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Current concepts of the viral etiology of the bovine malignant lymphoma

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CURRENT CONCEPTS OF THE VIRAL ETIOLOGY OF THE BOVINE MALIGNANT LYMPHOMA

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PREFACE

In the past few years, research and its concomitant ideas have become more favorably oriented to the concept of viral transmission of the leukemias and lymphomas in both man and the lesser animals.

This paper was promulgated as the aftermath of a summer fellowship in the Eppley Institute during which time, some statistical data were accumulated along with a growing interest in the relative paramedical associations with members of the agricultural and veterinarian associations who have also undertaken great studies in the development and furthering of this topic's popularity.

This paper will make no attempt to form a conclusion, but will be concerned primarily with review of the recent medical and veterinarian literature in order to bring the current ideas into focus.

INTRODUCTION: OCCURRENCES & INCIDENCES OF ANIMAL TUMORS

It has just been in the last few years since the increase in studies of the etiology of malignant lymphoma that some order has begun to be presented in these studies. To begin, many other names have been applied to lymphoid tumors of cattle, eg., leukosis, lymphoblastoma, lymphocytoma, lymphosarcoma, leukemia, lymphadenosis, pseudoleukemia, and malignant lymphoma.

At the W. H. O. conference from February 27 to March 4, 1961, it was officially decided and recommended that the term leukemia be used to embrace all proliferative diseases of the hematopoietic system. However, bovine malignant lymphoma is still the common term in usage in the United States at the present time.

During the last eight years in the United States, one billion animals have been slaughtered, with 80% of the animals being in plants under U. S. D. A. Meat Inspection Division supervision. These animals have all had ante and post mortem examinations. Examined meat was declared unfit for human consumption if the following criteria were met:

1. If the meat were derived from an animal suffering from an abnormal physiological state.
2. If the meat contained organisms or toxins dangerous to consumers.
3. If the meat contained morbid or neoplastic tissue; (a) metastatic tumors were considered evidence and the entire carcass was condemned, (b) carcasses with localized neoplasms are passed when the neoplastic part was removed. (1)

There are roughly one hundred million head of cattle in the United States, and approximately one third of these are slaughtered annually. From the available figures, approximately 8% of all animals slaughtered for food are condemned either wholly or in part. Further study also showed that V. M. I. D. diagnoses were 85% correct and it was noticed that years when the producers kept their stock to increase herd size, there was a decrease in the incidence of neoplasms. When these animals later reached the market (from 1-2 years later) it was found the neoplastic incidence increased.

From the national standpoint, the most common neoplasm of dairy cattle is malignant lymphoma, with cancer eye (or carc eye) which is epithelioma of the eye being the principal neoplasm of beef.

Hog neoplasms are found to be much lower with an incidence of 3.6/100,000 and equal 1.6% of the cattle incidence. This is thought to be because the farmers do not hold hogs a comparable length of time. Hogs have a high reproductivity and rapid growth with butcher hogs reaching the market at about 4½-6 months of age.

Sheep are found to be relatively free with an incidence of 1.7/100,000 condemned in whole or part. This equals 0.75% of the incidence in cattle. However, lambs are 95% of all sheep submitted for slaughter.

Horses apparently have a high tumor incidence with 50.6/100,000. Again, young horses are not slaughtered and

only the much older animals are sent to the abbatoir. In 1962, only 53,000 horses were slaughtered.

Goats show only 9/100,000 or 4% of the cattle incidence.

The most commonly reported tumors in cattle submitted for slaughter are as follows from a series of 293 condemnations and is representative of the United States Department of Agriculture figures:

1. malignant lymphoma.....177 cases
2. adenocarcinoma of the uterus.....116 cases
3. neurofibroma and neurolemmoma.....81 cases
4. adrenal tumors.....61 cases
5. epithelioma of the eye.....58 cases

(1)(2)(3)(4)

In an attempt to determine the picture of malignant lymphoma in the Omaha area slaughter houses for 1957-1960, the figures of the M. I. D. were tabulated and are shown as Table I. It was also attempted to determine the origin of the condemned animals, but due to the method of tabulation it was found that the several areas of more than one condemned animal from the same seller, turned out to be the area serviced by what is known as a "trader" to the buyers. This individual buys and sells livestock, rapidly sells the sick and weak animals, and for this reason, tracing the animal is impossible. It was also found that most individuals who had animals that were condemned were well aware of the animal's condition when it was brought in for sale.

There have been large numbers of monographs published on tumors in domestic animals beginning with early literature reviews by Sticker (1902), Folger (1917), and Teutschlander

Condemnations for Years 1957 - 1960
Omaha Packing Houses
Meat Inspection Division Figures

	YEAR	CATTLE	CALVES	HOGS	SHEEP & LAMBS
MALIGNANT LYMPHOMA	1957	5,418 12*	125 4*	918 5*	86
	1958	5,277	117	781	47
	1959	3,151	98	1006 1*	97
	1960	5,124 6*	131	1,014	189
EMACIATION	1957	6876	8460	918	17 167
	1958	5592	6854	781	16 822
	1959	1260	5785	1006	8 600
	1960	1771	2874	1014	20 000
TOTALS SLAUGHTERED & INSPECTED	1957	20142195		62238518	13 917 875
	1958	18529098		59202889	12 875 560
	1959	17320716		63870 419	12 804 157
	1960	18454318		70 494 437	13 447 780
					* = Ante mortem examination & condemnation

TABLE I. Personal tabulations from M.I.D. reports

(1920). Some of the early authors of original publications began with Murray (1908), Davis, et al, 1933, and many others including Moulton (1961). These reports, along with many others, indicate marked differences in occurrence and types of both common and uncommon neoplasms in domestic animals. It must also be brought out that these papers have a limited value because many of the animals do not live out their "normal" life span as they are marketed for slaughtering.

As some brief examples, it has been shown that epithelial tumors are commonly found in cattle, but relatively absent in the pig, and connective tissue tumors are common in the horse. Lymphosarcoma is found in all species, especially the bovine. Carcinoma of the eye is the most important neoplasm of the ox in the United States, and is seen in the horse, but is rare in other animals. Fibroma of the skin is found more often in the horse than other animals. Tumors of the skin adnexa are found more often in the ox than in other livestock. Melanomas are found most often in old gray horses, but are also occasionally seen in the pig and bovine as well.

