THE CYTOLOGY AND TERMINAL MANIFESTATIONS OF BOVINE LYMPHOSARCOMA

By

TALIB ABDUL AMIR MAKKAWI

Bachelor of Veterinary Science

Baghdad University

Baghdad, Iraq

1960

Submitted to the faculty of the Graduate School
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1965

THE CYTOLOGY AND TERMINAL

MANIFESTATIONS OF BOVINE

LYMPHOSARCOMA

Thesis Approved:

Andrew W. Lawler
Thesis Adviser

M E Crocker

Roger J. Panciera

O. L. Glenn

J. H. Boggs
Dean of the Graduate School

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and warm appreciation to Dr. A. W. Monlux, Professor and Head of the Department of Veterinary Pathology, for his thoughtful guidance and advice throughout the course of this study and for providing the facilities necessary for conducting the experiments and in the preparation of this paper.

Special thanks are extended to Dr. W. E. Brock of the Department of Veterinary Pathology for his assistance with the photography and guidance.

I am indebted to Dr. R. J. Panciera, Department of Veterinary Pathology for his assistance and cooperation.

I am also grateful to Dr. B. L. Glenn for his valuable suggestions and help.

Thanks are due to Dr. J. B. Corcoran, Dr. A. L. Malle, Dr. B. I. Osburn, Dr. R. R. Dahlgren and Dr. J. C. Hruska of the Department of Veterinary Pathology for their assistance during the course of this investigation.

Appreciation is expressed to the various members of the laboratory staff of the Department of Veterinary Pathology who rendered assistance in processing the laboratory specimens.

In addition, I would like to extend my sincere thanks to Dr. T. P. Piekunka, Dr. E. R. Benner and all staff members of the Department of Veterinary Medicine and Surgery for their kind assistance.

To my parents, I owe all the devoted encouragement and help throughout my life and in implanting in me the desire to learn and acquire knowledge.

To the Iraqi Government, I am extremely grateful for the financial support and nomination for this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I

INTRODUCTION

Bovine lymphosarcoma is a neoplastic disease which is believed to arise in lymph nodes or in other lymphoid tissues. In the early stages of the disease, there is often no recognizable neoplastic manifestation in the blood or bone marrow even though the tumor may be present in several lymph nodes or organs. This would suggest a multicentric origin for those cases with early multiple lesions. It is in the more terminal stages of the disease that neoplastic cells are apt to be recognized in the blood. Most investigators consider that metastases can occur in at least this terminal stage. Leukemia is used in this study to designate the presence of neoplastic cells in the blood.

This study was initiated to recognize this late stage and to compare the terminal alterations of the involved tissues with the hematological and clinical findings. Observations concerning the development of lymphocytosis, leukopenia, leukemia and anemia were included in these hematologic examinations. Cytologic groupings of neoplastic lymphoid cells into prolymphocytic, lymphoblastic, reticulum cell and lymphocytic types were made to allow a comparison of data in order to determine if there were variations in findings for each type. Hematologic smears as well as tissue sections were studied in making these groupings. In addition to conventional stains, preparations were made with acridine

orange to evaluate the use of nucleic acid differential staining in recognizing neoplastic cells. This staining was attempted to see if it would be helpful in differentiating atypical or immature lymphocytes since several investigators have concluded that such cells cannot be separated morphologically from neoplastic lymphoid cells.

It was desired also to establish the presence or absence of the neoplasms in anatomic sites other than lymph nodes in the late stage. This was considered important as a means of speculating whether there was a significant correlation between involvement of one or more sites and the terminal hematologic manifestations. A secondary correlation was made with the age and clinical observations on the animals as it was considered that there might be a relationship between age and the primary sites or metastases of the tumor.

CHAPTER II

REVIEW OF SELECTED LITERATURE

Lymphosarcoma is the designation used in this study to describe malignant tumors of lymphoid tissue of cattle. Other names that have been considered acceptable by some writers or used to depict some unusual feature manifested by tumors found in this group include lymphocytoma, malignant lymphoma, reticulum cell sarcoma, leukemia, leukosis and pseudoleukemia. (Moulton, 1961). A diagnosis of lymphoma or benign lymphoid tumor is not made in most studies, apparently, because there is early malignant transformation, and the lesions would be practically indistinguishable from lymphoid hyperplasia and other proliferations of lymphoid tissues. (Runnells et al., 1960; Smith, 1962; Moulton, 1961).

Incidence at Different Ages

All ages are susceptible to the disease, although it occurs more frequently in adult cattle than the younger ones. (Smith, 1962). The great majority of cases involves cattle between the fifth and eighth years of age. (Jubb and Kennedy, 1963). Moulton (1961) pointed out that the average age of animals with lymphosarcoma is 4 to 8 years.

Breed and Sex

Lymphosarcoma shows no breed prevalence, but it is seen more in

female than in male cattle. This sex difference is possibly due to the fact that more females are allowed to live to the peak age of tumor incidence. (Moulton, 1961).

Symptoms

The symptomatology manifested varies in accordance with the organs and parts of the body affected. (Smith, 1962). The disease may have an insidious onset with such variable symptoms as weakness, dyspnea, capricious appetite or digestive disturbance, abnormal cardiac function including right-sided failure, anemia, protrusion of the eyes, posterior paresis or paralysis, intermittent fever and enlargement of the superficial lymph nodes. (Cotchin, 1956).

The most significant manifestation is the pronounced enlargement of palpable lymph nodes, including the lumbar group which can be examined per rectum. Enlarged lymph nodes are noticed in more than half of the bovine cases presented for diagnosis. (Smith, 1962; Squire, 1964). The prescapular nodes appear to be particularly susceptible. Bilateral enlargement of superficial lymph nodes frequently occurs. (Cotchin, 1956). Jubb and Kennedy (1963) stated that generalized involvement of lymph nodes is less common in adult and aged cattle than in younger animals. In some cases, only one superficial node will be enlarged, or possibly the only enlargement is detected when rectal palpation is performed. Smith (1962) stated that enlarged nodes are firm, relatively painless and movable. Posterior paresis may occur due to infiltration of the lumbosacral region of the vertebral column and pressure on the spinal cord. (Monlux et al., 1956; Moulton, 1963; Jubb and

Kennedy, 1963; Innes and Saunders, 1962; Squire, 1964). Neoplastic infiltration of gluteal and sciatic nerves may also cause posterior paresis. (Moulton, 1961). Pressure on nerves from tumor masses in the abdominal and pelvic cavity is described. (Moulton, 1961; Cotchin, 1956; Squire, 1964).

Interference with respiration may occur due to enlargement of lymph nodes of the thoracic inlet. (Smith, 1962).

Obscure digestive disturbances may develop due to diffuse infiltration of the mucosa and submucosa of the abomasum and/or other segments of the gastro-intestinal tract with neoplastic cells of lymphoid origin. The animal may have diarrhea, varying degrees of bloating, and the feces may be watery and bloody due to ulceration of the abomasum. (Jubb and Kennedy, 1963).

Cardiac insufficiency due to invasion of cardiac muscle by neoplastic cells is observed. (Smith, 1962). This may lead to symptoms simulating traumatic pericarditis or active endocarditis with secondary lymphadenitis. (Cotchin, 1956; Jubb and Kennedy, 1963). The animal may show rapid, irregular pulse, distension and pulsation of jugular veins, chronic general passive hyperemia or edema. (Moulton, 1961). Furthermore, sinus tachycardia and presystolic and systolic murmurs have been described. (Squire, 1964).

Protrusion of the eyes may occur due to orbital invasion by tumor cells. (Feldman, 1932; Cotchin, 1956; Moulton, 1961; Jubb and Kennedy, 1963). This is almost always due to a deposition of neoplastic cells in the retrobulbar tissue. (Jubb and Kennedy, 1963).

Sites and Gross Appearance

Lesions are present in lymph nodes in about 98% of the affected animals. (Moulton, 1961; Squire, 1964). Both parietal and visceral lymph nodes are affected. The visceral nodes, particularly the internal iliac and mesenteric lymph nodes, are involved more frequently than the parietal nodes. (Moulton, 1961). In most severe cases, tiny, unnamed lymph nodes become sizeable nodes, and the named nodes in these cases become several times larger than normal. (Runnells et al., 1960). A few animals show massive enlargement of the thymus, posterior cervical or anterior mediastinal lymph nodes, with or without other lesions. (Moulton, 1961). Some cases had a massive tumefaction of sublumbar, renal and internal iliac lymph nodes with extension into adjacent sublumbar tissues. (Monlux et al., 1956). Moulton (1961) describes the consistency of affected lymph nodes as compact or putty-like. They are either grayish-white, pale yellow or pale pink in color. The enlarging parenchyma bulges greatly when the node is cut in half. The bulging parenchyma is grayish and pulp-like in consistency. Small hemorrhages, foci of grayish-yellow caseous necrosis and areas of mixed hemorrhage and necrosis may be prominent in these nodes. (Runnells et al., 1960). The initial gross changes seen in most lymph nodes are the development of discrete, bulging, white nodules in the cortex. These enlarge and coalesce to form a structureless mass which eventually ruptures the capsule and may become confluent with other nodes to form massive neoplastic growths. (Squire, 1964).

