TRANSPLANTATION OF LYMPHOID TUMORS IN THE BOVINE

by

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INTRODUCTION

The alarming increase of bovine lymphocytoma in recent years has prompted a number of workers to make detailed studies in an effort to determine the possible cause. Countless and varied opinions as to the etiology of this affliction have been expressed. At the present time the most plausible explanation for the malignancy is that is due to a viral infection.

A great number of investigations have been conducted with the idea of transmitting the disease to healthy, normal animals of the same and of different species. Most attempts at experimental transmission have entailed the use of lymph node suspensions and lymph node transplants. Results of such attempts have been for the most part rather discouraging; for in most instances inoculated neoplastic tissue has produced little or no change in the experimental animal.

This project was undertaken to determine whether it was possible to transmit lymphocytoma from a two-year-old heifer to five healthy cattle of different ages. Lymph node suspensions and transplants were inoculated by various routes, and the animals were observed for a period of over one year. Throughout the course of this investigation jugular blood and bone marrow specimens of each of the test animals were examined periodically for pathological alterations which might indicate transmission of the malignant disease.

REVIEW OF LITERATURE

Incidence

Bovine lymphocytoma is perhaps the most common malignant neoplastic disease in cattle (Feldman, 1930; Thompson and Roderick, 1942) and is increasing by what Kohler (1957) describes as "leaps and bounds". In the United States there is evidence to show that cases appear more often in certain areas, and within the past few years have increased at a tremendous rate. Tharp and Amstutz (1957) at the Ohio State Veterinary Clinic observed this affliction so frequently that they suggested that it might be responsible for many clinical cases with obscure symptoms. At the veterinary clinic, Kansas State University, Thompson and Roderick (1942) observed that since 1935 an average of ten lymphocytoma cases was diagnosed each year. At the present time it is understood that the rate has increased to 30 or more cases each year. Udall and Olafson (1930) noted that in 30 years only two cases had been diagnosed at the Cornell Veterinary Clinic. From this and other information it clearly illustrates that the disease is more prevalent, at least for the time being, in the midwestern states.

In regard to the incidence of lymphocytoma in cattle in Europe, Hjarre and Isakason (1950) found that the increase was more than twice the original rate in Sweden. Niepage (1953) described the overall incidence of the disease in one part of Germany and found that 0.5 percent of bovines were affected. However, he felt that in certain districts the incidence could very well be at least three times this figure. According to Bendixen (1959) leukosis occurs in about 400 herds in Denmark. Seventy of these herds furnish 80 to 85 percent of the annual number of cases. In other herds only occasional cases appear, and the blood picture of the remaining cattle is normal.

Although lymphocytoma appears to be on the increase in most countries, there are still those that remain relatively free from it. Verge and Drieux (1941) reported that leukosis rarely occurs in domestic animals in France and in the mountainous parts of Switzerland.

Age Susceptibility

In most instances lymphocytoma in cattle is seen during adulthood, but it is not altogether rare to find very young calves or even fetuses affected. In their leukosis studies Hjarre and Isaksson (1950) found that diseased cattle had a mean age of seven years and four months. However, they observed the disease in cattle of all ages. Udall and Olafson (1930) described the disease as aleukemic lymphadenosis in an animal six-months of age, and Buss (1938) called the affliction in a young calf leukemia. Schwirzke (1939) recorded an instance in which a calf was brought to slaughter at four weeks of age, and proved to have advanced leukosis. A detailed hematological study was conducted by Stasney and Feldman (1938) on a three-month-old calf with generalized enlargement of all lymph nodes. Montemagno (1959a) found an acute and fatal leukosis in a calf that was 45 days old. More recently Hatziolos (1960) gave an account of a case that was termed lymphoblastic lymphoma in a still-borne fetus from an apparently normal cow at the eighth month of pregnancy.

Breed Susceptibility

Most investigators are in agreement with the fact that breed is of little consequence in the susceptibility to lymphocytoma. Nonetheless Schaper (1938) described a breed that was considered highly susceptible. In support of this view a description of the incidence of the infection in the "Schwartzhunten Niederungsvich" breed was given.

Nomenclature

Undoubtedly, no other neoplastic disease has been subjected to such a variety of terms as bovine lymphocytoma. It has been known under such names as lymphadenoma, pseudoleukemia, malignant lymphoma, leukemia, lymphosarcoma, leukosis, to name a few. From such a conglomeration of names Feldman (1932) suggested the term lymphoblastoma to include both the benign tumor known as lymphoma and the malignant neoplastic disease as lymphocytoma. In the latter group were placed all the malignant types of neoplastic hyperplasia of which the immature lymphocyte is the cell type.

A rather confusing term Lubke (1939) that is used to a great extent by European investigators is leukosis. There is little reason to justify the use of this name other than the fact that lymphocytes are considered white blood cells, but so are the granulocytes. It would seem that leukosis suggests an increase or proliferation of granulocytes in the bone marrow, and not a neoplastic disease of which the immature lymphocyte is the most outstanding characteristic both in the blood and lymphoid tissues.

Thompson and Roderick (1942) made a study of the neoplastic disease of cattle, and concluded that there was little justification of a more complicated nomenclature for describing it. They felt that the term lymphocytoma was the most appropriate designation for the neoplastic process that involved both lymphoid cells and tissues. With this information in mind, it was decided that the neoplastic disease encountered in cattle in this investigation would be referred to as lymphocytoma.

Disease Characteristics

According to Frank (1952) Lymphocytoma is a malignant neoplasm arising from a proliferation of atypical lymphocytes. The disease may manifest

itself in several ways. It may originate in lymphatic structures including lymph nodes which may rapidly increase in size into large hyperplastic masses. Most all lymphoid tissue is involved in this case. From these masses the neoplasm may then infiltrate into the surrounding tissues. Another, though less common means, is that it may arise locally in an organ and metastasize to other organs such as the liver and the kidneys. Smith and Jones (1957) described the neoplastic disease as a transformation of lymphoid organs into neoplastic tissue rather than a spread through metastases as would be the case with other tumors.

Feldman (1932) described the gross characteristics of lymphocytoma as follows:

> Lymphocytoma consists of nodular or diffuse, irregular, fleshy masses or 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 or water-logged 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.

Tissue sections from the hypertrophied neoplastic glands show that the normal internal structure is obliterated (Runnells, 1954), and great numbers of immature lymphocytes including lymphoblasts are found. Few normal lymphocytes are seen dispersed throughout the glands but the normal arrangement is not seen.

Clinically the most commonly recognized symptom in lymphocytoma is the enlargement of all the superficial lymph glands (Frank, 1955). The affected animal may show a gradual loss of condition, but may exhibit a normal appetite. In some instances there may be a protrusion of one or perhaps both eyes due to the accumulation of lymphoid tissue in the orbital cavity. Not uncommonly a posterior paralysis may develop as a result of the pressure of the neoplastic tissue against the spinal cord in the vertebral canal. Other

symptoms, though of a more obscure nature, may be digestive and cardiac disturbances. Lymphocytoma may be confused with many conditions frequently observed in cattle. Tharp and Amstutz (1957) suggested that trauma to the eye, traumatic gastritis, chronic gastric tympany, ketosis, cystitis, enteritis, traumatic pericarditis, and various cirulatory disturbances have been diagnosed erroneously at various times in animals afflicted with lymphocytoma.

Hematological Studies

Although an increase in lymphocytes in the blood may be of some importance, it does not necessarily warrant a diagnosis of lymphocytoma. Niepage (1954) suggested that in cattle a not uncommon lymphocytosis was due to splenic stimulation of undetermined origin, and that a possible cause could be brucellosis.

Thompson and Roderick (1942) found total white cell counts varying from 6,300 to 142,000 in cattle affected with lymphocytoma. The differential white cell counts of ten cases showed an average of 65.24 percent lymphocytes. This figure was further subdivided into 31.4 percent large lymphocytes, 56.2 percent typical lymphocytes; and 12.4 percent small lymphocytes. A mild to marked anemia was associated with the disease. In regard to atypical lymphocytes, they stated the following:

> Morphologically the most predominate change is to a large-type cell with a bizzare-shaped nucleus and many large vacuoles in the cytoplasm. Less common are lymphocytes containing two or more nuclei and others that show several nucleoli.