Variations in occurrence are found not only in common neoplasms, but also in certain rare growths. For instance, intestinal carcinoma, which is common in man, is considered rare in domestic animals. Uterine carcinoma is found occasionally in the cow, and carcinoma of the stomach occurs in the horse, but is a rare neoplasm in the other

species. Prostatic carcinoma is common in man, but practically non-existent in animals. Bronchogenic carcinoma is common in man and dog, but rarely seen in domestic livestock. There are many other interesting facets to this, but they do not concern this report. (2)(3)(11)

The overall occurence of tumors in domestic animals is not known, but it is felt that tumors are probably more common in the cow and horse than in the pig and sheep. Again, marketing patterns have much to do with this incidence since pigs and sheep are killed at an early age before tumors have a chance to develpp. (See Fig. 2) From this table, it is quite obvious that tumors of marketable domestic animals have a dramatic impact on the economy of the livestock marketing loss. It is also quite obvious that the high rate of condemnation in the bovine is due to the large number of ocular carcinomas and lymphosarcomas. (2)(10)(11)

There are a few tumors of domestic animals that appear to be congenital in origin. These have been reported as embryonal nephroma in the pig, mesothelioma of the ox, and the rare multiple rhabdomyomas of the heart in the pig. (16)

Possible heredity factors have been well publicized and theorized as the etiology. However, due to the heterogeneity of animal population, careful analysis of the genetic role is difficult, and much more information is needed to establish this influence. At present, clear

NUMBER OF CARCASSES CONDEMNED FOR NEOPLASIA ON POST MORTEM INSPECTION 1961

	BOVINE	SHEEP	SWINE	HORSE
Carcinoma	1724	62	280	—
Epithelioma cancer eye	3203	—	2	1
Lymphosarcoma	3840	77	1093	1
Sarcoma	312	19	165	10
Miscellaneous	1082	82	475	28
Total Condemned (Neoplasia)	10,161	240	2005	40
Total Inspected Post Mortem	25 069 089	14 920 931	64 209 639	49394
Per Cent Condemned for Neoplasia	0.04	0.002	0.003	0.08

TABLE II

USDA - 1961

proof is lacking. Blackwell, et al, in 1956, found that predisposition to carc eye in cattle is inherited as a recessive character. Anderson found that he was able to produce the same results. (16)

Weischer, in 1944, found in a German study that 149 cases of lymphosarcoma were noted among 150 of the offspring of one affected bull. These tumors all developed between one and six years. A high incidence of lymphoma may be found in an apparently healthy herd of closely related cattle. As mentioned before, these animals often have periodic or persistent relative increase of lymphocytic atypical forms. This was recently disputed by Theilen, who 1962, reported on the preclinical form of the disease that was exhibited by 34% of the animals in a purebred California dairy herd.

Age has been expounded as an exciting factor but this is quite difficult to evaluate due to the incidence of early slaughter of many of the large domestic animals. (16)

Breed has also been discussed, but due to the various factors of the herds and their incidences for practical and economical reasons in areas of high tumor incidence, no pertinent conclusions were drawn. (16)(17)

Inso far as tumor sites of the various lesions in the different species, it was found that there does seem to be a certain predelection for a particular area in many of the neoplasms. Lymphosarcoma of the cow is frequent in sites such as the abomasam, heart, uterus, vertebra (Downer Syndrome) and the usual sites of the

lymph nodes and spleen. Another apparently entirely different form of lymphosarcoma develops in the skin and seems to manifest itself as predilection areas for metastases. (16)(17)(19)(20)(21)

Reisinger, in 1962, published one of the most extensive, comprehensive, and brilliant papers in regard to all the above questions as compounded in the United States. As before, a high incidence was found in certain areas or districts. Feldman and later, Karlson, reported that in the animal tumors, lymphatic neoplasms had a marked increase or frequency especially in the northern mid-western area. In addition to the aforementioned, a large study was undertaken by the Animal Disease Eradication Division, the Meat Inspection Division of the Agricultural Research Service, representation of the Atomic Energy Commission, and the Armed Forces Institute of Pathology for the epizootilogical study of malignant lymphoma and other internal neoplasms of cattle (excluding epithelioma). (See Figs. 3,4,5,&6)

Reisinger summarized his work by the acknowledgement that tumor frequency figures in slaughter cattle are related to many varying aspects. These include varying countrywide and regional marketing practices, economic factors and also climatic conditions. Due to the marketing practices of far and multiple movements of cattle from origin to destination of abbatoirs, it is quite difficult to ascertain true geographical foci. This is explained

