THE SIGNIFICANCE OF BONE MARROW EXAMINATION IN CERTAIN DISEASES OF THE BOVINE AND CANINE

by

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INTRODUCTION

The value of examinations of the peripheral blood as an aid to diagnosis and prognosis of diseases in domestic animals is well known. However, the identification of the cells in the circulating blood is considered but one view of a constantly changing picture. The vital importance of the bone marrow as the major organ of blood cell formation is recognized. An understanding of the normal and abnormal functions of the marrow are considered essential to permit an intelligent interpretation of the blood in health and disease.

The types and percentage distribution of the cells of the marrow of normal cattle and dogs have been determined. Research is meager with respect to the cellular structure of the bone marrow of diseased animals.

This project was undertaken to study the cellular structure of bone marrow in certain diseases of cattle and dogs, and to evaluate the methods and techniques of bone marrow examinations of the bovine and canine.

REVIEW OF LITERATURE

General Characteristics of Bone Marrow

The study of bone marrow has only recently come to the front in Veterinary Medicine. However, as early as 1906, Kulbs and Zuntz (Calhoun, 1954a) worked on the development of bone marrow using dogs as experimental animals. Varicak (1935) included the

cow in his study of the bone marrow of the domestic animals, and Holzel wrote a thesis on the bone marrow of the cow in 1943 (Calhoun, 1954b).

Examination of the bone marrow in human medicine has been widely recognized as a practical routine procedure, especially in the diagnosis of diseases of the blood and hematopoietic system. Jones (1938) summarized the advantages of bone marrow biopsies for hematological studies as follows: postmortem changes are avoided; successive examinations of the marrow can be made during the course of a given disease; a comparison of marrow with the type of response indicated in the blood stream can be made; and the source of pathologic forms encountered in the peripheral blood can be determined and their relationships established. While observations of the cells of the circulating blood are justifiably used as an index of hematopoiesis and subsequently as an indication of the existing pathological alterations of the tissue, it is well known that the peripheral blood does not always reflect the condition of the bone marrow. In addition, marrow cells are so sensitive in their response to the demand for blood cells that changes in the circulating cells of even moderate degrees can be detected in the changes of the pattern of maturation stages of the myeloid and erythroid cells. Furthermore, bone marrow studies give an exact knowledge of the relative integrity of this organ whenever any question arises regarding the interpretation of peripheral blood data. An intelligent understanding of the blood picture in the presence of disease depends

on knowledge of the blood-forming organs, especially the bone marrow, since it is the source of supply of the red cells, hemoglobin, granular cells, platelets, and possibly some of the lymphocytes and monocytes (Bloom and Meyer, 1944).

An evaluation of bone marrow (Bloom, 1945) indicates that in certain cases it is of distinct diagnostic importance; in others, it is of corroborative or confirmatory value, and in some instances, it is little or no aid. Even though the appearance of the marrow may not be specific, an intelligent correlation with the blood picture and clinical aspects gives further information and understanding of the pathology of the disease processes.

The marrow (Sisson and Grossman, 1953) occupies the interstices of the spongy bone and the medullary cavity of the long bones. There are two varieties in the adult--red and yellow. In the young there is only red marrow, but later this is replaced in the medullary cavity by yellow marrow. The red marrow contains several characteristic cells, and is a blood forming substance while the yellow is practically all adipose tissue.

In 1933, Fairman and Whipple observed that the red marrow, spleen, and liver were intimately related in the production, maintenance, and disposal of red cells and hemoglobin. There is evidence that the liver is essential for the production of parent substances which form the mature red cells and hemoglobin in the marrow. For this reason it is helpful to keep in mind the volumes or weights of these organs together with the circulating blood and plasma volumes. Fairman and Whipple (1933) made the

following illustration. A dog, of average nutrition, weighing 15 kilos (33 lbs.) should have a blood volume of 8 to 9 per cent; a plasma volume of 4 to 5 per cent; a liver weight of 3 to 3.7 per cent; a bone marrow weight of 2 to 2.5 per cent; and a spleen weight of 0.2 to 0.5 per cent. Marrow volumes are relatively higher in animals in poor nutrition and still higher when age introduces osteoporosis.

The liver-marrow ratio (Fairman and Whipple, 1933) is more stable for comparison as body nutrition has relatively little influence on the liver and bone marrow volume. The ratio of red to fat marrow is constantly fluctuating due to age, anemia, and other factors related to production, maintenance, and conservation of hemoglobin. The parent red cells in the red marrow under optimum conditions in a two-week period may produce many times their own volume of mature red cells.

In 1944, Osgood and Seaman described the human marrow as the "largest, most widely dispersed and least homogeneous organ in the body" with a volume one or two times that of the liver. Cowdry (1944) stated that the marrow makes up 5 per cent of the total body weight. Dietz (Calhoun, 1954a) gave some interesting statistics on the relative weight of rabbit bone marrow: one-third the weight of the skeleton, 2.2 per cent of the total body weight, two-thirds the weight of the liver, and 50 times that of the spleen.

Wintrobe reported in 1946 that the bone marrow consisted of a loose fibrous syncytium as well as reticulum cells with pale

nuclei poor in chromatin. Most of these cells are fixed, but they are capable of rounding off to become large free macrophages. No lymph vessels have been demonstrated in bone marrow, and nerves have been demonstrated only in connection with the larger divisions of the blood vessels. Lymph follicles like those in the spleen have been observed in bone marrow and are considered by most investigators to be normal.

The normal requirements for cells are met by division of differentiated precursor cells, and only under conditions of extreme stimulation are the primitive cells called into action. In a recent study, Craddock et al. (1960) found that the granulocytes spend an average of about 10 hours in the peripheral blood.

In discussing bone marrow cell metabolism in January, 1957, Laytha declared that it is only too clear that our knowledge of the metabolism and special functions of bone marrow cells is very deficient. Until recently this was due to inadequate techniques for experimental investigations. Recent advances in cytochemical methods, bone marrow culture techniques, use of radioactive isotopes, and such special methods as leucopheresis will significantly increase the amount of information. To date no essential biochemical difference has been demonstrated between identical cell types originating from normal or leukemic marrows. The well-known radiosensitivity of the bone marrow has given great impetus to research in recent years.

Epstein and Tompkins, in 1943, studied the variations in technique used by different workers in obtaining and preparing specimens of bone marrow which add considerably to the uncertainties which feature into any evaluation of differential counts of marrow. The techniques which have been employed in the preparation of specimens for counting may be classified into one of three general methods, namely, the use of fixed smears, of sections, and of supravital preparations.

The work of Epstein and Tompkins (1943) was planned to compare the counts made when these three general methods were employed by the same individuals, and applied to the same marrow. No technique was found for the preparation of fixed smears which yielded as representative a distribution of the cells, caused as little trauma to them, and permitted as delicate cytologic definition as could be obtained by the supravital and section techniques. Inadequate distribution and trauma were found to be inherent sources of error in the use of fixed smears. Differential counts made from them were therefore less dependable than by the other two methods. The difference between section counts of the same marrow by different observers is greater than between supravital counts under the same conditions. Both methods are sufficiently reliable for studies based on comparative, rather than absolute values.

Bovine Bone Marrow

The modern-day studies of the bone marrow of the cow have been made by Varicak (1935), Holzel (1939), Marcato (1941), and Hjarre (1943). The most recent work has been done by Calhoun (1946), Niepage (1952), Winqvist (1954), and Koehler (1957).

A number of variations in the cellular percentage of normal bone marrow in the bovine have been reported and are summarized in Table 1.

Varicak (Calhoun, 1954b) studied the bones of 300 cattle macroscopically and found that the deposition of fatty marrow occurred in the axial skeleton as early as nine months. He found some of the thoracic vertebrae to be completely filled with fatty marrow. The dorsal end of the ribs retained red marrow; the central part had both red and yellow marrow; and the sternal end became a reservoir for fat. The sternum also contained areas of fatty marrow, but with foci of red marrow.

Calhoun, in her studies of bovine marrow in 1954, chose the ribs as the most logical site from which to obtain the marrow. It had been reported by some authors to be convenient to execute a sternal puncture. The animal was confined in a prone position, and an especially constructed trocar was driven into the middle or back sternebra by a "light" tapping with a hard rubber hammer; however, Calhoun (1954b) found that no light tapping would succeed in penetrating the sternebra. Koehler, in 1957, used the sternum of the cow for his bone marrow studies.

Table 1. Percentage distribution of cells from normal bone marrow of cattle (Koehler, 1957).

Cells	Holzel (1939)	Marcato (1941)	Hjarre (1943)	Calhoun (1946)	Niepage (1952)	Winqvist (1954)
Myeloblasts	0.0- 3.0	---	1.5- 4.0	---	0.0- 1.0	---
N. Promyelocytes	1.0- 4.0	2.95	0.5- 3.0	1.51(0.0- 6.8)	0.5- 1.5	0.79(0.2- 1.4)
N. Myelocytes	5.0- 9.0	5.85	3.0- 9.6	19.39(10.4-32.0)	5.0-10.0	2.90(1.0- 5.0)
N. Metamyelocytes	1.0- 4.0	11.80	3.0-13.5		10.0-20.0	4.14(1.3- 7.4)
N. Stabs	10.0-21.5	---	7.5-18.1	5.73(1.2-12.2)	5.0-10.0	8.20(5.0-12.8)
N. Segmenters	10.0-21.0	---	6.5-19.5		0.0- 5.0	11.46(4.9-19.0)
E. Promyelocytes	---	---	---	---	0.5- 2.0	---
E. Myelocytes	0.5- 4.0	5.1	---	6.69(1.8-10.4)		0.73(0.1- 1.4)
E. Metamyelocytes	1.0- 4.0	5.79	---	---	0.5-15.0	2.09(0.7- 3.4)
E. Stabs and Segmenters	5.5-18.0	---	---	1.92(0.0- 7.6)		5.67(2.4-10.8)
Basophiles	---	---	---	0.34(0.0- 1.0)	0.0- 0.5	0.19(0.0- 0.8)
Lymphocytes	3.5-11.0	3.69	5.0-20.0	6.8 (1.4-16.8)	5.0-10.0 (small lymphocytes)	10.69(5.6-16,3)
Monocytes	0.0- 1.0	8.86	0.0- 1.0	2.64(0.0- 7.6)	0.0- 1.0	0.16(0.0- 0.4)
Plasma cells	0.0- 2.0	0.59	---	0.79(0.2- 2.0)	0.0- 1.0	0.99(0.4- 2.3)
Reticulocytes	0.0- 0.5	---	---	---	5.0-10.0 (large lymphocytes)	1.27(0.2- 2.9)
Megakaryocytes	0.0- 1.5	0.52	---	25.14 per 300 mm	0.0- 0.5	0.50(0.0- 0.2)
Stem cells	---	4.23	---	2.14(0.0- 5.0)	---	0.43(0.2- 0.8)
Proerythroblasts	1.0- 4.0	2.36	0.0- 1.0	---	0.0- 1.0	1.23(0.6- 1.7)
Erythroblasts	5.5-10.0	29.13	---	30.2 (11.8-42.8)	5.0-10.0	25.73(16.3-30.8)
Normoblasts	19.0-30.0	19.25	---	21.69(7.2-39.2)	30.0-40.0	23.28(10.4-35.3)

Canine Bone Marrow

The bone marrow of the dog was studied by Stasney and Higgins (1937), Bloom and Meyer (1944), and Meier (1955).

The average cellular constituents of the ilial bone marrow of normal dogs were studied by Meyer and Bloom (1944) and are reported in Table 2.

Table 2. Cellular constituents of ilial bone marrow of normal dogs (Bloom and Meyer, 1944).

Bone marrow cells	Average
Myeloblasts	0.58
Myelocyte neutrophils	3.76
Myelocyte eosinophils	0.26
Non-segmented neutrophils	23.5
Non-segmented eosinophils	0.12
Neutrophils	18.5
Eosinophils	1.56
Basophils	0.02
Heterophils	0.02
Megaloblasts	1.02
Erthoblasts	2.5
Normoblasts	35.18
Lymphocytes	9.8
Pathological lymphocytes	0.04
Monocytes	1.2
Monoblasts	0.14
Plasma cells	0.82
Hematogones	0.44
Reticulo endothelial cells	0.54
Megakaryocytes per ccm.	41.2
Myeloid-Erythroid ratio	1.36:1.00

A notable contribution in the study of the bone marrow of normal and diseased dogs was made by Bloom in 1945. He felt that in the normal animal there exists a triple mechanism of reserve to assure sufficient blood cells for emergency requirements. The

first consists of an immediately available supply of mature cells, which have been delivered from the marrow, and are idling in a relaxed spleen or in the less active recesses of the vascular bed. In the dog, the capacity of the spleen to serve as a physiological reservoir for blood cells is greater than in any other known animal. The "redistribution phenomenon" is a basic, physiologic mechanism that automatically meets the widely varying demands of normal physiologic activity. Secondly, when a cellular response greater than the physiologic reserve results from a pathologic condition, the bone marrow quickly delivers practically mature red and white cells to the active circulation.

In their studies of malignant lymphomas of the dog in 1945, Bloom and Meyer found that it was necessary to develop a technique for repeated bone marrow studies in the living animal. They first employed a method reported by Arinkin in 1927, which was a sternal aspiration. This method was found to be simple, and an unlimited number of successive biopsies could be made upon the same animal. This procedure was applied successfully in young dogs of medium and large size, but difficulty was experienced in small dogs due to the narrow medullary cavity and in older dogs due to an increase in density and thickness of the outer layer of compact bone. The method was then employed which was first described by Ho et al. in 1940, of aspiration of bone marrow from the crest of the ilium.