Lymphosarcoma in the lung is due to metastases or secondary growth of the lesion from adjacent lymph nodes. (Feldman, 1932).

The wall of the abomasum is the most frequent site of involvement in the gastro-intestinal tract. Moulton (1961) recorded abomasal involvement in 90% of the cases he reviewed. There is often a marked infiltration of the submucosa with frequent involvement of the mucosa and muscularis. The abomasal wall may become markedly thickened and irregular. The mucosa usually remains intact over the neoplastic tissue but focal ulceration may occur. (Squire, 1964). When any part of the digestive tube is invaded, the process seems to spread into the wall from the serosal side. (Fincher et al., 1956; Smith, 1962). Intestinal stasis or perforating gastric ulcer may occur due to extensive neoplastic invasion. (Smith, 1962). The abomasal growth may be diffuse or nodular. The diffuse infiltration produces a more or less uniform layer of tumor in the submucosa, especially in the fundus and body. The spiral plicae are converted into thick, nonpliable folds. The nodular formations may be 5 to 6 cm. or more in diameter. Cone shaped necrotic ulcerations may develop, especially in the pyloric region. (Moulton, 1961; Jubb and Kennedy, 1963). Cross sections of the neoplastic masses have the grayish-white appearance of fat but have a firmer texture. (Jubb and Kennedy, 1963). Ulcers are probably due to ischemia resulting from obliteration of submucosal blood vessels by the tumor. (Moulton, 1961).

Involvement of the liver is usually diffuse with marked infiltration of the portal areas, imparting a distinct macroscopic pattern to the lobular architecture. (Squire, 1964). In the final stages, a more generalized infiltration produces a greatly enlarged liver with rounded edges, a taut capsule and abnormal friability. (Jubb and

Kennedy, 1963; Squire, 1964). The hepatic infiltration in some cases appears as discrete nodules. (Jubb and Kennedy, 1963).

Moulton (1961) confirmed the impression of most investigators that splenic lesions are not as frequent as lymph node or heart involvement. He found splenic involvement in only 48% of his cases. Splenic lesions may be both focal and diffuse. (Moulton, 1961). The spleen may be greatly enlarged or may be entirely normal grossly and histologically. The size of the spleen is not a criterion of its involvement, since a spleen of normal size may be free of the neoplasm or diffusely neoplastic. When a neoplastic spleen is enlarged, it is firm, and the capsule is taut and may rupture with fatal hemorrhage. (Jubb and Kennedy, 1963). Cotchin (1956) pointed out that in East Prussia most cattle with lymphosarcoma had splenic rupture. The splenic changes often commence in the lymphoid follicles, resulting in grossly apparent, raised, pale nodules. (Squire, 1964). These follicular nodules are evenly scattered through the dark splenic pulp. (Smith, 1962).

The heart is frequently involved in the bovine species. Moulton (1961) suggests that cardiac involvement occurs in 76% of recognizable cases while Marshak et al. (1962) indicate that 89% of the cattle in their investigations had lesions in the heart. Both nodular and diffuse invasion of the atrial and ventricular myocardium occurs. (Moulton, 1961). The lesion is usually confined to the base of the heart and the wall of the atria with occasional extension to the ventricles. (Smith, 1962). The right auricle is most often affected. (Jarplid, 1964). The lesions sometimes are distinct nodules of soft, homogenous, whitish tissue or are represented by narrow pale streaks in the myocardium

between the muscle bundles. (Jubb and Kennedy, 1963). Jarplid (1964) pointed out that 7 of 9 calves with lymphosarcoma did not have macroscopic cardiac lesions in his study while only 6 of 21 older cattle were free of heart involvement.

The kidney may show interstitial infiltration resulting in either diffuse enlargement or discrete masses. (Squire, 1964). Moulton (1961) reports 53% of the animals with lymphosarcoma which he studied had kidney involvement. The tumor cells invade the interstitial spaces and eventually replace the tubules. The glomeruli are the last to disappear. (Feldman, 1932). When the nodular lesions are grossly visible, they are numerous and poorly defined, although occasionally they project hemispherically above the surface. The capsule is not adherent. If the involvement is diffuse, the organ is enlarged and has a uniform, white appearance. Peripelvic and periureteral infiltration may cause hydronephrosis. (Jubb and Kennedy, 1963).

The uterus is a frequent site for the tumor. Moulton (1961) indicates that 45% of the cases in his series had uterine involvement. The uterus may be greatly enlarged, and Feldman (1932) states that the majority of tumor masses are confined to the submucosa. Smith (1962) believes that tumor nodules appear beneath the serosal surface of the uterus and spread inward toward the mucosa. Multiple uterine lesions in 6 of 20 slaughtered cattle were reported by Monlux et al. (1956).

In the peritoneum, omentum and mesenteries, the tumor is represented by focal or diffuse masses of neoplastic tissue. (Moulton, 1961; Jubb and Kennedy, 1963).

Tumors involving the eye are common. The eye may protrude from its natural position and ultimately may be markedly displaced. This

almost always is due to deposits of neoplastic tissue in the retrobulbar region of the orbit. (Jubb and Kennedy, 1963). The condition may be unilateral or bilateral. (Smith, 1962).

The tumor in the spinal cord usually surrounds the nerve trunks and may fill the canal at a given level. (Smith, 1962). The lesion is commonly located in the last lumbosacral region. (Monlux et al., 1956; Cotchin, 1956). Cotchin (1956) believes these spinal lesions originate from adjacent lymph nodes and invade through the vertebral openings.

Lymphosarcoma of the skin is described as forming a diffuse thickening or multiple discrete nodular foci. The lesions may be located either in the dermis or subcutaneous tissue. (Cotchin, 1956). The nodular lesions may form masses of various sizes. (Jubb and Kennedy, 1963).

Invasion of skeletal muscle with lymphosarcoma is described. This may be due to direct extension or by hematogenous dissemination. (Jubb and Kennedy, 1963).

Histopathology

In the lymph nodes, during the earliest stages, Squire (1964) suggests that neoplastic cells may appear either in the sinusoids or in the germinal centers. Later, large segments of the cortex and medulla are replaced by neoplastic cells, and the infiltration may eventually extend throughout the entire node.

In the heart, neoplastic cells often infiltrate between the cardiac muscle fibers and may cause atrophy and degeneration of muscle fibers. (Moulton, 1961).

In the spleen, the tumor cells replace the normal parenchyma or form focal accumulations beneath the splenic capsule. (Feldman, 1932). Neoplastic cells may penetrate the capsule and trabeculae and grow beneath the endothelium of trabecular veins. (Jubb and Kennedy, 1963).

In the kidney, tumor cells invade the interstitial spaces and eventually replace the tubules. (Feldman, 1932; Squire, 1964).

Neoplastic infiltration of the liver is usually confined initially to portal triads and especially to the adventitia of hepatic veins. (Jubb and Kennedy, 1963).

In the hollow organs, e.g., the abomasum, uterus and intestine, the infiltration usually is initially confined to the submucosa and then rather rapidly spreads to the mucosa and muscularis. (Feldman, 1932; Moulton, 1961; Squire, 1964).

Histologically, the neoplastic cells may be lymphoblastic, prolymphocytic, lymphocytic or reticulum cell types according to the description by Squire (1965).

Hematology

There are no exact erythrocyte, hematocrit or hemoglobin levels to indicate an anemic state. Schalm (1961) places the normal erythrocyte count at 5 to 10 million with the average count at approximately 7 million. The hematocrit value (packed cell volume) in his text is listed at 24-48 volume per cent and an average of 35. Hemoglobin ranges are listed at 8-14 gram per cent with an average of eleven. While other writers give higher or lower figures, it is not considered important to make too exact an interpretation when there are so many other variable

factors. He stated that anemia may be associated with lymphosarcoma but is of variable occurrence.

It is apparently impossible to predict accurately the presence or absence of leukemia from the morphology or location of the tumors. The exception may be that extensive bone marrow involvement can almost invariably be associated with leukemia. (Squire, 1964).

The leukocyte count may increase in the early stages of the disease, but a steady decline to normal or subnormal levels may occur as death approaches. (Cotchin, 1956; Moulton, 1961; Jubb and Kennedy, 1963).

In attempting to diagnose lymphoid leukemia, chief reliance should be placed on total leukocyte count per cubic millimeter and on the percentage of lymphocytes in the differential count. The presence of, or even more satisfactory, the percentage of abnormal, atypical, or immature lymphocytes is an indication of possible leukemia. (Smith, 1962; Jubb and Kennedy, 1963). The total leukocyte count may show an increase up to 200,000 or more per cubic millimeter of blood. (Smith and Jones, 1961).