They suggested that in attempts to diagnose lymphocytoma by blood count emphasis should be placed on the percentage of lymphocytes classified as atypical.

Protein poisoning in young calves (Egehoj, 1943) produced a permanent increase in lymphocytes, but still there was no evidence to indicate that this increase was, in any way, associated with lymphocytoma. Hematological examination of one poisoned calf revealed the greatest number of leucocytes (47,000)

which was about four to five times the number occurring in healthy calves. Morphologically the predominant type of white blood cell was the lymphocyte. Numerous pathological cell types and juvenile lymphocytes occured commonly, but the picture identical to that of the malignant disease was never reproduced.

Jasper et al. (1946) found, not only an increase of ten to twenty percent lymphocytes above normal in most cases of lymphocytoma, but also found that many of the lymphocytes had nucleoli and a fine chromatin arrangement in the nucleus. They felt that a number of cells that were classified as lymphocytes might have actually been monocytes, but it was rather difficult to differentiate them from the atypical forms. A progressive anemia was noted in the affected animals. Stasney and Feldman (1938) showed that there was an increase in lymphocytes, and that there was also a definite correlation between the immature forms found in the peripheral blood and the cellular picture of the diseased lymph nodes.

For diagnosing leukosis on a herd basis Gotze et al. (1953, 1954) proposed the use of a leukosis key. This key involves the (1) estimation of total white blood cell counts; (2) the percentages of lymphocytes and lymphocyte-like cells; and (3) the determination as to whether or not the figures fall within a normal, suspicious, or leukotic range. They were of the opinion that in many instances where tumorous lesions were not present this system was of particular value. However, Ziegenhagen and Dohmen (1955) in a study of healthy and leucotic cattle found that the leukosis key could be used in diagnosing the disease in only about 90 to 95 percent of the cases. It was considered next to impossible to make a definite diagnosis from just the morphological study of the blood cells.

Bone Marrow Studies

In recent years there has been an increased interest in bone marrow investigations in the domestic animals. Among various conditions being studied lymphocytoma is one of prime importance. Recently Griffing (1960) among other diseases studied included lymphocytoma. He performed numerous bone marrow examinations on cattle with this affliction at the Kansas State Veterinary Clinic. Some investigators have made attempts to correlate the findings of the peripheral blood with bone marrow specimens in this disease very much the same way as in leukemic conditions in man. According to Kohler (1957) several workers, namely, Rohr (1949) and Schoen and Tischendorff (1950) found that chronic lymphadenosis in man could be easily diagnosed from bone marrow preparations along with other clinical findings. In cattle this does not appear to be the case, for even though the total number of lymphocytes including immature forms was increased in most bone marrow specimens several animals with high lymphocytic counts in the peripheral blood yielded very low bone marrow counts. From this information he stated the following:

> Therefore, neither the quantitative nor the qualitative composition of the lymphatic cells of the bone marrow alone are sufficient to establish the diagnosis of leukosis.

However, he considered that more than 30 percent lymphocytes in bone marrow specimens together with the findings of the peripheral blood might be of significant value in arriving at a diagnosis.

Etiology

Investigations into the cause or causes of lymphocytoma have been undertaken by many workers particularly the Europeans, and much interesting, if not significant, information has been made available through these studies. In a recent survey conducted by Bottger (1956) of the neoplastic disease in cattle,

he made the following observations: (1) The disease may be present in a herd for over 20 years. (2) Unrelated animals brought into affected herds may develop the disease. (3) Many herds remain free from leukosis in spite of being closely related by blood to some herds that are affected. From these observations he concluded that leukosis was not simply a hereditary trait stimulated by environmental factors. In turn Weischer (1944) stressed the importance of heredity. He felt that leukosis was transmitted by a fully dominant character. His conclusions were based on tracing the disease of 150 cows to the afflicted herd sire. In commenting on Weischer's article, Ulm (1944) felt that it would be unwise to conclude from one exceptional instance that leukosis was a true inherited condition, transmitted by a dominant gene, but rather there might be a possibility of a mutation or factors other than heredity involved.

According to Schwirzke (1939) cattle leukosis is closely related to an hereditary factor. He described advanced leukosis in a heifer which was the offspring of a cow which tended to produce abnormal calves not healthy enough to raise to maturity. Although the actual cause of death of the calves was not always determined, it was highly suggested that all might have succumbed to leukosis.

Fortner (1953) suggested that bovine leukosis tended to run in families, and that breeding affected animals should be discouraged. He compared bovine leukosis with the hereditary leukosis in mice and concluded that the disease was essentially identical in all aspects with mouse leukosis. Quoting certain field observations to support his view, Morretti (1953) also put forth the same idea. Svanberg and Aberg (1955) felt that the spread of lymphatic leukosis in Sweden might in some way be related to grazing animals on poor, weed-grown, phosphorus-deficient pastures. However, Gotze et al. (1956a) were of the opinion that poor nutrition or bad management had not been shown to be a fundamental

cause, but that their importance as contributory factors was highly probable. X-rays, radioactivity and chemical substances which are known to provoke leukosis in man and in laboratory animals, probably have the same effect on cattle.

Egehoj (1947) considered that the cause of leukemia was neither a virus nor chronic protein poisoning, and excluded soil and water as possible factors in the causation of the disease. Einert (1952) concluded that leukosis was caused by an infective and contagious agent with a variable incubation period, and that dietetic deficiencies, by lowering resistance to infection, were only of secondary importance. From their studies Hjarre, and Isaksson (1950) believed that the malady might well be related to a variety of factors: heredity, a virus, hormone action, or possibly environmental conditions. Gotze et al. (1956b) gave evidence that suggested that bovine leukosis could be transmitted. Seventeen young cattle and two cows were inoculated by various routes with blood, milk and neoplastic glandular tissue from infected animals. All inoculated animals were observed for a period of five years after which seven were found to be leukemic and three had tumor-like leukotic nodules. In three animals the blood picture was slightly changed, and the remaining six animals were apparently normal. Gotze et al. (1956a) proved placental transmission of the disease in two cases. They observed that the incubation period, from the time of infection to the appearance of leukemic changes in the blood, varied from two weeks to several months. From this study several interesting points were noted: (1) The length of time involved after inoculation before the appearance of the disease varied considerably. (2) All animals did not come down with the disease. (3) All animals did not show the typically enlarged external lymph glands. This picture might at least suggest the possibility of a virus as being the causative agent of leukemia; and even though leukemic changes are seen at an early date after inoculation, the disease becomes a reality only if certain unknown predisposing conditions are present.

Kohler (1957) indicated that leukosis in the bovine was a virus-caused disease with a long latent period. Evidence incriminating a virus as the etiological agent was provided by Montemagno, et al. (1957) and by Papparella (1958). Montemagno (1959b) made 20 serial passages in seven-day-old chick embryos with a filtrate of lymph node tissue from a calf with lymphatic leukosis. Embryo mortality rate varied from two to one hundred percent after 36 to 72 hours. Papparella (1958) prepared a lymph node suspension from the same calf and injected chick embryos. When 27 chick embryo passages had been made, he inoculated amnio-allantoic fluid into the allantoic cavity of nine-day chick embryos. After incubating at 38°C to 39°C for 72 hours all chick embryos were injected with influenza virus and incubated at 36°C for 20 hours. He found that the hemagglutination titer in control chick embryos was much higher than in embryos which had been inoculated with the bovine leukosis agent. From this evidence he concluded that the low hemagglutination titer was due to viral interference. In 1959, the same investigator (Papparella, 1959b) gave a description of the virus isolated from the leukemic calf, and provided three micrographs of the virus as further evidence.

Transmission Studies

Chronic protein intoxication has often been incriminated as causing leukosis by activating a latent virus. Egehoj (1943, 1947) attempted to reproduce this neoplastic condition by injecting indole into young healthy calves. This substance proved to be highly toxic and gave no significant results. Indole injections were discontinued and replaced with daily injections of 50 to 200 ml. of skim milk administered intravenously for several weeks. These injections produced a swelling of the superficial lymph nodes which later subsided, but blood studies indicated that there was a permanent increase in

lymphocytes. The general picture produced experimentally was considered to be one of exhaustion of the erythropoetic system and a marked stimulation of the reticulo-endothelial system. On necropsy it was noticed that the liver and kidneys were degenerated. He felt that these studies were in no way suggestive of leukemia. The results were not modified to any significant degree by simultaneous injections of streptococcal vaccine, serum of a cow affected with leukemia, and extracts of spleen or lymph nodes from animals with the disease. Transplants of malignant lymph nodes into four untreated calves was also without effect. In view of these findings, he concluded that a virus etiology was unlikely, and that the condition could possibly be of a hereditary nature.