Stasney and Higgins made a comparative study in 1937 of the bone marrow of the ribs, the proximal portion of the femurs, and

the middle of the femur of 35 dogs and found the cell distribution to be similar at all sites. Meier, in 1955, proposed the femoral puncture as a preferred practical clinical procedure for doing bone marrow biopsies on the dog.

Bovine Lymphocytoma

Among the neoplasms of the blood-forming tissues, those consisting of lymphoid cells are by far the most important. Indeed, this is the most frequently encountered tumor in American veterinary practice with none of the usual domestic species being exempt. Goats may be resistant (Smith and Jones, 1957).

Probably no neoplasm in pathology has been the victim of as many variations in nomenclature. The type cell of the malignant lymphoma (Smith and Jones, 1957) in some instances closely resembles the normal lymphocyte, in which case the neoplasm may be designated a lymphocytoma. In other instances, the type cell is larger with a vesicular nucleus and considerable cytoplasm resembling the maternal lymphoblast as seen in a germinal center of a lymph node. Such a tumor is sometimes given the name of lymphoblastoma. A still more undifferentiated type is occasionally called a reticulum-cell sarcoma. Gall and Mallory (1942) spoke of malignant lymphomas of lymphocytoma type, lymphoblastoma type, lymphosarcoma type, and certain others. Their lymphosarcoma type of malignant lymphoma carried the implication of some of the primitive lymphoid cells in the direction of fibroblasts, representing a form of the tumor which is sometimes seen.

The majority of the European workers, notably Koehler (1957), Goetze, Rosenberger, Ziegenhagen, and Merkt (Koehler, 1957), and Bendixen refer to the diseases as leukosis. Leukosis (Runnells, 1954) is characterized by a proliferation of the leukocyte-forming tissues of the body. Since leukocytes include both the myelocytes and the lymphocytes, it is obvious that the changes in leukosis may involve the bone marrow or the lymph nodes and spleen, or even both sets of leukocyte-forming organs. If the proliferative changes affect the bone marrow, the condition is called myelosis; if the lymph node and spleen, lymphadenosis. Leukosis then includes myelosis and lymphadenosis. If, in leukosis, the proliferating leukocytes appear in the general circulation in excessive numbers, a state of leukemia exists.

Thompson and Roderick, in 1942, noted that the term lymphocytoma seemed to be the most appropriate designation for that neoplastic process of animals primarily involving lymphoid cells and tissues. They said there seemed to be little justification for a more complicated nomenclature to supply a more descriptive terminology for variations in fundamentally the same malignant condition. Observations indicated moreover, that lymphocytomatosis is probably the most common neoplasm of cattle, and that if adequate means of recognition are utilized, it may be found to be a problem of economic significance.

Tharp and Amstutz, in 1957, used the term lymphocytoma in their discussion of the disease as diagnosed in the Ohio State University Veterinary Clinic. The term malignant lymphoma was

used in 1945 by Bloom and Meyer in their description of the disease in the dog. Mulligan (1949) has written that the most satisfactory inclusive name for cancer of lymphoid tissue is lymphosarcaoma.

After reviewing the nomenclature that has been used by various authors, the disease of the bovine has been termed lymphocytoma in this paper.

Lymphocytoma (Feldman, 1932) consists of nodular or diffuse, irregular, fleshy masses of tissue, flesh pink or grayish-white. The tissue is usually compact, but is seldom hard. If freshly cut, the exposed portion reveals a moist, glistening surface of soft, velvety texture. Not infrequently the tissue is markedly hemorrhagic, and occasionally the neoplastic elements and the adjacent nontumorous tissues present an edematous appearance. Encapsulation does not occur and the intimate, infiltrative relation of the metastatic, tumorous tissues with the adjacent nontumorous tissues is a feature of these malignant processes. If the masses are large, necrosis and other retrogressive changes may occur.

Lymphocytoma occurs in cattle of all ages, but is far more commonly encountered in animals five years of age and older (Feldman, 1932; Schalm, 1956). However, Udall and Olafson (1930) reported a case in a six-month-old calf. Bendixen (1959a), in eight new-born calves, and Hatziolos (1960) recently observed lymphocytoma in a bovine fetus.

Information from various sources indicated that lymphocytoma was more prevalent in the middle west than in the eastern part of the United States. Frank and Thompson, in 1940, described four cases of lymphocytoma and mentioned the increase in the number of lymphocytoma cases at the Kansas State Veterinary Clinic since 1935. Since that time the Kansas State Veterinary Clinic has averaged approximately 33 cases per year. Tharp and Amstutz (1957) reported that lymphocytoma is frequently diagnosed in bovines at the Ohio State University Veterinary Clinic. They found that the disease is observed so often that one should be alert to the fact that it may be responsible for many clinical cases with vague signs. Lymphocytoma increased in Germany, as Koehler (1957) puts it, by "leaps and bounds" during the last 10 years and Bendixen (1959) reported from Denmark that the disease occurs regularly year after year.

Lymphocytoma has been thought to be caused by a virus (Tharp and Amstutz, 1957; Koehler, 1957). Goetz and his co-workers (Tharp and Amstutz, 1957) made extensive studies of leukemia over a 10-year period and published the results in a series of articles. They worked out a key for the diagnosis of leukemia on a herd basis. The key was based on the white cell count and the percentages of lymphocytes, and proved to be a valuable diagnostic tool in their studies. Goetz (Tharp and Amstutz, 1957) was able to transmit leukemia from infected animals to healthy ones. Tharp and Amstutz (1957) were of the opinion that the disease could be transmitted.

Bendixen, in January, 1960, made an extensive study of bovine lymphocytoma in Denmark, and found that 80-85 per cent of the reported cases of lymphocytoma in that country originated from certain herds. In the disease-infected herds, a percentage of the animals always showed leukemic blood pictures. He observed that the disease developed through a subclinical (leukemic) phase to a clinical (tumorous) phase. These studies tend to validate the theory of virus etiology.

The most commonly recognized symptom of lymphocytoma is an enlargement of practically all of the external lymph glands. The affected animal may show rather obscure digestive or cardiac symptoms. When the digestive tract is involved (usually it is the abomasum, rarely, the reticulum), the animal may show digestive disturbances and chronic gastric tympany. The symptoms (Frank, 1955) may consist of gradual loss of condition even though the affected animal continues to eat a normal quantity of food. When tumorous tissue is present in the orbital cavity, the eyeball is extruded; either one or both eyes may be affected.

Other organs and tissues that may show metastasis are the heart, liver, spleen, lungs, kidneys, adrenals, pancreas, skeletal muscles, genital organs, and udder. Lymph nodes and metastatic neoplastic masses may become of such size as to cause paralysis of the hind limbs, due to pressure on regional nerves. Various other symptoms may occur as a result of pressure on vital organs (Schalm, 1956; Tharp and Amstutz, 1967; Jasper et al., 1946).

The total leukocyte count (peripheral blood) is variable and commonly within the normal range, but when elevated, total counts of 27,000, 42,000, 175,000 (Schalm, 1956), or even over a million (Coles, 1960) have been reported. The differential leukocyte count may show an elevation in percentages of lymphocytes, and the number of neoplastic lymphocytic cells may vary from just a few to 30 per cent or more of the cells making up the leukocyte counts (Thompson and Roderick, 1942). Tharp and Amstutz (1957) found that the differential count ranged from a normal number of lymphocytes to a high of 80 per cent or more.

The neoplastic cells invade the bone marrow, leading to displacement of marrow which may, in turn, crowd out some of the centers for erythropoeisis. In addition, erythrocytes produced by leukemic bone marrow were reported to have a shorter life than erythrocytes of normal marrow (Berlin et al., 1951). Daland, in 1959, pointed out that anemia in leukemic conditions may be the result of acute or chronic blood loss because of the thrombocytopenia or it may be a myelophthisic anemia showing nucleated red cells and myelocytes resulting from infiltration of the bone marrow.

Lymphocytoma may be confused with many commonly occurring diseases of cattle. Trauma to the eye, traumatic reticulitis, chronic gastric tympany, ketosis, cystitis, enteritis, traumatic pericarditis, and various circulatory disturbances are some of the diagnoses that have been made in animals suffering from lymphocytoma. Occasionally, rectal examination will reveal single

or multiple hard nodular enlargements involving the rectal wall, pelvic area, omentum, and mesentery.

Koehler, in 1957, expressed surprise that the bone marrow had not been included earlier in the circle of diagnostic aids for lymphocytoma. According to Rohr (Koehler, 1957), it is easily possible to diagnose the chronic lymphadenosis in man from the puncture exudate of the bone marrow. It was reported that the lymphatic leukosis of man is characterized by the appearance of a large number of mature lymphocytes in the peripheral blood; one cannot say this of bovine lymphocytoma.

Koehler (1957) expressed doubt that either the quantitative or the qualitative composition of the lymphatic cells of the bone marrow alone are sufficient to establish a diagnosis of lymphocytoma. However, an increase of lymphocytic cells of more than 30 per cent might, in connection with an examination of the peripheral blood, support the suspicion of lymphocytoma. The bone marrow (Koehler, 1957) of almost all cattle with lymphocytoma is poor in granulocytes with segmented nuclei; myelocytes dominate the field and a strong eosinophilia occurs frequently. It was suggested by Koehler (1957) that the reticulogenous cells had displaced extensively the specific cells of the bone marrow. The proliferated cells were thought to be reticulum cells because of the presence of reticulated fibrils. These matured proliferated cells have a similarity, but no identity with lymphocytes. With all required caution and reserve, a classification of lymphocytoma of the bovine animal as a reticulogenous hemoblastosis seemed to

be justified. It was felt that the mesenchyme, the tissue which is ubiquitous, proliferated and that the parenchyma of the bone marrow was affected only indirectly.

Canine Malignant Lymphoma

The terms lymphocytoma and lymphosarcoma are acceptable and descriptive. However, the term malignant lymphoma represents a more modern usage among authors.

Bloom and Meyer, in 1945, made a study of 10,000 dogs entering the small animal hospital of Frank Bloom. Twenty cases of malignant lymphoma were encountered, representing an incidence of 0.2 per cent. The age of affected animals varied from 5 to 12 years and averaged 9.08 years. The disease thus occurs in older animals, as the normal dog usually lives 10 to 14 years, barring death from accidents. Differential counts of aspirated marrow was compared with a study of the sections of bone marrow. They found that the marrow of some dogs showed scattered nodules of lymphoma cells. Surrounding these nodules were many megakaryocytes, a few erythroid cells, and an extensive hyperplasia of myeloid cells. It was the feeling that there were discrepancies in the aspirated marrow as the needle may miss the nodules. Conversely, the needle may be inserted into a nodule so that numerous lymphoma cells will be aspirated and an erroneous high lymphocyte count obtained. The evidence indicated that sectioned material gave a more accurate picture of the lymphomatous infiltrations in the marrow.

In the majority of cases of canine malignant lymphoma there is an accompanying leukocytosis and anemia. Bloom and Meyer (1945), who have contributed much toward the pathological studies of the bone marrow in the canine, stated in their work on malignant lymphomas:

> The mechanism for the leukocytosis is a speculative question and several possibilities can be considered. The presence of leukocytosis probably results from a combination of these factors; namely, the marrow involvement, the necrosis in the lymphomatous tissue, and the relatively rapid growth of lymphoma cells. The hyperplasia of myeloid cells in the bone marrow adequately explains the neutrophilic leukocytosis encountered in the peripheral blood. Although the replacement of normal marrow by lymphoid foci is considered an important factor in the production of the accompanying anemia in man, this explanation cannot be unreservedly applied to the canine disease. In the dog, the suppression of erythrogenesis results from the myeloid hyperplasia and only partially from the lymphomatous infiltrations which are both moderate and focal in extent. The anemia probably is similar to that occurring in many malignant tumors.

The existence of noncirculating lymphoma in dogs is accepted by all students of the subject. Mulligan (1949), in his survey of 1,000 neoplasms in dogs, does not include instances of leukemia. In his discussion of lymphosarcoma he stated that "significant and specific cells in the peripheral blood are uncommon features."

According to Reihart and Reihart (1953), malignant lymphoma is a highly malignant neoplasm which affects males somewhat more than females, usually occurs in the age range of five to ten years and terminates fatally in three to six months after onset. The physical examination usually reveals enlarged firm and freely

movable cervical mandibular and inguinal lymph nodes, but other lymph node systems may be involved. Soon the affected animal shows evidence of weakness, loss of appetite, listlessness, and sometimes respiratory embarrassment. In the later stage, vomiting, diarrhea, cough, ocular and nasal discharge, and anemia are prominent features. Hutyra et al. (1949), and Reihart and Reihart (1953) reported an elevated temperature (102° to 104° F.) in dogs affected with the disease.

A study of the peripheral blood of dogs affected by malignant lymphoma was made by Reihart and Reihart (1953), who reported that little was revealed in the early phases, but soon a hypochromic microcytic anemia was present. In most cases there was a leukocytosis, the total count varying from 15,000 to 80,000 per cmm. The differential counts revealed that from 50 to 90 per cent of the cells were neutrophils; the vast majority being segmented or juvenile forms. They reported there is nothing diagnostic about the peripheral blood study, but it was of more than passing interest because the neoplasm is lymphogenous and the peripheral blood shows only a response to a toxic condition and does not reflect a neoplastic invasion.

The spleen and liver are constantly the site of lymphomatous infiltration. Other areas of infiltration include the intestine, lung, kidney, prostate, gallbladder, genital organs, adrenals, periorbital fat, and pancreas (Reihart and Reihart, 1953).