Theilen et al. (1961) regarded total leukocyte counts up to 12,000 per cubic millimeter as normal for young cows and up to 8,000 cells per cubic millimeter as normal in animals 5-16 years old. The most consistent leukocyte values in cows which they designated as positive were total leukocytes of 12,000 or greater with absolute lymphocyte counts in excess of 9,000 per cubic millimeter.

Smith (1962) refers to Rosenberger's key of herd classification which places in a leukotic group all cattle having total leukocyte counts

of more than 18,000 per cubic millimeter with lymphoid cells representing 75% or more of the total white cells. Cattle with less than 10,000 total leukocytes (12,000 in animals younger than 2 years of age) and not more than 60% lymphocytes, are regarded as normal. Animals with total leukocytes and lymphocytes in the intermediate range between these two groups are classed as suspicious.

Schalm (1961) reviewed Goetze's and Bendixen's keys for classifying leukemia which are similar to that described above.

Cytology of Neoplastic Cells

Characteristics of neoplastic cell types in blood smears from leukemic cattle have been frequently described and are basically similar to the recent grouping made by Squire (1965). He grouped the cells as lymphoblastic, prolymphocytic, lymphocytic or reticulum cell types.

Generally, the lymphoblastic type is described as larger than a normal lymphocyte. Its cytoplasm appears as a thin rim around the nucleus and medium to deep blue color. The cytoplasm may appear more abundant in some cells with eccentric nuclei. Occasionally, there are small vacuoles in the cytoplasm. The nucleus is generally spherical, although there are some irregular shapes. The chromatin exists as fine, evenly distributed particles with no areas of clumping. Pale blue nucleoli are usually large and easily seen.

The neoplastic prolymphocytic type cells are usually not as large as the lymphoblastic type. The nuclear chromatin pattern is irregular. In some areas it remains fine with chromatin-parachromatin distinction. In others, it is clumped or coarse. Generally, the neoplastic cells

have both light and dark areas of chromatin. The nuclear membrane is coarse in the cells having pronounced clumping of chromatin, which is usually seen in the more mature cells.

Neoplastic cells of the lymphocytic type are usually spherical in shape and uniform in size. The cells are smaller than the other types and usually are only slightly larger or near the average normal lymphocyte size. The cytoplasm is scant. The nuclear chromatin is completely clumped and no pale area of chromatin-parachromatin distinction remain.

The reticulum cell type is large like the prolymphocytic type and has a large spherical nucleus. The nuclear chromatin exists as coarse, unevenly divided, violet particles which are distinct from the colorless parachromatin spaces. Nucleoli are pale blue spheres embedded in a chromatin-parachromatin network. The cytoplasm is pale staining, is scant and sometimes forms syncytial processes.

Bijlenga et al. (1962) found that acridine orange fluorescent staining of peripheral blood cells seems to be a promising procedure for a rapid diagnosis of lymphosarcoma in cattle. The cytoplasm and nucleolus of neoplastic cells show a flaming orange-red fluorescence while the nucleus has a yellow-green fluorescence. Increased fluorescence in neoplastic cells is due to increased ribonucleic acid (RNA) in the cytoplasm and increased desoxyribonucleic acid (DNA) in the nucleus. The marked increase in fluorescence of the cytoplasm was readily detected in neoplastic cells. A slight increase in fluorescence was seen in the cytoplasm of an occasional cell but could not be associated with neoplastic transformation.

Brock (1965) indicated that increased fluorescence of the cytoplasm of included cells were not seen in a survey of blood smears from

approximately 100 normal and diseased cattle. Neoplastic diseases were not represented in the group but many of the more common infections and metabolic diseases of cattle were compared to the apparently normal smears. Diseases were included in which hyperplastic lymph nodes would be prominent. Increases in fluorescence of the nuclei of cells were difficult to measure and were not used in the estimations.

CHAPTER III

MATERIALS AND METHODS

A total of 7 cows with lymphosarcoma were observed in this study. They were cattle presented for diagnosis or treatment at the Oklahoma State University Veterinary College during the period from August 4, 1964, to February 2, 1965 and represent the first 7 cases in Table I (M5067 through M5387). Data and preparations were also included from 11 selected cases in cattle studied at the College during the period from 1957 to 1963 which were adequately documented by hematologic and necropsy observations to be of comparative value (cases M1940 through M4491 in Table I). It was possible to compare 18 animals in this study as the procedures used in the preparation of specimens were basically the same as those outlined in this chapter for the 7 animals.

Hematology

A 10 ml. syringe with a size 15 gauge needle was used in taking samples of blood from the jugular vein of the 7 animals. Blood was immediately transferred into 10 ml. test tubes containing EDTA[1] anticoagulant. Blood films for differential counts were stained with

---

[1]EDTA is the abbreviation for the disodium salt of ethylenediamine tetracetic acid.

TABLE I

DISTRIBUTION OF MACROSCOPIC LESIONS OF LYMPHOSARCOMA
IN 18 CATTLE

Animal	Age in Years	Sex	Lymph Nodes	Abomasum	Heart	Liver	Spleen	Uterus	Kidney	Retro-bulbar	Lung	Bone Marrow	Vertebral Canal	Intestine
M5387	6	F	+	+	+	+	+	+	+	+	+	+		+
M5325	10 months	M	+				+				+	+	+	
M5275	7	F	+	+	+	+			+	+				
M5240	6	F	+	+	+		+	+			+	+		+
M5220	6	F	+	+	+		+			+	+			
M5134	11	F	+	+	+		+	+		+		+		
M5067	5-6	F	+	+	+		+					+		
M4491	4	F	+	+	+									
M4094	8	F	+					+				+	+	
M3811	7	F	+	+	+			+						
M3500	2	F	+	+				+	+					
M3240	1	F	+	+	+						+			
M3168	2	M	+	+										+
M2839	2	F	+			+								
M2750	7	F	+	+	+			+						
M2667	5	F	+	+	+									
M2449	7-8	F	+	+	+	+	+	+	+			+		
M1940	7	F	+	+	+	+			+		+			+
Total			18	15	13	5	7	8	5	4	6	7	2	4

Wright's stain. The count was made using high-dry and oil immersion objectives of the microscope. The various types of leukocytes were expressed as a percentage of the first 100 white cells encountered.

The total leukocyte and erythrocyte counts were made according to standard procedure. The diluting fluid for the leukocyte counts was composed of 1.0 ml. glacial acetic acid, 100 ml. distilled water and 1 ml. of 1% Gentian Violet. Gower's solution (12.5 gm anhydrous sodium sulfate, 33.3 ml. glacial acetic acid and 200 ml. distilled water) was used as the diluting fluid for the erythrocyte counts. A standard bright-line hemocytometer was used to make the cell counts.

The Spencer hemoglobinometer was used for determination of hemoglobin values. Grams of hemoglobin per 100 ml. of blood was read directly from the graduated scale of the instrument.

Hematocrit values were determined by the microcapillary tube method. A capillary tube was filled with blood to approximately two-thirds of its capacity, sealed at one end and centrifuged at 12,000 rpm for 5 minutes. The percentage of packed cells was obtained by using a microcapillary hematocrit tube reader.

Acridine Orange Fluorescent Staining

Fluorescence microscopy was used to compare neoplastic leukemic lymphocytes and normal lymphocytes in both blood and fixed tissue. The technique for fluorescent staining entailed the use of acridine orange and followed most closely the procedures outlined by Bertalanffy *et al.* (1956). Two kinds of blood films were made; conventional whole blood films and buffy coat blood films. The latter was prepared by filling Wintrobe sedimentation tubes with blood and centrifuging for 10 minutes.

Plasma was removed after centrifugation without disturbing the buffy coat. With a Pasteur pipette, a portion of the buffy coat was removed. One drop was transferred to a slide, and a thick smear was made. After drying, the smears and films were fixed in a mixture of equal parts 95% ethyl alcohol and 95% diethyl ether for 1 hour and then hydrated by dipping into 80%, 70% and 50% alcohol and distilled water. After hydration, the specimens were rinsed 5 times in 1% acetic acid and 5 times in distilled water. The smears were stained in acridine orange[1] (0.01% acridine orange in 1/15 M phosphate buffer; pH 6.0) for 3 minutes. The stained smears were rinsed 1 minute in the above buffer (5 Na_2HPO_4 and 45 KH_2PO_4 adusted to obtain a pH of 6.0 and checked with a pH meter before use). After that, they were differentiated with 0.1 M $CaCl_2$ (2.78 gms/500 ml. of H_2O) for 30 seconds. Excess dye was removed by washing in the above described phosphate buffer. A cover-glass was placed over the stained blood film using the phosphate buffer as mounting medium. The films were then examined with a fluorescence microscope. The only advantage of the buffy coat film is that it allows a concentration of a greater number of leukocytes when their number is minimal in the circulating blood. The fluorescent light was a HBO 200 mercury arc lamp with a dark field condenser. The exciter filter was a Zeiss BG12/4 which transmitted between 330 to 500 mμ with a maximum of 400 mμ. The same staining procedure was performed on paraffin

---

[1]The acridine orange stain was obtained from the National Aniline Division, Allied Chemical and Dye Company, 40 Rector Street, New York, N. Y., Catalogue No. 408.

embedded tissue except that xylol was used to remove excess paraffin, and then the procedure preparatory to staining was instituted by transferring to 80% alcohol.

