LYMPHOSARCOMA or LYMPHATIC LEUKAEMIA in the DOG and CAT

by

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The thesis is divided into three parts. The first is a description of lymphosarcoma or lymphatic leukaemia in the dog and cat. The second is a report of transmission experiments with lymphosarcoma of the cat. The final part is a description of a series of experiments designed to calibrate and evaluate an electronic particle counter for use in experimental haematology in the cat.

Part one of the thesis is in two sections. The initial section deals with lymphosarcoma in naturally occurring cases in the dog. This is one of the most important malignant conditions encountered in this species and is a neoplastic disease of the adult animal which is relatively short in duration. It is characterised by the abnormal proliferation of cells of the lymphoid series on sites of lymphoid tissue together with infiltration and invasion by tumour cells into many organs and tissues throughout the body.

One hundred and fifty dogs which had been admitted to the University of Glasgow Veterinary Hospital suffering from lymphosarcoma formed the basis of the study. Each animal had been subjected to a full post-mortem examination. The morbid anatomy, histo-pathology, haematology, blood biochemistry, radiology and clinical data in this group are tabulated and also described in the text. A classification of the disease is proposed which is based on the distribution of the major lesions. This classification is particularly useful from a clinical viewpoint, since the two common forms of the disease are associated with distinctive syndromes

and have thus to be differentiated from two different groups of disease. Three forms of lymphosarcoma are recognised. There is a multicentric type which is associated with bilaterally symmetrical enlargement of most of the superficial lymph nodes and with hepatosplenomegaly. An alimentary type is found which is characterised by tumour lesions along the alimentary tract and/or gross infiltration of the mesenteric node, but there is no infiltration or enlargement of the superficial lymph nodes. In both of these forms of lymphosarcoma other organs and tissues are involved to a greater or lesser extent. The third form of the disease, the so-called aberrant type, is relatively rare and tumour lesions are found in sites other than the superficial lymph nodes or the alimentary-mesenteric area. The multicentric and alimentary forms of the disease together account for approximately 94% of the cases in this series and they occur in equal numbers.

On the basis of a quantitative study of the post-mortem histology of affected lymph nodes and lesions of the alimentary tract, diagnostic criteria have been determined and are presented. The essential diagnostic markers in the common forms of the disease have also been established and are described.

Although abnormal changes in the peripheral blood are frequently observed, they are seldom specific. A true leukaemia, comparable to leukaemia in man, is uncommon in the dog, so that blood is of little value as a diagnostic tool. Furthermore, the useful correlations which

have been found in man between the duration of overt haemopoietic tumours and such factors as the degree of differentiation of the dominant tumour cell, mitotic rate and the age of the patient are not correlated in lymphosarcoma in the dog and are of no value in prognosis in this disease.

In the second section of part one there is a description of naturally occurring cases of lymphosarcoma in the cat. The methods of study and presentation are similar to those of the previous section. With certain exceptions the disease in the cat is similar to that in the dog. The three forms of the disease which are observed in the dog are again found. A fourth type is also recognised. This is a so-called thymic type, which is associated with a gross tumour mass in the anterior mediastinum at the site formerly occupied by the thymus. In this form of the disease, the tumour of the anterior thorax is the sole macroscopical lesion in the body and is accompanied by a characteristic respiratory syndrome. Space-occupying lesions in the anterior thorax are also observed in certain other cases, but because of the presence of superficial lymphadenopathy, these are included in the multicentric group. As in the dog, lymphosarcoma in the cat is essentially aleukaemic.

Part two of the thesis comprises a preliminary report on initiation experiments designed to transmit this disease by inoculating newborn kittens with preparations from spontaneous and experimental cases of the disease in this species. This is part of a long-term research programme which is being carried out at this Hospital to test the hypothesis that lymphosarcoma in the domestic mammals is caused by a virus.

Following the injection of newborn kittens with preparations from tumour tissues of two field cases, lymphosarcoma developed in animals in two experiments. The latent periods have been between 9 and 43 months. In further experiments, preparations from one of the experimental animals which developed a form of lymphosarcoma indistinguishable from a multicentric case were injected into newborn kittens. One kitten developed lymphosarcoma of all the marrow cavities at the age of 8 weeks. Other kittens which have died have shown cellular changes in the Malpighian corpuscles of the spleen which are comparable with early lesions of lymphosarcoma as observed in certain field cases of the disease in the cat.

Virus-like particles which are similar to those observed in experimental leukaemia in mice have been found on electron microscopical examinations of the tumour tissues of two of the experimental cats.

Part three of the thesis contains a description of the calibration and evaluation of an electronic particle counter for the estimation of total erythrocyte and total leucocyte counts in experimental leukaemia of the cat.

The experiments included a test of the reliability of the instrument and the determination of optimal threshold settings and electrical currents for the two types of blood cells. Comparisons were made between the electronic method and the traditional haemocytometer technique. The variations amongst electronic counts of the same cell suspensions and also the variations amongst counts of different cell suspensions

prepared from a given blood sample were next established Finally, the performance of different technicians with the same blood samples were compared.

The results of these experiments were subjected to statistical analyses and it was concluded that the electronic method was highly accurate for total erythrocyte counts. Whilst somewhat less accurate for estimating the total leucocyte counts, the new technique compared very favourably with the traditional method.

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PART ONE.

NATURALLY OCCURRING LYMPHOSARCOMA OR LYMPHATIC LEUKAEMIA OF THE DOG AND CAT.

SECTION I.

LYMPHOSARCOMA OR LYMPHATIC LEUKAEMIA OF THE DOG.

INTRODUCTION.

Lymphosarcoma is a neoplastic disease which is characterised by the abnormal proliferation of cells of the lymphoid series and is associated with visible changes in the size and structure of lymphoid tissue due to the formation of relatively uniform and infiltrative sheets of lymphoid tumour cells. Discrete metastatic tumours and diffuse infiltration by tumour cells may be found in many other organs and tissues. If this is accompanied by quantitative or qualitative changes in the lymphoid cells of the peripheral blood or infiltration of the bone marrow, the term Lymphatic Leukaemia is permissible.

Lymphosarcoma is one of the most important malignant conditions observed in the dog, Cotchin (1954), Meier (1957). It is a disease of comparatively short duration and it is fatal. Little is known of its pathogenesis, and even less of its aetiology.

Reports on this condition in the literature are somewhat confusing due to the application of terms borrowed from a rather ill-defined human nomenclature. There is, therefore, an advantage in starting with an outline of the disease complex in man, so that the veterinary nomenclature can be better appreciated.

HAEMOPOIETIC TUMOURS IN MAN.

At the present time, malignant disease of the haemopoietic tissue is divided into 2 apparently distinct groups:- 1) The Leukaemias and 2) Tumours of Lymphoid Tissue.

Leukaemia was a term first coined by Virchow in 1846, to describe a fatal disease in man, which was characterised by splenomegaly together with specific changes, both quantitative and qualitative, in cells of the peripheral blood. Later, it was established that this was a disease of the haemopoietic tissue associated with abnormal proliferation of blood cells, or blood cell precursors, in haemopoietic centres or other sites throughout the body, and that blood changes were not an invariable finding. The word leukaemia persisted, however, and gradually became a convenient non-specific generic term comparable to the term anaemia.

Since an essential feature of leukaemia is neoplastic growth of one or more of the cell types found in haemopoietic tissue, it has been found possible to classify it by the predominant tumour cell present. The following types have been recorded in man (Modified from Whitby and Britton, 1953):-

Myeloid leukaemia, acute and chronic.

Lymphatic leukaemia, acute and chronic.

Monocytic leukaemia, acute and chronic.

Atypical and mixed leukaemias.

Polycythemia Vera and Myelomatosis might also be included in this list.

Good correlations have been established between the duration of this disease, the maturity of the predominant tumour cell, and the age of the patient. When associated with an undifferentiated or stem cell, the course of the disease is short, tending to be fatal within a few weeks or months after onset. This type is most common in children and young adults. Where a more mature tumour cell is discovered, the duration is very much longer and patients are usually in an older age group. Because of these rather close relationships, the terms Acute and Chronic have been introduced, but it should be noted that these descriptions are essentially clinical and meaningful in prognosis. In both of these types, a very common finding is widespread involvement of bone marrow, although the peripheral blood picture may vary. When the absolute blood count of the leucocyte, which is incriminated in the neoplastic process, is much higher than normal this is described as leukaemia. If the absolute count is within normal limits the blood picture is aleukaemic and this state can also be termed pseudo-leukaemia. Subleukaemia apparently refers to one of two findings, a) aleukaemia where circulating immature cells are observed, i.e. one pattern of acute leukaemia, or b) a persistent white blood count of less than 30,000 per cu.mm. where the cells are all mature in type, i.e. a chronic type.

The phenomenon of an increased white blood cell count is termed leucocytosis. It is not associated solely with malignant disease of the haemopoietic system, but is frequently temporary in nature and accompanies various infectious processes. If both the neutrophil and the lymphocyte count is raised,/

raised, this is called an Absolute Leucocytosis. Where only the polymorph count is increased, the term employed is Neutrophilia; and Lymphocytosis refers to an absolute increase in lymphocytes alone. The so-called Leukaemoid blood picture is used to describe a more lasting rise in the absolute count which is often associated with toxic states, infective conditions or malignant diseases in general. It has been established that such non-leukaemic haematological findings can almost invariably be differentiated from true leuhaemia by bone marrow biopsies.

Leukaemias, therefore, have become classified in 3 ways, 1) according to the predominant tumour cell present, 2) from the degree of tumour cell differentiation, but actually because this is related to the duration of the illness, and 3) on haematological data.

Another most important group of malignant diseases in man is that of Tumours of Lymphoid Tissue. These are not so commonly associated with peripheral blood cell changes and have been classified almost entirely on a histological basis. Lumb (1954), in a monograph on a series of such conditions, which were observed at the Westminster Hospital, London, between 1940 and 1952, described 6 tumour types as follows: 1) Follicular lymphoma. 2) Lymphosarcoma, including a) lymphoblastoma, and b) lymphocytoma. 3) Reticulum cell sarcoma (lymphosarcoma of reticulum cell type). 4) Reticular lymphoma (Benign Hodgkin's disease). 5) Hodgkin's disease and 6) Anaplastic sarcoma of lymphoid tissue.