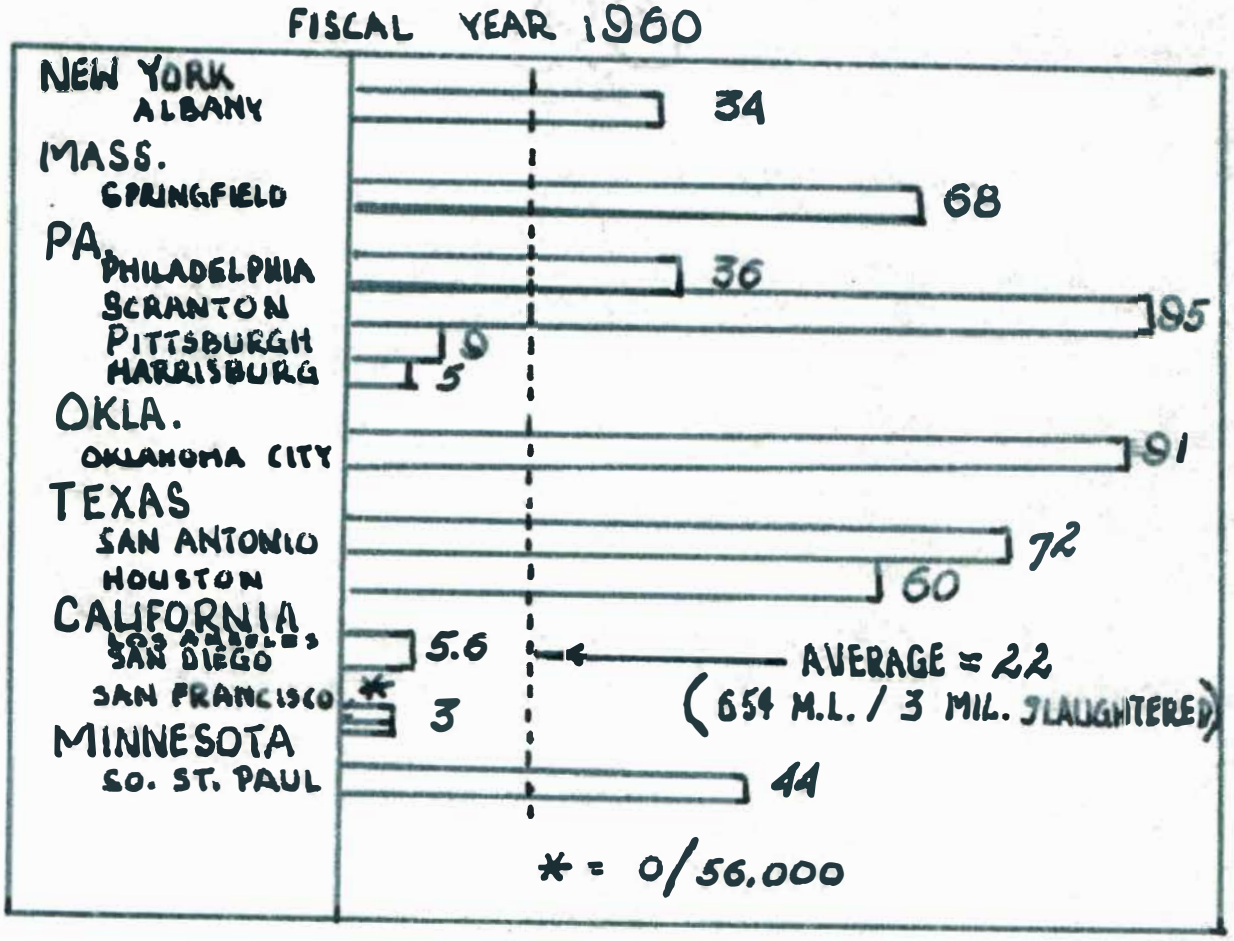

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TABLE III: REPRESENTS REPORTED FREQUENCY RATES (WHOLE CARCASS CONDEMNATIONS) OF MALIGNANT LYMPHOMA FOR 13 DIFFERENT M.I.D. STATIONS. CATTLE SLAUGHTER NUMBERS EXCLUDES CALVES.

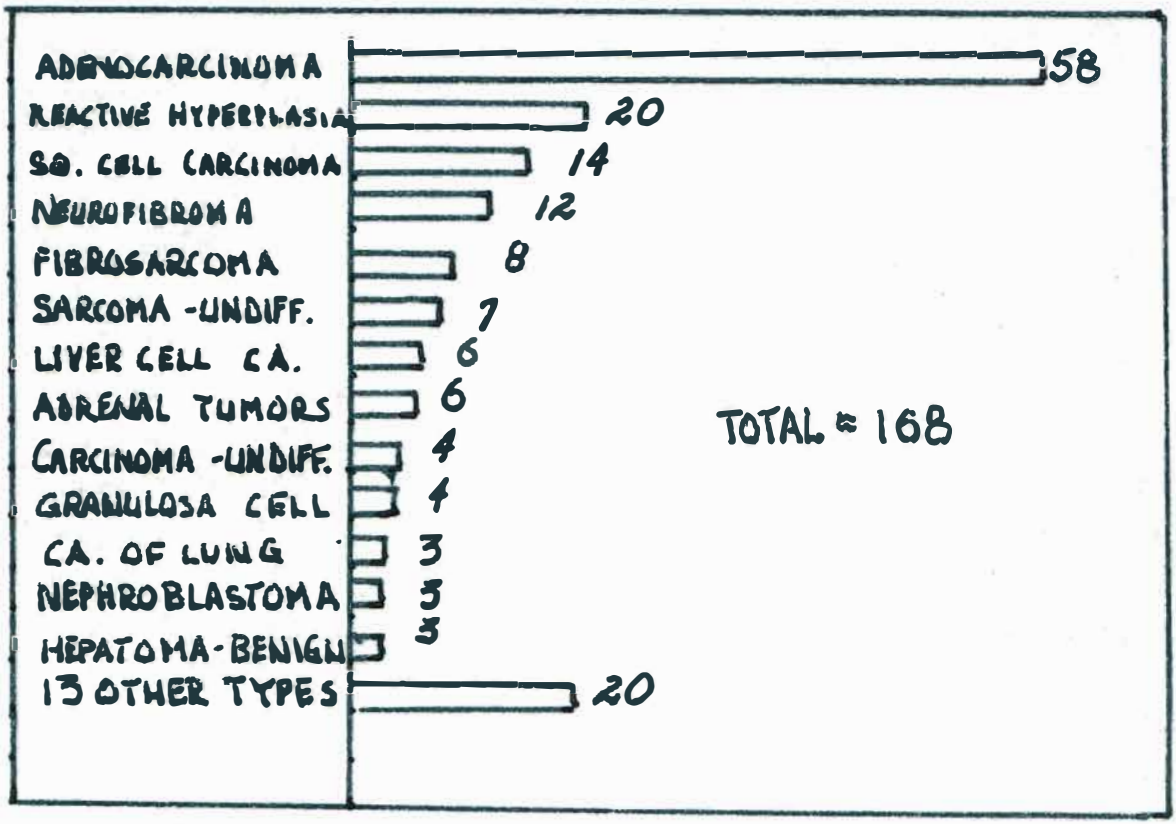

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FIGURE NO. 4: INCIDENCE OF NEOPLASMS (OTHER THAN MALIGNANT LYMPHOMA) AND REACTIVE HYPERPLASIA OF LYMPH NODES SUBMITTED FOR HISTOPATHOLOGICAL DIAGNOSIS.

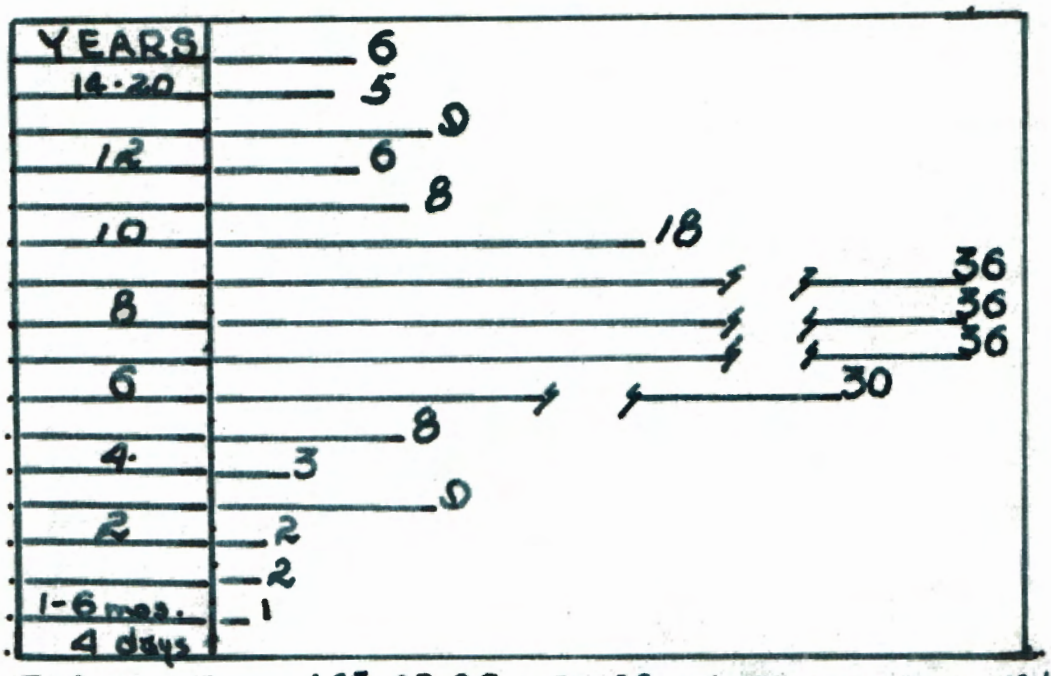

FIG. No. 5 AGE OF 257 CASES OF MALIGNANT LYMPHOMA
U.S.D.A. 213 + 7 Adults, 35 Aged, 2 No information