Canine Pyometra

In pyometra, the peripheral blood is characterized by (a) a pronounced neutrophilic leukocytosis, (b) a decided "shift to the left," and (c) toxic changes and disturbed maturation levels of the neutrophilic cells (Bloom, 1944).

The bone marrow changes in pyometra were described by Bloom in 1944. His studies indicated that the marrow shows (a) a marked hyperplasia, particularly of neutrophilic forms, (b) increased number of megakaryocytes, and (c) widespread toxic changes and disturbed maturation differentials of neutrophilic cells.

The mechanism for the leukocytosis in pyometra is similar to that of other inflammatory diseases where the demand for neutrophilic leukocytes is met by an increased production of these cells in the bone marrow. The hyperplasia of the marrow was due largely to the increased number of neutrophilic forms which was so great that the erythroid cells in the marrow were suppressed. It appears probable that the toxic changes which occur in the neutrophilic (peripheral) cells originate in the bone marrow as the neutrophilic marrow cells exhibited almost widespread degenerative changes (Bloom, 1944).

Other Canine Diseases

The bone marrow of diseased dogs was studied by Bloom in 1945. These studies included: streptotrichosis, subacute suppurative peritonitis, nervous distemper, and advanced filariasis.

These results are summarized as follows:

Streptotrichosis (pseudo actinomycosis). In this condition, both the clinical features and the qualitative and quantitative blood and marrow changes resemble those in pyometra. The toxic changes and anisocytosis of the neutrophilic cells are identical with similar alterations in pyometra. In streptotrichosis, the megakaryocytes in the marrow were in the normal range in contrast to their increase in pyometra.

Subacute Suppurative Peritonitis. In both the blood and marrow, there were moderate toxic cytoplasmic changes and many band cells were vacuolated. There was a hyperplasia of the myeloid strains in the marrow.

Nervous Distemper. The myeloid tissue of the marrow consisted principally of the immature varieties. The marrow was rich in cells and not aplastic, a finding confirmed by examination of the marrow sections at autopsy.

Advanced Filariosis. The marrow changes consisted of hyperplasia of erythroid cells with a decreased myeloid erythroid ratio. It is of interest that the stained marrow smears contained large numbers of microfilariae.

Chronic Kidney Disease. Isaac, in 1944, investigated the effect of the anemias on the bone marrow. Although his work was confined to humans, his findings should be of value in veterinary medicine. He remarked that little is known of the factors that influence the development and maturation processes of either white or red blood cells, but there are some clues with regard

to the latter. It is known that in chronic kidney disease and in some types of aplastic anemia, cell growth is blocked at the primitive erythroblast stage. Red cells that do mature under these conditions are normal in size, shape, and hemoglobin content, but they are inadequate in number.

MATERIALS AND METHODS

Obtaining the Bovine Sample

The animals used in this investigation were nine cows, two steers, and one calf presented for treatment at the Kansas State Veterinary Clinic.

After reviewing the findings by Calhoun (1954b) and Koehler (1957), the eleventh and twelfth ribs were selected as the site from which to obtain the marrow samples in the bovine. Examination of these ribs at postmortem revealed that the most satisfactory red marrow was obtained approximately two inches below the costovertebral articulation.

The animals were confined in a stock or restrained against the side of a stall.

The back and side of the animal were thoroughly scrubbed and by palpation, the rib with the least amount of tissue covering it was located. The operative site was clipped, shaved, and disinfected with antiseptic solution of alcoholic Roccal.[1]

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[1] Winthrop Laboratories, New York 18, N. Y.

Anaesthesia was obtained by injecting 10 ml. of a 2 per cent procaine hydrochloride solution, first into the skin, next into the fascia, and finally on the periosteum. An incision approximately two inches long was made through the above layers, attempting to avoid the latissimus dorsi and serratus dorsalis posterior muscles. A Sklar muscle spreader was employed to allow more freedom in the operation.

A No. 1221 Stanley[1] hand drill, equipped with a straight shank 3/32-inch jobbers' drill was used to bore into the marrow cavity. A point midway between the anterior and posterior borders of the rib was chosen for insertion of the drill to avoid missing the marrow cavity. When the medullary cavity was entered, there was a tendency for the drill to "give" a bit. Following removal of the drill, a 12-gauge blunted needle with a shaft 3/4 cm. long was inserted into the hole. The needle had the same outside diameter as the opening in the bone, which prevented peripheral contamination.

An airtight 10-ml. syringe was attached to aspirate the marrow or if a biopsy was preferred, a 14-gauge needle with a shaft 3 cm. long and a blunt end was inserted through the 12-gauge needle, and a plug of marrow obtained. On occasion, a Turkel 17-gauge, biopsy needle was inserted and with a twisting movement, a costal marrow sample secured. Regardless of whether an aspiration was performed or marrow was obtained by biopsy, fresh smears were made at the operative site.

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[1] Stanley Works, New Britain, Connecticut.

The muscles and fascia were sutured with catgut and the skin incision closed with a non-absorbable material.

A peripheral blood sample was collected from the jugular vein, using E.D.T.A. as an anticoagulant. The blood and marrow slides were immediately removed to the laboratory.

A postmortem examination was performed on five cows affected with lymphocytoma and five affected with other diseases. Samples of the marrow obtained at postmortem were taken as soon as possible after death to avoid autolysis. Two of the costal bones were preserved in Zenker's fixative, and four in formalin, until taken to the laboratory to be decalcified, sectioned, and stained.

Obtaining the Canine Sample

The animals used in this aspect of the investigation were five dogs presented for treatment at the Kansas State Veterinary Clinic, and one experimental dog.

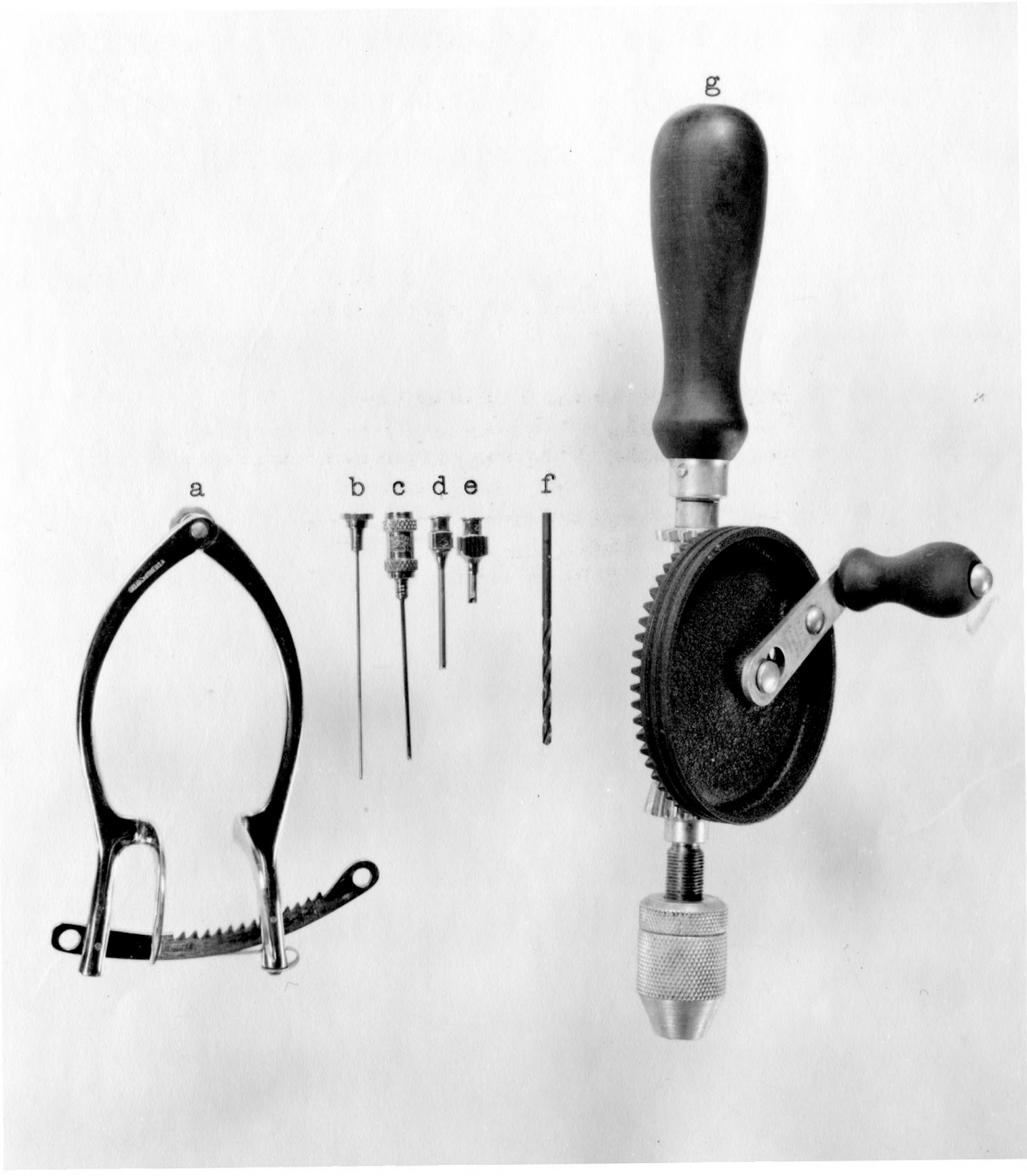
Bloom and Meyer (1943) reported that unquestionably the simplest and easiest method of obtaining marrow from living dogs consisted of aspirating marrow from the crest of the ilium. In view of these findings, the iliac crest was employed to secure samples of bone marrow from four of the dogs in this study. One sample was obtained from the femur and the other specimen was obtained at postmortem immediately after euthanasia of the experimental animal.

The technique for marrow puncture from the iliac crest was patterned after that described by Meyer and Bloom (1943).

EXPLANATION OF PLATE I

a. Sklar muscle spreader.
b. Turkel, 17-gauge, biopsy stylet.
c. Turkel, 17-gauge, biopsy needle.
d. 14-gauge blunted needle.
e. 12-gauge blunted needle.
f. 3/32-inch jobbers' drill.
g. Stanley hand drill.

PLATE I



The animal was administered a tranquilizer 15 minutes prior to the operation. A small area over the iliac crest was clipped, shaved, and alcoholic Roccal applied. Approximately 2 ml. of a 2 per cent procaine hydrochloride were infiltrated into the skin, muscles, and on the periosteum of the crest of the ilium.

The anterior border of the ilium was outlined with one hand, and a sterile No. 2076121[1] 13-gauge aspirating trochar and cannula 3 1/4 inches long were inserted through the skin and fascia into the thickest portion of the crest. The trocar was simultaneously pushed and rotated until the medullary cavity was reached. Occasionally, entrance was recognized by a sudden "give"; although this was not usual. The trocar was removed from the cannula and a 10-ml. syringe attached to aspirate the marrow. A marrow biopsy was performed by introducing a split Silverman needle through the cannula and securing marrow particles. Slides were streaked immediately with either aspirated or biopsy-obtained marrow.

The preoperative technique for the femoral puncture was similar to that of the iliac crest except that a general anaesthesia (Nembutal)[2] was used. A Kirschner intramedullary pin drill was used to introduce an intramedullary pin through the skin, muscles, and into the proximal portion of the femur, medial to the summit of the trochanter major.

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[1] Becton, Dickinson and Co., Rutherford, New Jersey.

[2] Abbott Laboratories, North Chicago, Illinois.

When the pin was introduced into the medullary cavity, a distinct "give" was felt. The pin was removed from the cavity and an aspirating trochar and cannula (No. 2076121, 13-gauge, 3¼ in. long) was introduced through the same tract. The trocar was removed, a syringe was attached to the cannula, and the marrow sample aspirated.

Peripheral blood samples were obtained from each dog for hematocrit readings, total white blood cell count, and differential counts.

Staining and Counting Technique

The marrow slides from 16 of the 18 animals and the peripheral blood slides were stained with C. L. stain.[1] The marrow from two animals was stained with Giemsa stain. Differential counts were made with a magnification of 980 times.

Following the recommendations of Stasney and Higgins (1937), Bloom and Meyer (1944), and Calhoun (1954a), 500 marrow cells were identified in each differential count.

The sections of costal bone obtained at postmortem were decalcified with a saturated aqueous solution of Sequestrene,[2] subjected to the standard histological procedure, and stained with Giemsa stain.