Follicular lymphoma (syn. Follicular lymphoblastoma, Giant follicular lymphoblastoma,/

lymphoblastoma, Lymphoid follicular reticulosis) is believed to be a tumour of low malignancy with a high potential. Localised enlargements of lymph nodes and splenomegaly are found. Histologically, the enlarged nodes have lost their usual cortical-medullary arrangement, becoming packed with large and irregular "follicles" which have compressed the sinuses. This condition bears a superficial resemblence to a reactive lymph node. Diagnosis is based on medullary invasion by follicles, absence of sinuses containing inflammatory cells, and, most important, the absence of macrophages and the rarity of mitotic figures within the so-called germinal centres.

Lymphosarcoma (syn. Lymphoblastoma and lymphocytoma) is associated with lymph node enlargement of the neck or abdominal nodes. Splenomegaly occurs in 50% of the cases whilst gross foci of lymphocyte proliferation in bone marrow is noted in 25%. Many other organs and tissues may be infiltrated including the skin producing the condition termed Mycosis fungoides. Histologically, lymph node architecture is lost and replaced by uniform sheets of lymphoblasts or lymphocytes arranged on a fine stroma of reticulin fibres with a supporting fine vascular network. Trabecular infiltration and invasion of the capsule and the peri-nodal regions is very common.

Reticulum cell sarcoma (syn. Lymphosarcoma-Reticulum cell type, Reto-thelsarcoma, reticulocytoma) is characterised, histologically, by uniform sheets of reticulum cells, which replace the normal tissue of an affected node. Multinucleate cells are rare. Fibrosis and necrosis may be features. The condition may start in nodes of the neck or in bone marrow and become multicentric. It is an uncommon, but highly malignant disease.

Reticular lymphoma (syn. Hodgkin's paragranuloma, Lympho-reticular-medullary-reticulosis, Early Hodgkin's disease, Benign Hodgkin's disease) is considered to be the primary stage of true Hodgkin's disease. It is also uncommon (5.1% of Lumb's series). Lymph nodes have been found to be the only site of this disease so far. The picture is one of sheets of lymphocytes dotted with isolated reticulum cells, some of which may be bi-nucleated. Capsule invasion does not occur and necrosis is rare. There is more evidence of collagen formation than in lymphosarcoma. The duration of this type may be up to 10 to 15 years, although it may quite suddenly become much more malignant and deteriorate into true Hodgkin's disease.

Hodgkin's disease (syn. Hodgkin's granuloma, Lymphadenoma, Lymphogranuloma malignum, Fibromyeloid medullary reticulosis) is by far the most important type of lymphoid tumour found in man and accounted for 46.1% of the Westminster series. Any or all of the lymph nodes may be enlarged and the spleen, liver and bone marrow are also often affected. Skin infiltration is common, resulting in another form of Mycosis fungoides. Lesions along the alimentary tract are seldom found in comparison to lymphosarcoma where alimentary tumour formation is a feature. The blood picture varies, but it is never diagnostic. Severe sub-terminal anaemia develops. Histologically, there are 1) destruction of the normal node architecture, 2) proliferation of reticulum cells and characteristic giant cells (Dorothy Reed-Sternberg cells) which are found in relatively high numbers, 3) Fibrosis in the lesions, and 4) the presence of eosinophils and plasma cells.

Anaplastic sarcoma of lymphoid tissue (syn. Lymphoblastic reticulosarcoma,

Anaplastic sarcoma of lymphoid tissue (syn. Lymphoblastic reticulosarcoma,
Hodgkin's sarcoma, Stem cell sarcoma). This is the final common pathway of the foregoing types which become anaplastic and completely undifferentiated. If there has been a long history, backed by lymph node biopsies, one may be more specific in the diagnosis e.g. Hodgkin's sarcoma, but it is doubtful if one can tell the precursor type in most cases. Lesions consist of large highly invasive non-encapsulated masses of tumour tissue composed of pleomorphic stem cells. Focal haemorrhages and areas of necrosis are common. Ulceration through the skin is noted frequently; a rare observation in the other types of lymphoid tumours. The condition is highly malignant and of short duration.

The outstanding features of this classification are not only the histological differences between one type and another, which permit a specific diagnosis in most cases, but also the fact that the histology and the duration of the disease are closely linked. Thus, from a histopathologist's report, it is possible to predict, to some extent, how long the patient is likely to live. In the Westminster series, the percentage of each type which survived for 5 years following diagnosis:-

Follicular lymphoma	62.5
Lymphosarcoma, over 35 years	22.22
Lymphosarcoma, under 35 years	12.50
Reticulum cell sarcoma	12.50
Reticular lymphoma	80.0
Hodgkin's disease	34.96
Anaplastic sarcoma of lymphoid tissue	5.26

Thus, from the human patient's viewpoint, follicular lymphoma or reticular lymphoma are vastly different diseases from lymphosarcoma or anaplastic sarcoma.

Earlier, in America, a fairly complex nomenclature and classification of tumours and tumour-like conditions of the lymphocyte, the myelocyte, the erythrocyte, and the reticulum cell had been created for the Lymphatic Tumour Registry of the American Association of Pathologists and Bacteriologists. A report based on this classification was issued by Callender (1934). This seems to have been an attempt by non-clinical and non-experimental pathologists to draw up a super classification to include every possible tumour and tumour-like condition. Three principal phenomena were recognised. These were 1) Reactions, 2) proliferations of neoplastic type and 3) malignant tumours.

The main criticisms of such a painstaking classification are that it was highly controversial, possibly aetiologically unsound and of little value to the clinician.

Gall and Mallory (1942), expressing dissatisfaction with the classification used by Callender, presented a list of lymphoid tumours which they termed malignant lymphoma, apparently in the hope that the innovation of this innocuous term might offend no-one. The types they listed on histological grounds were as follows:- 1) Stem cell lymphoma, which included a) a syncytial type and b) a discrete cell type. 2) Clasmatocyte lymphoma. 3) Lymphoblastic lymphoma. 4) Lymphocytic lymphoma. 5) Hodgkin's lymphoma. 6) Hodgkin's sarcoma. 7) Follicular lymphoma.

These workers noted that the first 4 in the list presented lesions of uniform cell sheets with centrifugal invasion. On the other hand, the final three types presented more complex cellular patterns and much less evidence of local invasiveness. Compared with Lumb's later classification, there is broad agreement. Again, the significance of Gall and Mallory's work is that the types they listed were meaningful for the prognosis of the individual case and they were all malignant tumours.

From all of the foregoing it is clear that, so far as neoplastic disease of haemopoietic tissue is concerned, the two groups of diseases, Leukaemias and Lymphoid tumours, are fairly distinct except those which involve lymphoblasts and lymphocytes, i.e. lymphatic leukaemia and lymphosarcoma. There is, today, wide agreement that, histologically, these two diseases are identical (Gall and Mallory, 1942 and Lumb, 1954). However, the view is still held by some that lymphosarcoma is a localised and very destructive disease process which is much more invasive than lymphatic leukaemic infiltrations (Robbins, 1962).

The term Reticulosis has been used to describe lymphoid tumours in some circles. Today this term is obsolete and non-specific. Lumb defines it as "a reaction to various stimuli of those mesenchymal cells found scattered throughout the body as well as being localised in certain well-define sites such as the lymph nodes, bone marrow, liver and spleen."

HAEMOPOIETIC TUMOURS IN THE DOG.

Tumours of Lymphatic Tissue Showing Cellular Uniformity.

The earliest report in the British literature was by McFadyean (1903).

He/

He described a disease which was associated with generalised lymphadenopathy and splenomegaly. Histologically, the lymph nodes and spleen were almost completely replaced by sheets of uniform lymphoid cells. Excellent photomicrographs were published confirming that the disease was undoubtedly comparable to the lymphosarcoma of Lumb or lymphocytic lymphoma of Gall and Mallory. Four dogs and one pig were studied, but in only one dog did McFadyean have an opportunity of carrying out a full post-mortem examination. This worker was fully aware that the disease was not Hodgkin's disease as found in man, and gave histological criteria for Hodgkin's which are perfectly valid today. Unfortunately, the title of the paper was "Five cases of Hodgkin's Disease in the Lower Animals."

Another case in the dog was described by Hobday (1911). This was confirmed as typical (of Hodgkin's) by McFadyean. It was reported as aleukaemic. Although the description Hodgkin's or Pseudo-Hodgkin"s has been condemned (White, 1945; Jennings, 1953), these terms are still in use, Irfan (1958), Wilkins (1958).

The term Leukaemia was used to describe a tumour in a bulldog by Share-Jones (1927). This was thought to be associated with a previous injury, but neither histological nor haematological data were given.

White (1945) used the term leukaemia for cases which were characterised by gross generalised lymphadenopathy, splenomegaly and infiltrations into other tissues. No details of post-mortem or histological examinations were given. Forty-four cases, all adult animals, had been found during the 2 year/

year period 1938-1939, giving an incidence of 1.8% of all autopsies carried out on dogs. The incidence of other tumours, particularly malignant tumours, was not given. 10 dogs, nearly 25% of his series, were Scottish Terriers. In some cases a "leukaemoid" blood picture (Neutrophilia) had been observed.