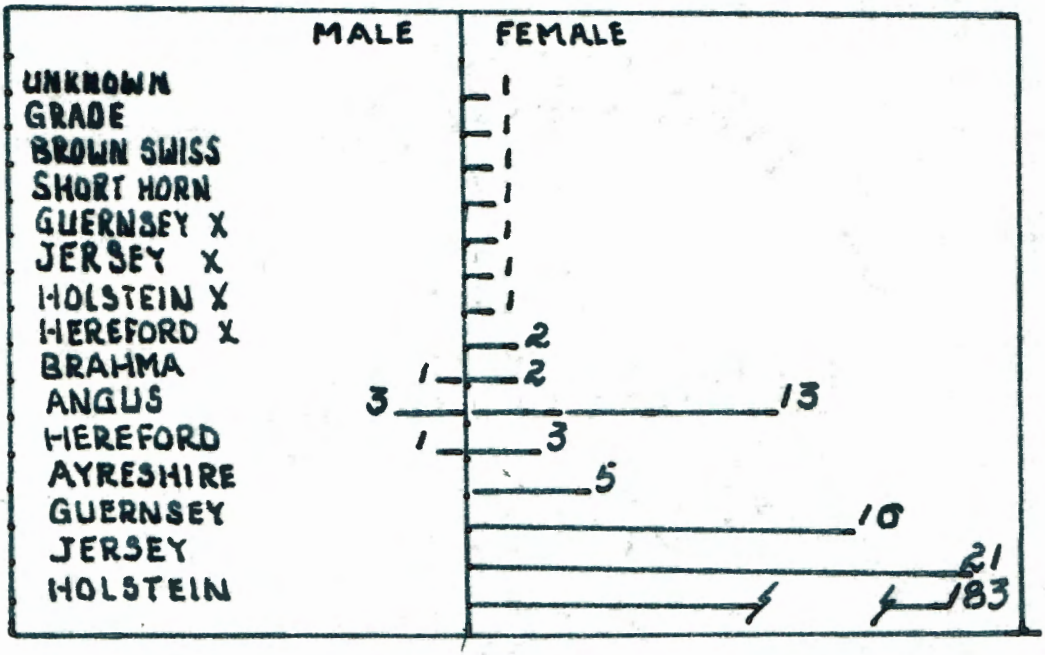

FIG. No. 6 BREED & SEX OF 257 CASES OF MALIGNANT LYMPHOMA

U.S.D.A. AGRI. RES. SER.

by the practice of buying, selling, trading, and speculating with the holding of animals for profit. Further, since not all tumor animals are sent to federally inspected plants, data is often difficult to compile.

Ninety per cent of malignant lymphomas found at slaughter in selected federally inspected plants occurred in cows five years old or older, with 98% in cows two years and older. When frequency data was related to cow slaughter, it was observed that the overall tumor frequency rate increased in inverse proportion to the number of cows slaughtered, and this frequency rate and cattle slaughter number was of a cyclic nature relating to previously described beef cattle cycles.

Available data do not indicate an increase in the frequency of carcass condemnations due to malignant lymphoma or other internal tumors from 1949 to the present time. (1)(10)(14)

Geographical occurrence is another interesting facet of the bovine lymphosarcoma, both as to country and individual farm. The neoplasm may have an enzootic occurrence or it may be sporadic. This tumor is common in Germany, Sweden, Denmark, and the United States. Winquist, In 1962, reported a 2-3% incidence of the disease in adult cattle in Southeastern Sweden, but a lesser percentage in the northern areas. In contrast, lymphosarcoma of the calf in the same country occurred at approximately the same time in all areas. There has been no evidence

of spread when the cattle were transferred from area to area. There seems to be an increase in the incidence of the neoplasm, but most authors feel this is due to more diligent examination and accurate diagnosis. Fortner, in 1944, had noted that the disease seemed to be doubling in Western Germany when in the ten years prior, it had originally been more prevalent in Eastern Germany. Sweden has noted their reported cases have doubled. Also, in the United States, there has been an increase of 150% in adult cattle (1.8/10,000 slaughtered in 1958) and a 34% in calves. (U.S.D.A. 1945-58). There is a notable farm animal incidence increase in the United States as opposed to range cattle. Bendixen, In 1957, noted the disease developed in twenty-five herds where diseased animals were introduced from affected herds. The interval between introduction and recognition of lymphosarcoma in these herds was approximately four years. (1)(5)(10)

Smith, in a summary of 301 canine neoplasms composed of lymphoid, myeloid and related tissues that were studied from the standpoint of incidence, clinical anatomical and histological characteristics, found they all had one thing in common, i.e., they appeared to arise from normal body cells that play an active and important part in reactions against irritants or inflammations. He found that in many instances, diagnostic differentiation from inflammation was made with great difficulty. He had also noticed that tumors of this kind often yielded some of their virulence to the same cortico-steroid therapy that inhibits inflammation. He further postulated that this kind of neoplasia might represent an excessive or abnormal stimulation

of reactive processes against an irritant or combination of irritants that are not unknown but in some other guise. (12)

STUDIES OF THE VIRAL ETIOLOGY OF THE BOVINE LYMPHOSARCOMA

A known viral papillomatosis of the skin is enzootic in some herds of cattle throughout the world. There is some speculation that the great numbers of carcinoma of the eye in cattle of the Southwestern United States may be due to a viral spread and/or the greater duration of periods of sunshine in their areas.

Olson and Cook, In 1951, reported that bovine papilloma virus produces a fibroma when injected into the skin of the horse. It has also produced a fibroma when injected into the skin of the penis of a mature beef animal (McEntee, 1951). Recovery from natural infections of skin papillomas is

common in the spring when the cattle are turned out to pasture. This probably develops from immunity to the virus and less chance of infection from a different strain. Another interesting fact is that animals may be vaccinated against this virus. Bagdonas and Olson, in 1953, postulated that transmission is by contact, possibly through skin abrasion. There is also good evidence that their particular viral papilloma is transmitted venereally from bull to cow and vice versa, and that breeding efficiencies of many bulls is hindered when this tumor occurs in a herd. Beard and Kidd, in 1936, showed there was no antigenic relationship between papilloma viruses of animals and man. (16)(18)

Other neoplasms that may be viral in origin are papilloma of the skin in sheep and goats and the lymphosarcoma and ocular carcinoma of the bovine. The prior mentioned enzootic nature of this tumor in certain countries and farms, the increased occurrence in winter, and the preleukemic blood picture found in herds with a large number of spontaneous tumors all suggest viral etiology. (17)

Paparella, in 1959, isolated an agent from a calf with lymphosarcoma, passed it through chick embryos, and then injected it into guinea pigs and mice, and was able to produce leukemic signs in these animals. He also managed to obtain electronmicrographs of the isolated agent from the chick embryos, and found it to measure 135 mu in diameter. (18)

Due to the many failures and difficulties of trans-

mitting bovine lymphosarcoma in susceptible animals, many authorities have speculated that the infectious agent might be carried through the germ ce lls from generation to generation in an inactive form. Egehøj, ln 1947, postulated that the agent would remain inactive until "triggered" by some unknown stimulus and then this caused the development of the neoplasm.