Creech and Bunyea (1929) inoculated guinea pigs, rabbits, a weanling calf, a sheep, and a cow with either jugular blood or composite samples of an emulsion of the enlarged lymph glands of a leukemic cow. Approximately one year after inoculation the experimental animals did not show any clinical evidence suggestive of leukemia, and on necropsy none exhibited any lesions characteristic of the disease. Bacteriological studies of the neoplastic tissues of the leukemic cow gave negative results. The inoculated cow was maintained under observation for one year, and a number of blood counts were made in order to note any possible alterations from the normal of the cell content which might give any indication of a leukemic condition. The authors concluded that no evidence was obtained to indicate that bovine leukemia was of an infectious nature.

In one project (Hoge, 1937) experimental animals were injected intravenously or intramuscularly with either normal serum or leucotic lymph node material. Those animals injected with normal serum alone had an increase in the number of erythrocytes, while those that were inoculated with normal lymph node material had a slight neutrophilic leucocytosis and an increase in erythrocytes. About five to ten days after intravenous inoculation with neoplastic tissue the

animals showed a decrease in erythrocytes. Lymphoid cells including Rieder's lymphocytes were observed in the blood. Splenectomized animals exhibited a much greater reaction when inoculated with the same materials. Attempts to block the reticulo-endothelial system with a ferric saccharate solution or second injections of neoplastic node tissue had no appreciable effect on transmitting the disease. Implantation of the leukemic tissue into the anterior chamber of the eye was without effect.

Transmission of leukemia in monkeys was attempted by Losch (1937) by means of injections of blood, serum, and lymphatic material from cattle affected with the disease. He observed young immature lymphocytes in the blood stream, with or without a lymphocytosis, only after three injections in one animal and four in another. However, the reaction was of a transient nature and led to the conclusion that a specific substance was present in the injected material which stimulated lymphoid tissue. It would seem that repeated injections of protein alone were responsible for this reaction.

Stasney and Feldman (1939) injected two healthy calves of the same breed both intravenously and subcutaneously with blood and lymph node emulsions. Before inoculation the spleen of one calf had been irradiated with a large dose of X-rays. Numerous blood examinations of both calves were made during the period that the experiment was in progress, but except for a transitory but marked leucocytosis that subsided ten days after the animals had received their injections, there were no significant changes. One of the calves was maintained under observation for 282 days and the other for 346 days after inoculation. Histological sections showed both to be essentially normal.

Jasper et al. (1946) attempted to transmit lymphocytoma into the same animal from which the neoplastic tissue had been removed. They injected the buffy coat from 250 ml. of blood into the subcutis. The left prescapular lymph gland was ground in sterile physiological saline, and a suspension of the cells

was also injected subcutaneously. A small transplant was inserted subcutaneously on the left side of the neck; a similar section was inserted intramuscularly on the left side. From this study they found no evidence of any activity other than suppuration at the inoculation sites. In fact, the transplants not only failed to "take" but approximately two weeks after inoculation they noted that they could easily be expressed manually.

Guinea pigs, both young and adult, were inoculated with amnio-allantoic fluid of 32nd passage in chick embryos by Papparella (1959c). The controls were injected with the same material which had been previously heated at 60°C for 30 minutes. Although the mortality rate of the inoculated animals was low, blood examinations revealed a lymphocytosis with occasional lymphoblasts and a sharp drop in erythrocytes. The virus was recovered from dead guinea pigs by chick embryo inoculation. In addition, gross and histological changes occured; and inclusions bodies were observed in neurones and microglial cells.

Montemagno (1959b) described what he considered to be the first effective experimental evidence that a virus is the etiological agent in the transmission of a lymphatic leukosis. He took two 70-day-old Holstein heifers and inoculated them with allantoic fluid from embryonated eggs that had been injected with a filtrate from lymphatic tissue of a young calf that had died from an acute lymphatic leukosis. One heifer was injected with allantoic fluid preheated to 60°C for 30 minutes, and the other had unheated fluid. Fifteen days later the heifer receiving unheated fluid showed an increase of up to 22,000 leucocytes per ml. There were 4.2 percent promyelocytes, 93.4 percent lymphocytes, and 5.3 percent neutrophiles. After more than 30 months had elapsed the same blood picture continued unchanged. Clinically, two months after inoculation the prescapular and prefemoral lymph glands were hypertrophied. Histological sections of lymph glands showed an increase of lymphoblasts

and promyelocytes with a decrease of normal lymphocytes. Bone marrow studies revealed lymphoblasts, prolymphocytes, and lymphocytes.

The second heifer never manifested clinically or hematologically any changes, and after three months' observation the animal was removed from the experiment.

From the several investigations of Montemagno and Papparella, reviewed above, it can almost be considered without question that the causative agent, at least in these two instances, is a virus.

MATERIALS AND METHODS

Experimental Animals

Cattle used in this project were a two year old Hereford heifer affected with lymphocytoma, six bulls and four steers ranging in age from one to three years. The heifer had been under observation at the Kansas State Veterinary Clinic prior to being purchased for this study by the Department of Pathology, and the experimental bovine animals were selected from the herd maintained at the Veterinary Research Farm at the same institution. A brief description of each of the ten experimental animals is as follows:

1. No. 76, Mixed breed, male, three years
2. No. 341, Holstein, male, one year
3. No. 342, Hereford, male, one year
4. No. 343, Mixed breed, male, one year
5. No. 413, Mixed breed, steer, three years
6. No. 254, Hereford, steer, one year
7. No. 256, Hereford, steer, one year
8. No. 317, Red polled, male, three years
9. No. 345, Hereford, steer, one year

10. No. 390, Hereford mix, male, one year.

Cattle having numbers 76, 341, 342, 343, and 413 were inoculated with malignant lymphoid tissue while those having numbers 254, 256, 317, 345, and 390 served as controls.

Obtaining the Neoplastic Tissue

The right prefemoral lymph gland from the heifer with lymphocytoma was selected as the material for the inoculation studies. It appeared to be the malignant tissue that was readily accessible and the removal required the minimum of preparation. From the beginning there had been evidence of a posterior paralysis, and the heifer tended to lie on the left side. In fact, it was due to the paralysis that no restraints were needed while the actual extirpation of the gland was performed.

The skin over the gland was thoroughly scrubbed, shaved, and disinfected with a 1:1000 solution of Roccal[1]. Local anesthesia was induced by infiltrating 40 ml. of a four percent solution of procaine hydrochloride into the skin and tissues surrounding the much enlarged neoplastic gland. After ten minutes elapsed, an incision approximately four inches long and parallel to the femur was made through the skin, and by blunt dissection the gland was isolated and removed. Blood vessels were ligated and the skin incision was closed with interrupted non-absorbable sutures.

Preparing the Tissue Homogenate

Within a few minutes following the extirpation of the malignant prescapular lymph gland, a portion of the capsule was reflected, and approximately

[1]Sterwin Chemicals, Inc., New York, New York

20 gm. of the fresh glandular tissue was withdrawn. A uniform homogenous suspension of this harvested tissue was prepared in a sterile Waring[2] blender with 50 ml. of phosphate buffer saline. The blender was in operation for approximately five minutes. At the end of this time to avoid contamination the homogenate was poured aseptically into sterile 30 ml. vaccine bottles from which it was removed as needed. Transplants were obtained from the remaining portion of the gland.

Preparing the Phosphate Buffer Saline

Freshly prepared phosphate buffer saline used in the preparation of the tissue homogenate was made by mixing 10 ml. of solution A with 0.1 ml. of solution B, and enough demineralized water was added to make a volume of 100 ml. Solution A was diluted before adding solution B to avoid precipitation. Stock buffer solutions A and B were prepared in the following manner:

Phosphate Buffer Saline Solution A.

NaCl.....20.000 gm.

KCl..... 0.500 gm.

Na_2HPO_4..... 3.125 gm.

KH_2PO_4..... 0.025 gm.

