall or vicinity.

Summary: A second case of Histomonas meleagridis infection with typical neurolymphomatosis lesions in the nerves.

The cell count presented a slight majority of lymphoid cells. The fibrous elements were relatively high in number and there was a focus of fibrous proliferation. Basophilic cells were rare.

The blood vessels did not offer findings of any significance in lumen or surrounding.

Case 23: Buff-Orpington Male, Six Months Old.

Clinical symptoms. General droopiness. No paralysis. Blood was not obtained.

Pathology - Nerve.

Gross findings. The sciatic nerves were not visibly enlarged.

Microscopic findings. Only one division of the sciatic nerve was affected. In the major part of this division, invading cells were scattered loosely. In several locations, however, the destruction of the pre-existing structures was complete. Some of these foci showed a tendency to round up, reminding somewhat of a lymph-follicle. Scattered throughout the specimen were dark, structureless fibroblast nuclei. The latter, however, did not accumulate to form circumscribed foci as in other specimens. They were most numerous at the border of the lymphoid foci.

Cell count: (100 cells).[18]

| | |
|---|---|
| Large cells | |
| The lymphoid series | 6 |
| The fibroblasts | 22 |
| Transition forms | |
| cells related to the lymphoid series | 8 |
| cells with hypertrophied nuclei | 9 |
| cells related to the fibroblasts | 2 |
| cells related to the endothelial series | 5 |
| Medium cells | |
| The lymphoid series | 18 |
| Transition forms | |
| cells related to the lymphoid series | 12 |

[18]Counted among scattered cells.

| | |
|---|---|
| cells related to the endothelial series | 3 |
| Small cells | |
| Lymphoid series | 15 |
| Dying cells | 3 |

In examining one of the roundish cell foci (consisting of more than 100 cells) it was noted that, excepting the marginal area, all cells belonged to the lymphoid series, or were transition forms related to it. In the periphery and surrounding of this focus, nuclei of the densely staining fibroblast character formed a more or less distinct layer.

Upon observation of the foci described in the preceding paragraph, the impression was gained that a few of the small lymphoid cells showed an increase in the basophilia of their nuclei until all intra-nuclear structures were lost; at the same time the scanty cytoplasm completely disappeared. Besides these round diffusely blue forms, there were others of angular, irregular and elongated shape and similar staining qualities. The elongated form was identical with the nucleus of the dark fibroblast. Although this one observation did not permit definite conclusions, it nevertheless suggested the possibility of a direct transition from the small lymphoid series to the proliferating fibroblasts.

Observations on blood vessels: Three capillaries and two pre-capillaries were examined. The lumina contained lymphocytes in varying numbers, a few red blood cells and no granulocytes. In the surrounding, lymphoid cells and their transi-

tion forms were always in the majority. Darkly staining fibrous elements occurred, but not regularly.

A remarkable feature of this slide was the apparent absence of basophilic cells. Elements which had been classified as dying lymphoid cells were more numerous in this section than in others.

Pathology - Tumor.

Gross findings. A tumor of the consistency of lymphoid tissue, whitish in color, was located in the region of the testis, one part of it being adherent to the spleen.

Microscopic findings (of the portion attached to the spleen). The neoplasm had broken the capsule of the spleen in only a small area and even there the destruction was incomplete. The cellular make-up was lymphoid in character. Some portions of the growth were well vascularized; little stroma was present. Numerous fat cells could be seen. Foci of darkly staining fibroblasts reminded of the proliferating areas as described in nerve lesions. A few circular areas were hyalinized.

An examination of the marginal zone suggested the attempted or beginning formation of a fibrous capsule. The cells involved in this process were: (a) dark structureless fibroblasts, (b) large cells of elongated irregular shape. Their nucleus was oval, eccentric, and contained one or two relatively heavy chromatin particles, and sometimes a moderately distinct linin structure; it was otherwise clear. The nuclear membrane was thick. The abundant, homogeneous cytoplasm stained violet

and extended into several processes which probably led to other cells of the same type. Some of these elements were vacuolated. Their outline was indistinct. Such cells compared very closely with one form of the cells with hypertrophied nuclei, (c) numerous lymphoid cells and transition forms related to the lymphoid series. A majority of the latter belonged to the large type, the cytoplasm being darker than the nucleus in nearly all of these forms. In addition, a few typical basophilic cells were present.