Innes et al (1946) investigated a series of 30 dogs presenting signs of superficial lymphadenopathy and hepatosplenomegaly. 17 of them were subjected to a post-mortem examination. No sex incidence was noted and only adult animals were affected. 10% of the dogs in this series were Scottish Terriers. A brief, but adequate, description of the syndrome was presented. It was observed that lymphadenopathy often preceded other clinical symptoms and the preterminal stage was attended by marked emaciation and localised oedema. They observed, on examination of blood, "true leukaemia," "aleukaemia" and a "leukaemoid picture." Anaemia was common. The morbid anatomy followed a distinctive pattern in each case, i.e. generalised lymphadenopathy, splenomegaly (except in one case) and hepatomegaly. Transudates into body cavities were seen. The kidneys and bone marrow were often involved. Thymic lesions did not occur. Affected nodes showed a uniform hyperplasia of cells of the lymphoblast type; there was a high mitotic rate. Focal haemorrhages and necrosis were present. In the spleen the usual structures were largely replaced by sheets of tumour cells. A correlation was claimed to exist between frank leukaemia and a severe marrow infiltration. In the case of the other blood pictures, the lesion in the marrow was thought to be different, but this difference was not described. Affected livers were characterised by infiltration of the portal triads and with/

with parenchymal foci. The displaced hepatic cells had undergone pressure necrosis and the sinuses were believed to be packed with tumour cells only when blood changes were present. Kidney lesions were mainly discrete cortical foci of lymphoblasts, or diffuse interstitial infiltration along the juxta-medullary areas. In their discussion, they made certain observations as follows:-

1) the need for more accurate data on the disease,
2) the terms acute and chronic could not be used,
3) the blood picture mirrored the course of the disease within the body,
4) leukaemoid blood pictures complicated the haemotological data,
5) the pathogenesis remained obscure,
6) infiltration of the nervous system was not observed in any case.

Jennings (1953) described 8 cases in the dog of superficial lymphadenopathy and splenomegaly which he termed Canine Leukaemia (Lymphadenosis). Five of the cases were subsequently examined post-mortem. Six of the animals in the series were Scottish Terriers. The age incidence was between 3 and 7 years and the sex ratio was 5 males to 3 females. Clinical observations were not given in any detail. A total of 19 blood samples was taken. All cases presented a normochromic and normocytic anaemia, associated with circulating normoblasts, except one which showed a macrocytic hypochromic type. The mean total white blood count was within normal range and the highest count was 25,000 cells/cu.mm. This was a leukaemoid picture (neutrophilia). Two dogs had a lymphocytosis and the remainder had normal proportions of leucocytes. No primitive cells were seen. Lymph nodes were enlarged/

enlarged up to 3" x 2" x 1". They were firm, smooth and mobile, sometimes with associated local oedema. On section, they were a greyish yellow colour and without cortico-medullary differentiation. Foci of necrosis and haemorrhages were found. The tonsils in all cases were enlarged. Spleens were plum coloured with grey markings and were up to 5 times the normal weight. The capsules were tense and the contents semi-liquid and greyish red. Malpighian corpuscles were absent macroscopically. In 2 cases, band infarcts were noted. In three of the dogs, the livers were enlarged with rounded edges which extended behind the costal arch. They were mottled and one showed unraised yellowish-grey lesions about $\frac{3}{8}$" in diameter. Ascites occurred in 2 of these cases. In all 5 dogs, the mesenteric lymph nodes were enlarged. Lung, kidney and marrow lesions were observed, but no changes were noted in the C.N.S. This worker emphasised that no evidence of fibrosis and no multinucleated cells were seen (cf. Hodgkin's disease). In the discussion, he made the following points:-

1) Leukaemia was lymphogenous in the dog and most of the earlier accounts were of an identical disease, although the blood picture varied somewhat.

2) He condemned the terms Hodgkin's or Pseudo-Hodgkin's for this condition.

3) He could find no authenticated report of myeloid leukaemia in the dog.

4) He also drew attention to the fact that blood examinations had little diagnostic or prognostic value, basing his opinion not only on his own small series, but on earlier continental work.

Cotchin (1954) classified 1,150 tumours from dogs examined during the period 1950-1953. If one accepts the now widely held view that lymphosarcoma and lymphatic leukaemia are indistinguishable, histologically, then 96 (8.3%) of the cases in his series fall into this category:-

Lymphatic leukosis	40
Thymic lymphosarcoma	5
Mesenteric node lymphosarcoma	5
Intestinal lymphosarcoma	33
Liver lymphosarcoma	1
Kidney lymphosarcoma	1
Cases where the terms intestinal lymphosarcoma, thymic tumour or lymphatic leukosis were not indicated	11
Total	96 cases.

This series included benign tumours as well as suspected and frankly malignant neoplasms. From a histological standpoint, this method is perfectly permissible, but, clinically, there is a distinct gulf between a disease which is ultimately fatal and one which is not. If one attempts to estimate the number of cases in the "lymphoid group" as a percentage of the probable malignant conditions, then the incidence of this highly fatal disease assumes great significance in Cotchin's data.

The largest single series of cases of lymphoid tumours in dogs studied in/

in depth to date in the U.K. was that reported by Irfan (1961b). He termed the disease lymphatic Leukosis. Fifty-six dogs were included. Forty-four lymph node biopsies were carried out, and 28 dogs were subjected to a post-mortem examination. All the patients were adults, 2 - 14 years of age, and the peak incidence was 6 years. Eighteen different breeds were affected, but approximately 15% (8 cases) were Scottish Terriers. The sex ratio, males to females, was 35:21. Clinically, all cases presented bilateral enlargement of palpable lymph nodes which were well defined, painless and mobile. The lymph nodes in the throat were usually the first to be found increased in size and an enlarged spleen was palpable in 10 cases. One case survived 12 months, but most were destroyed rather early, one suspects, from the mild syndrome which was described. Impression smears were made when lymph node biopsies were carried out. In such imprints the majority cell was a lymphoblast with 2 to 3 nucleoli within each nucleus. Mitotic figures were only seen in some cases. Irfan presented details of the haematological data and found three distinct blood pictures. There were 6 dogs which showed leukaemia, 17 dogs which showed leukocytosis and 20 dogs without leucocytosis. Although not clearly defining his terms, he classified the leukaemic cases into 3 types, sub-acute, chronic, and acute. In all three cases of the so-called chronic type, there were circulating immature lymphoid cells. No data were offered to suggest that these subdivisions were linked to the clinical picture in any way. Most of the leucocytotic cases had a neutrophilia and half of them had an accompanying absolute lymphocytosis. In most of the cases in the third main group, the normal range of total white blood/

blood cells was found, but 8 of them showed a moderate lymphocytosis. From the post-mortem data given, the disease appeared to be identical to that described in the literature earlier. However, this worker adopted the extreme view that Lymphatic Leukosis was only associated with a lymphoblast cell type, and considered that any case, where other lymphoid cells predominated, should not be included under this title. The lymph node imprint technique was strongly advocated as a diagnostic marker and an incidence of true leukaemia of only 30% was considered useful as an aid to diagnosis. Irfan observed 7 cases of lymphosarcoma. He classed them with those conditions, other than lymphatic leukosis, which caused enlargement of the lymph nodes and reported that "the enlargement was confined to the mandibular, prescapular, inguinal and mesenteric lymph nodes."

In the United States, the earliest authentic report of a case of this disease may have been made by Milks and Goldberg (1919). This was a dog presenting emaciation, external lymphadenopathy and abdominal masses. It was anaemic with a total leucocyte count of only 7,200/cu.mm. On post-mortem examination all lymph nodes were enlarged; there was splenomegaly and the marrow was infiltrated. Lesions were composed of sheets of uniform lymphocytes. Because of the blood picture, which was aleukaemic, this disease was termed Pseudo-leukaemia.

Spaulding (1920) described 6 cases of leukaemia and 7 cases of pseudo-leukaemia which had been diagnosed in 15,000 clinic dogs over a 3 year interval. There is little doubt that some of the cases were haemopoietic tumours, but

no/

no histological data were given. The sole haemogram which was described in detail, and believed to be leukaemic, would not be diagnosed as such at the present time. This article was severely criticised by Burnett (1920) who stated that "to be accepted the diagnosis must have been based on data sufficiently detailed and clear to be understood by other members of the profession." Not all have heeded this advice since then.

Feldman (1932) described Lymphoblastoma as a malignant disease in which superficial lymphadenopathy was usual. This worker "observed cases in which lesions were extensive in the thoracic and abdominal viscera, but without any apparent increase in the palpable lymph nodes of the body." In his opinion, such cases were exceptional. Leukaemia was regarded as one manifestation of the disease which occurred only in a small percentage of cases, but he recommended blood examinations to differentiate lymphoblastoma from myelogenous leukaemia and chloroma, both of which he regarded as rare conditions.

Reports of other individual cases which were diagnosed histologically have been published, Milks & Olafson (1929); Bradbury (1949); and Khuen (1947). Other reports on single cases have lacked the essential histological data for positive diagnosis. Examples of these are Collings (1939); Cherry (1940); Atkinson (1941) and Combo (1947). Rook (1947) described a case which may well have been lymphosarcoma of the mesenteric lymph node, but histological data were omitted. Konde (1947) described 2 cases from the same owner, one of which may not have been a haemopoietic tumour.

Bloom & Meyer (1945) described lymphoid tumours in a series of 20 dogs. Bilateral

Bilateral superficial lymphadenopathy occurred in each. Details were given of the clinical picture, haematology, lymph node imprints and marrow studies. They proposed 4 cytological categories for their cases:- 1) Lymphoblastic type, 6 cases, 2) Lymphosarcomatous cell type, 1 case, 3) Mixed cell type, 4 cases and 4) Lymphocytic cell type, 2 cases. Stem cell lymphomas, Hodgkin's disease and follicular lymphoblastoma were not encountered. This cytological classification was dependent on a careful lymph node imprint technique and by attempting to single one case our of 13 for a special class, Bloom and Meyer failed to acknowledge the great probability that this might simply be an example of biological, or even technical, variation.