The above quantities were dissolved in 250 ml. of demineralized water, and autoclaved at ten lbs. pressure for ten minutes.

Phosphate Buffer Saline Solution B.

$CaCl_2 \cdot 2H_2O$..... 6.645 gm.

$MgCl_2 \cdot 6H_2O$..... 5.000 gm.

The above quantities were dissolved in 50 ml. of demineralized water, and autoclaved at ten lbs. pressure for ten minutes.

[2]Waring Products Corp., New York, New York

Transplanting the Neoplastic Tissue

Different routes of inoculation were used in introducing neoplastic tissue into the various experimental animals. The route of inoculation for each of the five test animals can be briefly summarized as follows:

1. No. 342 was inoculated into the anterior chamber of the right eye with a glandular transplant.
2. No. 343 was injected in the jugular vein with 2 ml. of the tissue homogenate.
3. No. 413 had a small transplant inserted into the right prefemoral lymph gland, and 2 ml. of the homogenate was infused into the marrow cavity of the thirteenth rib.
4. No. 76 was injected with ml. of the homogenate in the muscles of the right thigh, and 2 ml. were infused into the bone marrow of the thirteenth rib.
5. No. 341 had a transplant placed in the anterior chamber of the right eye, and 2 ml. of the homogenate was infused into the bone marrow cavity of the thirteenth rib.

Inoculating the Anterior Chamber of the Eye. A small transplant of malignant lymphoid tissue was introduced into the anterior chamber of the right eye. This inoculation site was selected for the following reasons: First, any multiplication or growth of the inoculum could be observed with little or no difficulty. Secondly, the immunogenic response here to foreign protein is not so pronounced as in other tissues of the body.

For inoculating the anterior chamber the test animal was secured in a stock, and the head was completely immobilized. Infiltration anesthesia as recommended by Peterson (1951) was adopted for blocking the sensory nerves to the eye, and twitching of the eyelids was prevented by the procedure advocated

by Frank (1953). The anesthetic was allowed to act for approximately ten minutes before inserting the "plug" of anaplastic lymphoid cells.

Tissue forceps were attached to the skin of the upper eyelid and raised so as to expose the upper surface of the eyeball. At a point just above the corneo-scleral junction a sharp 14 gauge one and one-half inch needle containing aspirated glandular tissue was gently pushed through the sclera. With slight pressure on an attached ten ml. syringe the needle was next directed downward and lateral to the iris until the tip could be seen in the anterior chamber. The syringe was removed, and a wire that fit snugly the bore of the needle was inserted so that the tissue was expelled into the chamber. Both needle and wire were withdrawn, and no medication given. Loss of vitreous humor was practically negligible and hemorrhage into the chamber was only slight.

Inoculating the Prefemoral Lymph Gland. A transplant of the neoplastic gland was deposited in the right prefemoral gland of one steer. The animal was placed in a stock, and the skin over the gland was prepared for aseptic surgery. A similar surgical procedure was followed as in the removal of the neoplastic gland in the lymphocytoma case with the exception that the incision was just two inches in length and then only the lateral surface of the normal gland was exposed. A pair of hemostats was thrust through the gladular capsule and spread open. With another pair of hemostats a transplant, approximately two cm. square, was introduced and released allowing it to come in close contact with the normal lymphoid tissue. Both hemostats were withdrawn, and the opening was closed with absorbable sutures.

Injecting Tissue Homogenate into the Marrow Cavity. The rib selected for injecting tumor tissue was prepared in a similar manner as in the sampling of marrow. After the opening was bored through the bone a 12 gauge blunted needle was introduced, and two ml. of the homogenate were infused slowly into

the cavity. The needle was removed, and the wound closed with non-absorbable sutures.

Obtaining the Bone Marrow Specimen

On several occasions prior to euthanasia marrow specimens were aspirated from the ailing heifer. Likewise, marrow was withdrawn from the treated animals periodically. The method for obtaining marrow samples described by Griffing (1960) was adopted for this study. In general, the procedure was adhered to rather closely.

Obtaining Peripheral Blood Samples

At various intervals throughout the course of this investigation, blood samples were drawn from the jugular vein of each treated animal including the five controls. One drop of Sequester-Sol[1] placed in each bleeding tube was sufficient to prevent clotting of approximately three ml. of blood. In most instances smears were made within the hour that the blood was taken.

Obtaining Vitreous Humor

Although the lymphoid transplant was readily seen suspended in the anterior chamber of the eye, it became necessary to aspirate fluid to determine whether or not the anaplastic lymphoid cells were actually multiplying or undergoing retrogressive changes.

The eye was anesthetized and a three-fourths-inch 25 gauge needle attached to a tuberculin syringe was inserted into the chamber at the original inoculation site. Approximately 0.1 ml. of vitreous humor was withdrawn, and smears were made immediately.

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[1]Cambridge Chemical Products, Inc., New York, New York.

Staining and Interpretation

Bone Marrow. All bone marrow smears without exception were prepared with Wright's stain which was made in the following manner:

Wright's stain (dried precipitate)..... 0.1 gm.

Methyl alcohol, absolute.....60.0 ml.

The stain was allowed to age at room temperature for several weeks, and filtered before each set of smears were stained.

Air dried marrow preparations were fixed for one minute with the methyl alcohol solution of the stain. The stain was later diluted by adding an equal quantity of Sorensen's phosphate buffer of pH 6.5. A metallic sheen formed on the surface of the smear, and the diluted stain was allowed to stand for three to five minutes. The smear was then washed by flooding with phosphate buffer solution, and air dried.

Blood Smears. Most of the slide preparations in the course of this investigation were stained by Wright's method with the exception that in the earlier part C. L. stain[1] was used on a few smears.

Vitreous Humor. Vitreous humor from experimental animals inoculated with malignant lymphoid transplants in the anterior chamber of the eye was aspirated, and smears were stained according to Wright's method.

Biopsys. Crushed impressions smears of biopsy material were stained by Wright's method as outlined in the staining of bone marrow.

Interpretation. Procedures as recommended by Diggs et al. (1954) were followed in interpreting the stained hematological and marrow preparations.

Counting Technique

Bone Marrow Cells. Differential counts on bone marrow specimens were

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

made according to the procedures adopted by Griffing (1960). In each case a total of 500 cells were identified under a magnification of 980X, and percentages of different cells were recorded. In addition, the M/E and M/L ratios were determined for each marrow sample.

White Blood Cells. Differential counts on peripheral blood were recorded as absolute numbers. In each count 100 white blood cells were identified and the percentage of each kind determined. Next the total white blood cell count was multiplied with the percentage of each cell type.

Precipitin Tests on Experimental Animals

Approximately eight months after inoculation of the experimental animals interfacial ring precipitin tests were prepared for each using lymph node material from the lymphocytoma heifer as the antigen and the serum from the test animals.

Two-fold dilutions of the antigen beginning with a dilution of 1:10 were layered over undiluted serum in twelve Dreyer tubes. A thirteenth tube was added as a control. The tubes were incubated at 37°C for three hours during which time they were observed every 30 minutes for interfacial precipitation to occur. If there was no reaction taking place within that time then the tubes were incubated overnight in a refrigerator at 8°C. Following this incubation the tubes were examined.

RESULTS AND DISCUSSION

Results of the Lymphocytoma Studies in a Heifer

(Pathology #4661) Hereford, female, 2 years. The heifer was presented to the clinic on 11/14/60 with a history of having swellings in the shoulders. On examination it was revealed that all superficial lymph glands were enlarged. A biopsy of the left suprascapular node on 11/18/60 disclosed numerous large lymphoblastic cells with prominent nucleoli, and interspersed throughout the gland relatively few typically small lymphocytes were noticed. On rectal examination large firm nodular masses were palpated in the pelvic cavity. A diagnosis of lymphocytoma was made and an unfavorable prognosis given. Paracentesis of the enlarged right suprascapular lymph node was performed, and negative results were recorded.

On 12/28/60 the right prefemoral lymph node was excised aseptically, and prepared for transplantation into normal experimental animals.

Bone marrow specimens were obtained on two separate occasions. Although very cellular, the last sample withdrawn was abundant and fluid in consistency, and continued to ooze out from the opening into the marrow cavity forming a reddish discolored clot at the base.