Cell count: (100 cells, in a central area).

| | |
|---|---|
| Large cells | |
| The lymphoid series | 31 |
| The fibroblasts | 1 |
| Transition forms | |
| cells related to the lymphoid series | 14 |
| cells with hypertrophied nuclei | 1 |
| cells related to the fibroblasts | 3 |
| cells related to the endothelial series | 7 |
| Mitotic figures | 2 |
| Medium cells | |
| The lymphoid series | 18 |
| Transition forms | |
| cells related to the lymphoid series | 8 |
| cells related to the endothelial series | 1 |
| Mitotic figures | 1 |
| Small cells | |
| The lymphoid series | 13 |

Observations on blood vessels: The lumina of blood vessels contained great numbers of lymphocytes, erythrocytes and rare granulocytes. The neighborhood around the vessels did not differ essentially from the densely invaded areas of the nerve. Besides the lymphoid cells, there were a number of elements related to the endothelial series and the fibroblasts.

Count for the comparison of cellular activity in nerve and tumor: (200 cells).

| | Nerve | Tumor |
|---|---|---|
| Mitotic figures | 3 | 9 |
| Dying cells | 3 | 4 |

Summary: A second case of lymphoid tumor and neurolymphomatous lesions of the sciatic nerve.

The nerve showed follicle-like foci, which consisted nearly exclusively of lymphoid cells. Among the scattered elements, however, the lymphoid type had only a slight majority (the smaller cells, as usually, being more numerous). In contrast to previously described sections, darkly staining, undifferentiated fibroid nuclei were scattered almost through the entire section without forming a single circumscribed focus. It was suggested that a transformation from small lymphoid cells to these dark fibroblasts may have taken place. Basophilic cells were absent.

The tumor was chiefly composed of lymphoid cells (84 in 100). Of these, a majority belonged to the large type. In its periphery were signs of an attempted capsule formation.

Dark fibroblast nuclei could be seen in the marginal area. Blood vessels both in tumor and nerve contained mainly lymphocytes. Similar elements occupied the neighborhood.

Counting 200 cells for the comparison of cellular activity in tumor and nerve, the former showed a considerably higher number of mitoses and only a slightly higher rate of cell death. The values on mitosis found in the complete differential count of the tumor, were only slightly higher than in the nerve, showing the varying amount of cell multiplication occurring in different areas of growth.

Case 29: Exhibition Single Comb Rhode Island Red Hen, One Year Old.

(This bird was examined as a normal control)

Clinical findings. No signs of disease were observed.

Blood examination:

Total erythrocyte count: 4,000,000

Total leukocyte count: 16,000

Differential leukocyte count: (100 cells)

| | | Percent |
|---|---|---|
| Granulocytes | | |
| eosinophiles | rods | 33 |
| | granules | 3 |
| basophiles | | 9 |
| Monocytes | | 5 |
| Lymphocytes | | |
| large | | 22 |
| medium | | 21 |
| small | | 5 |

Unrecognized cells 2

There were neither "irritation forms" nor cells with azurgranulation among the lymphocytes.

Pathology.

Gross findings. No gross lesions were found.

Microscopic findings. Both sciatic and wing nerves showed a normal histological aspect.

Cell count in the sciatic nerve: (100 cells).

Large cells

The fibroblasts 80

The Schwann cells 13

Transition forms cells with hypertrophied nuclei 4

Medium cells

The lymphoid series 3

Observations on blood vessels: A majority of capillaries and pre-capillaries showed no findings of significance. Now and then a medium sized lymphocyte could be seen in their vicinity; the lumina contained a number of erythrocytes, lymphocytes and rare granulocytes. In the neighborhood of a few capillaries, lymphoid cells of medium and small size were moderately numerous. At the junction of two pre-capillaries a group of six cells was located close to the vessel wall. Morphologically they could be classified as large transition forms. Two of these were basophilic elements, while the other four appeared to be related to the lymphoid series. One of the latter had

two nuclei. All of the cells had pseudopodia-like protrusions, the cytoplasm of the basophilic types was not homogeneous.

Summary: A clinically healthy hen at a susceptible age was examined as a control.

The total erythrocyte count was at the upper limit of the normal. The total leukocyte count was low. The differential count showed average values.

The cytological elements of this apparently healthy portion of the sciatic nerve consisted of a vast majority of fibroblasts, the cells with hypertrophied nuclei in this case being active fibroblasts exclusively. Schwann cells could be recognized by their localization.

A few lymphoid cells of medium size appeared to be scattered in the vicinity of blood vessels. Active transition forms could be observed in only one location at a vessel junction.

## DISCUSSION OF RESULTS

After having reported on the cytology of the cases obtained in this investigation, an attempt will be made to evaluate their significance in relation to the pathological processes under observation.