The two main sources of reference used in identifying and describing cells were "The Morphology of Blood Cells" by Diggs,

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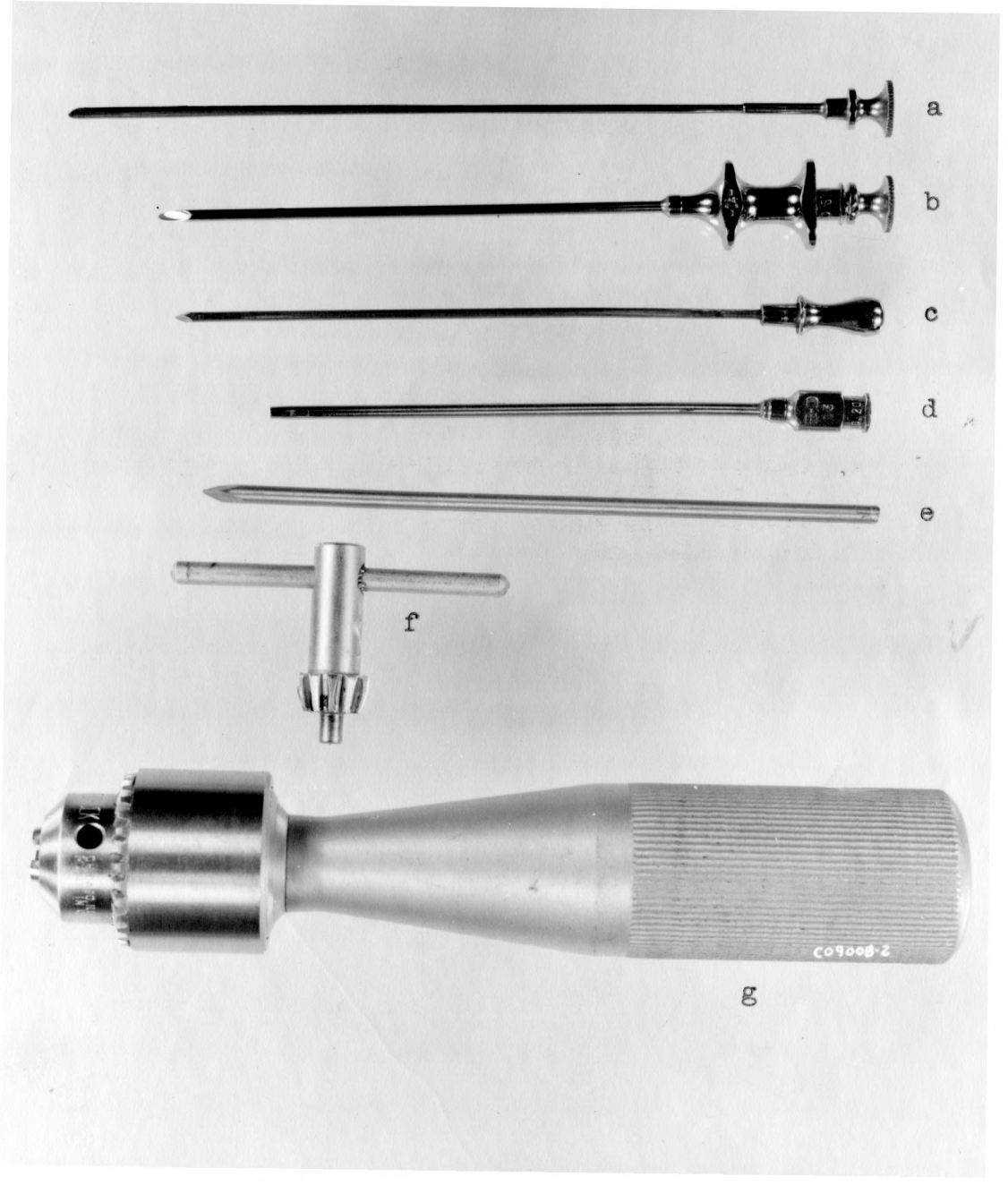
[1] C. W. Alban Co., St. Louis, Missouri.

[2] Geigy Industrial Chemicals, Ardsley, New York.

EXPLANATION OF PLATE II

a. Split Silverman needle.
b. Silverman biopsy needle with stylet.
c. No. 2076121, 13-gauge, aspirating trochar.
d. No. 2076121, 13-gauge, aspirating cannula.
e. Jacobs-type chuck wrench.
f. Intramedullary pin drill.

PLATE II



Sturm, and Bell (1954); and Daland's (1959) "Color Atlas of Morphologic Hematology."

Terminology

A classification and definition of terms was urgently needed for many years. The report of Osgood and his associates (1949) considered the clarification of the nomenclature of cells and diseases of the blood and blood-forming organs. Bloom and Meyer (1944) discussed the morphology of cell types, and Calhoun (1954b) employed a relatively simple, useful terminology.

After analyzing the various nomenclatures, the following classification was used.

Nucleated Erythrocytes. This category included the megaloblasts, erythroblasts, and normoblasts. The earliest red cells that could be identified were large cells with a deep blue cytoplasm without granules. The nuclei were large and had a characteristic loose, finely-meshed chromatin network that somewhat resembled a sieve. As the cells matured, the nuclei lost their nucleoli and stained darker, due to a clumping of the chromatin. The cytoplasm changed in color from blue to grey with a tinge of green as hemoglobin began to appear. In the final stages of this category, the cytoplasm is acidophilic or polychromatophilic with a pyknotic nucleus that stains a deep purple.

Myeloblasts, Promyelocytes, and Myelocytes. The most primitive cell of the myeloid series (myeloblast) was a large cell with an eccentric, round or oval nucleus in which the chromatin

was finely reticulated and contained no specific granules. Usually two or more nucleoli were visible.

As maturation progressed (promyelocyte) the cytoplasm became lighter, but the deep blue primitive cytoplasm was retained at the periphery. At this stage, non-specific granules began to appear in varying numbers throughout the cytoplasm. There was some condensation of the chromatin, producing a slightly mottled appearance to the nucleus.

The neutrophilic myelocytes were cells containing specific neutrophilic granules. The eosinophilic myelocytes and basophilic myelocytes were grouped with the eosinophils and basophils, respectively. The myelocytes were normally smaller than the progranulocytes and contained a relatively larger amount of cytoplasm. The nuclei were round, eccentric, and the nucleoli were indistinct or absent.

Metamyelocytes. The nucleus of this cell was round, indented, or kidney-shaped and there were small pinkish-blue granules in the cytoplasm. As a class, they were smaller than the myelocytes and had a relatively smaller nucleus and less well-defined chromatin structure.

Band Cells. As the metamyelocytes matured, the nuclear indention became more marked until it could be described as a curved or coiled band. The granules of band cells were small, evenly distributed, and stained various shades of blue and pink.

Segmented Cells. The nucleus of these cells was separated into definite lobes with a narrow filament connecting the lobes.

EXPLANATION OF PLATE III

Fig. 1. Bone marrow smear (Wright's stain, x 1000).

a. Nucleated erythrocytes.

Fig. 2. Bone marrow smear (Wright's stain, x 1000).

a. Myelocyte.
b. Band cell.

PLATE III

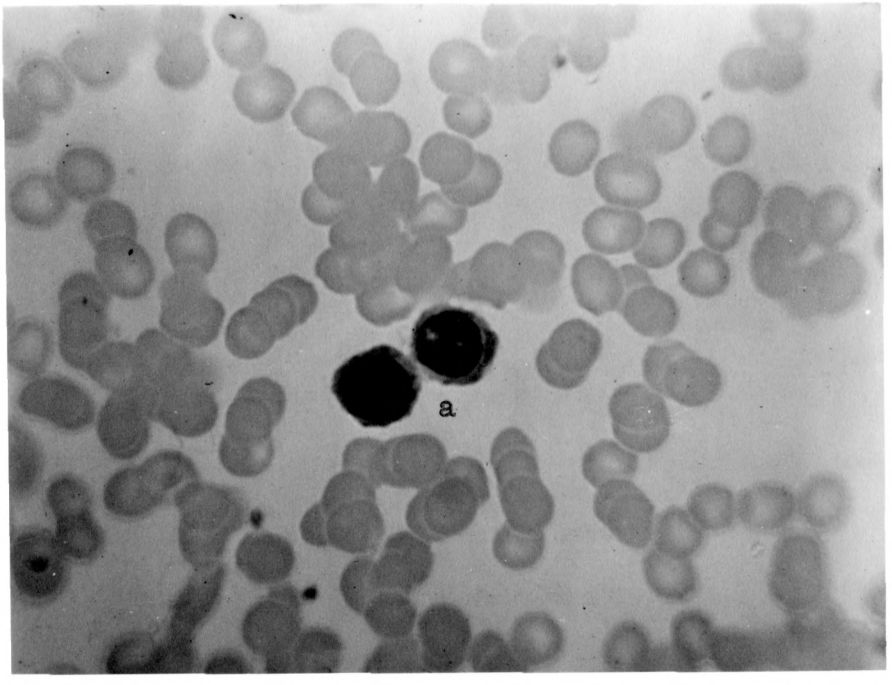


Fig. 1

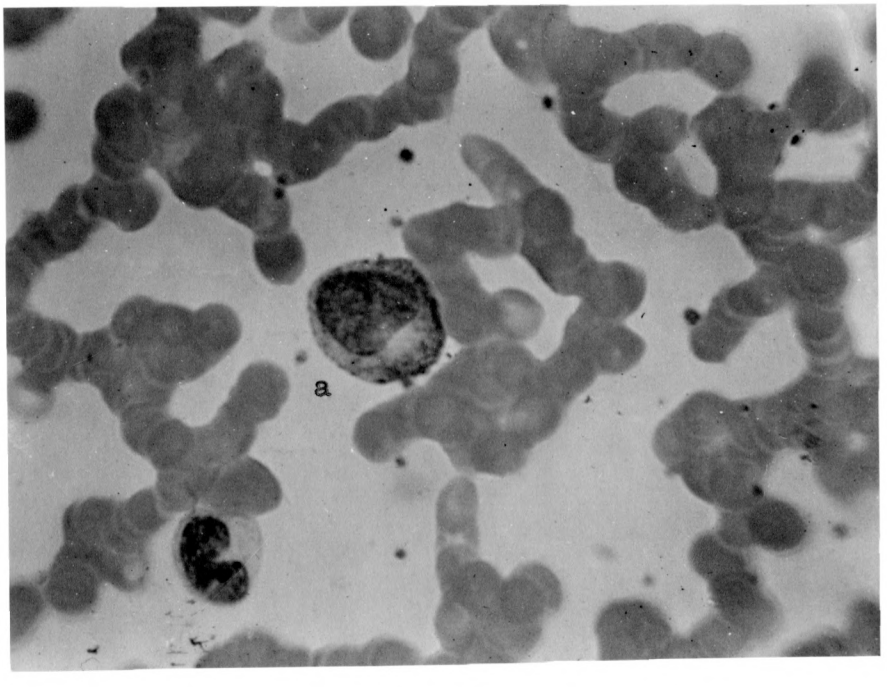


Fig. 2

The cytoplasm was a light pink and the granules had a light pink to bluish-black color.

Eosinophils. The developmental stages were similar to those of the neutrophilic series. They were readily recognized by their eosinophilic granules which frequently obscured the blue-grey cytoplasm. The nucleus went through a succession of shapes similar to the neutrophil.

Basophils. The reddish purple nucleus of the basophil was usually obscured by the deeply-stained basophilic granules which filled the cell. The nuclei exhibited maturation by changing from an oval shape to segmented forms.

Lymphocytes. The lymphocytes included all of the cells in the lymphocytic series. The lymphoblasts had a relatively large, reddish nucleus with fine chromatin structure and one to several nucleoli. The cytoplasm was bluish and non-granular. The adult lymphocytes varied greatly in size and were termed large and small.

The nucleus of the small lymphocyte was round and deep purple. The light blue cytoplasm formed a narrow rim around the well-defined periphery of the nucleus.

The nucleus of the large lymphocyte was larger and irregular in shape. The cytoplasm of the large lymphocyte was a light blue and was proportionally greater than that of the small lymphocyte.

Monocytes. The monocytes included all of the cells of the monocytic series. These cells were larger than the neutrophils and had a relatively greater amount of gray-blue cytoplasm in

EXPLANATION OF PLATE IV

Fig. 1. Appearance of bone marrow smear (Wright's stain, x 450).

Fig. 2. Bone marrow smear (Wright's stain, x 450).

a. Lymphocyte.
b. Nucleated erythrocytes.