From the time the enlarged external nodes had been noticed on this heifer there had been a gradual loss of weight, and there was evidence of a posterior paralysis. Nonetheless, the appetite remained normal and on 1/1/61 euthanasia was performed.

Necropsy examination revealed the subcutis to be edematous, and the submaxillary, prescapular, prefemoral, mediastinal, mesenteric, supramammary, and hepatic lymph glands were hypertrophied and edematous. When freshly cut, the exposed grayish-white surfaces were moist and glistening, and although the

glands were very much enlarged necrosis was not evident macroscopically. The spleen and kidneys were greatly enlarged, and the former contained large circumscribed swellings, the largest of which measured 6 cm. in diameter. The right and left kidney weighed 2060 Gm. and 1917 Gm. respectively.

A five-month-old fetus was present in the uterus and on necropsy no gross lesions were noticed. Histological sections of the fetal heart, liver, spllen, lung, and kidney disclosed no evidence of any pathological alterations.

Tissue sections of the spleen and lymph nodes of the heifer revealed that the normal structure had been replaced by neoplastic lymphoid cells. The walls of the alveoli in the lungs were greatly thickened with infiltrated lymphocytes, and all blood vessels contained an increased number of lymphocytes.

Malignant lymphoid cells were extensively dispersed throughout the kidney, and large areas of intertubular stroma were filled with lymphocytes. Many of the renal tubules and some glomeruli were completely obliterated.

Discussion of Lymphocytoma Studies in a Heifer

Tables in the "Appendix", page 35, show the results obtained from the peripheral blood and bone marrow studies of the lymphocytoma heifer. The peripheral blood counts were recorded in absolute numbers.

From Chart II, it is apparent that there is an increase of both neutrophiles and lymphocytes. Atypical lymphocytes were observed in great numbers, and in some instances were confused with monocytes. Toward the terminal stages of the disease an anemia was indicated by the declining hemoglobin and hematocrit readings. The blood findings corroborated closely the bone marrow picture. In Chart 12, the bone marrow showed a myeloid-erythroid ratio greater than one. This increase in granulocytes was at the expense of the erythroid series. The increase in lymphocytes in the marrow was indicated

by a myeloid-lymphoid ratio greater than one. The normal myeloid-erythroid ratio is usually better than three. From this study some rather prominent points were noted: (1) Near death an animal with lymphocytoma may exhibit an anemia and a neutrophilia. (2) A diagnosis of lymphocytoma in the bovine depends to a great extent on the morphological aspects of the lymphocytes found on hematological examinations. The atypical lymphocyte may have nucleoli, vacuoles both in the nucleus and cytoplasm, a bizzare-shaped nucleus, and other abnormal characteristics setting them apart from the healthy cells. (3) Bone marrow findings may serve as an aid in the diagnosis of lymphocytoma only when closely correlated with the findings of the peripheral blood and clinical examinations.

The peripheral blood reflects what is usually occuring in the marrow, and in this regard examination of marrow samples might be of inestimable value particularly where a question exists as to the true nature of a blood disease. However, a great variation in cell content even of the normal marrow occurs from samples withdrawn at different intervals from the same animal, and smears of the same specimen will also vary. For this reason it would be unwise to rely solely on bone marrow for arriving at a diagnosis in a disease condition. Moreover, if poor techniques are employed in obtaining a specimen, peripheral blood contamination may occur and the true picture distorted. Even on a marrow smear the investigator should select different fields in making cell counts; for in many instances a cell type may occur in "pockets of cells" giving misleading results. It is felt that for diagnosing lymphocytoma of the bovine the clinical examination along with peripheral blood findings will suffice. If necessary a biopsy of an enlarged external lymph node if present will yield on a crushed impression preparation or histological section significant information. PlateIII.

Experimental Results

Tables in "Appendix", page 35, show in detail the results obtained from the inoculated animals. The experiment was conducted for a period of one year during which time jugular blood and bone marrow specimens before and after inoculation were examined and recorded. Included in the "Appendix" are the blood findings of the five bovine controls. No marrow was withdrawn from the latter groups since these animals had not been inoculated, and the total white blood cell count and the differential count were within normal limits.

Discussion of Experimental Studies

Examination of blood and marrow preparations revealed that there were no apparent signs to indicate that transmission of lymphocytoma to the experimental bovines was accomplished. In general, peripheral blood findings of each animal could be correlated with their respective marrow preparation (Tables 1 through 13). With the exception of experimental animal No. 413 there were no appreciable differences in the blood findings and marrow specimens of the animals before and after inoculation. This case exhibited an increase in total white blood cells particularly lymphocytes. The average of the absolute lymphocyte counts before injection of malignant lymphoid tissue was 10,484.5 per ml. compared with 15,983.3 per ml. after inoculation (Table 14). Although the predominate type of white blood cells was the lymphocyte a picture identical to those found in bovine lymphocytoma was never noted. Very few if any atypical cells could be found only on detailed study of the smear.

A biopsy of the right prefemoral lymph gland of which case 413 had been previously implanted with a neoplastic transplant revealed on a crushed impression preparation as well as on a histological section that the gland was

essentially normal. None of the external lymph glands were enlarged, and from all outward appearances the animal was healthy.

The permanent increase in lymphocytes parallels the findings in the studies of protein poisoning in calves conducted by Egehoy (1947). He was of the opinion that a permanent increase in this type of mononuclear cell without any obvious pathological alteration might suggest an inherited labile reactivity of the hemopoetic tissues influenced by unknown stimuli.

Approximately eight months after injecting the test animals interfacial ring precipitin tests were conducted on each using lymph node material from the lymphocytoma heifer as the antigen and the serum from the affected animals. Two fold dilutions of the antigen starting with a dilution of 1:10 were layered over undiluted serum in twelve Dreyer tubes. The tubes were incubated at 37°C for three hours. Then placed in a refrigerator overnight. There were no positive reactions in any of the tubes indicating that the specific precipitin antibody for the lymph node tissue was not present in the serum of the animal at that time.

Examination of vitreous humor from the animals that had transplants introduced into the anterior chamber of the eye revealed that the malignant lymphocytes failed to multiply, but instead were undergoing regressive changes evidenced by (1) the presence of vacuoles in the cytoplasm; (2) finding red and black staining cytoplasmic granules; (3) observing light staining cytoplasm; and (4) rupture of the very fragile cell membranes with consequent out-pouring of the cytoplasm. Both animals injected intraocularly at the conclusion of this investigation exhibited slight scar tissue formation, and apparently had no extensive visual impairment.

At the conclusion of this investigation all animals including the controls were sold for slaughter. All appeared healthy and were in good physical condition.

SUMMARY

Injections of anaplastic lymphoid tissue from a clinical case of lymphocytoma were made intraocular, intramuscular, subcutaneous, intravenous, and intramedullary into five healthy cattle, ranging in age from one to three years. The inoculated test animals were observed closely for a period of one year during which time blood and bone marrow were examined periodically for pathological alterations indicative of lymphocytoma.

In four of the test animals blood and marrow specimens revealed no outstanding changes. However, in case 413 a permanent lymphocytosis with an absolute increase of more than 5,000 lymphocytes per milliliter was noticed (Table 14). In view of the fact that there is apparently no pathological or accidental cause for this lymphocytosis, perhaps it is due to an unknown stimulus causing a lability of the hemapoetic tissues evidenced by an abundant formation of cells particularly lymphocytes (Egehoy, 1947). In spite of a lymphocyte increase no atypical cells as would be expected in a lymphocytoma affected animal were found on routine examination. Intraocularly treated cattle showed evidence of a regression of the malignant lymphoid cells, and scar tissue formation which was only slight did not produce any extensive visual impairment. Precipitin tests performed with serum of the test animals and glandular tissue from the lymphocytoma affected heifer approximately eight months after treatment were negative. From all the assembled information it was concluded that no evidence was available to denote successful transmission of lymphocytoma in the bovine.

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APPENDIX I

Table 1. Total and differential leucocyte counts (absolute numbers), control animal No. 256.