It is proposed to analyze: first, the cellular elements and their origin; secondly, their role in the processes under observation; and finally, to discuss the character of the lesions.

To avoid confusion, the cells will be listed in the sequence in which they have been arranged in the chapter on tissue cytology. It may be helpful to reproduce the adopted scheme.

Large Cells

The Lymphoid Series
The Fibroblasts
The Schwann Cells
Transition Forms
    Cells related to lymphoid series
    Basophilic cells
    Cells with hypertrophied nuclei
    Cells related to the fibroblasts
    Cells related to the endothelial series

Medium Cells

The Lymphoid Series
Transition Forms
    Cells related to the lymphoid series
    Basophilic cells
    Cells related to the endothelial series

Small Cells

The Lymphoid Series

Transition Forms

Basophilic cells

Dying Lymphoid Cells

Strong evidence was found for the identity of the lymphoid cells with the lymphocytes of the blood. The lymphocytes within the vessel-lumina in nerves and of various other materials stained by the same method, closely resembled the cells of the infiltration. The rare wandering lymphoid cell[19] of the normal mesodermal nerve-sheaths had an appearance identical with the lymphoid series of the lesion. Furthermore, no cell in an unaltered nerve (besides the wandering lymphoid cell) has been known to resemble a lymphocyte or to have the faculty of transforming into an element of lymphocyte appearance.

It could be assumed, therefore, that the series which was designated as "lymphoid" had originated either from "wandering lymphoid cells," or from lymphocytes immigrated into the affected area from the blood. Considering the great number of elements involved in the lesion and the relatively low rate of multiplication, the lymphoid series was probably mainly derived from blood-lymphocytes and less from the scarce "wandering lymphoid cells."[20]

---

[19] This cell is commonly considered to be identical with the lymphocyte.

[20] In order to avoid an abrupt change of nomenclature in the last chapter, the term "lymphoid series" will be further used, with the understanding that these cells have been identified with lymphocytes.

The cells listed under the heading "fibroblasts" comprised elements normally present in the nerve sheath, and other cells which had been formed under the abnormal stimulation. Within the latter group two morphological forms could be distinguished: (a) fibroblast with a nucleus of normal appearance, (b) cells with dark, structureless, elongated nuclei, present mainly in accumulations (in which they were not counted), but also scattered throughout the section (note especially case 23).

The local origin of cells of the fibroblast series formed under the abnormal stimulus is doubtful. No evidence of a direct multiplication of fibroblasts could be found. Although mitotic figures seemed to occur in round cells and endothelial cells, none were observed in the fibroblasts.

Considering the tissue culture work done by Maximow (30) and Bloom (8), the transformation of lymphoid cells into fibroblasts had to be considered. In view of the fact that "natural" conditions could never be expected to yield pictures as conclusive as the controlled experiment, one appears to be justified in considering certain transition forms in such a development. Among the cells related to the lymphoid series elongated elements with an oval nucleus and light, more or less scattered chromatin and fibrillar cytoplasm came under observation. They may have represented one step in the transition towards the fibroblast. Secondly, the cells related to the fibroblasts could be mentioned as another phase in which the stainability of the cytoplasm had been lost. In addition

to this, it will be remembered that case 23 offered findings which were indicative of a direct transformation of small lymphoid cells into dark fibroid elements.[21]

As has been mentioned in the outline on the cytology, this staining method did not permit a clear morphological differentiation of the Schwann cells. They could only be classified with some degree of certainty by their localization, and have therefore been excluded from the counts, except in the normal control. Their possible role will be discussed in connection with the cells with hypertrophied nuclei.

The first subgroup listed within the transition forms was defined as cells related to the lymphoid series. This subgroup was linked to the lymphoid series by a chain of continuous transitions. With a number of elements, it could hardly be decided whether they were still unaltered lymphoid cells or whether they were cells of this type which had begun to undergo transformation. In view of these observations, it may be assumed that these cells were mainly of lymphoid origin.

In this place, mention must be made of the "monocyte."[22] In none of the nerve specimens were cells found which could have been classified as unaltered monocytes. It seemed, nevertheless, quite possible that some of the transition forms listed in this group might have originated from immigrated

---

[21] The direct proliferation of fibroblasts can, of course, not be ruled out.

[22] The term refers to the respective blood cell which may participate in the formation of the exudate.

monocytes. In view of the uncertainty regarding the stem cell of the monocyte itself, and in consideration of the fact that some authors believe it to be closely allied to the lymphoid system, a separate classification appeared to be undesirable.