They found an incidence of 0.2% in a group of 10,000 dogs. Ages varied from 5 to 12 years, averaging 9.08 years. No sex differences were found. Seven Scottish Terriers were involved (30% of the group). The duration of the illness averaged 99 days from onset to the time of death. In the earliest stages of the disease "lumps" were found on the surface of the body, but the animals were otherwise in good health. Later, patients became listless and alimentary disturbances were seen. Thirst, polyuria, coughing and heavy breathing occurred. Terminally, the animals were emaciated with pale mucous membranes and purulent oculo-nasal discharges. Blood findings showed progressive anaemia with circulating normoblasts. There were no signs of an acute or chronic leukaemia as seen in man. In all the dogs, except one, there was an absolute neutrophilia (Leukaemoid picture). A discrepancy was noted between bone marrow biopsy results, of which 7 cases were positive and the results/

results of histological examinations of sections, where 11 cases were positive. This was attributed to the focal nature of the tumour infiltrates and it was thought that sections were the more accurate means of finding evidence of tumour infiltration into the marrow. Good descriptions were given of the main tissues affected and histological details were given of the lymph nodes, spleen, liver, gall bladder etc. In all cases the tonsils were enlarged and affected, but no lesions were found in the C.N.S. Bloom and Meyer found no justification for the term leukaemia and dismissed earlier claims that myelogenous leukaemia had been diagnosed. They noted the aggressive tendencies, such as capsular and perinodal infiltrations in the condition they described, and saw a similarity to lymphosarcoma. However, they considered that other features, including spleen and marrow infiltration, were not typical findings in lymphosarcoma. They, therefore, adopted the term Malignant Lymphoma although the term chosen by Gall and Mallory referred to other specific disease entities, including Hodgkin's disease. Bloom and Meyer considered that malignant lymphoma was the most common type of haemopoietic tumour found in dogs, and that it was a sub-acute disease irrespective of the predominant tumour cell type found.

Meier (1957) presented a review of neoplastic diseases of the haemopoietic system (so-called Leukosis Complex) in the dog. This was based largely on data from the files of the Department of Pathology of the Angell Memorial Animal Hospital, Boston, Massachusetts., U.S.A. This author classified these diseases as follows:-

a) The lymphomas

1) Malignant lymphoma - lymphatic leukaemia.

2) Lymphosarcoma.

3) Hodgkin's sarcoma - Hodgkin's disease.

b) Myelogenous leukaemia

c) Monocytic leukaemia

d) Myelomatosis

Fifty-two cases of malignant lymphoma were subjected to post-mortem examination and details of the anatomical, clinical and haematological results were tabulated. This data were in agreement with that of earlier workers. However, 7 of the 52 cases did not present superficial lymphadenopathy, tumour lesions being confined to the abdomen and/or thorax. The tumour cell types recognised were the reticulum cell, the lymphoblast and the lymphocyte, but no relationship was found between the duration of the disease and the predominant cell type. The term leukaemia was admissible on blood examination in only 14 cases. When lymphocytosis and circulating lymphoblasts were observed, no differences were found between the lymphocytes in such cases and those in the blood of healthy dogs, except that the larger cells were much more fragile. The remaining 38 cases were aleukaemic, but granulocytic leucocytosis was common. Anaemia was rare in the series. Only patchy leukaemic infiltrates were observed in the bone marrow, although myeloid hyperplasia was outstanding. Meier stated that the diagnosis was seldom advanced by a blood examination.

He was in considerable difficulty when differentiating this disease from lymphosarcoma. Similarities were found, but he finally accepted that lymphosarcoma was a more expansive growth than malignant lymphoma and was less widespread throughout the body. Surprisingly, after the wealth of detail on malignant lymphoma, this author passed very lightly over the 20 cases of Lymphosarcoma in the files of the Angell Memorial.

The histopathology and clinical features of ocular lesions in 10 dogs with malignant lymphoma have been described by Cello and Mutcherson (1962). The cases were observed during a survey lasting for 3 years, but theincidence of eye lesions was not given. In 7 of the dogs, the haematological findings were reputed to be subleukaemic and diagnostic, but the criteria employed in this aspect of the study were not included. These authors regarded eye changes in this condition as part of the penultimate stage of the disease process which was preceded by other obvious clinical signs. Consequently, infiltrative lesions of the eyes were not regarded as useful early diagnostic markers in this species.

Reviews on malignant lymphomas in animals and leukaemic neoplasia in the dog have been reported by Smith (1962, 1963). In a survey of 301 cases in the dog, 5 classes were recognised; 1) a lymphoma group, 2) a Myelocytic group, 3) a Hodgkin's group, 4) a Mast-cell group and 5) a CVTV group (sic) = standing for contagious transmissible venereal tumour. In the lymphoma group, there was some evidence that the incidence of the disease tapered off with advancing years, but no sample of the background population was published/

published to permit a statistical evaluation of this viewpoint. Smith was doubtful if there was any relationship between incidence and breed, but again, no sample of the background breed incidence was included. This was not possible since the cases were derived from the whole country and a few from abroad. In classifying the lymphomas on the basis of the cell types, it was acknowledged that they ranged from stem cells to well differentiated lymphocytes and, although there was a marked contrast between the two extremes, exact classification was a matter of personal judgement. Of the 186 cases reported, the dominant tumour cell was found to be a stem cell in 58 cases, a histiocyte in 73, a lymphocyte in 21, a poorly differentiated lymphocyte in 19 and mixed or unclassifiable in 15 cases. There were insufficient clinical data to relate cell type to the clinical outcome of the disease as had been demonstrated in lymphoid tumours in man by Gall and Mallory (1942).

A review of the literature on haematopoietic tumours of domestic animals was presented by Squire (1964). In a description of lymphosarcoma of the dog, he ended "As in cattle, lymphatic leukaemia may also occur as a primary disorder without lymphosarcoma," but gave no references. Later, Squire (1965), in a cytological study of malignant lymphoma, reported that 2 out of 20 dogs, which he had investigated, presented lymphatic leukaemia as opposed to lymphosarcoma. This distinction was based on the assumption that leukaemia was typified by widespread neoplastic infiltration, whilst lymphosarcoma was characterised by discrete or invasive tumour formation. Histological details of these cases were not given. The cytological classi-

fication/

classification of malignant lymphoma included the following types:- 1) Histiocytic; 2) Lymphocytic, including lymphoblastic, prolymphocytic, and lymphocytic types; 3) Plasmacytic; and 4) Hodgkin's. Cell types could only be identified confidently by examination of cell imprints. Although this author admitted that 2 or more cell types might be found in the lesions, he was of the opinion that one of them invariably constituted a clear majority. He believed that any classification of this disease must rest on cell morphology, instancing the work of Gall and Mallory, but omitting to stress the fundamentally clinical basis of their classification of the lymphoid tumours of man. Squire considered that a cell type classification was justified, since there might be a multiplicity of aetiological agents which might be related to the tumour cell type. He further speculated that a cytological classification might prove of value in the domestic animals as it had in man, with regard to therapeutic response.

In some of the early European reports, the phenomenon of leucocytosis, which appeared to indicate a myelogenous rather than a lymphoid leukaemia, led to some confusion. Weil and Clerc (1904; 1905) were confident of the lymphoid nature of the condition when the blood picture was aleukaemic, but probably termed the disease myelogenous when a neutrophilia was observed. Wirth (1920) also misinterpreted the leucocytosis initially, but, on re-examination of data, it was recognised that leukaemia in the dog was mainly lymphatic, and this included those cases where a neutrophilia was observed, Wirth and Baumann (1933).

Hutyra et al (1949) gave a resumé of the generalised disease compiled from European sources. They considered that it was relatively common in the dog and was predominantly of the lymphatic type. It occurred in adults and aged animals, and males predominated. The clinical features included gross, painless, bilaterally symmetrical enlargements of most superficial lymph nodes with hepato-splenomegaly. Dyspnoea and/or alimentary disturbances were common, together with fever and anaemia. At a later stage of the disease, therewere depression, wasting and sometimes oedema which were followed by death. The blood picture showed 3 relatively distinct types 1) Leukaemic, 2) Subleukaemic and 3) a leucocytotic blood picture. The anatomical pattern was one of generalised lymphadenopathy, splenomegaly with infiltration of bone marrow, liver, kidneys and the gastro-intestinal tract. Histologically, node structure was replaced by sheets of lymphoid cells showing infiltrations into the trabeculae, capsules and peri-nodal areas. The spleen was characterised by hyperplasia of follicles or the formation of diffuse lymphoid cell sheets. Severe infiltration of the portal triads of the liver together with streams of tumour cells in sinuses were noted. The tumours of the wall of the gastro-intestinal tract were expansive and stenotic. Hutyra et al considered that, to establish a diagnosis of this condition, it was essential to examine a stained preparation of blood, but they failed to explain how one reached a diagnosis of leukaemia when the blood picture was consistently subleukaemic or leukaemoid. Here, they believed, repeated blood examinations might prove helpful. They stressed however, that, in the differential diagnosis from other/

other conditions where lymph node enlargement is found, few other conditions produce a gross painless and bilaterally symmetrical enlargement of lymph nodes.

Another lymphoid tumour was described which started in the mediastinal lymph nodes and spread metastatically to the spleen, liver and bone marrow. This condition, thought to be common, was regarded as "occupying an intermediate position between generalised lymphadenosis of pseudo-leukaemic type and malignant tumours." This disease they termed Lymphosarcomatosis.

Recently, Bäckgrën (1965) presented an epizootiological, clinical and haematological report on 60 cases of lymphatic leukosis in dogs examined at clinics throughout Sweden over an 18 month interval. The overall incidence was believed to be 13 per 100,000 at risk. Boxers and Scottish Terriers were found to be statistically over-represented in the leukosis group, and Dachshunds were under-represented. The disease described was similar to that found in the United States, the United Kingdom and other European countries. Bäckgrën observed three distinct clinical phases of the condition and thought that the haematological and biochemical examinations were related to the clinical phases. In the earliest phase, lymph node enlargement was the main clinical feature. Later, there were loss of condition, anaemia and alimentary disturbances. Finally, the animal became moribund. In 20% of the dogs, circulating immature or abnormal lymphocytes were observed as the disease advanced. This was accompanied by lymphocytosis, but cases of frank leukaemia were rare. Bone marrow aspirates showed an increase in the "blast" cell count as the disease progressed. A correlation was established between the peripheral lymphocyte/

lymphocyte count and the lymphoid content of the marrow and also between the degree of splenomegaly and thedegree of tumour cell infiltration of the spleen. Lymphocytosis was also positively correlated with infiltration of the spleen and liver. Serum proteins were depressed due to a fall in albumen. Terminally, the alpha-globulin levels increased, but there was variation in the gamma-globulin fraction. The latter was especially low where concomitant infections were found. The protocols of this study may have included the enlargement of one or more superficial lymph nodes as diagnostic markers, since no cases of a non-generalised nature was described. Indeed, this type of study may specifically exclude such cases.