Postmortem Examination

Postmortem examinations were performed on the 7 cows observed in this study in the pathology necropsy room. Lesions and tissues of particular interest were fixed in 10% buffered formalin solution. These tissues were allowed to fix for a minimum of 36-48 hours and then embedded in paraffin. Sections were cut at 5 microns thickness and stained with hematoxylin and eosin stain.

CHAPTER IV

RESULTS AND DISCUSSION

Clinical Observations

Elevations in body temperature to levels as high as 104° F were present in 4 animals. Signs referable to disturbance in the cardiovascular system were present in several animals. Pulse rates of 80, 96, and 120 were recorded in 3 animals, distention of the jugular veins was observed in 4 animals and edema of the brisket was present in three.

Anorexia was noted in 8 of the 18 patients. Three animals were bloated, 2 intermittently and 1 persistently for 4 weeks. One animal had a severe diarrhea at terminus.

Enlargement of superficial lymph nodes was present in 3 cows, and nodes in the sublumbar region were palpably enlarged in five. Protrusion of the eyeball was observed in 4 patients.

Four of the 18 patients in the study were presented for examination because of posterior paresis. One animal had a cough which had been present for several weeks. The visible mucous membranes of 5 were pale.

Postmortem Observations

External Examination. The external examination showed that 6 animals were in poor nutritional condition, 4 were in fair nutritional condition and 6 were in good nutritional condition. One animal had a

grayish-brown, mucoid discharge from the vagina, and extensive vaginal tumors were found at necropsy.

Internal Findings. The subcutaneous fat in 10 animals was represented by moist, gelatinous and edematous tissue which was yellow to yellowish-brown in color. This appeared to be related to the poor nutritional state of the animals. Body fat was minimal to moderate in amount in 9 animals. Ten animals had moderate to excessive, clear to cloudy fluid in the peritoneal cavity. Excessive, red tinged fluid was present in the pleural cavities of 5 animals, and hydropericardium was noted in 5 animals. Cardiac involvement was present in each of the latter 5 and probably contributed to the occurrence of the transudate. Fibrous adhesions between the liver and adjacent structures were present in 3 animals. Nodules and more diffuse, plaquelike masses of neoplastic tissue were present in the omentum of 4 animals. Nine animals had enlarged prescapular and prefemoral lymph nodes, and 7 had enlarged supramammary nodes. On cut surface the nodes were pale gray in color and lacked the usual architectural division and detail of normal structures. Similar enlargements were noted in the mesenteric lymph nodes of 8 animals. The mesentery was tumefied in 4 animals, and 3 animals had neoplastic involvement of the diaphragm. In 2 animals the diaphragmatic masses were pale, firm plaques of tissue just beneath the peritoneal membrane extending into the diaphragmatic muscle. In the other animal, the tumor was located entirely within the musculature. Growth of comparable nature was present beneath the costal pleura of 3 animals. The sublumbar lymph nodes were involved in 6 cases.

Heart. Thirteen animals had cardiac lesions. In 7 of them the lesions

were encountered in each of the cardiac chambers. The lesions were grayish, raised, nodular masses or linear streaks or both. The nodules and streaks were visible through the epicardial surface and sometimes extended throughout the myocardium to the endocardium. The streaks were sometimes slightly raised areas about 1 to 2 cm. in length on the epicardium. Four animals had lesions in the atria. In 3 of these, the lesions extended to the left ventricle. One animal had lesions in the right atrium which extended down to the right ventricle. In one case, the lesions were found only in the ventricles. It was observed that the heart lesions were found in only 1 of 5 animals under 4 years of age. In this case, lesions were limited to the ventricles.

Urinary Tract. Renal lesions, recognized in 5 animals, were scattered in the cortex and in some instances extended to the medulla. The lesions ranged from 1 mm. to 5 cm. in diameter. Two animals had involvement of the right ureter; in 1 the lesion was 5 cm. in diameter. Involvement of the bladder was noted in 2 animals. The lesions extended through the wall of the bladder.

Genital Organs. Eight animals had neoplastic lesions in various segments of the genital tract. Lesions in the uterine wall were both diffuse and nodular and were seen in all 8 animals. Two animals had diffuse lesions, 1 had diffuse and nodular lesions, and 5 had nodular lesions only. The lesions extended through the uterine walls. On cut surfaces the growths were yellowish-gray, of homogeneous texture and soft in consistency. The thickness of the nodular uterine lesions ranged from 3 to 15 cm. In 1 case, neoplastic growths were observed in the ovary. It was enlarged and whitish in color. On cut surface,

ovarian tissue was replaced by soft, grayish tissue. The fallopian tube was affected in 1 animal. The vagina was affected in 3 animals. In each, its wall was thickened and diffusely infiltrated with neoplastic tissue. In 1 animal, the cervix contained a lesion about 3 cm. in diameter in the submucosa.

Liver and Gall Bladder. Four of 5 animals with hepatic involvement had scattered focal lesions in the parenchyma of the liver. These livers were only slightly enlarged. In the other case, diffuse involvement existed, and the liver was approximately 60 cm. long, 32 cm. wide and 9 cm. thick. The gall bladder was affected in 2 animals. The wall was thickened and contained grayish, plaquelike masses.

Spleen. Seven animals had scattered, nodular lesions in the spleen. The spleens were slightly to markedly enlarged. The cut surface of the spleens bulged and exhibited a dark red colored parenchyma with firm, nodular, neoplastic growths.

Lungs. The lungs were affected in 6 animals. Four animals had dark red, doughy infiltrations in the lung while 2 had nodular lesions scattered in the parenchyma. On cut surface, the nodules were represented by yellowish, cheesy centers surrounded by firm, white capsules.

Gastrointestinal Tract. Two animals had lesions in the rumen, 3 in the reticulum, 15 in the abomasum and 4 in the intestine. The involved wall of each affected rumen and reticulum was thickened and firm. On cut surface, whitish-yellow, homogenous tissue was seen. The abomasal walls were greatly thickened in 10 of the 15 affected cattle. The thickness was up to 10 cm. and was represented by deep infiltration of neoplastic tissue throughout the wall and submucosa of the abomasum.

In 6 animals, the lesions were limited to the pyloric region of the abomasum and in 1 to the fundic region. Ulceration of the abomasal mucosa was observed in 3 animals. On cut surface, the abomasal lesions were soft, grayish-yellow, homogenous masses.

Bone Marrow. In each of the 7 animals necropsied by the writer, tissue sections of femoral bone marrow were made. Neoplastic cells were observed in 5 of the 7 cases. There was a record of examination of the marrow of only 2 of the other 11 animals, and in both of these, neoplastic cells were found (M4094 and M2449). These findings suggest that bone marrow involvement is frequent in at least the terminal stages of bovine leukemia.

It was mentioned in the clinical observations that 4 animals had posterior paresis. In 2 of the 4, the vertebral canal contained neoplastic growth. Invasion of the canal in 1 of the former, a 10 month old male Hereford, appeared to extend from the neoplastic growth within the body of the underlying vertebra. The body of vertebra T-6 was fractured and contained a reddish, friable material in the marrow area. Vertebra T-9 contained a smaller lesion, about 3 cm. in diameter, which extended through the bone into the right lateral portion of the vertebral canal. (Fig. 1). Infiltration of neoplastic cells was noted also in the marrow and cortex of the right femur and tibia indicating extensive marrow involvement in this case. While the bone marrow was found to be involved in most of the recently acquired cases, this is the only one in which a gross lesion was recognizable.