In addition to the foregoing, the tumor cells of the bovine ocular carcinoma have also been shown to exhibit viral-like particles by electron microscopy (Dmochowski, et al, 1958). (16)

The factors of parasites, carcinogens and irradiation have all been explored as various etiologic agents, but have fallen short of any promising leads. (17)

Trauma has been suggested, but has been ruled out as the singular cause of this cancer. However, chronic irritation may cause and has caused neoplasia in man and some experimental animals. (17)

Several years ago, Heubner, in a lecture on "Common Colds and Cancer" designed for a heterogeneous audience, suggested that modern observations on the potential variability of viral pathogenesis made it easy to imagine that cancer viruses might already be reposing in the well-stocked deep freezers of modern "virus-hunters". He further postulated that a virus causing mild respiratory disease in one individual or species might cause a fatal cancer in another. This supposition has biological merit and too,

known cancer viruses are themselves not much "different" from "ordinary" viruses. As a matter of fact, he further states that the behavior of human adenovirus 12 and 18 in hamsters implies that oncogenic viruses may in some cases be conventional viruses--even "common cold" viruses. Since some viruses tend to become species specific to another, usually in one direction, it is therefore possible to postulate that certain cancer viruses might spread only within species, but that others may also spread from one animal species to another, and therefore, behave like zoonoses. (19)

Certain of the cancer viruses, particularly the avian and murine leukemias are constantly associated with malignant cells, the latter producing large amounts of leukemia virus particles, while at the same time, they carry out normal (or supernormal?) activity. The ability of transplantable tumors to carry extraneous viruses (even oncogenic viruses) is notorious. A question that has not been answered and would answer the myriad questions of viral activity and involvement is, if the leukemia virus were eliminated from the cells and body of the infected mouse or chicken, would the disease (leukemia) disappear, undergo remission, or continue unabated? Rubin, in 1962, reported that the RNA of rous sarcoma (and also probably avian leukosis) is not associated with the genome of the infected cell, and thus, does not behave like the DNA of temperate bacteriophages. This constitutes an important and encourag-

ing thought as this might make the presence of a leukemic virus continuously important to oncogenic activity and therefore, more amenable to chemotherapy. However, more important are the implications provided by the mere existence of such different viruses as the avian sarcoma and leukemias, RNA viruses, and the DNA viruses (including adenoviruses, mouse polyoma and pox viruses.) From these facts, it is quite imperative that thinking must accomodate more than one concept of viral oncogenesis. We must also accomodate our thinking to the fact that oncogenesis may be only one of several pathologic manifestations of a given virus.

Heubner also makes a rather interesting, but unrelated viewpoint to this writing that quite possibly viruses like polyoma (and other relatively nonpathogenic latent viruses) might provide survival advantages to the very young and contribute in an additional way to the survival of the species by interference with lethal effects that readily kill the younger species. He deems it totally unnecessary to consider all viruses pathogenic and an undesirable experience. (19)

Hoag, in an evaluation of Dr. Heubner's paper, pointed out that host-agent-environment dynamics must be thoroughly evaluated as well as the fact that the animal population interspersed among the human cases must be thoroughly investigated for a complete epidemiological study. He also reiterates that one should not make the

mistake of assuming that a particular tumor in man should cause the same tumor type in an animal, or further, to assume that any type neoplasm would result. Horsfall, in 1962, pointed out that under natural conditions, tumor viruses in an animal population frequently cause infections that do not evolve into cancer. Ferrth has pointed out that the various influences of host factors on the growth of neoplastic cells and aptly referred to inducers and promoters of tumors and also the the importance of dosage and/or virulence factors in the manifestation of disease. (19)(20)

With the above for background, we may now go on to the actual physical studies where proof of the viral etiology of lymphosarcoma (or malignant lymphoma) was attempted.

Dutcher and his group have been the big leaders in the United States in this endeavor. In his paper presented in February 1963, he made reference to the following: "European workers believe that the incidence of lymphosarcoma, or "leukosis" of cattle has increased since World War II in the cattle populations of Denmark, Sweden, and Germany. They also believe that "leukosis" is a slowly advancing infectious disease with a long incubation period in which the initial sign is an increase in the absolute lymphocyte count. An infectious etiology for lymphosarcoma has also been suggested by Goetze, et al, in 1956; by the reported isolation of a virus from "leukosis" cattle by Thorell, in 1957, Montemagno, et al, in 1957, and Paparella, in 1959; by the cattle transmission experiments of Rosenberger,

Jarrett, in 1962, and Sorenson, in 1962. However, proof that an infectious agent is involved in the etiology of bovine lymphosarcoma is still lacking.

Dutcher, in early attempts to demonstrate a transmissible agent in cattle with lymphosarcoma, inoculated embryonated eggs, laboratory animals, tissue cultures, and newborn calves with leukemic materials. Indirect approaches included tissue culture, virus interference and flourescent antibody studies. Preliminary work on the characterization of the virus spectrum of cattle with lymphosarcoma has also been undertaken. (21)(22)

A more dynamic study was conducted with the maintenance of a herd of 48 cattle that were offspring from cows that died of lymphosarcoma and animals which, as newborn calves, were injected by multiple routes of various lymphoma tissues. Though some of the animals have been observed as long as three years at the time of publication, none had developed signs of lymphosarcoma. (25)

This group found a chick embryo toxic factor present in bovine lymphosarcoma tissues, but were unable to equivocate a mortality effect. Attempts to inactivate the hemorrhagic and mortality inducing effect by heating were also not fruitful. Their attempts to inoculate laboratory animals were fraught with failure also, Grace, in 1960, and Delong, in 1960, reported on the oncogenic properties of cell free filtrates of human tumors and the production of leukemia in mice from cell free filtrates of human

leukemias respectively.