PLATE IV

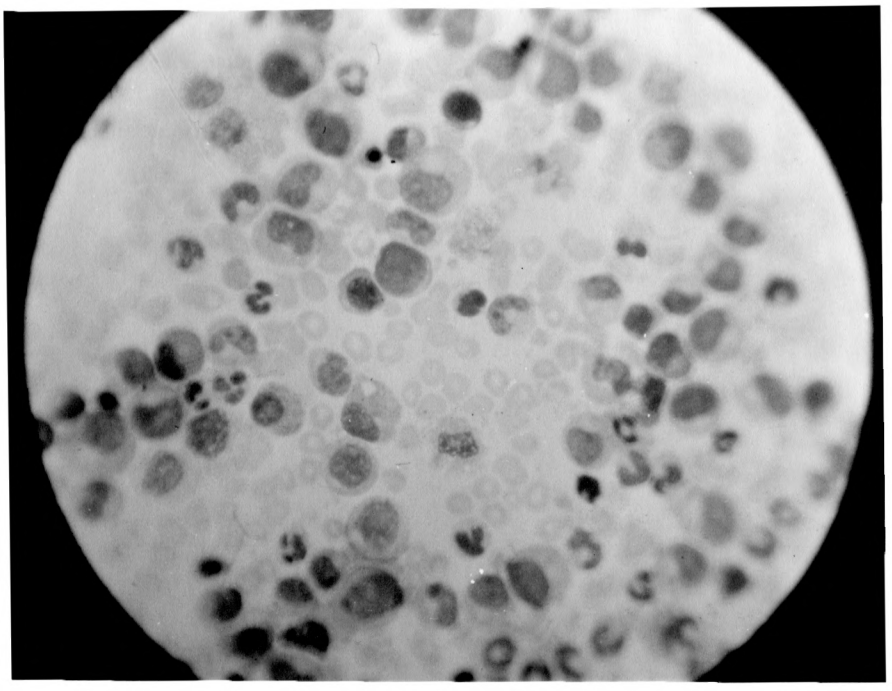


Fig. 1

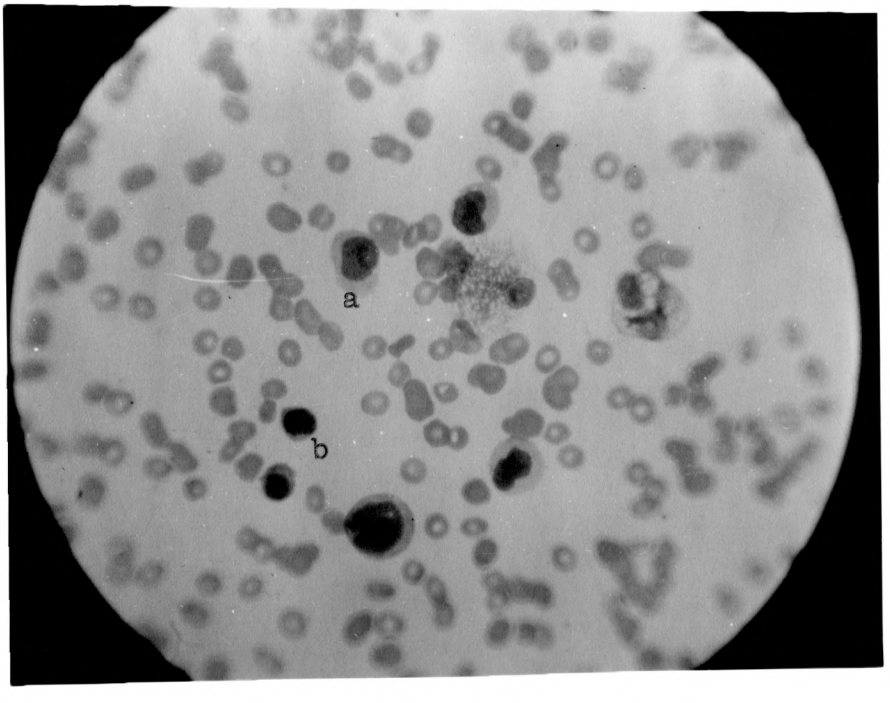


Fig. 2

relation to the nucleus. The nuclei of the monocytes were round or kidney-shaped, but had deep indentions which gave the appearance of brain-like convolutions. The nuclei appeared to be stained a lighter purple than the nuclei of other cells.

Plasma Cells. These cells included all of the plasmacytic series. The plasma cells were large in size with an intense blue-violet, eccentric nucleus that was round or oval in shape. There was a heavy chromatin network which had a radial arrangement resembling the spokes of a wheel. The cytoplasm was blue and vacuolated. A light perinuclear zone was present.

Megakaryocytes. These were the largest cells found in the bone marrow. The blue cytoplasm usually contained a number of reddish-purple granules. The nucleus was lobulated, dense, and stained a deep blue-purple.

Myeloid-Erythroid Ratio. The myeloid-erythroid ratio (M/E) was determined by adding the percentages of developing granulocytes and dividing this figure by the sum of nucleated erythrocytes.

Myeloid-Lymphoid Ratio. The myeloid-lymphoid ratio (M/L) was determined by adding the percentages of developing granulocytes and dividing this figure by the sum of the lymphocytes.

EXPLANATION OF PLATE V

Fig. 1. Bone marrow smear (Wright's stain, x 1000).

a. Eosinophil.
b. Nucleated erythrocyte.
c. Segmenter.

Fig. 2. Bone marrow smear (Wright's stain, x 100).

a. Megakaryocyte.

PLATE V

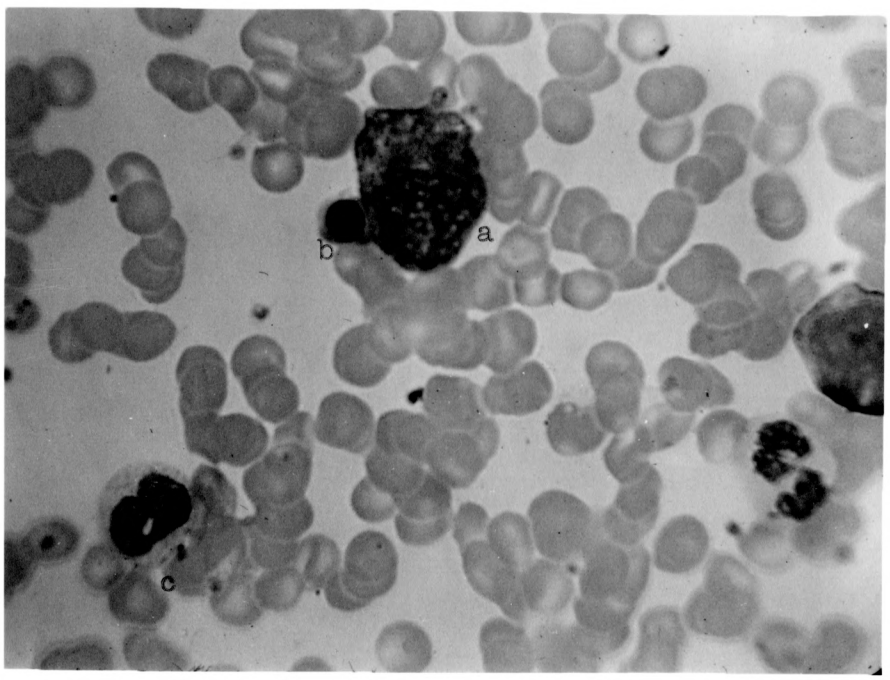


Fig. 1

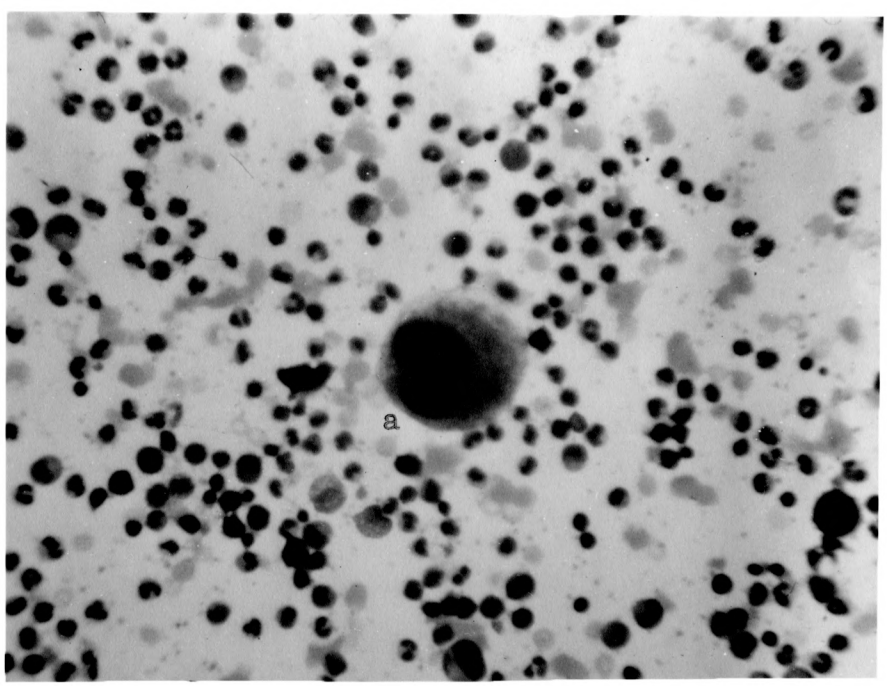


Fig. 2

RESULTS AND DISCUSSION

Results of Bovine Lymphocytoma Studies

Case 1. (Pathology #14723) Jersey, female, 10 years.

The cow was presented to the clinic with a history of gradually losing weight, but the appetite was normal. The temperature was 101.5° and the pulse was 78. The animal aborted three weeks before admission and was affected with a severe diarrhea. Rectal examination revealed the left kidney to be two to three times normal size. A diagnosis of lymphocytoma was made and an unfavorable prognosis given. Euthanasia was performed on 11/24/59.

Peripheral Counts	(11/23/59)	(11/24/59)
W.B.C.	11,300	11,800
Hematocrit	28	30
Bands	1	--
Segmenters	16	31
Lymphocytes	75	63
Monocytes	3	1
Eosinophils	5	5
		anisocytosis

Bone Marrow Count	(11/24/59)
Myeloblasts)	
Promyelocytes)	1.4
Myelocytes)	
Metamyelocytes	4.4
Bands	4.8
Segmenters	10.8
Eosinophils	7.6
Lymphocytes	48.6
Monocytes	.2
Plasma cells	.4
Megakaryocytes	.2
Nucleated erythrocytes	21.6
M/E ratio	1.34/1.00
M/L ratio	.596/1.00

At necropsy, the subcutis was edematous and the joints contained an excess of fluid. The right ventricle of the heart was dilated and the lymph nodes of the respiratory system were edematous and hypertrophied. The visceral peritoneum was edematous and the duodenum, jejunum, ileum, cecum, and colon exhibited a catarrhal enteritis.

The peritoneal cavity contained five gallons of straw-colored fluid and the liver and mesenteric lymph nodes were swollen. The kidneys were enlarged and infiltrated with lymphoid tissue. The brain was edematous.

A histological examination was made of the liver, spleen, heart, and abomasum. In each tissue, evidence of lymphoid infiltration was found. The necropsy diagnosis was lymphocytoma.

Case 2. (Pathology #14738) Mixed, female, 6 years.

The cow had been stiff, appeared lame, and had been losing weight since parturition two months previously. The urine ketone test was negative. Lymphocytoma was diagnosed and an unfavorable prognosis given. Euthanasia was performed on 12/1/59.

Peripheral Counts	(11/25/59)	(11/30/59)
W.B.C.	18,000	20,800
R.B.C.	---	5,100,000
Hematocrit	23	26
Bands	---	2
Segmenters	37	20
Eosinophils	2	2
Lymphocytes	60	75
Monocytes	1	1
	anisocytosis	4 Nuc.RBC/100 WBC

Bone Marrow Count	(11/30/59)
Myeloblasts)	
Promyelocytes)	1.2
Myelocytes)	
Metamyelocytes	1.6
Bands	6.8
Segmenters	13.2
Eosinophils	6.4
Lymphocytes	45.0
Megakaryocytes	.2
Nucleated erythrocytes	25.6
M/E ratio	1.15/1.00
M/L ratio	.649/1.00

At necropsy, the joints contained an excess of fluid. The pericardium was adhered to the epicardium. A lymphoid mass, the shape of a walnut, was attached to the right auricle. The wall of the pulmonary artery was thickened and infiltrated with lymphoid tissue. An old abscess, 4 cm. in diameter and 10 cm. in length which contained inspissated pus, was observed in the left apical lobe of the lung.

The lymphatics of the mesentery were edematous, and the Malpighian corpuscles of the spleen were very evident. The inguinal and retro-pharyngeal lymph nodes were swollen. Foci of necrosis were observed in the prescapular lymph nodes.

Sections of the spleen, mesenteric lymph node, and the mass from the right auricle were examined histopathologically. Each tissue exhibited an infiltration of lymphocytes. The necropsy diagnosis was lymphocytoma.

Case 3. (Pathology #14750) Holstein, female, 9 years.

The cow was admitted to the veterinary clinic with a history of general debility, chronic gastric tympany, and cessation of milk

production. The temperature was 101.2°. The rumen was greatly distended and the feces scanty and soft with a "putty-like" consistency. Large nodular masses were palpated in the abdominal cavity and the reproductive tract. A diagnosis of lymphocytoma was made and an unfavorable prognosis given. Euthanasia was performed on 12/3/59.

Peripheral Counts	(12/1/59)	(12/2/59)	(12/3/59)
W.B.C.	23,900	24,900	16,600
R.B.C.	---	6,410,000	6,080,000
Hematocrit	32	33	30
Bands	---	1	2
Segmenters	45	39	65
Lymphocytes	55	57	32
Monocytes	---	3	1
			atypical lymphocytes

Bone Marrow Count	(12/3/59)
Myeloblasts)	
Promyelocytes)	2.2
Myelocytes)	
Metamyelocytes	2.4
Bands	3.0
Segmenters	7.6
Eosinophils	5.4
Lymphocytes	32.0
Monocytes	.6
Megakaryocytes	.2
Nucleated erythrocytes	46.6
M/E ratio	.442/1.00
M/L ratio	.644/1.00

Necropsy examination revealed the prescapular, inguinal, mediastinal, and mesenteric lymph glands to be swollen and edematous. The pleural cavity contained 1,000 ml. of serous fluid. The heart was dilated, the valves thickened, and the myocardium invaded by neoplastic tissue.

The abomasal wall was greatly thickened and the mucous membrane infiltrated with lymphoid masses, which were undergoing

necrosis. The gall bladder was hyperplastic and there were masses of neoplastic growths in the oviducts and uterus. The right ovary was cystic. The necropsy diagnosis was lymphocytoma.

Case 4. (Pathology #14954) Hereford, female, aged.

The cow was affected with posterior paralysis when admitted to the clinic. The appetite appeared to be normal. Euthanasia was performed on 2/22/60.

Peripheral Blood Count	(2/12/60)
W.B.C.	11,700
Hematocrit	34
Bands	1
Segmenters	71
Eosinophils	1
Lymphocytes	27

Bone Marrow Count	(2/22/60)
Myeloblasts) Promyelocytes) Myelocytes)	.8
Metamyelocytes	.2
Bands	.8
Segmenters	1.2
Eosinophils	13.8
Lymphocytes	50.4
Monocytes	.2
Plasma cells	1.0
Nucleated erythrocytes	31.6
M/E ratio	.531/1.00
M/L ratio	.333/1.00

The musculature of the pelvic limbs was degenerated, hemorrhagic, and edematous. The bronchial, mediastinal, and mesenteric lymph nodes were enlarged and edematous. A histological examination of the nodes confirmed the necropsy diagnosis of lymphocytoma.