The basophilic cells very much resembled the cell designated as "polyblast" by Maximow (30) and Bloom (3). As Maximow stated, this element may originate from two sources: (a) the "resting-wandering cell;" and, (b) the immigrated lymphocyte. The observations on nerves did not permit any conclusions in regard to the origin of basophilic elements from the "resting-wandering cell" since none of the latter had been seen in endo and perineurium of the examined specimens. The derivation from lymphocytes appeared more probable as indicated by the fact that groups of basophilic cells were usually localized near blood vessels showing in rare instances a close relation to cells related to the lymphoid series. (see case 29, page 71). A number of elements within the basophilic subgroup could be identified as "plasma cells," and a general consensus has been reached on the lymphocytic origin of this form.

The origin of the cells with hypertrophied nuclei was most obscure. As has been mentioned above, some of the smallest nuclei were probably active fibroblasts. They were included in this subgroup in the belief that they may have illustrated an initial step towards the development of the larger forms, especially those with homogeneous or finely reticular violet cytoplasm with processes extending in one or more directions.

Similar cells were seen and described in the marginal zone of the tumor in case 23, in which place they were considered as taking part in the attempted or beginning formation of a capsule. In spite of the conventional refusal by certain authorities to acknowledge morphological changes in fibroblasts which had been submitted to abnormal stimuli, the thought of a fibroid derivation of the above mentioned cell could not be discarded.

The plasma configuration reminded one strongly of pictures given by v. Moellendorf in his treatise on the plasmatic configurations of irritated connective tissue. The nuclear structure in some of the cells could well be characterised as a fibroblast nucleus in the process of losing its basophilia.

Last, but not least, there were no conspicuous morphological relations to any other cell group, except, perhaps, the Schwann cells. Schwann cells, as has been said, could not be recognized in infiltrated nerve tissue. Case 18, however, may have constituted an exception in this respect. Cells with hypertrophied nuclei were seen located at the surface of the nerve fiber. The nearly homogeneous, blue cytoplasm of such cells seemed to envelop portions of the nerve, and could well have represented a stainable portion of the Schwann sheath. Unfortunately the information obtained on the Schwann cells in regard to their stainability with eosin-methylene blue was insufficient to state definitely whether or not the illustrated cells in case 18 belonged to this group.

The other type of cytoplasm observed with hypertrophied nuclei was honey-combed, in most cases disappearing at some distance from the nucleus so that the cell limits were not visible. Observations made on this type of cell were no more conclusive than those concerning the elements with homogeneous cytoplasm. It could be noted that a process of vacuolization with (in especially the largest forms) gradual cell disintegration was well under way. The origin of the cell, however, was not clear. Here again, fibroblasts seemed to be the most probable stem-cell if v. Moellendorf's illustrations were considered. The cytoplasmic change could also be brought in accord with Hassin's (20) outline on the transformation of Schwann cells in secondary nerve degeneration, whereas the vesiculation of the nucleus as observed in this investigation was contrary to the observations of the latter author.

Cells related to the fibroblasts have been mentioned above as a possible intermediate stage in the formation of new fibroblasts from elements of the lymphoid series. In part, however, elements of the discussed subgroup, seemed to be identical with adventitial structures, scattered through the break-up of the vessel walls in foci of heavy infiltration.

Cells related to the endothelial series were seen in sufficient numbers in the vicinity of blood vessels, sometimes in direct contact with the endothelium, to suggest their direct relation to the vessel wall. In dense cell accumulations, they may have represented the endothelium originating from destroyed

blood vessels.

No cell types different from those listed above were found within the groups of medium and small size. It may be repeated, however, that with loss of size the nuclei of the lymphoid series stained increasingly more like those of lymphocytes in blood smears.

The second part of this analysis was proposed to deal with the role of the previously described cells in the pathological process under observation. For this purpose it was considered helpful to introduce a table comparing some of the numerical values[23] found in the tissue counts. (See Table 2)

In the interpretation of the tabulated findings, the situation as it has been observed in the "normal" control will be taken as a basis. In that case, a total of ninety-seven non-lymphoid elements compared to only three cells of the lymphoid series.[24] The observation of only three cells of the latter type well illustrated their rarity in normal nerve tissue. Correlated to the fact that they were relatively much more numerous in the immediate surroundings of blood vessels, the former finding further indicated that these elements did not normally show a tendency to "invade" the nervous structures.

In all diseased cases except no. 5, cells of the lymphoid

---

[23] It has been explained in the chapter on methods that figures obtained in tissue differential counts represented rough values only. They should not be considered as "percent."

[24] The immediate surrounding of blood vessels was avoided in this count.