Dutcher did find cytoplasmic and perinuclear changes in cultured cells derived from cattle with lymphosarcoma that resembled changes reported in cells derived from human leukemia patients. Also, syncitial-like structures or giant cells were observed, reportedly developing in tissue cultures derived from lymphosarcoma. This is similar to the characteristic cytopathic effect of para-influenza-3 virus on tissue culture cells. Flourescent antibody studies suggest that serum globulins from some cows with lymphosarcoma combine specifically with cytoplasmic components in cultured lymphosarcoma cells.

Probably the most promising aspect of their study was that resistance to vesicular stomatitis virus by cultured lymphosarcoma cells, but not by cells derived from "normal" cattle supports the premise that a virus may be present in the tissues of cattle with lymphosarcoma. Attempts are being made to transfer these effects, i.e., resistance to VSV, the syncitial cytopathic effect and immunofluorescence to "normal" bovine tissue cultures.

McKercher, et al, reported the use of virologic (21)(22) techniques in exploratory studies. In their early studies, viral isolations were made from a leukemic cow and also a leukemic horse while none were obtained from leukemic free cattle. The strictest techniques and criteria were used. Permission to import Paparella's strain of virus was granted by the Animal Inspection and Quarantine Branch of U. S. D. A. The isolate was obtained in yolk sac culture

and in compliance with the U. S. D. A.'s request, it was cultured immediately on arrival to insure that mycoplasms had not inadvertantly been introduced. Due to the fact that recent reports indicate that highly fastidious mycoplasma organisms have been hitherto classified as "viruses", great care must be taken in virological studies to be certain the virus is in actuality a "virus".

After completion of screening qualitatively for antibody to the original bovine isolate, it was then tested quantitatively. The relationship of the McKercher's bovine isolate to Paparella's agent and to the equine isolate was also determined by qualitative SN testing, using antisera produced by experimental means from rabbits. SN tests on 18 leukemic cattle showed only three with neutralizing antibody to the bovine isolate. Of thirty-three cattle free from leukemia, none showed any neutralizing antibody to the virus. They feel at this time, with the tests to date, that the three isolates are closely related, if not identical. No claim is made that this report has any association with bovine leukemia, but with present knowledge, it is unwise to call it unrelated.

Their explanation of the low viral recovery rate is the fact that as in the case of the minority of birds with leukosis, all or most leukemic cattle carry both virus and homologous circulating antibody. Therefore, isolation is possible only when the virus is unassociated with the antibody. The failure to demonstrate antibody to the isolate in the serum of most leukemic cattle suggests that there

may be several antigenically distinct types of this virus associated with bovine leukemia. (21)(23)(25)

Paparella has had the most conspicuous success in viral research with isolates from a calf affected with lymphatic leukemia. He makes mention of the fact that little study has actually been done on the incidence of the disease in Italy.

At any rate, in 1956, Paparella attempted to isolate a transmissible agent from a 45 day old calf that had died from lymphatic leukemia. From the lymph node of this calf, a virus like entity was isolated from this cell free filtrate. This was adapted in the yolk sac of chick embryos, showing for the first twenty passages an average mortality of 40%. The possible presence of avian leukosis was discounted by the fact that chicks surviving for three months did not develop any pathological lesions. Furthermore, this filtrate did not produce any alteration when injected into a young chicken and observed for one year. The virus was successfully passed serially for 71 times. Control eggs were inactivated at 60 degrees for thirty minutes. Both these showed low or no mortality and were within biological limits. The embryos killed by the virus showed generalized hemorrhagic and parenchymal lesions. The virus has never shown hemoagglutinating capacity on red cells of the rabbit, chicken, calf, guinea pig and sheep, both at 4 degrees Centigrade and room temperature.

The virus has been successfully adapted to cultures

of calf kidney, employing for the first passage the homogenate of the 45th passage of chick embryos.

The LD 50 for chick embryos of the fluid obtained from the 16th passage on tissue cultures has been compared with that obtained from the 66th passage on chick embryos. The activity of the tissue culture fluid was significantly higher than that from chick embryos with a P value less than 0.05.

The author also inoculated one hundred seven newborn Swiss mice with 0.1 ml. of the viral material obtained from the 11th and 12th passages of calf kidney tissue cultures; 102 newborn mice were similarly injected with heat inactivated virus (68 animals) or with allantoic fluid obtained from untreated chick embryos (34 animals). A total of 222 mice were untreated. All the animals were kept in and under identical environmental conditions and observed daily for three months. Autopsies were performed on every animal. It was found that the inoculated animals had a tendency to develop breast cancer with a latency period of 2-17 months; several of the group had lung mestastases. There were no tumors observed in the control group. Attempts to recover the virus directly from the tumors have been unsuccessful so far.

During a year's period, the author also inoculated 28 newborn calves with viral material and control fluids in his usual disciplined manner. Histological techniques were done on lymph node biopsies. The material was fixed with ether, 10% formalin, or absolute alcohol and stained

with H and E, hematoxillin, Van Gieson, Giemsa stain, Gomori's reticulum stain, and Mallory-Vannucci's stain for connective tissue. Formalin fixation was preferred for all methods except Giemsa for which absolute alcohol was preferred. Blood red and white cell counts, hemoglobin determinations, and blood films have shown no significant changes between experimental and control animals at approximately two years after injection.

The histological examinations of the lymph nodes revealed that, in 14 out of 18 of the experimental animals, the cortex appeared to be thicker, the follicles were decreased and often absent. The borderline between the cortex and the medulla disappears, whereas, it is clearly evident in the controls. The sinuses of the medulla were completely filled with lymphocytic type cells that show hyperchromatic nuclei with narrow or absent cytoplasm. The sinuses of the controls were empty. Reticular cells are frequent while plasma cells, normally numerous, disappear. The hyperplasia of the cortex and medulla results in the disappearance of the borderline between them. Lymph node imprints were made, but as yet, have not been studied and reported on.

Paparella also reported that he had recovered the virus from the lymph nodes of the affected calves and had a Chi square val e of P $\leq$ 0.001. This to him meant significant positive results.

Also, since the viral particle he has seems to be

capable of producing mammary tumors in a strain of non-inbred mice, he wishes to explore the possible immunological relationships of his virus with the oncogenic powers of viruses that have already been demonstrated. He has been able to carry out cross neutralization tests with Rauscher's and Friend's viral antisera. No appreciable loss of the pathogenic power of the virus for chick embryos has been demonstrated. To date, Paparella and his group have been the most successful or even, in fact, the only consistently successful group to isolate, pass, and even to ship their cultures to the United States for further studies in this country.