In summarizing the necropsy findings, Table I shows that 18 animals had lesions in 1 or more lymph nodes. The abomasum was

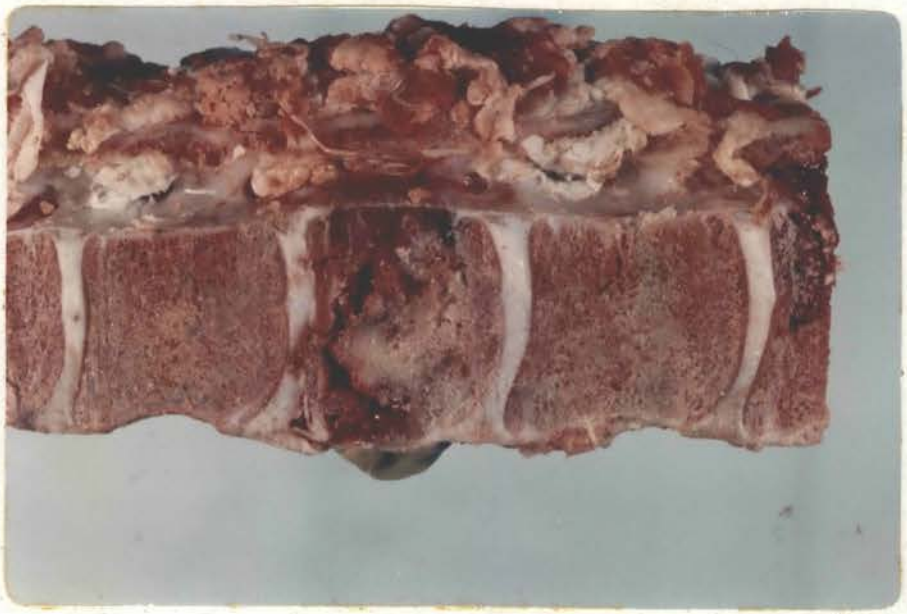

Fig. 1 Midsagittal section of thoracic vertebrae. Section of the vertebra T-9 is fractured and contains a reddish friable growth in the spongy portion of the bone. M5325.

involved in 15 animals, the heart in 13, the liver in 5, the spleen in 7, the uterus in 8, the kidney in 5, the retrobulbar tissue of the eye in 4, the lung in 5, the bone marrow in 7, the vertebral canal in 2 and the intestine in four. From these data, it became clear that the lymph nodes, the abomasum and the heart were most frequently involved. These findings are in agreement with those of other investigators.

It was of interest, further, that only 1 of the 5 animals less than 4 years old included in this study had lesions involving the heart, and in this one the atria were spared. If cardiac involvement is infrequent in the young animal, it could be a useful criterion in differential diagnosis.

Microscopic aggregates of neoplastic cells were present in the bone marrow in a high percentage of animals in the terminal stages of leukemia.

Age

Animals 5 to 8 years of age were most prone to develop lymphosarcoma in the cases studied. Five animals were 1-2 years of age, 1 was 4, 10 were 5 to 8, and 2 were 8 or older (Table I). The age range of 5-8 years is in agreement with most of the published reports. However, cases do appear in young animals and occasionally in aged animals, and this is indicated in the cases upon which this study is based.

Hematology

Table II lists the erythrocyte counts, hematocrit values and recorded hemoglobin for the 17 cattle compared in this survey. Using

TABLE II

TERMINAL ANEMIA ASSOCIATED WITH LEUKEMIA
IN 17 CATTLE

Animal	Hemoglobin (gm/100 ml)	Hematocrit (Vol. %)	Erythrocyte	Dates of Hemograms	Bone Marrow Lesion	Date of Necropsy
M5387	8.0	28.0	----	2-2-65	+	2-8-65
	8.5	28.5	----	2-5-65		
	8.0	24.5	----	2-6-65		
M5275	12.0	31.0	5,800,000	12-5-64		12-5-64
M5240	7.5	22.5	4,990,000	11-14-64	+	11-21-64
	6.5	19.5	3,800,000	11-16-64		
	7.0	22.5	4,250,000	11-17-64		
	7.0	21.5	4,280,000	11-20-64		
M5220	8.5	24.0	----	11-3-64		11-8-64
	6.5	20.0	3,740,000	11-7-64		
M5134	5.0	15.5	3,190,000	9-14-64	+	9-16-64
M5067	5.5	19.0	2,320,000	8-4-64	+	8-10-64
	5.0	16.0	----	8-5-64		
	5.0	16.0	2,570,000	8-6-64		
	4.0	13.0	2,160,000	8-8-64		
M4491	8.5	25.0	----	10-30-63		11-4-63
	7.0	20.0	2,970,000	11-1-63		
M4094	12.0	33.0	5,550,000	2-26-63	+	3-16-63
M3811	9.2	21.0	3,670,000	10-27-62		11-1-62
M3500	8.0	26.0	5,120,000	5-21-62		5-26-62
M3240	11.5	33.0	7,800,000	1-20-62		2-13-62
	10.0	32.5	7,350,000	1-27-62		
M3168	11.0	32.0	6,650,000	1-8-62		1-9-62
M2839	8.5	26.0	6,420,000	6-29-61		7-10-61
M2750	10.0	31.0	5,800,000	5-14-61		5-16-61
M2667	8.0	22.0	6,000,000	3-31-61		4-1-61
	8.0	22.0	4,850,000	4-1-61		
M2449	7.5	22.0	6,100,000	12-14-60	+	12-22-60
	7.0	18.0	3,870,000	12-22-60		
M1940	7.0	19.0	4,100,000	1-25-60		2-9-60

the values suggested by Schalm (1961), it would be concluded that 8 were definitely anemic, 5 were borderline (M3500, M2750, M1940, M4094 and M5387), and 4 (M3240, M3168, M2839 and M5275) were not anemic. These findings are consistent with the observations that some animals with lymphosarcoma will maintain normal erythrocyte values. Five animals developed anemia associated with the known infiltration of bone marrow by neoplastic cells. If marrow involvement was extensive, there could be a direct relation to the anemia. Anemia could be associated with the presence of internal bleeding such as could result from abomasal ulcers noted in 3 of our cases. Other causes of anemia in bovine lymphosarcoma in a nonleukemic or leukemic state are not completely understood, and these include relation to an autoimmune hemolytic process. Anemia was seldom seen in the younger animals under 4 years of age.

The only reliable confirming indication of leukemia in all of the 17 animals in which blood smears and tissue sections were available (Table III) was the presence of atypical, neoplastic lymphocytes. In 1 calf, the smears were misplaced. Total leukocyte and differential counts of the leukemic animals were made to determine the relationship between the percentage of lymphocytes and the total leukocyte counts of peripheral blood in the terminal stage of the disease. A second differential count was performed on 7 animals in this study. A third and fourth count was made on 3 and 2 animals respectively. (Table IV). Since only a few days separated most of these counts, the results were quite informative. In the terminal stages of the disease immediately before death, the leukocyte count decreased rather consistently and

TABLE III

LAST RECORDED LEUKOCYTE COUNT COMPARED WITH TYPE OF NEOPLASTIC CELLS IN SMEAR AND TISSUE SECTION IN 17 CATTLE

Animal	Age Years	Lymphocyte %	Leukocyte Count/ Cu. mm.	Blood Smear			Tissue Section		
				blast	prolymph	lymphocyte	blast	prolympho-cytic	lymphocytic
M5387	6	98	172,000	+			+		
M5275	7	56	10,400	+			+	+	
M5240	6	25	7,900	+			+	+	
M5220	6	89	108,500	+	+		+	+	
M5134	11	49	3,550		+	+		+	+
M5067	5-6	82	8,700	+	+		+	+	
M4491	4	97	68,000	+	+		+	+	
M4094	8	45	6,200		+			+	+
M3811	7	97	90,000		+	+		+	+
M3500	2	70	13,250		+			+	
M3240	1	90	8,750		+			+	
M3168	2	38	9,600		+			+	
M2839	2	69	7,600	+	+		+	+	
M2750	7	60	16,000	+			+		
M2667	5	93	43,000	+			+		
M2449	7-8	98	230,000	+	+	+	+	+	+
M1940	7	41	10,300	+			+		

TABLE IV

COMPARISON OF THE TOTAL LEUKOCYTE COUNT AND PERCENTAGE OF LYMPHOCYTES IN 7 CATTLE*

Animal	Age (yrs.)	Date	Lymphocyte %	Leukocyte Count per mm^3
M5387	6	Feb.-2-1965	98	171,400
		Feb.-5-1965	96	191,700
		Feb.-6-1965	98	172,000
M5240	7	Nov.-14-1964	9	15,300
		Nov.-16-1964	14	8,100
		Nov.-17-1964	15	7,550
		Nov.-20-1964	25	7,900
M5220	6	Nov.-3-1964	97	115,950
		Nov.-7-1964	89	108,500
M5067	5-6	Aug.-4-1964	77	13,800
		Aug.-5-1964	62	28,900
		Aug.-6-1964	78	17,850
		Aug.-8-1964	82	8,700
M4491	4	Oct.-30-1964	95	69,000
		Nov.-1-1964	97	68,000
M3240	1	Jan.-20-1962	81	16,300
		Jan.-27-1962	90	8,750
M2449	7-8	Dec.-14-1960	96	292,500
		Dec.-22-1960	98	230,000

*Data is given on cases with more than one recorded differential count.

sometimes very markedly. The percentage of lymphocytes increased during this stage, but their absolute number decreased with the decrease in total number of leukocytes. (Table IV). The decrease in leukocytes may be due partially to bone marrow involvement or it may be entirely associated with approaching death. Study of additional cases would be helpful in clarifying this point. A noticeable increase in neoplastic cells in blood smears during the days immediately prior to death typified the majority of the cases.