Table 2. Distribution of lymphoid and non-lymphoid cells.

| Character of infiltration in area of count | Case No. | Lymphoid series | Total lymphoid series | Fibroblasts | Total non-lymphoid cells |
|---|---|---|---|---|---|
| Scattered between intact nerve fibers | 15 | 46 | 54 | 41 | 46 |
| | 16 | 75 | 84 | 5 | 16 |
| | 16 | 40 | 61 | 22 | 38 |
| | 17* | 55 | 80 | 12 | 19 |
| | 18 | 46 | 71 | 24 | 29 |
| | 18 | 85 | 96 | 3 | 4 |
| Scattered between intact nerve fibers and fibrous proliferation | 23 | 39 | 59 | 22 | 41 |
| | 5 | 15 | 30 | 42 | 70 |
| | 19 | 80 | 86 | 13 | 14 |
| | 22* | 38 | 56 | 32 | 44 |
| All nerve elements destroyed | 15 | 67 | 78 | 4 | 21 |
| All nerve elements destroyed and fibrous proliferation | 19 | 54 | 70 | 13 | 26 |
| Normal control | 29 | 3 | 3 | 80 | 97 |

*Signifies the presence of Histomonas meleagridis

Total lymphoid cells: The lymphoid series

Transition forms
Cells related to the lymphoid series
Basophilic cells

Total non-lymphoid cells: The fibroblasts

Transition forms
Cells related to the fibroblasts
Cells with hypertrophied nuclei
Cells related to the endothelial series.

series and those related to them well outnumbered the fibroblasts and the total of the non-lymphoid cells. These figures were believed to be a proof for the major role of the lymphoid element in the process under observation. The situation as it had been noted in the normal nerve could only have been reversed by an immigration or new-formation of lymphoid cells in excess of the pre-existing and newly formed elements of non-lymphoid character.

"Exceptions" like case 6 were probably characteristic of a phase of increased fibroblast activity. These cases were grouped under one heading in order to demonstrate the shift of cellular balance which took place wherever numerous proliferating fibroblasts[85] occurred. Except in case 19, these figures were significant of a relative increase in fibrous elements compared to the lymphoid series. It should be noted, however, that even with a definite acceleration of fibroblast activity, the participation of the lymphoid series remained intensive.

Cases 19, 23, and 15 served to illustrate the fact that, wherever extensive destruction had taken place the lymphoid elements played a dominant role, regardless of the presence of proliferating fibroblasts (the latter never covered more than a fraction of the area invaded by the lymphoid cells.)

---

[85] It should be repeated that fibrous proliferation grouped in circumscribed foci consisted of mainly dark, structureless nuclei. The amount of fibrillar stroma varied.

Table 3 has been introduced to demonstrate the constant numerical superiority of small and medium elements of the lymphoid series over the large forms as it had been observed in the nerve cases. The findings in blood vessels and their surroundings showed in most cases a similar predominance of the smaller elements. These observations may have been another proof for the derivation of the lymphoid cells from the blood lymphocytes among which a similar prevalence of medium and small forms had been found; in contrast, the tumors (case 19, 23) showed relatively higher values for large cells.

In regard to the further role of the lymphoid series, it has been outlined above in which manner they may have transformed into fibroblasts.

The most remarkable alteration due to the activity of fibroblasts was the formation of areas of dense fibrous cell proliferation. In these foci, elements other than fibroblasts were absent. Among the latter, cells with darkly staining elongated nuclei void of internal structure were in the majority. Although the cells were accumulated in great numbers they occupied a small area of the section only due to the lack of fibrillar stroma. An exception in this respect was case 5, in which a moderate amount of intercellular substance could be seen to lie between the fibroblasts.

The transition forms may have consisted of: (a) elements having the potentiality of changing into a new cell-type, and (b) of others which served as "buffers" for the injurious

Table 3. Comparison of the involvement of large, medium and small lymphoid cells

| Case | Large | Medium | Small |
|---|---|---|---|
| 5 | 4 | 5 | 6 |
| 15 count 1 | 4 | 10 | 53 |
| 15 count 2 | 0 | 4 | 42 |
| 16 count 1 | 11 | 26 | 37 |
| 16 count 2 | 9 | 10 | 21 |
| 17 | 20 | 22 | 13 |
| 18 count 1 | 2 | 33 | 11 |
| 18 count 2 | 8 | 27 | 47 |
| 19 count 1 | 14 | 44 | 22 |
| 19 count 2 | 24 | 17 | 13 |
| 19 tumor | 21 | 8 | 6 |
| 22 | 6 | 19 | 13 |
| 23 | 6 | 18 | 15 |
| 23 tumor | 31 | 18 | 13 |

agent, in an attempt to protect the tissue by sacrificing the viability of certain of its constituents.