It should be noted that the peripheral count was performed 10 days before the marrow examination.

Case 5. (Pathology #14935) Angus, female, 5 years.

The cow was admitted to the clinic one week following parturition. The animal exhibited a partial paralysis and an exophthalmos of the left eye was noted. Inappetance was evident. The temperature was 102.4° and the pulse rate 84. The cow died in the stall, 2/17/60.

Peripheral Count	(2/16/60)
W.B.C.	65,400
Hematocrit	13
Metamyelocytes	5
Bands	4
Segmenters	16
Lymphocytes	75

Bone Marrow Count	(2/16/60)
Myeloblasts) Promyelocytes) Myelocytes)	.4
Metamyelocytes	.2
Bands	.4
Segmenters	2.8
Eosinophils	13.6
Lymphocytes	51.2
Plasma cells	.4
Nucleated erythrocytes	31.0
M/E ratio	.554/1.00
M/L ratio	.320/1.00

The bronchial, gastric, and hepatic lymph nodes were hypertrophied and edematous. Lymphoid tissue was evident in the orbital cavity. The abomasum showed infiltration of lymphoid tissue, the wall being 5 cm. thick in areas. A necropsy diagnosis of lymphocytoma was indicated.

Case 6. (Clinical Pathology #1021) Guernsey, female, 8 years.

The cow was admitted to the clinic with inappetance and enteritis. A tentative diagnosis of lymphocytoma was made, a guarded

prognosis given, and the cow sent home. Two weeks later the animal was returned to the clinic. Routine blood counts and a bone marrow examination were performed. The owner was advised to send the cow to slaughter.

Peripheral Counts	(4/23/60)
W.B.C.	14,700
Hematocrit	13
Metamyelocytes	1
Bands	3
Segmenters	47
Eosinophils	2
Lymphocytes	46
Monocytes	1

15 NRBC/100 WBC

mild anisocytosis, poikilocytosis, RBC show polychromasia and basophilic stippling, segmenters show toxic granules

(5/10/60)

W.B.C.	13,000
Hematocrit	13
Bands	9
Segmenters	48
Eosinophils	3
Lymphocytes	37
Monocytes	3

75 NRBC/100 WBC

anisocytosis and polychromasia

(5/12/60)

R.B.C.	1,610,000
W.B.C.	5,300
Hemoglobin	3.8 gr/100 ml.
Hematocrit	11
Metamyelocytes	1
Bands	8
Segmenters	45
Eosinophils	1
Lymphocytes	43
Monocytes	2

89 NRBC/100 WBC

polychromasia, anisocytosis, poikilocytosis, and basophilic stippling

Bone Marrow Count	(5/10/60)
Myeloblasts)	
Promyelocytes)	.4
Myelocytes)	
Metamyelocytes	2.4
Bands	5.2
Segmenters	7.6
Eosinophils	2.0
Basophils	.4
Lymphocytes	44.2
Plasma cells	.2
Nucleated erythrocytes	35.8
M/E ratio	.503/1.00
M/L ratio	.407/1.00

Discussion of Bovine Lymphocytoma Studies

It was pointed out by Bloom (1945) that a common error is to attach undue significance to variations in the percentage distribution of the various blood cells. He remarked that the bone marrow is so variable that caution must be exercised in the interpretation of minor quantitative cellular changes.

The correlation of bone marrow examination and a diagnosis of lymphocytoma were evident in case No. 1. The peripheral blood differential count was essentially normal, with a slight increase in the lymphocyte count. However, a marked increase in the lymphocyte percentage of the marrow was noted. This increase was at the expense of the erythroid series. The myeloid series was depressed, but the erythroid series was depleted sufficiently to allow the M/E ratio to be greater than one.

Anemia was evident in case No. 2, as indicated by the hematocrit reading and the appearance of nucleated erythrocytes in the circulating blood. The bone marrow examination confirmed

this finding and also showed that the increase in per cent of lymphocytes in the peripheral blood was corroborated in the marrow. The myeloid-erythroid series in case No. 2 was also greater than one, since the nucleated erythrocytes were depleted 50 per cent.

The nucleated erythrocytes were only moderately depressed in case No. 3. However, the lymphocyte percentage was increased sufficiently to lower the myeloid percentage. This case had the lowest M/E ratio of those studied. A pronounced variation in the peripheral blood differential count was noted on the day the bone marrow examination was performed. There was a drop in total white blood cells, atypical lymphocytes were noted, and a neutrophilia was observed.

Cases No. 4, 5, and 6 had similar M/E ratios. The lymphocytic percentage was elevated nearly five times which caused a subsequent depression of the nucleated erythrocytes to approximately the same level in each case. Cases No. 4 and 5 had almost identical bone marrow counts. Eosinophils were the predominant granulocytes in the marrow of these cases. There was almost a complete aplasia of the neutrophilic series. It was significant that both of these cows were greatly debilatated and near death when the marrow aspirations were performed. It appeared that the neutrophilic cells became exhausted, and little remained except the erythrocytic and lymphoid cells. It has been proposed that the hyperplasia of eosinophils is stimulated by toxic products.

An obvious anemia existed in case No. 6, as demonstrated by the hematocrit reading and the increased number of nucleated

erythrocytes observed in the circulating blood. The per cent of neutrophils in the peripheral blood was above normal blood value and the lymphocyte percentage decreased. The nucleated erythrocytes of the marrow were expelled prematurely into the circulating blood which accounted for the lowered erythrocyte percentage of the marrow. The myeloid series was greatly suppressed and the lymphoid percentage elevated.

Examination of bone marrow sections taken from the ribs of cases No. 1, 2, 3, 4, and 5 indicated some inconsistency between marrow aspiration smears and marrow sections. The sections were taken from the same rib and level as the aspirations. The bone marrow sections demonstrated the accumulation of the cells of a series into groups, while the cells of the bone marrow smear were more evenly distributed.

A number of eosinophils were present in the section slide of case No. 1. A considerable number of megakaryocytes were viewed and the nucleated erythrocytes were located in "nests."

There were numerous nucleated erythrocytes present in the marrow section of case No. 2. This contrasted with the relatively few nucleated erythrocytes observed in the aspiration smear. Sections of cases No. 2 and 3 contained large numbers of eosinophils. A greater number of lymphocytes were seen in the sections of cases No. 3, 4, and 5. There was a quantity of disintegrated cells in the section of No. 4. The bone marrow cells deteriorated rapidly after death. There was a prevalence of megakaryocytes in the section of case No. 5.

A study of bone marrow smears and sections from cattle affected with lymphocytoma indicated that the myeloid and erythroid cells of the marrow were replaced by lymphoid cells as evidenced by the depression of the nucleated erythrocytes. This depression caused an anemia. There was a suppression of the neutrophils in the marrow and elevation of the lymphocyte percentage of three to five times the normal. An eosinophilia was apparent near death in two of the cases. Caution must be exercised in placing emphasis on the myeloid-erythroid ratio (Table 3) and on the myeloid-lymphocyte ratio (Table 3). In general, however, the M/E ratio is below normal and the M/L ratio is markedly below normal.

Table 3. A comparison of the myeloid-erythroid and myeloid-lymphoid ratios of the bovine and canine.

Case No.:	Disease	M/E ratio	M/L ratio
	Bovine		
	Normal	.678/1.00	3.33 /1.00
1	Lymphocytoma	1.34 /1.00	.596/1.00
2	Lymphocytoma	1.15 /1.00	.649/1.00
3	Lymphocytoma	.442/1.00	.644/1.00
4	Lymphocytoma	.531/1.00	.333/1.00
5	Lymphocytoma	.554/1.00	.320/1.00
6	Lymphocytoma	.503/1.00	.407/1.00
7	Chronic pneumonia	1.016/1.00	1.64 /1.00
8	Uremia	.782/1.00	.919/1.00
9	Traumatic pericarditis	.742/1.00	.388/1.00
10	Pyelonephritis	.873/1.00	.955/1.00
11	Anemia	.218/1.00	.115/1.00
12	Liver abscess	.795/1.00	.708/1.00
	Canine		
	Normal	1.36 /1.00	4.91 /1.00
13	Malignant lymphoma	1.05 /1.00	2.94 /1.00
14	Malignant lymphoma	.797/1.00	2.00 /1.00
		.521/1.00	2.36 /1.00
15	Bacteremia	2.22 /1.00	1.68 /1.00
16	Fibrosarcoma	1.07 /1.00	1.22 /1.00
17	Malignant melanoma	.575/1.00	1.37 /1.00
18	Chronic nephritis	.849/1.00	.622/1.00

Results of Other Bovine Diseases

Case 7. (Pathology #14782) Shorthorn, male, 2 years.

The steer entered the clinic with a history of having a chronic cough for one and one-half years. The animal's appetite was fair, but a diarrhea and progressive emaciation were noted. The animal had lost considerable weight on pasture last year, but had gained on a ration of grain during the winter months.

No rales were heard on auscultation of the lungs. A fecal sample was positive for Haemonchus, trichostrongyles, and coccidia. A diagnosis of chronic purulent pneumonia was made and an unfavorable prognosis given. Euthanasia was performed 12/11/59.

Peripheral Counts	(12/3/59)	(12/8/59)
W.B.C.	10,300	7,700
R.B.C.	5,070,000	--
Hematocrit	18	21
Bands	2	2
Segmenters	43	21
Eosinophils	1	--
Lymphocytes	51	73
Monocytes	3	4
	microcytic anemia anisocytosis	atypical lymphocytes

Bone Marrow Count	(12/3/59)
Myeloblasts) Promyelocytes) Myelocytes)	5.8
Metamyelocytes	1.8
Bands	4.2
Segmenters	19.4
Eosinophils	7.2
Lymphocytes	23.4
Megakaryocytes	.4
Nucleated erythrocytes	37.8
M/E ratio	1.016/1.00
M/L ratio	1.64 /1.00

Dehydration and hypoproteinemia were observed on necropsy. The pericardium was adhered to the epicardium, and 30 ml. of pericardial fluid was present. The right side of the heart was dilated.

The larynx, distal one-third of the trachea, and bronchi were filled with a purulent exudate. Grey hepatization was observed in both lungs, and the cardiac and apical lobes of the lungs had

extensive areas of encapsulated purulent exudate. The pleura was adhered to the right rib cage and the mediastinal lymph nodes were hypertrophied and edematous.

The peritoneum contained four gallons of fluid. The necropsy diagnosis was chronic purulent pneumonia.

Case 8. (Pathology #14785) Angus, female, 7 years.

General emaciation, chronic tympany, and inappetance had been noted by the owner of the animal for a month. The cow was affected with a posterior paralysis on the morning of admittance to the clinic. No enlarged lymph glands were found on rectal examination; however, an eight-month, viable fetus was palpated. The cow died in the stall, 12/14/59.

Peripheral Counts	(12/10/59)	(12/11/59)
W.B.C.	6,500	5,000
R.B.C.	5,200,000	4,780,000
Hematocrit	27	27
Bands	1	1
Segmenters	60	59
Lymphocytes	37	38
Monocytes	2	2

Bone Marrow Count	(12/11/59)
Myeloblasts) Promyelocytes) Myelocytes)	3.2
Metamyelocytes	1.4
Bands	1.2
Segmenters	19.4
Eosinophils	4.2
Lymphocytes	32.2
Monocytes	.4
Megakaryocytes	.4
Nucleated erythrocytes	37.6
M/E ratio	.782/1.00
M/L ratio	.919/1.00

On postmortem examination, the joints had an excess of blood-tinged serum. The right side of the heart was dilated and there were extensive hemorrhages on the pericardium and epicardium.

Red hepatization of the lungs was evident as were ecchymotic hemorrhages of the larynx and edema of the lymph nodes of the respiratory system.

The wall of the omasum and the mesenteric lymph nodes were edematous. The liver was lighter in color and some fibrin was adhered to the pancreas. The kidneys were small, sclerotic, and exhibited a thin cortex. The necropsy diagnosis was uremia.

Case 9. (Pathology #15051) Hereford, female, 4 years.

The cow exhibited general debility, weakness, and was markedly incoordinate in the rear quarters when admitted to the clinic. The animal had to be driven to the feed rack, but maintained an appetite. The cow was nursing a three-month-old calf. The cow died in the clinic, 4/12/60.

Peripheral Counts	(4/5/60)	(4/6/60)
W.B.C.	12,800	13,200
Hematocrit	27	31
Bands	3	4
Segmenters	40	54
Eosinophils	--	1
Basophils	--	2
Lymphocytes	55	38
Monocytes	2	1
	toxic granulation of segmenters	calcium 10.4 mg./ 100 ml. serum

Bone Marrow Count	(4/6/60)
Myeloblasts)	
Promyelocytes)	2.2
Myelocytes)	
Metamyelocytes	.8
Bands	3.0
Segmenters	6.8
Eosinophils	7.4
Lymphocytes	52.0
Megakaryocytes	.6
Nucleated erythrocytes	27.2
M/E ratio	.742/1.00
M/L ratio	.388/1.00

The pericardium was adhered to the heart, and a fistulous tract was observed extending from the reticulum to the pericardium. The heart was hypertrophied and dilated. The pleura was adhered to the rib cage, and the lungs slightly congested.