The neoplastic cells which appeared in the blood films were of lymphoblastic, prolymphocytic and lymphocytic types. In no case was the reticulum cell type encountered.

Cytology

In the 17 animals in which both blood smears and tissue sections were available (Table III), 4 had mixed lymphoblastic-prolymphocytic cell types, 6 had lymphoblastic cell types, 4 had prolymphocytic cell types, and 2 had mixed prolymphocytic-lymphocytic cell types and 1 had a mixture of all 3 types in the blood smears examined. It was observed that when the leukocyte count was very high, the lymphoblastic type was the neoplastic cell identified in the blood.

In this study, it was found that the animals which had lymphoblastic or prolymphocytic type cells in blood smears generally had the same type of cells in the tissue sections. However, 2 cases possessing lymphoblastic type cells in peripheral blood had prolymphocytic cells as well in tissue sections, and 1 case characterized by prolymphocytic cells in blood smears had lymphocytic cells, as well, in tissue sections.

Mixed lesions of lymphoblastic-prolymphocytic and prolymphocytic-lymphocytic type cells were seen also in the same animals which had the mixture in the blood smears. (Table III).

The lymphoblastic type cells in the fresh blood smears which were taken from leukemic animals were large (the nuclei measuring up to 10 or 12 μ in diameter). The chromatin was delicate and appeared as finely divided particles with no area of clumping. The parachromatin was uniform and was represented as colorless spaces between the chromatin particles. The cytoplasm usually formed a distinct band around the nuclei and appeared weakly basophilic and sometimes vacuolated. The nuclei were round, oval, vesiculated or irregularly shaped. One to three large, pale blue nucleoli were prominent in many of the nuclei. (Fig. 2). Mitotic figures were seen rather frequently. (Fig. 2).

The prolymphocytic type cells were variable in size, ranging from that of normal large lymphocytes to that of the smaller lymphoblastic cell types (5-9 μ in diameter). Their cytoplasm was less abundant than that in the lymphoblastic type and was pale and basophilic. The nuclei were spherical, oval or bizarre in shape and had clumped areas of chromatin. (Fig. 3). The nucleus generally presented 1 or more ringlike structures representing remnants of nucleoli.

The lymphocytic type cells were within the size range of normal lymphocytes. The nuclei were usually spherical, and the chromatin was heavily clumped. Areas of chromatin-parachromatin were not distinct. Nucleoli were not seen in these cells, and the cytoplasm was minimal.

The cells in tissue sections had a slightly different appearance than those in blood smears. The cell types in the tissue sections were generally smaller than the cells in the blood smears, and the

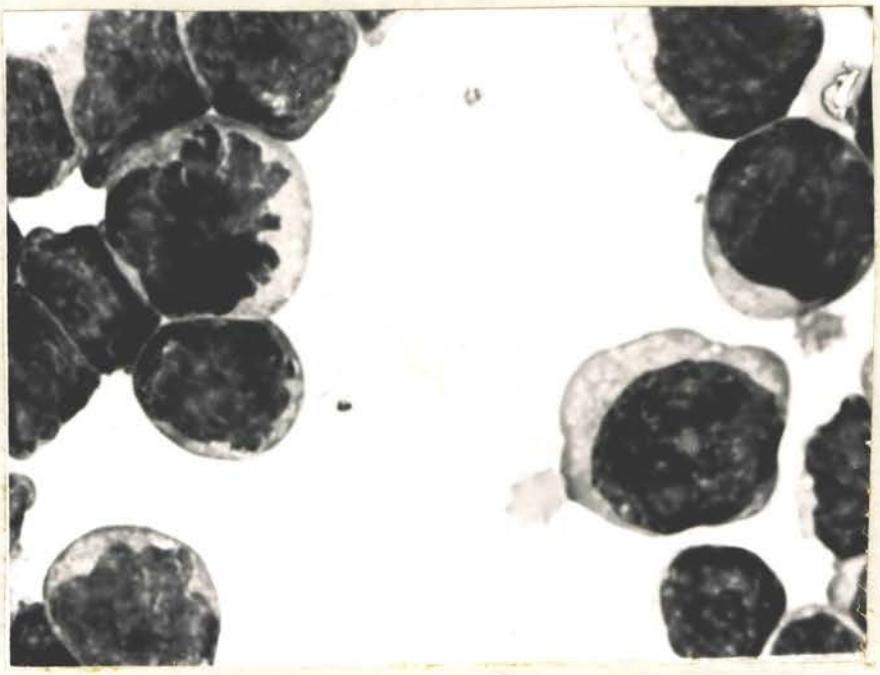

Fig. 2 Buffy coat smear of peripheral blood from a case of lymphosarcoma in cow M5387; pale nucleoli and mitotic figures are noted in this group of cells of the lymphoblastic type. Wright's stain. Magnification 400x.

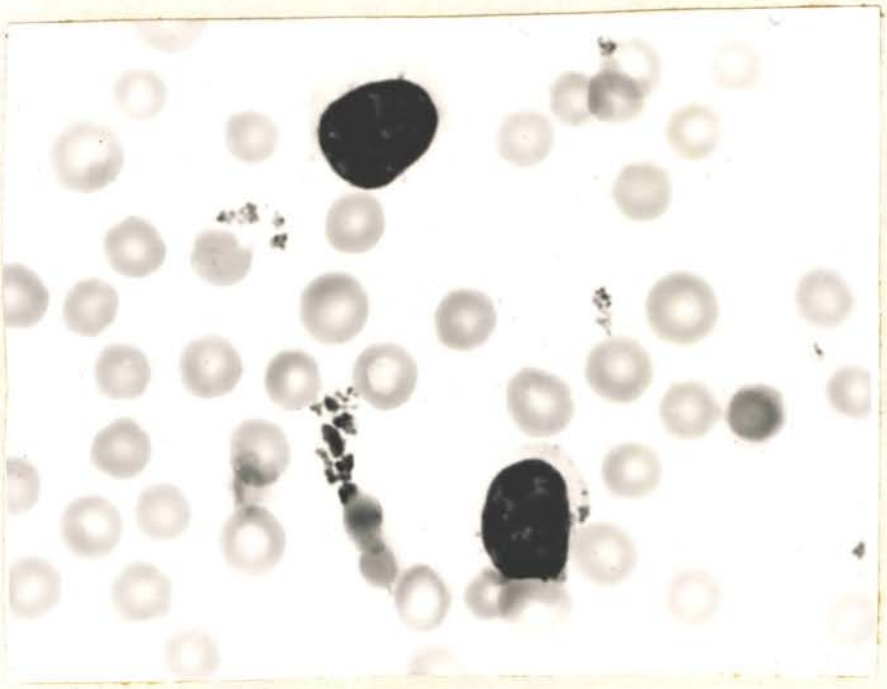

Fig. 3 Peripheral blood smear from cow M5134 with lymphosarcoma. Notice the clumping of chromatin in nuclei in these two neoplastic cells of the prolymphocytic type. Wright's Stain. Magnification 400x.

cytoplasm was more meager and prone to take a more basophilic stain. Nuclear staining was similar in both the blood smears and tissue sections.

The lymphoblastic type cells in the tissue section were relatively large in comparison to the other types of neoplastic cells. The nuclei were large and rounded, vesiculated or indented. The nuclear membranes were prominent and deeply stained. The nucleoli were obvious and large. (Fig. 4). Frequently, mitotic figures were numerous in a section.

The prolymphocytic type cells were smaller than the lymphoblastic. Nucleoli were either not recognizable or barely discernable (Fig. 5).

The lymphocytic type cells were small and uniform. The cytoplasm was scanty. The nuclei were round in shape with uniform distribution of chromatin.

Acridine Orange Staining

Acridine orange staining was used in order to distinguish between lymphocytes from leukemic animals, apparently normal animals, and a group of animals afflicted with non-neoplastic diseases.