Das (1951) found 8 cases of lymphoid leukaemia in dogs in Calcutta. This was largely a review article covering the literature and the aetiological factors which have been the subject of speculation. This worker wrote "it is generally stated that the blood picture usually shows a heavy increase in white blood corpuscles mainly due to a comparative increase in the number of lymphocytes" which is not exactly in agreement with the references he gave.

Tumours of Lymphatic Tissue Presenting Complex Cellular Patterns.

There had been no authentic reports in the literature on Hodgkin's disease in the dog before 1934 when MacMahon reported a case in a mongrel. The tumour was a localised mass of matted cervical lymph nodes which, histologically, satisfied most of the criteria of Hodgkin's including the presence of many Reed-Sternberg cells. Stalker et al (1936) described a more generalised disease involving the mesenteric, mediastinal and bronchial lymph/

lymph nodes as well as the lungs, liver, spleen and pancreas. There were fibrosis in the lesions and many polymorphonuclear leucocytes. Some Langhan Giant Cells were found and a few cells identical to Reed-Sternberg cells. Eosinophils, reticulum cells and plasma cells were prominent. Bloom (1952) also described a generalised form in which mesenteric and mediastinal lymph nodes, spleen and liver were affected. A similar histological picture was observed, but fibrosis was less of a feature and the diagnostic multinucleate cells were sparse. Darchfield (1953) reported similar lesions in the liver and lungs of a young bitch. Erichsen (1955) described a localised tumour causing ulceration on the back with involvement of associated pelvic lymph nodes. The lesions appeared to fulfill the criteria of Hodgkin's including the presence of large numbers of Reed-Sternberg cells. Six cases were recorded by Meier (1957). All were localised superficial tumours. In only two of the cases did the histology resemble those reported before. The others are very doubtful cases, and may simply be forms of chronic granulomata. A good review of the literature was given by Moulton and Bostick (1958). They described a case of an ulcerative lesion of a paw with apparent spread to the prescapular and mediastinal lymph nodes and the spleen. Histologically, the lesions in the paw and the nodes fulfilled the criteria of human Hodgkin's, although, as in some of the former cases, not many diagnostic cells were observed. It must be stated that none of these reports are really convincing.

Smith (1963) found 7 cases in dogs out of a series of 301 leukaemic tumours. In 5 of the cases, the spleen was grossly enlarged and, in 4 of these/

these, the liver was also grossly involved. Cells resembling Reed-Sternberg cells were recognised in each case, but, principally on the grounds that similar cells had been observed in 12 cases per 1,000 of malignant lymphoma in cattle, he was not entirely convinced that true Hodgkin's disease was an entity in the dog.

In man there is a distinct sex ratio in Hodgkin's disease, males far out-numbering females. In the reports on the dog, however, more females have been found than males.

A condition in the dog which could be diagnosed as Hodgkin's disease has not been recognised at this hospital.

One is tempted to compare this condition, as reported in the dog, with Benign Hodgkin's in man, but the differences are marked, since reticular lymphoma has never been found except within lymph nodes and the cellular pattern is dissimilar.

Giant follicular lymphoblastoma has not yet been described in dogs, although it has been suspected (Jones and Berg, 1960).

Other Leukaemias.

The range of leukaemic diseases which has been reported in man does not appear to occur in the dog. Whilst there have been many cases of the lymphatic type reported, other types are rare. Only one case of basophilic leukaemia has been described (Romanelli, 1953). This worker presented good photographic evidence to support his diagnosis. Wirth at Baumann (1933) admitted/

admitted that cases which had been regarded earlier as myeloid leukaemia, on the evidence of the blood picture, were in fact lymphatic leukaemia with a leukaemoid blood picture. Bloom and Meyer denied that any genuine case of myelogenous leukaemia had been described in the literature, prior to 1945. The files of the Angell Memorial Hospital, Boston, offered evidence that the disease did occur (Meier, 1957). Eight cases were reported. Lucke and Sumner-Smith (1963) and Medway & Rapp (1962) have also recorded cases in the dog. The characteristics of this type were the absence of marked lymphadenopathy and the presence of an enormously enlarged spleen which was regarded as almost diagnostic. Histological sections revealed infiltrations of cells which, from their variation and similarities to the range of myeloid cells, supported the diagnosis.

Cases of myelomatous neoplasia were observed by Smith (1963) in 28 of the 301 dogs with leukaemic neoplasia. Myeloma, plasmacytoma, myelogenous leukaemia, granulocytic leukaemia, or some equivalent term was employed within this group. Smith was of the opinion that "just what fundamental differences exist between the lymphoid and the myeloid neoplasms will ultimately have to be decided on aetiological grounds."

Meier (1957) also recorded a possible case of monocytic leukaemia with circulating monocytes and monoblasts. There is, however, too much controversy over the so-called monoblast to accept this without reservations.

Myelomatosis.

Only a few instances of plasma cell myelomas have been described in dogs, (Bloom,/

(Bloom, 1946; Jennings, 1949). There are also two cases in the files at this hospital, but this condition in the dog does not appear to be at all common.

Briefly, one might summarise the literature by stating that the consensus of opinion has incriminated the lymphoid cell series in the majority of leukaemic tumours of the dog. True leukaemia, which is characterised by specific changes in the circulating blood cells, is relatively uncommon, and there is still insufficient evidence to relate the dominant tumour cell in the tissues to the clinical outcome of the disease with the degree of confidence which is shown in the lymphoid tumours of man.

MATERIALS AND METHODS.

This report was based on data which were collected from 130 dogs admitted to this hospital between the years 1954 and 1964. Each animal was subjected to a detailed autopsy. Clinical examinations were carried out on 115 of these dogs and haematological and biochemical tests were performed on the venous blood of 91 dogs in the clinical group.

The morbid anatomical appearances of affected organs and tissues in each case were recorded at the time of autopsy. Blocks of tissue for histological examination were taken from most of the major organs and all tissues which appeared to be affected with the disease. The tissues were fixed in Formol-sublimate, dehydrated and cleared in alcohol-amyl acetate-benzol series and double embedded in celloidin and paraffin. Sections were stained with Haematoxylin and Eosin. In the later stages of the survey, imprint smears were prepared from affected lymph nodes from 14 dogs immediately after death and these were stained with Leishman's stain.

Haematological examinations were carried out on uncoagulated venous blood. The Erythrocyte Sedimentation Rates were estimated by the method of Wintrobe and Landsberg (1935). Two methods were used to estimate Packed Cell Volumes. Up to 1962, the technique employed was that of Wintrobe (1933). Thereafter, the Hawksley Haematocrit was introduced, with a standardised centrifugation interval of 6 minutes after Fisher (1962). The Oxyhaemoglobin levels were calculated according to the method of Bell et al (1945). Until 1962/

1962, the traditional methods of estimating the Total Erythrocytes and Total Leucocytes per cu.mm. of blood were employed, using Thoma pipettes and Improved Neubauer Haemocytometers, Dacie (1963). Subsequently, an electronic particle counter, Coulter Counter, Model "D", was calibrated and used for routine erythrocyte and leucocyte counts. A thin blood film was prepared and stained with Leishman's stain from the majority of blood samples. A Differential Leucocyte Count was carried out according to the method described by Dacie (1963). In addition, Differential Lymphoid Cell Counts were performed on selected blood films.

Biochemical examinations were carried out on heparinised venous blood. Plasma Protein levels were estimated by a modification of the original Biuret method after Weichselbaum (1946). The Albumen Globulin ratios were carried out by electrophoresis using data from Martin and Franglen (1954), Jenkks et al. (1955), Henry et al. (1957). Blood Urea estimations were carried out by the Urease and Nesslerisation method described by Harrison (1947) and later by Varley (1958). Three methods were used for the estimation of Serum Alkaline Phosphatase levels. Until 1957, the method was after King et al. (1951); between 1957 and 1963 after King and Wootton (1956); thereafter, by the method of Bessey et al. (1946). Serum transaminase estimations were carried out using a colorimetric method adapted from Sigma Tech. Bull. No. 505. The Bilirubin levels were calculated until 1963 by the method of King et al. (1950), and then by the methods of Jendrassik and Cleghorn (1936) and Jendrassik and Grof (1938).

Radiological examinations were carried out on 72 of the dogs.

Normal Values of Haematological and Biochemical Tests on the Blood of Adult Dogs.

Haematological Data. After Irfan (1961a).

Haemoglobin	17.5 ± 1.5 gms./100 ml.
Total Erythrocyte counts	6.11 ± 1.05 millions/cu.mm.
Packed Cell Volume	52.0 ± 6%
M.C.V.	86.0 ± 13 cu.μ
M.C.H.C.	33.0 ± 5%

Biochemical Data. Hospital figures.

Serum Protein	5 - 7.8 gm.
A/G ratio	0.6 - 1.2
Blood urea	30 (0-45)
Alkaline phosphatase	1 - 15 K.A. units
Serum transaminases	< 40 S.F. units
Bilirubin	0.2 - 0.6 mgm./100 ml.

CLASSIFICATION OF LYMPHOSARCOMA OF THE DOG.

An arbitrary classification of this disease was made on the basis of the anatomical distribution of the gross tumour lesions. Each case was assigned to one of three forms:1)a Multicentric type; 2) an Alimentary type; 3) an Aberrant type.

Each of the forms was associated with a relatively distinct clinical syndrome and thus the classification was also justified from the viewpoint of differential diagnosis.