The following cells could be listed under (a): a portion of cells related to the lymphoid series, basophilic cells (except plasma cells and those with disintegrating cytoplasm), cells with hypertrophied nuclei (showing a homogeneous cytoplasm), and cells related to the fibroblasts. It may be added that the basophilic cells enumerated in this group were those which have been identified with Maximow's (30) "polyblasts." In the cells with hypertrophied nuclei, a well stained cytoplasm was present. It occupied a relatively large area and had slender extensions. This morphological picture was highly suggestive of an actively transforming phase of this cell, especially when Mollendorf's concept was taken into consideration.

Elements classified under (b) were: (1) those having vacuole formation and disintegration of cytoplasm (some basophilic cells, and cells with hypertrophied nuclei); (2) plasma cells which by most authors have been described as incapable of further development; and (3) cells related to the endothelial series. The latter were believed to have originated by proliferation of abnormally stimulated endothelium. They may, however, have come from other elements of the vessel wall, as the "Gefaesswandzellen" defined by Marchand (28).

The final discussion in this chapter was to deal with the characterization of the lesion.

As has been mentioned, two opinions have been expressed in regard to the nature of the infiltration. The older school of Marek (29), Van Der Walle-Winkler Junius (40) and others applied the term "neuritis" by which they classified the lesion as inflammatory in nature. More recent investigators as Furth and Breedis (17), Stubbs (38) with a tendency to consider the nerve lesion as part of a general lymphatic disorder preferred to define it as a "neoplastic process."

In order to decide upon the significance of the reported findings in regard to such classifications, it was thought helpful to characterize an inflammatory, as well as a neoplastic process, and then to correlate the definitions with the observations made in the course of this investigation.

Mallory (27) described an inflammatory alteration as taking place in three stages (quoted from page 34).

(1). Circulatory disturbances.

(2). Inflammatory exudation.

(a) Exudation of lymph (including formation of fibrin).

(b) Emigration of leukocytes (chiefly of the polymorphonuclear leukocytes).

(3) Proliferation of emigrated endothelial leukocytes[26] and lymphocytes, of fibroblasts, of vascular endothelium, and of epithelial cells, if included in the lesion.

---

[26]Mallory's term for blood-monocytes.

In regard to neoplasms, Mallory said of a true tumor, (quoted from page 252):

....a new formation (usually a more or less circumscribed collection) of cells which proliferate continuously and without control; tend to differentiate as the cells from which they arose would do under normal conditions; serve no useful function; lack an orderly structural arrangement; and have, at least at the present time, no assignable cause for their existence.

Analyzing the observed changes in respect to their inflammatory nature, the following points had to be considered.

(1) The initial stage of circulatory disturbance was not observed.

(2) Exudation was very limited although authors who examined nerves with major gross changes generally mentioned the presence of "edema."

(3) Emigration of polymorphonuclear leukocytes was nearly absent; it had not been ascribed a major role by any of the previous investigators.

(4) The emigration was nearly restricted to lymphocytes including perhaps a small number of monocytes.

(5) The proliferating cells were lymphoid cells and fibroblasts. None of the observed lesions were "acute" in the sense of inflammation. They had, however, certain of the characteristics of chronic inflammatory alterations.

Undoubtedly more evidence was present for the neoplastic character of the lesion. As the counts have shown, the lymphoid cell in either unchanged or somewhat modified form constituted the predominant element. Mitotic figures were present which,

when compared to concurrent lymphoid tumors (case 19 and 23) demonstrated an activity approximately comparable to that of a major portion of the growth. The elements forming "lymphomas" were morphologically closely related to those present in the nerve; the main difference lying in the fact that the medium and small cells of the lymphoid series played a predominant role in the nerve, while in the tumor large elements of that group were relatively more numerous.

A further point of importance was the behaviour of the fibroblasts. As has been mentioned, foci of apparent fibrous proliferation which consisted mainly of dark, homogeneous nuclei were formed. In spite of the dense arrangement of cellular constituents, mitotic figures could not be seen. The latter observation made a neoplastic reaction of the connective tissue elements improbable. On the other hand, the density of arrangement, as well as the extreme basophilia of a majority of the individual nuclei was decidedly abnormal. If, furthermore, the peripheral position of such cell accumulations in regard to the lymphoid foci was considered, (as for instance in cases 19 and 23) this condition would well be compared to the chronic irritation commonly present in the areas surrounding the neoplastic processes.