In the examination of blood smears from 4 leukemic animals in the group (5220, 5240, 5275, 5387), it was observed that neoplastic cells of lymphoid origin had pale green nuclei whereas the cytoplasm and nucleoli had a flaming orange-red fluorescence. The fluorescence is believed due to higher concentrations of RNA in the cytoplasm and nucleoli and of DNA in the nuclei. (Fig. 6). Unfortunately, only 4 animals were admitted for study while the stain was available, but these positive findings were consistent with previously reported studies. Controls were not conducted on the blood smears, as Brock

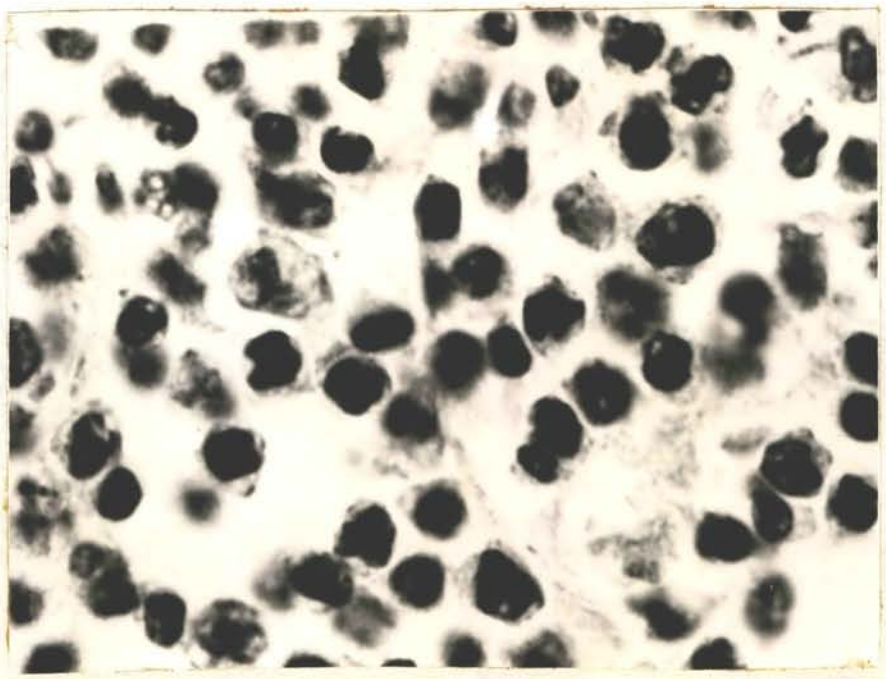

Fig. 4 Tissue section of lymphosarcoma of a lymph node; note the many nucleoli in the lymphoblastic type cells. Hematoxylin and Eosin Stain. M5387. Magnification 400x.

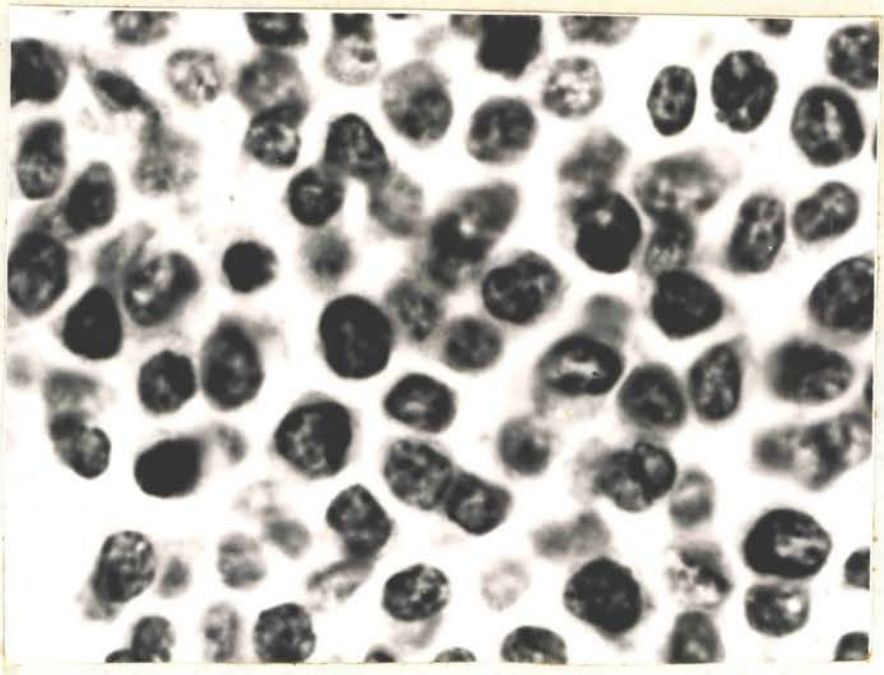

Fig. 5 Tissue section of lymphosarcoma of a lymph node in cow M5134. Most of these cells are neoplastic and of the prolymphocytic type. Hematoxylin and Eosin Stain. Magnification 400x.

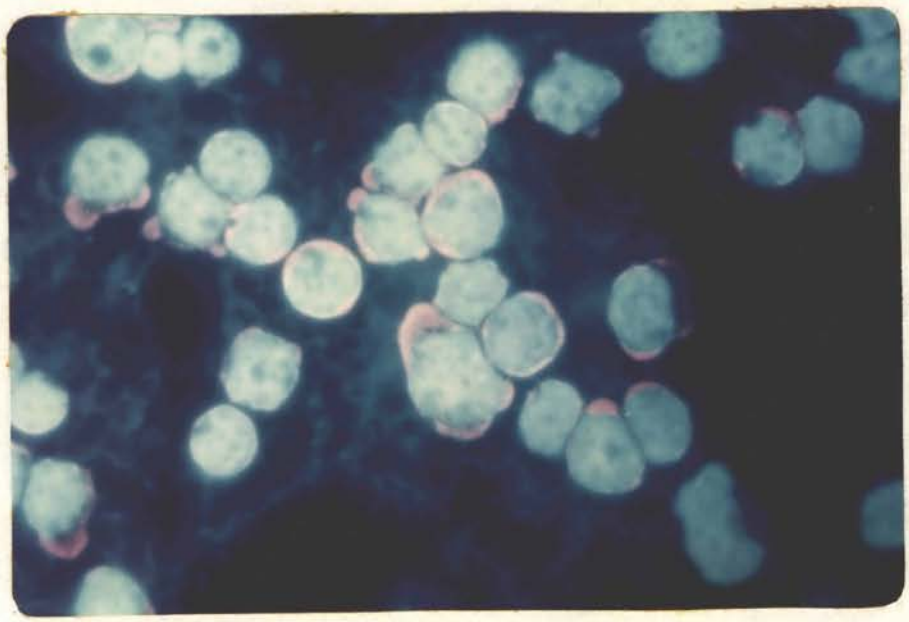

Fig. 6 Neoplastic cells from buffy coat smear of peripheral blood. A flaming orange-red fluorescence of cytoplasm and a pale green fluorescence of the nuclei are seen. Acridine Orange staining. M5387. Magnification 256x.

(1965) had recently completed his survey in this same laboratory on acridine orange stained smears from approximately 100 cattle which were apparently normal or afflicted with non-neoplastic diseases. These smears had not shown increased fluorescence.

Acridine orange stains were also carried out on tissues from normal and hyperplastic lymph nodes. The hyperplastic lymph nodes were obtained from cases of pneumonia, parasitism and septicemia. It was observed that lymphocytes from normal lymph nodes had no apparent fluorescence in the cytoplasm and only slightly fluorescent yellowish-green nuclei, whereas the lymphocytes in the tissue from hyperplastic lymph nodes had the same characteristics as neoplastic lymphocytes. (Fig. 7). The increased fluorescence in neoplastic and hyperplastic cells would support the previously reviewed paper where it was indicated that it was related to an increase of RNA in the cytoplasm and DNA in the nucleus. The similarity in fluorescent properties of hyperplastic and neoplastic cells considerably limits the value of acridine orange in the diagnosis of lymphosarcoma.

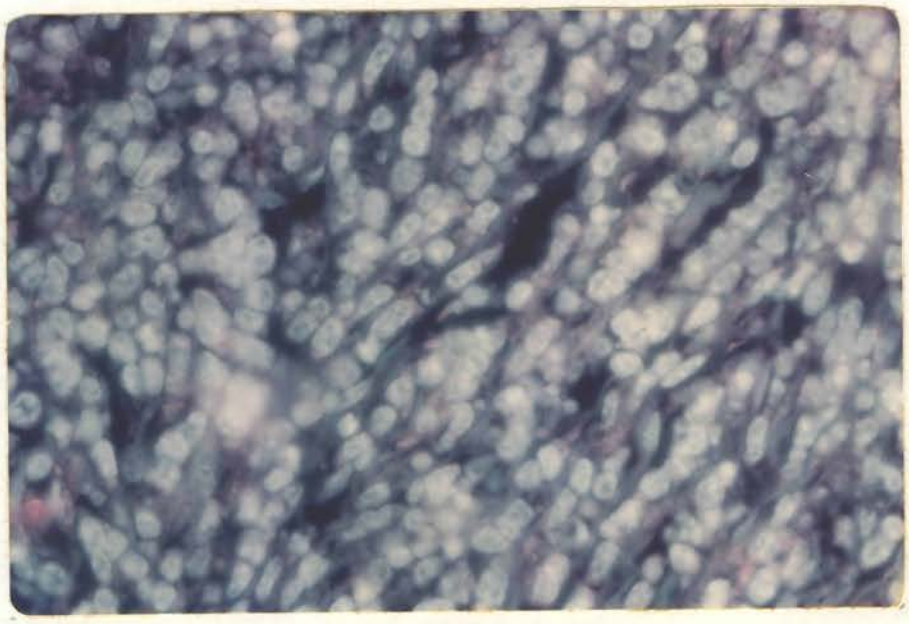

Fig. 7 Neoplastic cells in tissue section of a lymph node of a leukemic animal. Red fluorescence of cytoplasm and pale green nuclei are seen. Acridine Orange staining. M5387. Magnification 160x.