Details of the distribution of the lesions and the number of dogs in each group are given below.

THE PATHOLOGY OF THE DISEASE.

THE ANATOMICAL DISTRIBUTION OF LESIONS.

A summary of the distribution of the lesions of lymphosarcoma in the cases is given in Tables 1 and 2.

The lesion complex followed 2 main trends. One major group was characterised by bilaterally symmetrical enlargement of almost every superficial lymph node together with hepato-splenomegaly. Most of the deeper nodes were also affected. Other tissues were also involved, including the alimentary system, kidneys, lungs, heart, tonsils, pancreas and bone marrow. Occasionally the skin, eyes and voluntary muscle were affected, but lesions in the central nervous system were not recorded except in a single case. There were 61 dogs in this group and this pattern was termed the "multicentric type."

TABLE 1

Distribution of the Main Lesions in 130 Autopsied Cases.

	Multicentric	Alimentary	Aberrant	Total
Lymph nodes.				
Superficial	61	0	1	62
Thoracic	42	6	1	49
Anterior Sternal		14		14
Mesenteric	49	50	0	99
Other abdominal	56	18	4	78
Liver	50	38	7	95
Spleen	52	5	3	60
Alimentary tract	19	47	0	66
Kidney	26	12	4	42
Lung	14	6		20
Heart	11	1		12
Tonsil	25	1		26
Marrow	14	1		15
Total	61	61	8	130

TABLE 2

The Distribution of Minor Lesions in 130 Cases of Canine Lymphosarcoma.

	Multicentric	Alimentary	Aberrant	Total
Number of cases	61	61	8	130
Abdominal fluid	1	4	1	6
Adrenals	3	1		4
C.N.S.	1			1
Eyes	3			3
Hydrothorax	2	2		4
Jaundice	4	3		7
Lachrymal gland	1			1
Mammae	1			1
Oesophagus	1			1
Pancreas	6	3		9
Peritoneum		1		1
Prostate	3	1	1	5
Skin	3			3
Scrotum			1	1
Thymus	4	4		8
Thyroid	1			1
Urinary bladder	3	1		4
Uterus & ovary		1		1
Voluntary muscle	1			1

The second group also comprised 61 dogs and was termed the "alimentary type." It was clearly differentiated from the multicentric type because the superficial lymph nodes were never affected and splenomegaly was very seldom found. These animals all had tumour lesions of thegastro-intestinal tract or in the mesenteric lymph node. Frequently both sites were involved. Less commonly, other organs within the abdominal and thoracic cavities were infiltrated. In 14 cases, gross enlargement and tumification of the anterior sternal lymph nodes were noted, and in several cases, this was the sole extra-abdominal lesion which was recognisable macroscopically.

The small number of cases which remained formed no clearcut anatomical pattern, so no attempt was made to subdivide them although infiltration of the liver was observed in 7 of the 8 dogs. This was described as the "Aberrant type."

THE INCIDENCE OF LYMPHOSARCOMA IN THE POST-MORTEM ROOM.

Out of 630 cases where dogs died or were destroyed due to the presence of malignant disease, 140 were found to be suffering from lymphosarcoma; an incidence of 22.2%.

HISTO-PATHOLOGICAL FINDINGS.

Very few differences were observed in the macroscopical and microscopical appearances of individual tumour lesions in the three forms of the disease and the condition will be considered here as a single entity. Points of contrast will be indicated and described in the text, where this is necessary.

THE LYMPH NODES.

Observations of the lymph nodes of healthy dogs. Palpation of the superficial lymph nodes of adult dogs, showing no signs of clinical disease, revealed that the submaxillary, prescapular and popliteal nodes were frequently larger than hazel nuts, whilst the axillary and superficial inguinal nodes were smaller and much less readily palpable.

In 20 dogs, the superficial nodes were examined soon after death and it was confirmed that the axillary and superficial inguinal nodes were small and difficult to locate, especially if there was much subcutaneous fat present. A typical node in these animals lay in a bed of loose connective or adipose tissue and was bean shaped or roughly spherical with a slight hilar indentation. Such nodes could be cut open without difficulty and the pale straw coloured cortex could be clearly distinguished from the much darker central medulla.

The majority of these nodes presented a histological pattern similar to the description of human lymph nodes given by Ham (1961). Some nodes, however, particularly the prescapulars and popliteals, were much firmer than the others and were more difficult to section due to central fibrosis, which, microscopically, was the scarred sequel of earlier inflammatory responses.

Lymph nodes in the tumour series. Distribution of affected lymph nodes in the series is given in Table 1. In the multicentric type, virtually every superficial lymph node was affected, although not all were markedly increased in size. For example, the nodes of the head and the prescapulars were often enormously/

enormously enlarged whilst the popliteals and superficial inguinals in the same carcase were only moderately so. In certain cases, less than 10%, only moderate enlargement of all the superficial nodes was observed and each one was found to be infiltrated on section. A recurring feature of this latter pattern was palpable enlargement of the maxillary lymph nodes. A constant finding was bilateral symmetry of the superficial nodes.

In the alimentary type, the lymph node most frequently affected was the mesenteric node and other abdominal nodes were involved to a much lesser extent. A noteworthy feature of this group was infiltration of the anterior sternal nodes in a small number of the cases.

Infiltration of lymph nodes was uncommon in the Aberrant form of the disease.

Morbid anatomy. Affected nodes were enlarged from 2 up to 10 times their modal sizes. They were easy to identify on the carcase and were discrete, mobile and appeared to be encapsulated. In only rare instances had groups of nodes become confluent.

No distinction could be made between affected lymph nodes from alimentary types and those from the multicentric group.

The surfaces of tumified nodes were usually dark straw coloured, but a few were congested. On section most had lost all evidence of cortico-medullary differentiation and presented a uniform greyish-yellow, highly cellular, soft tissue which bulged from the cut surface. Immediately after death,/

death, cloudy lymph could be freely expressed from this surface. Fine haemorrhagic points and etchings were often visible on this tissue, especially over its central aspect. Frequently, these had become exaggerated into ill-defined congested areas. In certain nodes, there was intense congestion throughout and, in these, round yellow foci or strands of necrotic tissue could be observed. In others, the tumour tissue was arranged as tightly packed cream coloured foci not quite obliterating the former gross nodal structure, but standing out clearly on section. In a minority of cases the entire node was distended with greyish-pink tissue which was so soft and cellular as to be almost fluid in character. Such nodes tended to collapse completely on section.

Although most of the lymph nodes affected on a given canine carcase presented a rather similar macroscopical appearance, it was possible to find considerable variation from node to node in some cases. Figure 1.

Histological findings. A detailed study of the histological features of 163 sections from 109 cases of the disease in the dog was done with experimental diagnostic markers in view. The results of the survey are given in Table 3 and Table 4.

The recurring lesion in affected lymph nodes was loss of the usual follicular arrangement in the cortex and the formation of relatively uniform sheets of lymphoid cells on a background of fine reticulum fibres and a fine vascular network. These sheets of tumour cells were locally invasive and led to disruption and destruction of the dense connective tissue framework of the node with obliteration of cortico-medullary differentiation.

TABLE 3

A Summary of the Main Histological Features of Lymph Nodes Affected with Lymphosarcoma in the Dog.

Number of sections examined		163
General architecture		
Preserved	12	
Loss of	151	163
Cortico-medullary differentiation		
Visible	18	
Lost	142	
Medulla absent	3	163
Perinode area		
Negative	16	
Slight infiltration	27	
Moderate infiltration	57	
Marked infiltration	63	163
Capsule		
Intact	33	
Laminated	76	
Fragmented	22	
Obliterated	32	163
Subcapsular sinus		
Patent, in parts	12	
Flooded, visible	84	
Obliterated	67	163
Cortical trabeculae		
Intact	16	
Laminated	46	
Fragmented	12	
Obliterated	89	163
Follicles		
Germinal centres	4	
Vestigial follicles	20	
Cell sheets	132	
Tumour follicles	7	163
Cortical sinus		
Patent, in parts	5	
Flooded, visible	65	
Obliterated	93	163

Medullary trabeculae		
Intact	14	
Laminated	37	
Fragmented	8	
Obliterated	101	
Medulla absent	3	
		163
Medullary pegs		
Tumified, visible	28	
Cell sheets	132	
Medulla absent	3	
		163
Medullary sinus		
Patent	11	
Flooded, visible	37	
Obliterated	112	
Medulla absent	3	
		163

TABLE 4

Additional Features of Lymph Nodes Affected with Lymphosarcoma in the Dog.

Number of sections examined	163
Marked vascular congestion	9
Haemorrhages	12
Necrosis	
Diffuse	19
Focal	13
Focal and diffuse	4
Reticulo-endothelial hyperplasia	6
Foci of neutrophils	4
Foci of eosinophils	0
Multinucleated cells	0
Phagocytosed carbon	2
Blood vessels packed with leucocytes	6
Erythrophagocytosis	2
Haemosiderin	1
Diffuse fibrosis	6
Marked fibrosis	1

Invasion of the loose connective tissue around the lymph node, the peri-nodal area was observed in the great majority of sections, and in only 16 of the 163 sections was this area completely free from tumour cells. In some cases, only a narrow rim of relatively non-active lymphoid cells was found immediately outside the capsule, Figure 2. This was often associated with heavy lymphocyte packing of the afferent lymph vessels. However, in most cases, the invasion was on a moderate to massive scale. Often in these sections, the main evidence that the tumour cell sheet was outwith the original capsule was the presence of groups of fat cells which were only rarely observed within the node itself, Figures 3, 4 and 5. Encapsulation of the extra-capsular infiltration was never particularly marked, but condensation of the immediately adjacent loose connective tissue was observed and this appeared to form a rather loose secondary capsule around many nodes, Figure 3.

The capsule was usually involved in the process and three stages of capsular infiltration could be determined, 1) lamination, 2) fragmentation and 3) obliteration.