A wire was located in the reticulum. The liver and spleen were friable, and cloudy swelling was evident in the liver. A slight metritis was observed in the uterus.

The necropsy diagnosis was traumatic pericarditis, causing a toxemia, which accounted for the incoordination. Poor heart action was attributed to anoxia.

Case 10. (Pathology #15045) Shorthorn, female, 6 years.

The cow was admitted to the clinic with a history of gradual emaciation for six months. The primary symptoms were frequent urination and the passing of small amounts of blood in the urine. A diagnosis of pyelonephritis was made and an unfavorable prognosis given. Euthanasia was performed 4/7/60.

Peripheral Count	(4/7/60)
W.B.C.	4,800
R.B.C.	4,210,000
Hematocrit	21
Bands	2
Segmenters	58
Lymphocytes	37
Monocytes	3
	B.U.N. 300+ mg./100 ml.

Bone Marrow Count	(4/7/60)
Myeloblasts)	
Promyelocytes)	10.4
Myelocytes)	
Metamyelocytes	3.2
Bands	2.6
Segmenters	7.6
Eosinophils	4.0
Basophils	.2
Lymphocytes	39.8
Megakaryocytes	.2
Nucleated erythrocytes	32.0
M/E ratio	.875/1.00
M/L ratio	.955/1.00

At necropsy, the heart was dilated and a small abscess was found in the wall of the reticulum. Extensive peritonitis was evident and the kidneys were enlarged. The normal lobulation of the kidneys diminished and the capsules were easily removed. The ureters were enlarged and irregular and the bladder contained a mixture of blood and pus.

A metritis was observed. The postmortem findings confirmed the diagnosis of pyelonephritis.

Case 11. (Pathology #15178) Hereford, female, 3 months.

This calf was from a group of 106 calves that were on pasture. Four calves had died since being turned out to grass. The calf exhibited general debility, inappetance, weakness, and

a white diarrhea. The temperature was 104°. The calf was extremely anemic, and euthanasia was performed on 6/13/60.

Peripheral Count	(6/13/60)
W.B.C.	7,600
Hematocrit	14
Segmenters	8
Lymphocytes	91
Monocytes	1
	mild anisocytosis and poikilocytosis

Bone Marrow Count	(6/13/60)
Myeloblasts) Promyelocytes) Myelocytes)	3.4
Metamyelocytes	1.2
Bands	1.4
Segmenters	.4
Eosinophils	.6
Lymphocytes	61.8
Nucleated erythrocytes	31.2
M/E ratio	.218/1.00
M/L ratio	.115/1.00

The mucous membranes of the mouth and nostrils were pale; the musculature was very pale; and the joints contained an excess of fluid.

The diaphragmatic lobes of the lungs were slightly congested and the mediastinal lymph nodes were edematous.

The mucous membranes of the digestive system were pale. The rumen contained dirt, dried feed, and hair balls. The duodenum was filled with yellow to orange-tinged exudate, and the walls of the jejunem, ileum, and cecum were thickened. The colon was enlarged, had little tone, and the mesenteric lymph nodes were hypertrophied and edematous.

Hemolytic Escherichia coli was isolated from the intestine, liver, and lymph node cultures. The Reinsch test was negative for heavy metals. The necropsy diagnosis was anemia.

Case 12. (Clinical Pathology #1192) Hereford, male, 2 years.

The steer had been listless for two weeks and refused to eat when admitted to the clinic. The temperature was 103°.

An exploratory rumenotomy was performed and an enlarged liver palpated. A diagnosis of liver abscess was made and a guarded prognosis given. The animal was released five days later; the temperature and appetite having been normal for three days.

Peripheral Count	(7/1/60)
W.B.C.	11,900
Hematocrit	28
Segmenters	23
Basophils	6
Lymphocytes	69
Monocytes	2

Bone Marrow Count	(7/1/60)
Myeloblasts) Promyelocytes) Myelocytes)	1.0
Metamyelocytes	2.0
Bands	8.0
Segmenters	12.2
Eosinophils	3.2
Basophils	.8
Lymphocytes	38.4
Monocytes	.2
Nucleated erythrocytes	34.2
M/E ratio	.795/1.00
M/L ratio	.708/1.00

Discussion of Other Bovine Diseases

The examination of the peripheral blood of case No. 7 did not reveal an elevated white blood cell count; however, a lymphocytosis existed which correlated with the findings of a chronic infection. The atypical lymphocytes recognized in the peripheral count prompted the examination of the bone marrow. The myeloid series was slightly above normal; the lymphocyte percentage was increased two times the normal; and the nucleated erythrocytes were depressed, which accounted for the anemia as evidenced by the hematocrit reading.

A bone marrow section of case No. 7 revealed large numbers of nucleated erythrocytes and eosinophils.

The posterior paralysis of case No. 8 prompted a bone marrow examination. The myeloid and lymphocyte series percentage was found to be nearly the same. The nucleated erythrocytes were suppressed. The low white blood cell count of the peripheral blood was explained by the necropsy diagnosis of uremia.

The bone marrow findings of the case of traumatic pericarditis (No. 9) were similar to those of lymphocytoma, particularly cases No. 4 and 5. The myeloid and erythroid series were suppressed and the lymphocyte percentage elevated. The M/E ratio corresponded with the cases of chronic infection rather than the lymphocytoma cases.

A comparison was made between case No. 10 and those of the lymphocytoma series. The nucleated erythrocytes were suppressed; the myeloid series was near normal; but there was a hyperplasia

of the myeloblasts. The lymphocyte percentage did not reach the elevated proportions found in lymphocytoma, and the M/E ratio of the case of pyelonephritis (No. 10) was nearly one to one.

Case No. 11 was an example of extreme anemia. The low hematocrit reading and the lymphocytosis of the peripheral blood count prompted a bone marrow examination. The lymphocyte percentage was of great magnitude which caused a severe depression of the myeloid series and a moderate lowering of the erythroid series. Evidence of lymphocytoma was negative at necropsy. The hyperplasia of the lymphocytes was thought to be the result of the overwhelming disease produced by the hemolytic Escherichia coli organisms.

There was a striking resemblance between the peripheral blood counts of the case of liver abscess (No. 12) and case No. 1. The white blood cell counts were similar and the lymphocyte percentages were in the same range. A bone marrow examination of case No. 12 indicated that the myeloid series was slightly below normal, but there was a moderate depression of the erythroid series as compared with the marked lowering of the nucleated erythrocytes in the bone marrow of case No. 1. The M/E ratio was slightly above normal.

Bone marrow studies of chronic bovine diseases, other than lymphocytoma, indicate that the myeloid series was not suppressed of the magnitude of the lymphocytoma cases. The erythroid series was depressed below normal, but the M/E ratio remained above normal in all of the cases with the exception of the anemic calf.

The lymphocyte percentages were elevated in the cases of chronic pneumonia (No. 7) and uremia (No. 8), but not as high as in the cases of traumatic pericarditis (No. 9). The myeloid-lymphocyte percentage (Table 3) was lower than normal in each case studied. In general, the M/E ratios of chronic infections were above normal while those of lymphocytoma were subnormal.

The bone marrows studied indicated there was a lymphocytic percentage increase in all of the diseases of the bovine, with a corresponding depression of the myeloid and erythroid series. The peripheral white blood cell counts of the lymphocytoma cases were usually normal or above normal whereas the peripheral white blood cell counts of the other diseases were usually normal or subnormal.

Results of Canine Studies

Case 13. (Pathology #14970) Boxer, female, 8 years.

The dog had been admitted to the clinic from time to time for two months prior to euthanasia. A biopsy of a lymph node was performed when the animal was examined initially. A diagnosis of malignant lymphoma was made and an unfavorable prognosis given.

Treatment included Cytoxan,[1] antibiotics, and vitamins. No response was recognized and euthanasia was advised.

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[1] Mead Johnson and Co., Evansville, Indiana.

Peripheral Counts	(12/19/59)	(12/22/59)
W.B.C.	12,500	11,700
R.B.C.	---	5,000,000
Hematocrit	38	31
Segmenters	76	74
Lymphocytes	24	26

B.U.N. 24 mg./100 ml.

	(12/28/59)	(12/31/59)
W.B.C.	8,700	5,900
Hematocrit	45	--
Bands	--	2
Segmenters	78	70
Lymphocytes	22	24
Monocytes	--	4

	(1/11/60)	(1/18/60)
W.B.C.	5,700	4,100
R.B.C.	5,450,000	--
Hematocrit	44	46
Segmenters	49	54
Eosinophils	24	4
Lymphocytes	27	42

	(1/20/60)	(1/26/60)
W.B.C.	10,200	11,200
R.B.C.	6,720,000	--
Hematocrit	43	36
Bands	--	1
Segmenters	72	81
Eosinophils	1	6
Lymphocytes	27	12
		slight anisocytosis, some toxic granulation in segmenters

	(2/27/60)
W.B.C.	23,600
Hematocrit	36
Bands	1
Segmenters	74
Eosinophils	1
Lymphocytes	24

lymphocytes smeared

Bone Marrow Count	(2/27/60)
Myeloblasts) Promyelocytes) Myelocytes)	3.2
Metamyelocytes	3.8
Bands	15.0
Segmenters	21.0
Eosinophils	--
Lymphocytes	14.6
Monocytes	.4
Plasma cells	.8
Megakaryocytes	.2
Nucleated erythrocytes	41.0
M/E ratio	1.05/1.00
M/L ratio	2.94/1.00

The cervical, mandibular, and inguinal lymph nodes were hypertrophied as were the mediastinal lymph nodes. The intestinal tract, from the duodenum to the colon, appeared to be infiltrated with lymphoid tissue and was edematous. The mesenteric lymph glands were hypertrophied.

The liver was friable, but of normal size. The spleen was enlarged. The kidneys were normal in size, but appeared to be infiltrated with lymphoid tissue.

Two tumors approximately 3½ cm. in diameter were located just anterior to the bladder. The adrenals were enlarged and the caudal pair of mammary glands were interspersed with lymphoid tissue.

A histological examination was made of the liver, spleen, duodenum, kidneys, bladder, and lymph nodes. Lymphoid infiltration was recognized in these tissues. A necropsy diagnosis of malignant lymphoma was made.

Case 14. (Pathology #15195) Boxer, male, 8 years.

The dog had been affected with malignant lymphoma for approximately two months before euthanasia was performed. The animal had been admitted to the clinic six times for peripheral blood tests and one bone marrow examination. Another bone marrow examination was performed on the day of euthanasia.

The lymph glands were swollen, but the dog maintained a satisfactory appetite. Cytoxan was the choice of treatment.

Peripheral Counts	(5/3/60)	(5/5/60)
W.B.C.	28,000	17,900
Hematocrit	48	45
Bands	1	1
Segmenters	89	85
Eosinophils	--	1
Lymphocytes	8	13
Monocytes	1	--
	B.U.N. 25 mg./100 ml.	sedimentation rate
	hypersegmentation	1 mm.-15 min.
		11 mm.-30 min.
		28 mm.- 1 hour

	(5/16/60)	(5/23/60)
W.B.C.	6,100	8,300
Hematocrit	38	47
Segmenters	67	64
Eosinophils	4	13
Lymphocytes	29	23
	hypersegmentation	Howell-Jolly bodies
	sedimentation rate	polychromasia
	1 mm.-30 min.	anisocytosis
	4 mm.- 1 hour	
	8 mm.- 2 hours	

Peripheral Counts (concl.)	(6/3/60)	(6/17/60)
W.B.C.	7,200	13,400
Hematocrit	41	44
Bands	1	2
Segmenters	84	81
Eosinophils	1	5
Lymphocytes	14	11
	one Nuc.RBC/100 WBC	sedimentation rate
	one Howell-Jolly body	0 mm.-15 min.
	polychromasia	4 mm.- 1 hour
	anisocytosis	12 mm.- 2 hours

Bone Marrow Counts	(5/3/60)	(6/17/60)
Myeloblasts)		
Promyelocytes)	1.0	.4
Myelocytes)		
Metamyelocytes	4.0	1.8
Bands	8.8	7.6
Segmenters	16.0	16.4
Eosinophils	6.0	3.4
Basophils	.4	.2
Lymphocytes	18.0	12.6
Megakaryocytes	.4	.4
Nucleated erythrocytes	54.4	57.2
M/E ratio	.797/1.00	.521/1.00
	2.00 /1.00	2.36 /1.00

The popliteal and inguinal lymph glands were hypertrophied and edematous. The heart was extremely dilated and there was approximately 5 ml. of fluid in the pericardial sac.

A tumor 3 cm. in diameter was loosely attached near the base of the heart. The lymphatics of the digestive and respiratory tracts were hypertrophied. The walls of the duodenum, jejunum, and ileum were edematous.

The spleen was grossly enlarged, two to three times its normal size and the liver swollen. Evidence of mild nephritis was found in the kidneys. The bladder and prostate were infiltrated with lymphoid tissue.

The liver, spleen, duodenum, kidney, bladder, and prostate were examined histologically. These tissues were found to have a pronounced increase in lymphoid tissue. The necropsy diagnosis was malignant lymphoma.

Case 15. (Clinical Pathology #1019) Walkerhound, male, 5 years.

The dog was admitted to the clinic in a greatly emaciated state. Inappetance, jaundice, and nephritis were noted. The animal had an enlarged spleen and evidenced pain when palpated over the liver area. A tumor had been removed from the chest four months previously. The dog was found to be parasitized with Trichuris vulpis. The dog was released after two weeks of hospitalization. A diagnosis of bacteremia of unknown etiology was made.