Paparella states that the interesting finding of the apparent behavior of the virus in mice is at variance with the known behavior of viruses studied so far. There is not at present sufficient evidence to imply the observed tumor growth in the mice is induced by this agent and it is not absolutely certain that this could not be a viral agent latent in the mice that, reacting with their viruses, gives rise to an oncogenic effect. Too, the reactions of the lymphoid tissues from the introduction of the virus was interesting, but no conclusion can be drawn at this time. (23)

Another report from Hatziolos and Chang revealed that cells of five sub cultures obtained from the original culture of affected animals cell free isolate in Eagles medium supplemented by 10% calf serum preserved their morphologic characteristics which differentiated them from the cells of normal lymph node tissue cultures, also growing in the same

medium. The main characteristics of the cells in question were (1) irregular growth in all directions and piling up of the cells in superimposed layers, (2) irregular size and shape of the nucleus, being rounder and larger than in the normal lymph node tissue culture cells, (3) more abundant and prominent structure of the chromatin network, (4) large and numerous prominent nucleoli, (5) more abundant, dense and less vacuolated cytoplasm which was less affected by large vacuoles, and (6) cytoplasmic inclusions which appeared to be of two types. These appeared to be granular, and flourescence of the small yellowish-green bodies seems to indicate the presence of predominant amounts of DNA. Sequence of cellular changes suggestive of the presence of a cytopathogenic agent in the lymphosarcoma tissue extracts of two cows in dilutions up to 10^{-4} were also observed. Intense mitotic activity in cells located around the sloughing areas of the monolayer culture of normal bovine lymph node, cytoplasmic inclusions, and large mononuclear or giant cells were observed in the second successive passage on this monolayer, suggesting multiplication of the agent in serial passages. (26)

Blood reaction of twelve one-day old calves inoculated with cell emulsion, tissue cultures, and extract of tissues taken from an affected cow, was, according to Bendixen's classification, positive for five, suspicious for three, and negative for four. The calves under observation were between the first 4-8 months of life and those with the

positive reactions were the ones inoculated with the tissue cultures.

Of 435 mice inoculated, a high incidence of lymphocytic leukemia with a relatively short latency was observed among the mice of a group inoculated intracerebrally with extract. These mice were the progeny of parents who had also been inoculated intracerebrally with extract from the same cow. Longer observations and more laboratory tests are being awaited for further definite conclusions. (26)

One of the more recent studies in tissue cultures was performed by Dutcher, et al, in 1963, where they successfully made normal susceptible calf tissues resistant to VSV by inoculating them with supernatant fluids from resistant tissue cultures in series. Tissue cultures from lymphosarcomatous animals, their progeny, nursing calves of lymphomatous mother and normal cattle were used. It was found that there was interference with all but the normal animal tissue cultures. In one instance, resistance was conferred upon susceptible lymph node tissue cultures.

Giant cells and multilobed and fragmented nuclei were observed in lymph node tissue culture from cattle with lymphosarcoma. A cytopathogenic factor was passed twice in normal lymph node, Hela and Chinese hamster cells from a VSV-resistant tissue culture in which giant cells persisted throughout its passage life. (33)

Theilen and Sorenson have done the most recent work on the electron microscopy studies. Montemagno (1959) was

one of the earliest to report on his findings in this same endeavor.

The neoplastic cells of bovine lymphosarcoma possess ultra-structural features common to normal bovine lymphocytes as well as to normal and neoplastic lymphocytes from a variety of other species. In general, the cytologic features of the neoplastic cells are somewhat similar to those in immature lymphoid cells. These observations are in agreement with studies of the ultra-structural characteristics of malignant neoplasms including lymphosarcoma in man. The cytoplasmic inclusions observed were interesting, but difficult to interpret. Certain features of the inclusion particles such as the size, shape, clusters and variation in appearance which may represent stages of development are consistent with characteristics of known viruses. It must be remembered that if these are virus particles, they may still be a coincidental infection or a passenger virus. Too, it must constantly be remembered that the sampling difficulties of electron microscopy may miss what could possibly be focal changes in the neoplastic cells that are not manifested at the same time. It is also theoretically possible that if one could examine the lymphomatous tissue at the right stage of the disease (whenever this might be), one would more regularly find the "virus". (25)(27)(31)(33)

ATTEMPTS TO CORRELATE VIRUS ETIOLOGY OF LEUKEMIA IN ANIMALS AND MAN

Many workers in the last century had long felt that leukemia was a disease that could possibly have an infectious etiology. There was no basis of proof, and these ideas were largely conjectural in nature. The ideas were postulated because of the clinical similarity of the disease in man and animals and the clinical picture that was seen in infections. This idea was gradually repressed until the last few years, and especially the last thirty years, has brought forth a renewal of interest.

Mulligan, in an extensive study and concomitant publication, reported on his findings of 2112 tumors in 1828 dogs. This study had started in 1949, and 20% of his findings were from the autopsy table and 80% at operation. His findings are largely in tabular form with the most important conclusions being that verruca vulgaris in man and the dog had a possible viral inter-relationship and, of interest to this writing, that a more intensive search for the possible viral etiology of canine histiocytoma be undertaken and whether this disease might not bear a causal relationship to human Hodgkins's disease. (13)(15)

Probably one of the most widely quoted and extensive early publications in the United States was that of Marshak, et al, In 1960. These authors tried to correlate the resemblance between human and bovine leukemia. Both diseases are characterized by localized neoplastic proliferation of lymphoid tissue. There is diffuse organ infiltration and/or the formation of discrete tumor masses. There is a monotonous growth of cells resembling either lymphocytes or lymphoblasts, but

more often, a combination of both.

The symptomatology in cattle and humans is about the same and there is usually the presence of a definable mass.

CATTLE

1. high incidence of cardiac involvement
2. low incidence of cutaneous involvement
3. increased serum uric acid
4. increased SGOT
5. increased SGPT
6. increased LDH
7. lymphocytoma

HUMANS

1. increased serum alkaline phosphatase
2. increased serum uric acid

Death results from:

1. tumor cachexia 38.6%) especially
2. infection 35.8%) humans
3. anemia
4. mechanical encroachment
5. cardiac failure

The incidence for humans is estimated to be 14,000 lymphomas per year, and 11,000 leukemias per year. The age ranges from infancy to a recorded 107 years.

There are no reliable figures for cattle on overall incidence. In 1958, the ratio was approximately 17.6/100,000 animals and primarily in mature animals. There is a sex difference, but bulls are not kept in dairy herds and therefore, this difference lacks validity. (5)

Karlson, a European worker, in 1897, reported the occurrence in related animals and in 1915, the disease became epizootic in East Prussia. There have been many workers trying to relate the disease to heredity factors. One study by Weisches, "An Outbreak of Cattle Leukosis" (5), (the common European terminology), had 149/150 daughters sired by an affected bull succumb to the disease. Starr and Young, in the United States, followed several families with four successive

generations of affected cattle. (5)

More recent European studies have discounted the hereditary factors. They now seem to favor the concept of lymphoma as a slowly advancing infectious disease. However, it is also generally acknowledged that the spread of disease parallels the exchange of breeding animals. Bendixen, in his well publicized studies, showed quite adequately.