Small streams of tumour cells in single file moving between the collagen bundles of an otherwise compact capsule was the earliest phase seen. This was followed by an increase in the number and width of these streams with a resulting disruption and marked separation of the parallel collagen strands of the original capsule, Figure 5. Thereafter, the laminated capsule became frayed and torn as torrents of tumour cells swarmed into the nearby peri-nodal areas. A combination of lamination and fragmentation appeared to lead to/

to virtual elimination of the original tissue. However, in 20% of the sections, infiltration had not taken place and the capsule was well preserved despite invasion of the perinode. In other sections, the capsule had become markedly stretched whilst the node was enlarging and this had resulted in the formation of a fine undulating vestige of former capsule which was clearly outlined deep within the new tumour tissue.

At post-mortem examination, affected nodes were contained within a capsule and, from histological observations, it appeared that the original stretched and partially shattered capsule was supported by condensed loose connective tissue, which had formed a secondary capsule for the tumour mass around the original node.

A similar sequence of lamination, fragmentation and obliteration was noted in the trabeculae of the cortex and medulla, Figure 6. In 2 instances, distinct hyperplasia of the trabeculae had taken place, but early lamination was in progress. In more than half of the sections in the series there was very little sign at all of the former trabeculae.

In many sections, the subcapsular, cortical and medullary sinuses had become completely flooded with tumour cells so that the former sinuses could no longer be identified. In others, the reticulo-endothelial boundaries of the sinuses were still visible, Figures 8 and 10, and sometimes the sinuses contained a cell type rather more mature than those of nearby tumour cell sheets, Figure 6. In one or two sections, sinuses were distended with pus or macrophages, especially those which were close to necrotic patches. Completely patent sinuses were observed very occasionally.

In every section which proved to be affected with lymphosarcoma, lesions in the cortex were noted. There were total loss of follicle patterns and the formation of uniform sheets of tumour cells in 152 out of the 163 sections. In 20 of these sections, compact groups of lymphocytes were observed within the tumour cell sheets. These were often "marginated" towards the former capsule and were believed to represent vestigial follicles, Figure 9. In only 4 sections was there evidence of secondary centres and, in these sections, there were sufficient additional signs to justify a diagnosis. In 7 sections, the cortex and medulla were replaced by large round foci of immature or stem cells which were termed tumour follicles, Figures 6 and 7. In some areas, these had almost formed cell sheets, but elsewhere they were expanding and compressing adjacent sinuses which contained somewhat more mature cells than those in the follicles, Figure 6. Small lymphocytes were found in the centre of some of these tumour follicles.

In 132 sections, the medulla could not be clearly distinguished from the cortex, but in 28 sections this was possible. In the centre of some nodes, there were large round foci containing tumour cells and these were thought to represent tumified pegs or tumour follicles. In other sections, the medulla did not, at first inspection, appear to be involved in the tumour process. There were good cortico-medullary differentiation, delicate pegs, well formed trabeculae and only slight flooding of the sinuses. Such pegs, however, were found to contain a cell population which was identical to that of the cortical sheets which were streaming towards the pegs, Figure 11. Lymphoid cells in the adjacent sinuses were also identical to the tumour cells of the cortex.

The tumour cell sheets were either compact or diffuse. Compact sheets were composed of lymphoid cells, each in contact with its neighbours. In most of these sheets, cells such as reticuloendothelial cells and macrophages were difficult to identify although, in some affected bronchial lymph nodes, there were large amounts of carbon particles which pointed to a background population of macrophages not apparent in the tumour cell sheet. These compact sheets tended to be composed of the more immature cell types such as blast cells and large lymphocytes. Stem cells, with abundant cytoplasm, were observed singly in the cell sheets in some sections.

Some of the diffuse cell sheets were so loosely arranged that cells were clearly separated from their neighbours. Within such sheets, there was a much greater variation in cell types. Relatively more reticuloendothelial cells, active macrophages, stem cells, neutrophils and plasma cells could be recognised compared to the compact type. This type of cell sheet was more often associated with mixed or mature lymphoid tumour cells.

The Histological Diagnosis of Lymphosarcoma From Examination of Lymph Nodes. Diagnosis of the disease was comparatively simple when lesions were composed of immature lymphoid cells for there was no other lesions known to simulate this. A problem arose where the original pattern of the node persisted to some extent, and where the tumour cell type was mixed or mature.

Three groups of characteristics were believed to be of importance in diagnosis and these were termed primary, secondary and tertiary characteristics.

Two cellular patterns were observed as primary characteristics, an afollicular and a follicular form:-

1) An afollicular form which included,

   a) sheets of immature lymphoid cells which were usually compact.

   b) sheets of mixed or mature lymphoid cells, often diffuse.

2) A follicular form which included,

   a) vestigial follicles, often marginated.

   b) germinal follicles.

   c) tumour follicles.

Where vestigial follicles were associated with an immature tumour cell sheet, the diagnosis was uncomplicated, but secondary diagnostic criteria had to be considered where the cell sheet was composed of mixed or mature tumour cells. When germinal centres persisted in a node, lymphosarcoma was indicated by the development of streams and sheets of tumour cells elsewhere in the node and the presence of most of the secondary characteristics.

Six secondary characteristics were found to be of importance. None of them, at first inspection, might appear to be particularly significant, but two or more were noted in the great majority of affected lymph nodes. These were:

1) Perinodal infiltration or invasion in 90% of the sections.

2) Flooding or elimination of sinuses in 97% of the sections.

3) Capsular infiltration or elimination in 79% of the sections.

4)/

4) Trabecular infiltration or elimination in 90% of the sections.

5) Loss of cortico-medullary differentiation in 87% of the sections.

6) Diffuse or focal necrosis in 22% of the sections.

The tertiary characteristics included the important negative findings. In none of the sections was there evidence of the widespread and marked fibrosis observed in Hodgkin's disease in man, nor were multinucleated cells resembling Dorothy Reed-Sternberg cells or even eosinophils encountered, except very rarely. No abscess formation was noted, other than secondary to necrosis in the nodes and foci of neutrophils were found in a few instances only.

THE LIVER.

Ninety-five dogs showed lesions of lymphosarcoma in the liver and histological sections from 89 of them were available for study. Table 1 gives the numbers of affected livers in each of the forms of the disease.

Morbid anatomy. Infiltration of a liver with tumour cells was, in most cases, accompanied by an increase in size of the organ and a change in its macroscopical appearance. Its edges became rounded, it thickened antero-posteriorly and extended behind the costal arch for a variable distance depending on the degree of hepatomegaly. Livers varied in colour from the normal red-brown to a milk chocolate or even a putty colour. Occasionally, the organ was distinctly bronze and a minority was congested. Capsules of affected livers often presented a greyish mottled effect and on section, there/

there was a well defined, delicate, greyish coloured exaggeration of the portal triads throughout the parenchyma. Less commonly, distinct and only rarely raised, round grey or pale yellow foci of tumour infiltration up to 1.0 to 1.5 cm. in diameter, were found scattered over the liver surface. On sections, these areas were seen to be discrete, non-encapsulated, round lesions scattered in the liver parenchyma, Figure 12.

Histological findings. A summary of the main lesions is given in Table 5.

In most of the sections, the typical microscopical lobulation of canine liver tissue was well preserved. The main cause of absence of liver lobulation was the widespread or diffuse nature of some of the lymphosarcoma lesions.

The most common site of infiltration was the portal triad, Figure 13. This region was affected in 96% of the sections. Infiltrated triads tended to be rather uniform in size and profile throughout a given section. This uniformity was not a constant feature and there were a few sections where both affected and unaffected triads were present side by side. In only 8 sections was evidence found of lesions of lymphosarcoma without any apparent triad involvement. Triad profile varied and, in the heaviest infiltrations, assumed a roughly circular outline which was often quite well defined except where infiltration into adjacent sinusoids had taken place.

All but 10 sections presented some evidence of infiltration into the sinusoids. In the main, this comprised small foci of lymphoid cells lying scattered/

TABLE 5

A Summary of the Liver Lesions in Dogs with Lymphosarcoma.

Number of cases reported positive at post-mortem examination		93
Number of cases with sections available for histological study		89
Typical lobulation		
preserved	75	
absent	14	89
Subcapsular infiltration		
slight	27	
moderate	15	
marked	4	
absent	31	
capsule absent	12	89
Portal triad infiltration		
slight	29	
moderate	25	
marked	28	
variable	4	
negative	3	89
Sinusoidal infiltration		
slight	54	
moderate	1	
marked	8	
streams of cells	16	
absent	10	89

Peri-central venous aggregates		
slight	43	
moderate	19	
marked	10	
absent	17	
		89
Hepatic cord cells.		
Fatty changes		
central zone only	15	
central and mid-zone	13	
peripheral zone only	1	
all zones	6	
Necrotic changes		
central zone only	1	
No marked abnormalities	53	
		89

scattered throughout the lobules, Figure 14. In only 10% of the sections were foci large enough to displace or replace adjacent cord cells. Rows of tumour cells streaming from the triads towards the central veins were fairly common.

Another recurring lesion was a collection of tumour cells within the sinusoids immediately round the central veins. These central venous "cuffs" were usually rather narrow, but in 29 sections these areas of tumour cells were comparable in size and shape to the lesions within triads. In many cases it was difficult to distinguish one from the other, particularly where compression of the bile ducts in the triads had taken place, and also where degenerative changes in cord cells were obscuring the radial effect of the hepatic cords away from the central veins.

Some degree of subcapsular infiltration was observed in many section, but this was only severe in a very small number.

Degenerative changes in the hepatic cord cells were observed in more than 1/3 of the sections. This was principally a central or a central and midzonal lesion. However, all the zones were involved in 6 sections. Central necrosis was seen in one section; and degenerative changes, which were confined to the peripheral zones alone, were noted in one other case.

Less common features included haemosiderosis, which was observed in 7 sections and high numbers of neutrophils in the triads. This neutrophil content was observed in 8 cases and was sufficiently high in some areas to call/

call into question the diagnosis of lymphosarcoma. However, in each of these cases, there was ample evidence of the lymphoid character of the tumour elsewhere in the body.