CHAPTER V

CONCLUSIONS AND SUMMARY

A study was conducted on the manifestations of the terminal stage of bovine lymphosarcoma. In addition to 7 animals presented for observation and eventual necropsy during the period of the study, there were 11 cases in the files of the Department of Veterinary Pathology which were adequately documented so that their data could be used.

In 17 of the 18 animals, at least 1 blood smear was made before death. While all of these cattle were visibly ill at the time of presentation, it was surprising that neoplastic cells were identifiable in all of the smears.

In 4 of these animals, acridine orange stains of the smears were made, and the neoplastic cells were easily identified by the increased affinity of their cytoplasm for the stain. It apparently is unusual to find this acridine positive staining in blood smears except in neoplastic diseases such as the leukemia described in this study. Neoplastic lesions in lymph nodes could be stained with acridine orange from tissue sections, after the death of the animal, and they also showed increased cytoplasmic affinity for the stain. However, hyperplastic lymph nodes such as are seen in parasitic and infectious diseases also reacted positively to the stain. This ruled out the possibility of the acridine orange stain being very helpful in

confirming a diagnosis of lymphosarcoma in a suspicious lymph node lesion as the difficult distinction is between hyperplastic and neoplastic lymph nodes.

The recognition of leukemia in all of the 17 animals indicates that, perhaps, most cattle with lymphosarcoma will show a persistent leukemia before death. While few cattle would show a leukemia in the early stages, it may be a rather consistent finding when symptoms such are described in this study become prominent. The acridine orange stain should be investigated further to establish its true value in identifying suspicious cells or screening smears.

Association of bovine lymphosarcoma with cows 5-8 years of age in 10 of the 18 cattle followed the findings of other investigators. There were 8 cases in younger and older cattle to support published observations that the disease may appear at any age and in both sexes. The lack of macroscopic involvement of the base of the heart with neoplastic growths in the 5 animals under 4 years of age was of interest; particularly because 12 of the 13 older cattle had lesions in this area. If true in a larger group of young animals, it would lead one to expect not to find clinical signs of cardiac involvement in the majority of young animals in which the disease was suspected. Other reports that were reviewed did not show a consistent relation although more cases of cardiac involvement were noted in the older group and fewest in calves. A conclusion that calves, but not cattle over 2 years with the disease frequently do not show the cardiac involvement is probably more correct. The presence of lesions in both ventricles of the heart of one of these 5 animals illustrate that some calves would have

cardiac involvement and perhaps detectable signs of disease. In contrast to the late appearance of cardiac lesions, the abomasal lesions appeared in 3 of 5 of the young animals and probably are not so late in appearing. Anemia was associated with most of the cases of lymphosarcoma in older cattle but did not appear in more than 1 (questionable) in the 4 animals under 4 years of age. The failure of most calves to develop anemia in the terminal stage also would be important in a differential diagnosis.

Lesions were too scattered in various anatomic sites to make any but the above conclusions in regard to relation of symptoms to heart and abomasal lesions. Lesions were found most frequently in the abomasum and heart if the lymph node growths which were found in every case are not considered. If there are true metastases rather than further appearances of lesions at multicentric points of origin in this terminal stage, there does not appear to be a consistent pattern to their location. The great variety of clinical signs relating to the location of lesions support this observation.

The cytologic grouping of the neoplastic lymphoid cells in both the smears and tissue sections into prolymphocytic, lymphoblastic and lymphocytic types has some value in identifying questionable cells. These types of cell had no readily apparent association with symptoms or lesions seen in the study. If a cell type appeared in the blood smear, it also was found in the tissue sections except in some of the cases where mixed types were present. Lymphoblastic types of leukemia were more apt to have a higher leukocyte count than the other types, but this was not a consistent finding.

In several cases, it was noted that the total leukocyte count decreased to normal or subnormal levels in the days immediately prior to death even though the percentage of lymphocyte increased. A total leukocyte count by itself would be of questionable value in diagnosis in such cases unless it was significantly elevated. The number of neoplastic cells increased noticeably in the later smears in several of these cases in the days immediately prior to death.

SELECTED BIBLIOGRAPHY

Bertalanffy, L. V., F. Masin, and M. Masin. 1956. Use of Acridine-Orange Technique in Exfoliative Cytology. Science, 124: 1024-1025.

Bijlenga, G., D. Cohen, L. Ferrigan, and K. P. Altera. 1962. The Use of Acridine Orange Fluorescent Staining of Peripheral Blood Cells as a Rapid Method for the Detection of Abnormal Proliferation of Haematopoietic Tissues. Bull. World Health Org., 26: 688-692.

Brock, W. E. 1965. Personal Communication.

Cotchin, E. 1956. Neoplasms of the Domesticated Animals. Review Series No. 4, Commonwealth Agri. Bur., Farnham Royal, England.

Fincher, M. G., W. J. Gibbons, Karl Mayer, and S. E. Park. 1956. Diseases of Cattle. American Vet. Publications, Inc., Evanston, Ill.

Feldman, W. H. 1932. Neoplasms of Domesticated Animals. W. B. Saunders Company, Philadelphia and London.

Innes, J. R. M. and L. Z. Saunders. 1962. Comparative Neuropathology. Academic Press, New York and London.

Jarplid, B. 1964. Studies on the Site of Leukotic and Preleukotic Changes in Bovine Heart. Pathologia Veterinaria, 1: 366-408.

Jubb, K. V. F. and P. C. Kennedy. 1963. Pathology of Domestic Animals. Academic Press, New York and London.

Marshak, R. R., L. L. Coriell, W. C. Laurence, J. E. Croshow, H. F. Schryrer, K. P. Altera, and W. W. Nichols. 1962. Studies on Bovine Lymphosarcoma, Clinical Aspects, Pathological Alterations and Herd Studies. Cancer Res., 2: 202-217.

Monlux, A. W., W. A. Anderson, and C. L. Davis. 1956. A Survey of Tumors Occurring in Cattle, Sheep, and Swine. Am. J. Vet. Res., 17: 646-677.

Moulton, J. E. 1961. Tumors in Domestic Animals. University of Calif. Press, Berkeley and Los Angeles.

Runnells, R. A., W. S. Monlux, and A. W. Monlux. 1960. Principles of Veterinary Pathology. The Iowa State University Press, Ames, Iowa.

Schalm, O. W. 1961. Veterinary Hematology. Lea and Febiger, Philadelphia.

Smith, H. A. and T. C. Jones. 1961. Veterinary Pathology, 2nd. ed., Lea and Febiger, Philadelphia.

Smith, H. A. 1962. Malignant Lymphoma in Animals. Am. J. of Clinical Path., 38: 75-87.

Squire, R. A. 1964. Hematopoietic Tumors of Domestic Animals. The Cornell Vet., 54: 97-150.

Squire, R. A. 1965. A Cytologic Study of Malignant Lymphoma in Cattle, Dogs, and Cats. Am. J. Vet. Res., 26: 97-107.

Theilen, G. H., O. W. Schalm, and V. Gilmore. 1961. Clinical and Hematologic Studies of Lymphosarcomata in a Herd of Cattle. Am. J. Vet. Res., 22: 23-30.

VITA

Talib Abdul Amir Makkawi

Candidate for the Degree of

Master of Science

Thesis: THE CYTOLOGY AND TERMINAL MANIFESTATIONS OF BOVINE LYMPHOSARCOMA

Major Field: Veterinary Pathology

Biographical:

Personal Data: Born in Mussayab, Iraq, May 22, 1933, the son of Abdul Amir and Himzzia Makkawi.

Education: Attended primary school at Mussayab, and high school at Baghdad, Iraq; graduated from Karkh High School in 1954-1955; received the Bachelor of Veterinary Science from Baghdad University in June, 1960; completed requirements for the Master of Science degree in May, 1965.

Professional Experience: Served in Iraqi Army Veterinary Corps as a meat inspector and Director of Veterinary Army Hospital, Agra-Mosul from August, 1960 to December, 1961; Provine Veterinary officer, Deyala State from January, 1962 to November, 1963.

Professional Organizations: Member of Professional Medical Association, Baghdad, Iraq; member of Iraqi Veterinary Association.