DATE	TOTAL W.B.C.	NEUTROPHILES	BANDS	LYMPHOCYTES
12/14/60	8,450	2,112.5		5,577
12/23/60	7,650	2,371.5		4,819.5
1/30/61	10,050	3,110.5		6,633
2/18/61	10,550	2,848.5		6,330
3/21/61	9,450	2,835		5,575.5
4/ 7/61	11,050	3,094		7,072
4/28/61	8,300	2,407		4,980
5/ 7/61	8,350	2,755.5		4,926.5
1/ 7/62	9,300	2,790		6,231
2/ 3/62	9,000	3,510		5,220
2/27/62	8,900	3,204		4,628

Table 2. Total and differential leucocyte counts (absolute numbers), control animal No. 317.

12/14/60	9,450	2,173.5		6,615
12/23/60	10,850	2,712.5		6,727
1/30/61	10,200	2,448		7,446
2/18/61	11,100	1,998		7,770
3/21/61	8,300	1,660		6,142
4/ 7/61	9,050	1,991		6,063.5
4/28/61	8,950	2,327		5,459.5
5/ 7/61	8,850	1,504.5		5,929.5
1/27/62	10,100	3,232		6,666
2/ 3/62	9,950	2,487.5		6,865.5
2/27/62	12,350	3,828.5		7,904

Table 3. Total and differential leucocyte counts (absolute numbers), control animal No. 390.

12/14/60	10,550	3,376	105.5	6,541
12/23/60	9,850	3,053.5		6,402.5
1/30/61	11,050	2,762.5		6,961.5
2/18/61	9,250	2,405		5,180
3/21/61	8,700	2,088		5,742
4/ 7/61	9,650	2,605.5	193	5,886.5
4/28/61	11,650	3,495		7,106.5
5/ 7/61	10,600	2,438	106	7,102
1/27/62	11,100	2,775		6,438
2/ 3/62	12,900	3,612		7,611
2/27/62	7,450	2,458.5		4,246.5

hemoglobin percentage, and hematocrit values of jugular blood samples of

BASOPHILES	EOSINOPHILES	MONOCYTES	HB.	HT.
	338	422.5	12	34
	76.5	382.5	13	35
	1,005	301.5	11.5	31
	738.5	633	12	33
	189	850.5	12.5	33
	331.5	552.5	13	36
	415	498	13.5	32
	417.5	250.5	12	32
		279	12	34
	90	180	12.5	36
	445	623	12.5	31

hemoglobin percentage, and hematocrit values of jugular blood samples of

	283.5	378	12.5	32
	759.5	651	14	36
		306	12	33
	444	888	13	33
	166	332	12.5	31
90.5	452.5	452.5	14	38
	537	626.5	12.5	34
88.5	354	973.5	12	34
		202	13	38
	199	398	13.5	36
	123.5	494	12.5	36

hemoglobin percentage, and hematocrit values of jugular blood samples of

	211	316.5	13	33
	98.5	295.5	12	31
110.5	773.5	442	12	33
	1,017.5	647.5	14	36
	522	348	12.5	38
	386	579	12	34
	815.5	233	13	36
	848	106	12	34
	1,110	777	12.5	34
129	903	645	14	35
	298	447	13	32

Table 4. Total and differential leucocyte counts (absolute numbers), control animal No. 345.

DATE	TOTAL W.B.C.	NEUTROPHILES	BANDS	LYMPHOCYTES
12/14/60	12,350	2,593.5		7,657
12/23/60	11,650	1,281.5		8,155
1/30/61	13,650	2,730	136.5	9,691.5
2/18/61	8,150	1,385.5		5,623.5
3/21/61	9,950	1,890.5		6,368
4/ 7/61	10,050	3,015		5,326.5
4/28/61	13,000	2,990		7,930
5/ 7/61	9,050	1,267		6,244.5
1/27/62	11,550	2,310		7,969.5
2/ 3/62	10,850	1,302		7,595

Table 5. Total and differential leucocyte counts (absolute numbers), control animal No. 254.

12/14/60	8,150	2,200.5		5,379
12/23/60	14,100	6,486	282	6,768
1/30/61	9,850	2,462.5		7,092
2/18/61	10,950	4,161	219	5,803.5
3/21/61	11,600	4,988		6,032
4/ 7/61	9,650	2,798.5		6,465.5
4/28/61	10,550	3,270.5		6,963
5/ 7/61	8,450	2,704		5,239
1/27/62	8,100	2,106		5,589
2/ 3/62	7,900	1,580	79	5,846

Table 6. Total and differential leucocyte counts (absolute numbers), and after inoculation of experimental animal No. 341.

12/10/60	10,000	3,700	100	5,800
12/12/60	10,350	3,208.5	103.5	6,624
12/23/60	9,900	1,683		7,128
12/29/60	Animal Inoculated			
1/ 6/61	8,700	1,218	87	7,308
1/30/61	10,800	4,320	108	5,616
2/10/61	8,750	2,800	175	4,987.5
2/18/61	11,000	2,640		7,590
3/ 3/61	8,450	2,873		5,239
3/27/61	8,000	1,680		5,760
4/ 7/61	7,900	2,291		5,214
4/28/61	9,750	2,827.5		5,557.5
5/ 7/61	8,000	3,040		4,480
1/27/62	12,050	5,543	241	5,784
2/ 3/62	12,800	3,840		8,064
2/27/62	9,950	2,686.5		6,368

hemoglobin percentage, and hematocrit values of jugular blood samples of

BASOPHILES	EOSINOPHILES	MONOCYTES	HB.	HT.
	1,111.5	988	15	36
	1,165	1,048.5	13.5	34
	546	546	12	31
	652	489	12	32
	1,094.5	597	12.5	36
	1,005	703.5	13	33
	1,430	650	14	32
	905	633.5	12	34
	577.5	693	12	32
	1,519	434	14	33

hemoglobin percentage, and hematocrit values of jugular blood samples of

		570.5	12	31
	282	282	11.5	31
		295.5	14	34
	109.5	657	13	36
	116	464	12	32
		386	12.5	34
105.5		211	15	37
	169	338	12	35
	243	162	13.5	33
	79	316	12.5	32

hemoglobin percentage, and hematocrit values of jugular blood samples before

100	200	100	12.5	41
	207	207	13	38
	792	297	12	33
	87		12	35
	432	324	13	36
	525	262.5	14	35
	550	220	13	35
	84.5	253.5	12	31
	320	240	13.5	36
	79	316	15.5	37
	682.5	682.5	15	35
		480	13	38
	241	241	12.5	33
	128	768	13	31
	298.5	597	12	31

Table 7. Total and differential leucocyte counts (absolute numbers), and after inoculation of experimental animal No. 342.

DATE	TOTAL W.B.C.	NEUTROPHILES	BANDS	LYMPHOCYTES
12/10/60	10,200	1,632	102	8,058
12/12/60	12,050	1,807.5		9,640
12/14/60	14,450	3,179		10,404
12/23/60	12,500	3,500		8,625
12/29/60	Animal Inoculated			
1/ 6/61	16,400	2,132	164	14,104
1/30/61	9,900	1,287		8,415
2/10/61	10,050	1,206		8,140.5
2/18/61	8,000	560		7,120
3/ 3/61	9,950	2,288.5	199	7,064.5
3/27/61	10,850	2,712.5		7,378
4/ 7/61	9,600	1,920		7,488
4/28/61	7,500	1,575		5,400
5/ 7/61	7,550	1,283.5		5,813.5
1/27/62	7,600	3,040		4,408
2/ 3/62	9,100	2,002		6,643
2/27/62	8,700	2,436		5,829

Table 8. Total and differential leucocyte counts (absolute numbers), and after inoculation of experimental animal No. 343.