After consideration of all the recorded observations, quotations and conclusions, it appeared to be permissible to attempt the following characterization of the process in question.

The reaction observed in the peripheral nerves was essential neoplastic. The stem cell of the tumorous process was not pre-existent in the nerve but invaded the latter from the blood stream.

In several cases, dark fibroblast nuclei were observed which had a tendency to form accumulations; such foci were, with one exception void of collagenous fibrils. This reaction was believed to represent the response of fibrous tissue to the chronic irritation of a neoplastic process.

The blood counts in the examined birds did not show manifest changes indicating leukosis. Deviations from the average quantitative and qualitative appearance of the chicken blood, which came to observation in some cases, were too slight or irregular to be conclusive.

The degeneration of nerve fibers was due to pressure-atrophy.

A possible, though unproved, explanation of this situation could be given by assuming the elective invasion of peripheral nerves by an injurious agent having the potency of reversing an inflammatory defense reaction into a neoplastic proliferation of certain elements, namely lymphocytes.

Those cells among the transition forms which were classified as "buffers," may have represented residues of the abortive inflammatory phase.

# SUMMARY AND CONCLUSIONS

The lymphoid accumulations were initiated by emigrated lymphocytes mainly, which developed neoplastic activities within the nerve.

The connective tissue proliferation present in some lesions appeared to be a response to the chronic irritation caused by the neoplastic process.

The blood counts did not permit conclusions as to the involvement of the blood forming organs.

It was suggested that an injurious agent with affinity for peripheral nerves might have initiated the observed process.

ACKNOWLEDGMENT

Indebtedness is acknowledged to Dr. L.D. Bushnell, Head of the Department of Bacteriology, for advice in the experimental work and for counsel in the preparation of the manuscript; to Dr. M.J. Twiehaus for aid in obtaining the material; to Dr. L.M. Roderick, Dr. W.W. Thompson, Dr. J.H. Whitlock and Miss Alice Kimball for advice and assistance in the technical procedure; and to Mr. C.L. Gish for furnishing the major part of the material.

## LITERATURE CITED


*(1) Aschoff, L.
Das retikulo-endotheliale System. Ergeb. D. inn. Mediz. u. Kinderhlk., 26:1. 1924.

*(2) Aschoff, L. and Kiyono, K.
Zur Frage der grossen Monomuklaeren. Fol. haemat., 15:383. 1913.

(3) Barber, C.W.
A study of fowl paralysis. Cornell Vet. 27:44-51. 1937.

(4) Bayon, H.P.
The comparative pathology of anemia and leucocythemia in fowl. Jour. Compar. Path. and Ther. 43: 18-204.

(5) _____
Acute neurolymphomatosis gallinarum in a strain of Rhode Island Red fowls. Vet. Rec. Sept. 5, 1931.

(6) _____
Notes on pathology and treatment of neuro-lymphomatosis gallinarum observed on fowls during eight outbreaks. Vet. Rec. Dec. 5, 1931.

(7) Bensley, R.R. and Bensley, S.H.
Handbook of histological and cytological technique. Chicago. University of Chicago Press. 167 p. 1938.

(8) Bloom, W.
Mammalian lymph in tissue culture. From lymphocyte to fibroblast. Arch. f. exp. Zellforschung, 5:269-307. 1928.

(9) Bushnell, Leland D.
Poultry practice. Chicago. Veterinary Magazine Corporation. 160 p. 1940.

*(10) Cajal, S.R.
Degeneration and regeneration of the nervous system. Transl. by R.M. May. London. Oxford Univ. Press. 1928.


---

*Original paper not seen.

(11) Carrel, A. and Ebeling, A.H.
Pure cultures of large mononuclear leucocytes. Jour. Expt. Med. 36:365-377. 1922.

(12) Cowdry, E.V.
Histology. Philadelphia. Lea & Febiger. 503 p. 1934.

(13) Doyle, L.P.
Neuritis or paralysis in chickens. Amer. Vet. Med. Assoc. Jour. 72:585-587. 1928.

(14) Evans, H.M.
The macrophages of mammals. Amer. Jour. Physiol. 37:243-258. 1915.

(15) Fischer, A.
Sur la transformation, in vitro, des gros leucocytes mononucleaires en fibroblastes. Soc. de Biol. Compt. Rend. 92:109-112. 1925.

(16) Foot, N.C.
The endothelial phagocyte, a critical review. Anat. Rec. 30:15-51. 1925.