Marshak, et al, in their studies, showed that the probability of chance of the animals contracting the disease calculated to P=0.0000441, stretching credulity.

The established viral etiology of avian leukosis and lymphocytic mouse leukemia gives impetus to studies of lymphoid tumors in other species. As the authors state, bovine lymphosarcoma should be particularly interesting because it so closely resembles the disease in man. (5)(6)(7)(8)(9)(10)(30)(3

The development of highly inbred strains of mice with high and low leukemic strains from selective breeding has evinced great interest in this disease again. It was thought that genetic factors were the determining cause of the murine leukemia, but it was eventually shown that, although genetic factors play a part, infection is primarily responsible for the development of the leukemia and the infectious agent is a virus. This virus has been extensively studied, characterized, photographed, and finally accepted as the murine leukemia agent.

This inevitably brought about the question of whether human leukemia is also virally induced. It has been well documented and proven that human leukemia tissue extracts will accelerate the disease in the high leukemic strains of mice and will induce leukemia in the low leukemic strains. In other animals, these extracts have produced a "leukemia-like" disease. Viruses similar to those seen in the mouse leukemias have been recovered from the blood of patients with leukemia while these same type of particles, by electron microscopy,

are but rarely found in the "normal" population or the non-leukemic individuals. (27)

Other factors have also evidenced much interest in this aspect of etiology. Two of these which are now of common knowledge are the "lymphoma belt" in Africa and the increasing awareness of "leukemia clusters" as that popularized in Niles, Illinois.

At the present time, no satisfactory explanation for this has been made. The only explanation so far is that they represent a foci of infection, the mode, route, incubation, susceptibility and/or resistance all being unknown. (34)(35)(36)(37)

The current techniques for the demonstration of leukemia antibodies apparently gives confirmation to the thesis that leukemia is an infectious disease and that antibodies may form in a manner common to other bacterial and viral diseases. It is well known that some viruses produce chromosomal abnormalities and are oncogenic in laboratory animals. Viruses may also evoke malignant transformations in human tissue cultures and these facts are used in data to correlate leukemia etiology to virus.

It has been suggested that human leukemia is due to a virus of low pathogenicity and wide prevalence. It is also thought to be blood borne, but the method and cause of virulence has not been determined. (39)

In mice and chickens, leukemogenic viruses are transmitted vertically from mother to offspring, but a world

review of literature by Fraumeni, in 1964, revealed that, of about three hundred women with leukemia during pregnancy, in only one case was the child reported to have leukemia. Burch, in 1963, has pointed out that vertical transmission of a virus should be more frequent through the mother than the father, but his publications reveal an equal incidence in the lineage of mothers and fathers. (38)

Straub and Amiotto, in 1960 and 1953, in two different publications showed that no cases of leukemia were found in the mothers of 324 leukemic children in one series, and in another series, there were 855 leukemic persons of all ages in which only one of the mothers was affected. (37)(38)

Probably one of the most interesting reports that has come out just recently is the one by Wheelock and Dingle in which they reported on their observations of viral agents. Since the reports of Dmochowski, in 1963, of electron-microscopy studies of host-virus relationships, Almeida, et al, in 1963, and his report on virus like particles in two human leukemic patients and Burger, et al, in 1964, who reported on the same subject, the authors theorized therapy should be presented in terms of viral inhibition. They reasoned that therapy with an anti-viral agent such as interferon or viruses that stimulate the production of interferon or directly interfere with the "hypothetical virus" should be a rational and sound approach to anti-leukemic therapy. They theorized that if selective inhibition of a leukemia virus genome is integrated into the cellular DNA and divides with the cell, inhibition would be difficult.

They employed six different viruses and noted an unexpectedly prolonged course together with decreases in both the number of myeloblasts and size of the spleen and lymph nodes in a patient that had acute myelogenous leukemia. The viruses used were Sendai, Newcastle disease, PR8 and Lee strains of influenza, Semliki Forest and Sindbis. The administration of the viruses was associated with fever, but no other unusual signs or symptoms. Semliki Forest was the only virus that showed subsequent viremia and infectious evidence. The 95% blastic composition of the bone marrow was unchanged during the study until the death of the patient when less than 1% myeloblasts were seen. One of the exciting aspects of the study was that, on two occasions during life, interferonwas detected in the patient's serum and during post mortem examination of samples of the patient's lymph nodes, spleen and bone marrow.

This study, as so many others, is not conclusive due to the paucity of subjects, but it adds one more log to the fire of knowledge regarding viral etiology and activity in the lymphomas and leukemias. (40)

DISCUSSION AND SUMMARY

The foregoing pages have attempted to review the early thinking and work in the area of the malignant lymphomas and leukemias. A brief history of the disease and its impact on our economy was also presented. Only those articles that were pertinent were reviewed and these included some that were quite recent. Most of the publications have not observed their materials long enough to

draw valid conclusions at this time, and many of them did not have enough material to warrant dogmatic statements. It must be said that each added its small token of information to the lengthening list that seems to implicate viral origin or activity in the etiology of the lymphomas and leukemias.

Many studies are currently in progress to further unlock bits of information that may someday solve this enigma. Currently, there is a study of familial leukemic incidence in Nebraska and an attempt to correlate these with areas of increased bovine malignant lymphoma.

At present, no pertinent conclusions are drawn from these studies except the well known fact that, in the Kearney, Nebraska area, there is an apparently marked increase in the number of leukemic and lymphomatous patients. This is, of course, an area that is relatively high in the census of cattle.

It may well be that the answer will someday be shown as something that has been viewed and recorded many times, but that, as yet, we have not recognized in its various guises.

In a recent seminar at the Eppley Institute, Dr. A. A. Werder related to the assembled group how they had grown the high leukemic strain of mice in a germ free environment and not one of them had developed leukemia, even though, under normal conditions, this particular strain of mice would be expected to have a 30% incidence of the disease. These animals were delivered by Cesarean operation

to insure a germ free condition, and from this procedure it was assumed that the viruses do not cross the placenta, or in fact, are not born with the virus. If, however, these animals were nursed by leukemic mothers or placed in close contact with leukemic animals, they also contracted the disease. Germ free animals in the general area did not get the disease, and it was deduced from this that the virus is not air-borne.

Another extremely interesting aspect of their work was the inoculation of germ free mice with bone marrow from human leukemia patients. The mice then developed a leukemia although it was different from that seen in the mice that developed leukemia without the benefit of inoculation. Perhaps in the near future, with the benefit of experiments such as these, and even more sophisticated experiments, a positive correlation between the bovine malignant lymphoma and human leukemia will become reality and we may be able to answer one of the Vice-President of the Union Stock Yard's statements that was made during initial investigation of lymphoma incidence. As our program was unfolded to him, he stated, "I'm certainly glad to see you here and this program undertaken because we have often remarked ourselves that it seems as though a lot of our stock yard employees die from leukemia!"

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