12/10/60	12,400	2,728	124	8,308
12/12/60	11,800	2,478	236	8,850
12/14/60	11,800	2,714		8,142
12/23/60	10,050	1,909.5		7,135.5
12/29/60	Animal Inoculated			
1/ 6/61	9,450	1,606.5		7,087.5
1/30/61	11,100	1,554		8,769
2/10/61	7,550	2,038.5		4,605.5
2/18/61	11,300	2,599		7,571
3/ 3/61	11,750	2,467.5		6,815
3/27/61	8,950	1,611		6,354.5
4/ 7/61	10,550	2,110		7,174
4/28/61	9,800	1,372		7,154
5/ 7/61	11,550	4,158		6,121.5
1/27/62	7,250	3,697.5		3,335
2/ 3/62	9,700	5,238		4,171
2/27/62	8,250	3,960		4,207.5

hemoglobin percentage, and hematocrit values of jugular blood samples before

BASOPHILES	EOSINOPHILES	MONOCYTES	HB.	HT.
	204	204	13	38
	361.5	241	13	36
	144.5	722.5	12.5	35
		375	12	33
			12	34
		198	11	30
	201	502.5	12.5	32
	160	160	12	33
	199	199	12	32
		759.5	11	31
		192	13	35
	225	300	13	34
	302	151	12	36
	76	76	12	32
	273	182	12.5	31
	348	87	11	32

hemoglobin percentage, and hematocrit values of jugular blood samples before

	496	744	16.5	39
	118	118	14	36
	354	590	13.5	37
	301.5	703.5	13	36
		756	11.5	32
	444	333	13.5	35
	528.5	377.5	12	33
	565	565	14	36
	1,057.5	1,410	14	36
	716	268.5	13	34
	633	633	15	36
	1,078	196	12.5	33
	808.5	462	12.5	36
	217.5			
	97	194	13.5	34
	82.6		14	35

Table 9. Total and differential leucocyte counts (absolute numbers), and after inoculation of experimental animal No. 76.

DATE	TOTAL W.B.C.	NEUTROPHILES	BANDS	LYMPHOCYTES
12/10/60	19,400	6,984		9,894
12/12/60	15,950	3,668.5		9,889
12/14/60	19,250	4,042.5		11,550
12/23/60	17,200	4,644		9,116
12/29/60	Animal Inoculated			
1/ 7/61	25,800	5,676		18,576
1/30/61	15,450	4,326	154.5	9,579
2/10/61	14,450	5,238		8,439
2/18/61	16,800	4,032		9,912
3/ 3/61	15,000	3,750		9,300
3/27/61	19,800	7,920		9,306
4/ 7/61	15,750	4,725		9,765
4/28/61	19,650	4,519.5	196.5	13,951.5
5/ 7/61	13,650	3,412.5		9,555
1/27/62	18,200	6,552		10,192
2/ 3/62	20,400	8,364		10,608
2/27/62	15,050	5,117		8,578.5

Table 10. Total and differential leucocyte counts (absolute numbers), and after inoculation of experimental animal No. 413.

12/10/60	16,900	4,056		12,168
12/12/60	16,150	2,584		12,758.5
12/14/60	16,050	3,691.5		11,395.5
12/23/60	7,200	1,152		5,616
12/29/60	Animal Inoculated			
1/ 7/61	15,250	4,880	152.5	9,760
1/30/61	19,600	4,704		14,112
2/10/61	21,550	4,525.5		15,300.5
2/18/61	19,900	2,786		16,119
3/ 3/61	21,850	3,714.5		17,698.5
3/27/61	22,250	2,892.5		18,245
4/ 7/61	25,550	3,832.5		21,206.5
4/28/61	22,300	3,791		16,948
5/ 7/61	25,050	7,264.5		17,034
1/27/62	23,850	4,293		18,603
2/ 3/62	20,350	3,052.5		15,059
2/27/62	17,750	4,792.5		11,715

Table 11. Total and differential leucocyte counts (absolute numbers), heifer with lymphocytoma*.

11/14/60	19,200	11,904	384	4,992
11/16/60	17,500	12,425		4,375
11/24/60	25,250	11,110	3,282.5	10,100
11/26/60	29,450	15,608.5	883.5	9,129.5
11/28/60	Peroxidose stain yielded 70% granulocytes and 30% lymphocytes			
1/ 3/61	58,000	36,540	4,060	15,080

*atypical lymphocytes

hemoglobin percentage, and hematocrit values of jugular blood samples before

BASOPHILES	EOSINOPHILES	MONOCYTES	HB.	HT.
	1,940	582	12.5	32
	2,073.5	319	12	31
	2,117.5	1,540	10.5	33
	2,064	1,376	11	33
	1,032	516	10	30
	927	463.5	10.5	31
	436.5	436.5	9	29
	1,848	1,008	11	33
	1,800	150	12	32
	1,584	990	12	32
	157.5	1,102.5	11	30
	786	196.5	7	17
	273	409.5	9	27
	1,456		10	31
	612	816	11	29
	903	451.5	11	27

hemoglobin percentage, and hematocrit values of jugular blood samples before

	169	507	10.5	32
	161.5	646	10.5	33
	160.5	802.5	10.5	34
	144	288	12	34
	305	152.5	11	31
	588	196	11.5	33
	862	862	12	34
	398	597	12	32
	437		11.5	33
	667.5	445	11	30
		511	12	33
	1,338	223	12.5	35
	501	250.5	10.5	31
	715.5	238.5	12	34
	1,017.5	1,221	12	33
	532.5	710	11	31

hemoglobin percentage, and hematocrit values of jugular blood samples of the

	576	1,344	9.5	
	525	175	9	28
	252.5	505	7.5	21
	2,356	1,472.5		
	1,160	1,160	8.2	25

Table 12. Percentage distribution of cells from bone marrow from the heifer with after inoculation.

	NO. 76				
	LYMPHOCYTOMA HEIFER		BEFORE INOCULATION	AFTER INOCULATION	
CELLS	11/18/60	12/26/60	12/23/60	4/12/61	11/27/61
Myeloblasts	.2	.8	.2	.2	.8
Promyelocytes	.6	1.6	.2	.6	1.6
Myelocytes	1.6	2.0	.6	.6	1.8
Metamyelocytes	3.4	3.2	2.4	1.8	2.2
Bands	25.4	17.6	7.4	9.4	10.4
Segmenters	6.2	9.2	10.4	14.0	13.6
Eosinophiles	7.0	2.8	10.4	9.8	8.2
Basophiles					
Lymphocytes	27.6	32.0	24.0	16.8	17.4
Monocytes	1.8	5.4	4.4	1.4	.4
Plasmacells			.2		.6
Megakargocytes	.2	.2	.2		
Nucleated erythrocytes	26.0	25.2	39.6	45.4	43.0
M/E ratio	1.70/1.00	1.47/1.00	.797/1.00	.801/1.00	.897/1.00
M/L ratio	1.60/1.00	1.16/1.00	1.31 /1.00	2.16 /1.00	2.21 /1.00

Table 13. Percentage distribution of cells from bone marrow of experimental

	NO. 341			NO. 343		
	BEFORE INOCULATION	AFTER INOCULATION		BEFORE INOCULATION	AFTER INOCULATION	
CELLS	12/23/60	4/13/61	11/20/61	12/19/60	4/14/61	11/23/61
Myeloblasts	.2	.4	.8	.8		.4
Promyelocytes	.6	.8	1.4	1.4	.6	1.0
Myelocytes	.4	.8	1.0	4.0	.6	1.4
Metamyelocytes	1.6	1.6	1.6	3.0	1.2	2.2
Bands	12.4	15.2	10.4	9.0	18.0	12.4
Segmenters	4.0	6.8	11.2	8.4	7.6	11.2
Eosinophiles	15.6	11.0	18.0	6.6	12.4	8.8
Basophiles	.4					.6
Lymphocytes	11.6	14.4	17.0	24.8	23.6	20.6
Monocytes	2.0	3.8	1.6	1.6	1.8	2.2
Plasma/cells	.2	.2	.6	.4	.2	.6
Megakargocytes				.2	.2	.4
Nucleated erythrocytes	51.0	45.0	36.4	39.8	33.8	38.2
M/E ratio	.690/1.00	.813/1.00	1.21/1.00	.834/1.00	1.17/1.00	.994/1.00
M/L ratio	3.03 /1.00	2.54 /1.00	2.61/1.00	1.33 /1.00	1.70/1.00	1.84 /1.00

lymphocytoma, and experimental animals No. 76 and No. 413 before and

NO. 413		
BEFORE INOCULATION 12/23/60	AFTER INOCULATION 5/7/61	11/25/61
.8	.2	
.6	.2	.6
.4	.4	1.4
.6	.6	1.2
4.4	7.2	7.6
14.4	10.8	16.2
5.0	10.8	6.6
		.2
25.4	23.0	23.8
2.6	2.2	.8
		1.0
45.8	44.6	40.6
.572/1.00	.677/1.00	.832/1.00
1.03 /1.00	1.31 /1.00	1.42 /1.00

animals No. 341, No. 342, and No. 343 before and after inoculation.