(17) Furth, J. and Breedis, Chs.
Lymphomatosis in relation to fowl paralysis. Arch. Path. 20:379-428. 1935.

(18) Gibbs, C.S. and Johnson, C.G.
Differentiation of the pathological cell in neurolymphomatosis from lymphocytes of the blood. The differentiation of neurolymphomatosis from lympholeukosis. Mass. Agr. Expt. Sta. Bul. 327:77. 1936.

(19) Gradwohl, R.B.H.
Clinical laboratory methods and diagnosis. 2nd Ed. St. Louis. C.V. Mosby Co. 1607 p. 1938.

(20) Hassin, George B.
Histopathology of the peripheral and central nervous systems. Baltimore. William Wood. 491 p. 1933.

(21) Johnson, E.P.
A study of lymphomatosis of fowl. Va. Agr. Expt. Sta. Tech. Bul. 44. 22 p. 1932.

(22) Johnson, E.P. and Connar, B.V.
Blood studies of fowls with various forms of lymphomatosis (fowl paralysis). Amer. Vet. Med. Assoc. Jour. 83:326-343. 1933.

(23) Knupp, B.F.
The anatomy of the domestic fowl. Philadelphia. W.B. Saunders Co. 373 p. 1918.

(24) Lee, C.D., Wilcke, H.L., Murray, Chs., and Henderson, E.W.
Fowl leukosis. Jour. Infect. Dis. 61:1-20. 1937.

(25) Lewis, M.R. and Lewis, W.H.
Transformation of mononuclear blood cells into macrophages, epithelioid cells and giant cells in hanging drop blood cultures from lower vertebrates. Carnegie Inst. Wash. Pub. 18:121-147. 1926.

(26) Mallory, F.B.
A histological study of typhoid fever. Jour. Expt. Med. 3:611-639. 1898.

(27) Mallory, Frank B.
The principles of pathologic histology. Philadelphia. W.B. Saunders Co. 677 p. 1914.

*(28) Marchand, F.
Die oertlichen reaktiven Vorgaenge. Handb. d. allg. Pathol. v. Krehl-Marchand, 4:1. 1924.

*(29) Marek, J.
Multiple Nervenentzuendungen (Polyneuritis) bei Huehnern. Deutsch. Tierarztl. Wochenschr. 15:417-421. 1907.

(30) Maximow, A.A.
Cultures of blood leucocytes. From lymphocyte and monocyte to connective tissue. Arch. f. exp. Zellforschung, 5:169-268. 1928.

(31) Maximow, Alexander A. and Bloom, William
A textbook of histology. 3rd ed. Philadelphia. W.B. Saunders and Co. 668 p. 1938.

(32) Moellendorf, v. W. and Moellendorf, v. M.
Das Fibrocytennetz in lockeren Bindegewebe; seine Wandlungsfaehigkeit und Anteilnahme am Stoffwechsel. Ztsch. f. Zellforschung und mikroskopische Anatomie, 3:503-601. 1926.

(33) Nageotte, J.
Role des corps granuleux dans la phagocytose du neurite, au course de la degeneration wallerienne. Soc. de Biol. Compt. Rend. 71:251-255. 1911.

(34) Pappenheimer, A.M., Dunn, L.C. and Cone, V.
Studies on fowl paralysis (neurolymphomatosis gallinarum). I. Clinical features and pathology. Jour. Expt. Med. 49:63-86. 1929.

(35) Patterson, F.D., Wilcke, H.L., Murray, Chs. and Henderson, E.W.
So-called range paralysis of the chicken. Amer. Vet. Assoc. Jour. 81:747-767. 1932.

(36) Sabin, F.R., Doan, C.A. and Cunningham, R.S.
Discrimination of two types of phagocytic cells in the connective tissues by the supravital technique. Carnegie Inst. Wash. Pub. 16:125-162. 1925.

(37) Seagar, E.A.
The pathology of fowl paralysis. Cellular reactions in the blood in neurolymphomatosis gallinarum (fowl paralysis). Vet. Jour. 89:557-561. 1933.

(38) Stubbs, E.L.
Lymphomatosis and leucosis. Quoted from Bushnell (9).

*(39) Timofejewski, A. and Benewolenskaja, S.
Prospective Potenzen des Myeloblasten auf Grund von Explantationsversuchen. Virch. Arch. 263:719. 1927.

*(40) Van der Walle and Winkler-Junius
Tijdschr. vergelijk. Geneesk. Enz. 10:34. 1924.

(41) Warrack, G.H. and Dalling, T.
So-called "fowl paralysis." Vet. Jour. 88:23-43. 1932.