Peripheral Counts	(4/22/60)	(4/25/60)
W.B.C.	45,000	24,000
Hematocrit	27	26
Bands	11	16
Segmenters	85	72
Lymphocytes	4	12
		B.U.N. 30 mg./100 ml.
	(4/28/60)	(5/1/60)
W.B.C.	30,200	19,900
Hematocrit	29	27
Bands	11	3
Segmenters	73	73
Eosinophils	2	3
Lymphocytes	13	21
Monocytes	1	--

Bone Marrow Count	(4/25/60)
Myeloblasts)	
Promyelocytes)	.2
Myelocytes)	
Metamyelocytes	2.0
Bands	13.2
Segmenters	29.8
Eosinophils	3.6
Lymphocytes	29.0
Megakaryocytes	.2
Nucleated erythrocytes	22.0
M/E ratio	2.22/1.00
M/L ratio	1.68/1.00

Case 16. (Pathology #15143) Doberman Pinscher, female, 8 years.

The dog was admitted to the clinic with symptoms of general listlessness and inappetance. Exploratory surgery was performed and a metastatic neoplasm observed. Euthanasia was suggested and performed on 5/25/60.

Peripheral Count	(5/21/60)
W.B.C.	30,500
Hematocrit	42
Bands	1
Segmenters	90
Lymphocytes	8
Monocytes	1

B.U.N. 15 mg./100 ml.

Bone Marrow Count	(5/21/60)
Myeloblasts)	
Promyelocytes)	1.0
Myelocytes)	
Metamyelocytes	1.8
Bands	9.4
Segmenters	20.0
Eosinophils	3.2
Basophils	.6
Lymphocytes	29.4
Megakaryocytes	.2
Nucleated erythrocytes	34.4
M/E ratio	1.07/1.00
M/L ratio	1.22/1.00

Evidence of neoplastic metastasis was found in the lungs, pleura, liver, and kidneys. A very small amount of normal splenic tissue remained due to the infiltration of the neoplasm. A histopathological examination indicated that the neoplasm was a fibrosarcoma.

Case 17. (Pathology #15148) Doberman Pinscher, male, 7 years.

The dog was admitted to the clinic due to a growth approximately 3 cm. in diameter located on the hard palate. A similar tumor had been removed from the post pharyngeal region four months previously. A diagnosis of malignant melanoma was made and an unfavorable prognosis given. Euthanasia was performed 5/27/60.

A peripheral blood count was not considered necessary; however, a bone marrow examination was made at the time of euthanasia. Traces of melanin were seen in the marrow.

Bone Marrow Count	(5/27/60)
Myeloblasts) Promyelocytes)	6.0
Myelocytes)	
Metamyelocytes	2.4
Bands	5.8
Segmenters	12.4
Eosinophils	2.2
Lymphocytes	21.0
Nucleated erythrocytes	50.2
M/E ratio	.575/1.00
M/L ratio	1.37 /1.00

A malignant melanoma was evidenced as the neoplasm was not limited solely to the pharynx. Four large and several small melanomas were found in the lungs. Metastasis was recognized in the mesentery, and the spleen had a nodule approximately 1 cm. in diameter.

Histopathological examination of the pharynx and the liver confirmed the diagnosis of malignant melanoma.

Case 18. (Experimental #10) Beagle, male, 6 months.

This animal was inoculated in the right kidney with an agar culture of proteus. Euthanasia was performed three months later on 6/30/60.

Peripheral Count	(6/30/60)
W.B.C.	12,550
R.B.C.	4,630,000
Hematocrit	37
Segmenters	82
Eosinophils	2
Lymphocytes	16
	B.U.N. 22.5 mg./100 ml.
	blood sugar 93 mg./100 ml.
	plasma calcium 9.6 mg./100 ml. of serum
	hemoglobin 10.5 gr./100 ml.

Bone Marrow Count	(6/30/60)
Myeloblasts)	
Promyelocytes)	3.6
Myelocytes)	
Metamyelocytes	2.6
Bands	7.0
Segmenters	12.4
Eosinophils	.6
Basophils	.2
Lymphocytes	42.4
Nucleated erythrocytes	31.2
M/E ratio	.849/1.00
M/L ratio	.622/1.00

The postmortem lesions were limited to the urinary tract. The capsule of the right kidney was adherent and the cortex pale. The left kidney was hypertrophied and the cortex dark. The bladder exhibited evidence of traumatic cystitis. The diagnosis was interstitial nephritis.

Discussion of Canine Studies

A bone marrow examination was performed on two dogs (cases No. 13 and 14) affected with malignant lymphoma. Case 13 had an initial total white blood cell count that was within the normal range. The dog was treated with Cytoxan, and its effect on the subsequent peripheral blood counts was noted. There was a distinct lowering of the total white blood cell count. Evidence of myeloid disturbance was seen by the increased percentage of eosinophils and the absolute decrease in neutrophils. Examination of the bone marrow on the day of euthanasia revealed that there was a slight depression in the myeloid series as compared to the normal. The lymphocyte percentage was moderately increased and the erythroid series was essentially normal.

Bloom and Meyer (1945) found that leukocytosis apparent in the peripheral blood of dogs affected with malignant lymphoma was due to ulcerative and proliferative processes and hyperplasia of the myeloid cells in the bone marrow. They found an erythrogenic suppression in the bone marrow due to myeloid hyperplasia and only partially from lymphomatous infiltration. This was not observed in the marrow of case No. 13. It was believed that the effect of Cytoxan altered the findings. The M/E ratio (Table 3) was found to be only slightly below normal, while the M/L ratio was low.

A bone marrow aspiration was performed on case No. 14 when first admitted for treatment and a second aspiration made on the day of euthanasia. The initial peripheral blood count showed a

leukocytosis with a neutrophilia. Treatment with Cytoxan lowered the peripheral white blood cell count.

The lymphocyte percentage in the first marrow examination was increased almost two to one; the myeloid series was depressed; and the erythroid series was slightly elevated. The second examination of the marrow showed the lymphocyte percentage to be only slightly above normal; the myeloid series greatly depressed; and the erythroid series hyperplastic. The Cytoxan treatment apparently caused a pronounced depression of the leukocytes. The M/E ratio and M/L ratio of case No. 14 was lower in both instances than case No. 13.

A bone marrow examination was made in case No. 15, because of the leukocytosis observed in the peripheral blood count. The dog was extremely anemic as evidenced by the hematocrit reading. The myeloid series was normal; the lymphocytic percentage greatly increased; and the nucleated erythrocytes markedly depressed. This suppression explained the anemic condition of the animal. The M/E ratio of this dog was higher than any of those examined.

The total white blood cell count in case No. 16 was an example of the leukocytosis that occurs with many malignant neoplasms. The lymphocytic percentage was increased, but the myeloid series was lower than normal.

An increase in the nucleated erythrocytes and lymphocyte percentage was found in the marrow examination of case No. 17. The increase was at the expense of the myeloid series as evidenced by the low M/E ratio. The M/L ratio of the dog affected

with fibrosarcoma (No. 16) was comparable to the malignant melanoma case (No. 17). It was believed that traces of melanin were found in the marrow smear of case No. 17, confirming the metatasis of the neoplasm to the bone marrow.

The myeloid series was depressed in the bone marrow examination of the chronic nephritis case (No. 18). The peripheral white blood cell differential count did not preclude the marked lymphocyte percentage found in the marrow. The M/E ratio was below normal, but the M/L ratio was greatly lowered.

In general, it was found that the dogs affected with neoplastic diseases had a subnormal M/E ratio (Table 3) as compared to a marked elevation of the ratio in bacteremia. The M/L ratio (Table 3) of the malignant lymphoma cases was below normal, although consistently higher than the M/L ratio of the other diseases studied.

SUMMARY AND CONCLUSIONS

The most desirable location for a bone marrow examination in the bovine was found to be approximately two inches below the costovertebral articulation of the eleventh or twelfth rib. The technique employed for femoral puncture in the dog was considered to be the most satisfactory, due to the narrow iliac crest in small dogs and small quantity of red marrow found in the crest of older dogs.

The bone marrow was found to be so variable in its content that prudence should be exercised in attaching significance to

minor variations in the percentage distribution of marrow cells. It was found that a variability exists between the marrow smear and section. The degree of cellularity in the marrow was difficult to ascertain in the smear, but could be evaluated by a histological section.

It was found essential to examine the smears thoroughly and to move the slide frequently when making a marrow count because of the uneven distribution of the cells.

A study of the bone marrow smears and bone marrow sections from cattle affected with lymphocytoma indicated that the myeloid and erythroid cells of the marrow were replaced by lymphoid cells. The depression of the nucleated erythrocytes resulted in an anemia. The percentage of lymphocytes in the marrow was increased three to five times. An eosinophilia was apparent in two of the cases near death.

Caution must be exercised in placing emphasis on the myeloid-erythroid ratio and on the myeloid-lymphocytic ratio in the diseases of the bovine. The M/E ratio of the cows affected with lymphocytoma was below normal in four of the six cases, and the M/L ratio was markedly below normal in all of the cases.

Bone marrow studies of chronic bovine diseases indicated that the myeloid series was not suppressed to the extent that it was in the lymphocytoma cases. The erythroid series was below normal, but the M/E ratio remained above normal in all of the cases with the exception of the anemic calf. The lymphocyte percentages were elevated in the cases of chronic pneumonia and

uremia, but were not as marked as in the cases of traumatic pericarditis, pyelonephritis, and liver abscess. Lymphocyte percentages that resembled those of lymphocytoma were found in the cases of anemia and traumatic pericarditis.

There was a lymphocytic percentage increase in all of the diseases of the bovine, with a corresponding depression of the myeloid and erythroid series. However, the peripheral white blood cell counts of the lymphocytoma cases usually were normal or above normal as compared to the peripheral white blood cell count of the other diseases, which were usually normal or subnormal. This indicated the importance of a peripheral blood count performed in conjunction with the bone marrow examination. The bone marrow differential count of the traumatic pericarditis case closely simulated the marrow findings of some of the cases of lymphocytoma.

An evaluation of bone marrow examinations in the canine malignant lymphomas indicated that the myeloid series was decreased; the lymphocytic series elevated one and one-half to two times; and the erythroid series elevated. This was in contrast with the extensive hyperplasia of the lymphocytes and depression of the nucleated erythrocytes found in the marrow examination of bovine lymphocytomas. The effect of Cytoxan on the bone marrow of the dogs affected with malignant lymphoma probably caused depression of the granulocytes and lymphocytes.

It was found that dogs affected with malignant lymphoma, fibrosarcoma, malignant melanoma, and chronic nephritis had a

subnormal M/E ratio as compared to a marked elevation of the ratio in bacteremia. The M/L ratio of the malignant lymphoma cases was below normal, although consistently higher than the M/L ratio of the cases of bacteremia, fibrosarcoma, malignant melanoma, and chronic nephritis.

In the animals studied, a bone marrow examination was found to be of diagnostic aid when intelligently correlated with a peripheral blood count and clinical symptomatology.

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THE SIGNIFICANCE OF BONE MARROW EXAMINATION IN CERTAIN DISEASES OF THE BOVINE AND CANINE

by

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This project was undertaken to study the cellular structure of bone marrow in certain diseases of cattle and dogs, and to evaluate the methods and techniques of bone marrow examinations of the bovine and canine.

The 12 bovine samples were obtained from the eleventh or twelfth ribs approximately two inches below the costovertebral articulation. A drill was employed to gain entrance into the medullary cavity of the rib. An aspiration was performed or marrow was obtained by biopsy. Peripheral blood samples were collected in conjunction with the marrow examination to correlate the findings. Sections of costal bone, obtained from six of the cows at postmortem, were examined histologically.

Bone marrow samples were obtained from the iliac crest of four dogs and from the femur of two dogs. The technique employed for femoral puncture was considered to be the most satisfactory due to the narrow iliac crest in small dogs and the small quantity of red marrow found in the crest of older dogs. An intramedullary pin drill was used to introduce a pin into the marrow cavity of the femur.

The marrow and peripheral blood slides were stained, and differential counts performed on each. Five hundred cells were enumerated in the differential count of the marrow. The cells were classified according to a nomenclature which defined the basic characteristics of each type of cell.

The bone marrow was found to be so variable that prudence should be exercised in attaching significance to minor variations

in the percentage of marrow cells. It was found there exists a variability between the marrow smear and section. The degree of cellularity in the marrow was difficult to ascertain in the smear, but could be evaluated by a histological section. A comparison of the myeloid, erythroid, and lymphoid series percentages of the marrow were established as ratios.

A study of the bone marrow smears and sections of six cows affected with lymphocytoma indicated that the myeloid and erythroid cells of the marrow were replaced by lymphoid cells. The depression of the nucleated erythrocytes resulted in an anemia.

Bone marrow studies of chronic bovine diseases indicated that the myeloid series was not suppressed to the extent that it was in the lymphocytoma cases. The lymphocyte percentages were elevated in the cases of chronic pneumonia and uremia, but not as marked as in the cases of traumatic pericarditis, pyelonephritis, and liver abscess. Lymphocyte percentages that resembled those of lymphocytoma were found in the cases of anemia and traumatic pericarditis.

An evaluation of bone marrow examinations in the canine malignant lymphomas indicated that the myeloid series was decreased, and the erythroid series elevated. The dogs affected with malignant lymphoma, fibrosarcoma, malignant melanoma, and chronic nephritis had a subnormal M/E ratio as compared to a marked elevation of the ratio in bacteremia.

In the animals studied, a bone marrow examination was found to be of diagnostic aid when intelligently correlated with a peripheral blood count and clinical symptomatology.