NO. 342		
BEFORE INOCULATION 12/23/60	AFTER INOCULATION 4/4/61	11/23/61
.6	.4	.8
1.0	.8	1.2
1.2	.2	1.6
1.6	.8	1.4
5.8	13.4	10.8
9.8	10.4	8.6
8.6	13.8	9.6
	.2	.4
23.0	21.0	22.2
1.6	1.2	1.8
		.6
.6	.4	
46.2	37.4	41.0
.619/1.00	1.06/1.00	.839/1.00
1.24 /1.00	1.90/1.00	1.54 /1.00

Table 14. Average of total leucocyte counts, and average lymphocyte counts (in absolute numbers) before and after inoculation.

Experimental Animal	Before Inoculation		After Inoculation	
	Total W.B.C.	Lymphocytes	Total W.B.C.	Lymphocytes
76	17,950	10,112.25	17,508.3	10,646.8
413	14,075	10,484.5	21,270.8	15,983.3
341	10,083.3	6,517.3	9,679.1	7,626.6
342	12,300	9,181.75	9,600	7,364.9
343	11,512.5	8,108.8	9,766.6	6,113.79

Table 15. Average of total leucocytic counts, and average lymphocyte counts (absolute numbers) of control animals.

Control Animal	Total W.B.C.	Lymphocytes
345	11,025	7,256.05
254	9,930	6,117.7
256	9,163	5,635.67
317	9,924.5	6,689.8
390	10,250	6,292.5

EXPLANATION OF PLATE I

Fig. 1. Tissue section of kidney from heifer with lymphocytoma (hematoxylin and eosin stain, x 125).

a. Lymphocytic infiltration.
b. Renal tubules.

Fig. 2. Tissue section of kidney of heifer with lymphocytoma (hematoxylin and eosin stain, x 125).

a. Glomerulus.
b. Lymphocytic infiltration.

PLATE I

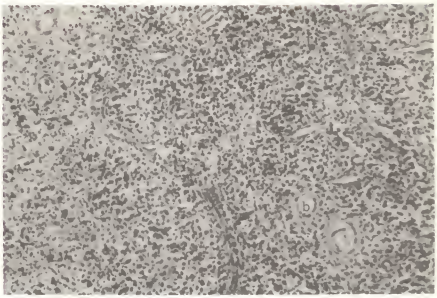

Fig. 1

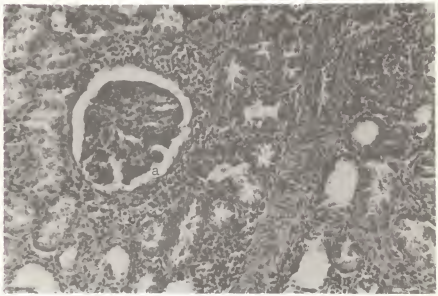

Fig. 2

EXPLANATION OF PLATE II

Fig. 1. Crushed impression smear of normal lymph node (Wright's stain, x 125).

Fig. 2. Crushed impression smear of normal lymph node (Wright's stain, x 500).

PLATE II

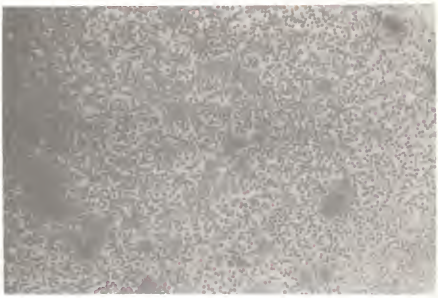

Fig. 1

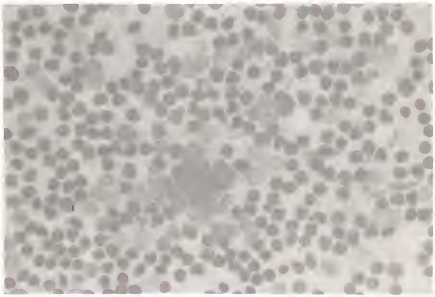

Fig. 2

EXPLANATION OF PLATE III

Fig. 1. Crushed impression smear of lymph node from heifer with lymphocytoma (Wright's stain, x 125).

Fig. 2. Crushed impression smear of lymph node from heifer with lymphocytoma (Wright's stain, x 500).

a. Lymphoblasts
b. Cell undergoing mitosis

PLATE III

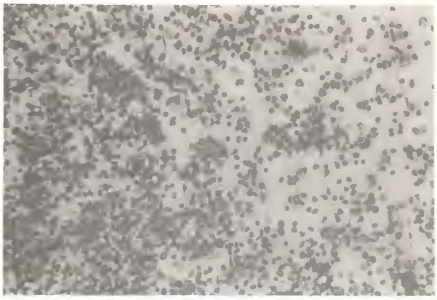

Fig. 1

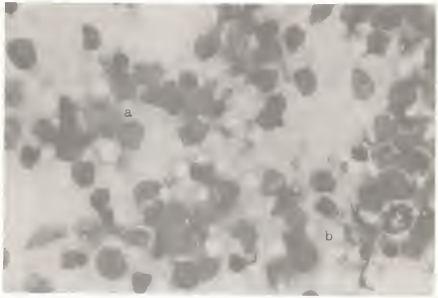

Fig. 2

EXPLANATION OF PLATE IV

Fig. 1. Vitreous humor with neoplastic lymphoid cells which are undergoing retrogressive changes (Wright's stain, x 500).

Fig. 2. Vitreous humor with neoplastic lymphoid cells which are undergoing regressive changes (Wright's stain x 500).

a. Erythrocyte.
b. Mitotic cell with granules and vacuoles.

PLATE IV

Fig. 1

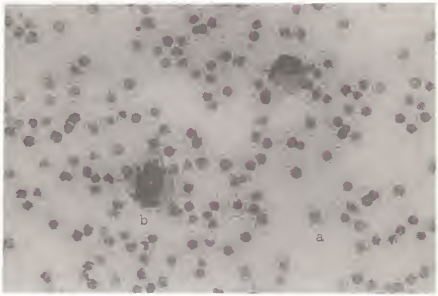

Fig. 2

TRANSPLANTATION OF LYMPHOID TUMORS IN THE BOVINE

by

THEODORE VERA

B. S., Kansas State University, 1956
D. V. M., Kansas State University, 1956

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1962

This investigation was undertaken to determine whether or not lymphocytoma of the cattle could be transmitted from a two year old Hereford heifer afflicted with the disease to five healthy normal cattle ranging in age from one to three years. Tissue homogenates as well as small glandular transplants from the extirpated right prefemoral lymph gland of the heifer were inoculated intramuscularly, intravenously, intraocularly, subcutaneously, and into the medulary cavity of the thirteenth rib. Different routes of inoculation were used for the various animals.

All test animals were observed closely for approximately one year. During this period of time bone marrow and jugular blood specimens were withdrawn periodically and stained following Wright's method. In most instances the peripheral blood could be correlated with the marrow findings. Hematological examination of particularly one bovine, No. 413, revealed a permanent absolute increase of lymphocytes. This blood picture parallels the findings in protein poisoning of calves in which a permanent lymphocytosis is attributed to a peculiar reactivity or lability of the hemopoetic tissues elicited by unknown stimuli. Few if any atypical lymphocytes could be seen and then only after thorough search of any single blood preparation from this case. However, a blood picture identical to those observed in lymphocytoma was never reproduced. In addition it must be noted that in this experimental bovine the pre-inoculation blood counts were greater than normal as well as in case No. 76. However, in the latter case the post-inoculation blood specimens yielded an absolute average of lymphocytes very nearly the same as before inoculation. In none of the bovines were there any enlarged superficial lymph glands.

Two weeks after an animal had been inoculated in the anterior chamber of the eye with lymphoid tissue "plugs" vitreous humor was aspirated, and it was revealed that the transplanted cells were undergoing retrogressive changes. At

the conclusion of this study there was slight scar tissue formed and apparently no visual impairment was observed.

Precipitin tests on the serum of the injected animals were negative.

From all the information available from this investigation it is concluded that inoculation of neoplastic lymph node material from a lymphocytoma affected heifer was without effect on five experimental bovines, and that in no case was the neoplastic disease as seen clinically in the bovine transmitted to normal cattle by the methods used in this study. All bovines including the controls were sold for slaughter at the conclusion of this study, and prior to shipping all appeared in excellent health.