Unusual lesions were present in the livers of 2 cases of the aberrant type, Case no. 10513 and Case no. 19424. The lesions in these cases consisted of massive foci of lymphoid cells which were non-encapsulated, expanding, but non-invasive, and which were stretching and distorting the hepatic cords peripherally. Some of these foci were raised and umbilicated. Case no. 19424 was associated with similar lesions in both kidneys.

THE SPLEEN.

Of the 60 cases in which the spleen was reported as affected at the post-mortem examination, sections were available from 50 of them for detailed histological study. The group distribution of affected spleens is shown in Table 1.

Morbid anatomy. Spleens which were affected with lymphosarcoma had distinguishing features which may be considered under the following headings, 1) the size and shape of the organ, 2) its colour, 3) structural changes and 4) its environment.

1) Most affected spleens were moderately to grossly enlarged. This increase in size was in all dimensions, so that the entire organ was involved; its edges were distinctly rounded, but its profile was preserved, Figure 15. The spleen was readily palpable in life and was usually found lying antero-posteriorly/

antero-posteriorly towards or along the lower aspect of the abdomen on the left side. In cases of extreme splenomegaly, the organ extended as far back as the entrance to the pelvic cavity. Few signs of surface erosions or ulcerations, which could be attributed to the disease process, were found. In one instance, rupture of the spleen had preceded death and was due apparently to overenthusiastic palpation. A noteworthy difference existed between a typically affected spleen from the multicentric and aberrant groups and those few which were found in the alimentary type. In this latter form, out of the 5 affected organs, only one was increased in size enough to merit the term splenomegaly.

2) Immediately after death, most spleens were purple or plum coloured, but many of these became pale bluish pink after refrigeration for 24 hours. Other spleens showed straw-coloured stripes with occasional small infarcts on their surfaces, Figure 16.

3) On section, infiltrated spleens were found to have well-defined capsules which were often thin and tense, but there was loss of the characteristical internal structure with the disappearance of the typical trabecular networks. In most cases there was complete replacement of the red and white pulp by a fairly uniform, soft and highly cellular, reddish purple tissue. In some cases, however, the spleen showed marked hyperplasia of the Malpighian bodies which appeared as large numbers of spherical, bulging, white and glistening foci.

Infarcts and foci of necrosis which were visible on the surface also involved the deeper areas. Areas of gross haemorrhage, with islands of tumour tissue, were occasionally found.

4) Apart from the very largest spleens, most were relatively mobile within the abdominal cavity and not attached by inflammatory or neoplastic adhesions to adjacent structures or to the omentum. There were never gross extensions of the tumour process from the spleen into its visceral mesentery.

Histological findings. Most of the cases in which the spleen was involved were of the multicentric form, 52 of the 60 cases. Although there were morbid anatomical differences between affected spleens of the alimentary group and those of the other two, they were found to be indistinguishable histologically and were considered as a single series.

Table 6 gives a summary of the histological findings. Obliteration of the internal trabecular framework, due to stretching or infiltration, was observed in 80% of the sections. Most of the other sections presented an earlier phase of splenic involvement which included marked hyperplasia of the Malpighian corpuscles with peripheral infiltration into the adjacent red pulp.. Well defined, discrete, raised tumours were found in a single case. This was a multicentric case, which showed an unusual pleomorphic lymphoid stem cell in the lesions.

Five sections in the lymphosarcoma series showed evidence of tumour infiltration of the capsule, but only 10 sections failed to show a similar or a more intensive invasion of the trabeculae.

In a small number of sections, there was invasion of the sub-intima of the trabecular blood vessels and aggregates of tightly packed lymphocytes lay within the vessels. Megakaryocytes were observed in 50% of the sections; they/

TABLE 6

Summary of the Histological Features of Spleens Affected with Lymphosarcoma.

Total number of sections.	50	
Capsules		
Absent from the section	1	
Non-infiltrated	44	
Infiltrated	5	50
Trabecular/pulp ratio		
Low	36	
Moderate	9	
High	5	50
Trabecular infiltration		
Absent	10	
Mild	26	
Moderate	11	
Marked	3	50

Infiltration of trabecular vessels

	Sub-intimal infiltration	Vessels packed with lymphocytes	Both sites	
Mild	6	2	8	
Moderate	3			
Marked				
Absent			31	50

The splenic pulp

Diffuse white/red infiltration	39	
Malpighian hyperplasia with invasion	9	
Malpighian hyperplasia with moderate invasion	1	
Well-defined metastases	1	
		50

Megakaryocytes

Negative on sections	23	
+ small numbers	25	
++ moderate numbers	1	
+++ numerous	1	
		50

they were observed as isolated cells and some contained phagocytosed leucocytes. High numbers were only noted in one case. Occasionally, erythromyeloid activity was also seen.

THE ALIMENTARY SYSTEM.

With the exception of the tonsils which will be considered under a separate heading, all tumours of lymphosarcoma in the alimentary system were found in the stomach or intestines.

Distribution of the lesions. Table 7 shows the distribution of lymphosarcoma of the alimentary system amongst the three groupings, i.e. multicentric, alimentary and aberrant forms. It will be noted that there is a disparity between the numbers of cases where tumours were diagnosed at autopsy and those which were later confirmed histologically. There are two reasons for this. Firstly, some tissue blocks were taken from regions immediately adjacent to the tumour mass and were found to be negative microscopically. Secondly, where the tumours were necrotic, tissue blocks were selected from the grossly affected mesenteric nodes which offered better diagnostic material.

The 47 cases of the alimentary type in Table 7 belong to a larger group of dogs with lesions of the alimentary-mesenteric-lymphatic complex. Altogether, 61 cases with the major lesions in this area were found. These are shown in Table 8. From this table it will be noted that, in 53 cases (87%), mesenteric lymph node involvement was a feature of the tumour process. However, in 8 of the animals (13%), the mesenteric node was not affected. In Table 9 there is a breakdown of the lesions in those 8 dogs. In only 3 of them were circumscribed tumours found on the alimentary tract without

any/

TABLE 7

The Distribution of Tumours of the Alimentary System Amongst the Three Anatomical Groupings.

Anatomical group	Confirmed histologically	Diagnosed at autopsy
Multicentric	10	19 (29%)
Alimentary	38	47 (71%)
Aberrant	0	0
Total	48	66 (100%)

TABLE 8

Lesions Throughout the Alimentary-Mesenteric Area in the Alimentary Form of Canine Lymphosarcoma.

Alimentary tumours plus mesenteric node infiltration	39	(64%)
Alimentary tumours without mesenteric node infiltration	8	(13%)
Mesenteric node infiltration without alimentary tumours	14	(23%)
Totals	61	(100%)

TABLE 9

The Distribution of Lesions in 8 of the Alimentary Cases of Canine Lymphosarcoma in which the Mesenteric Lymph Node was Unaffected.

Case No.	Sites	Liver	Spleen	Kidney	Other tissues
2804	Duodenum	+			
2939	Stomach				
3565	Ileum				
4548	Duodenum, Ileum and colon	+			
13304	Jejunum, Ileum	+		+	Lungs
14841	Colon				Drainage nodes
14945	Duodenum				
21993	Small intestine			+	

any microscopical extension elsewhere. Therefore, only 5% of the dogs in the entire alimentary group could be regarded as cases which were suitable for surgical intervention; in the remaining 95%, the lesion complex included enlargement and infiltration of the mesenteric lymph node or lesions in other organs.

Morbid anatomy. Expanding mural tumours, either single or multiple, were the principal lesions found on post-mortem examination of the alimentary system. Examples were found in stomach, duodenum, jejunum, ileum, colon and rectum. A typical tumour mass was spherical or elongated, often with a smooth, greyish-yellow surface bearing a stippled or fine vascular pattern. In its centre, the tumour was frequently necrotic and, on occasions, this had led to perforation, especially along the paramesenteric borders. Such lesions were sometimes associated with the presence of haemorrhagic or purulent fluid in the abdomen; more often, there were inflammatory and neoplastic adhesions to other parts of the tract or to the omentum. On section, tumour masses often partly or wholly occluded the lumen, especially in the small intestine; here the mucous membrane was often ulcerated. Many mural tumours had remained apparently well defined and immediately adjacent parts of the tract were macroscopically normal. In a minority of cases, there was diffuse lymphosarcomatous infiltration along lengths of the canal; this appeared to extend from Peyer's Patches in the small intestine. The wall was cream coloured, thickened and on section, showed an apparent increase in width of all layers. In one or two instances, infiltration on to the tract from an affected mesenteric lymph node was observed, Figure 17.

Histological findings. In 66 dogs, lesions of lymphosarcoma were found along the gastro-intestinal tract. Sections were available from 48 animals for study and there were 4 sections from biopsies. A summary of the histological findings is given in Table 10.

Histologically, lymphosarcomatous tumours of the stomach and intestine were highly invasive and intensely destructive. Usually all the layers were infiltrated, and frequently, there was extension of the tumour process on to the mesentery,,or subserous area. Infiltration of the muscle bundles took the forms of mild to severe lamination, fragmentation, and finally obliteration. In some sections, the only evidence that the area was formerly smooth muscle of the intestine was the presence of isolated groups of nerve cells of Auerbach's or Meissner's plexi. Infiltration of the mucous membrane was usually accompanied by fairly marked distension of the inter-glandular tissue, Figure 18. This progressed to heavy infiltration and destruction of the glandular elements and then to complete elimination of the mucous membrane. In the cases where the mucosa alone was infiltrated by mature lymphoid cell types, it was not always a simple matter to determine that the lesion was neoplastic. High numbers of mitotic figures and active destruction of the glandular elements by the round cells were considered to be important guides.

Neither encapsulation of tumour masses nor internal fibrosis were prominent, but there was a greater concentration of reticular stroma than in tumour masses in other organs.

TABLE 10

Summary of the Histological Features of Alimentary Lesions in Canine Lymphosarcoma.

Mucous membranes			
	Intact	4	
	Necrotic or fibrotic	4	
	Infiltrated	14	
	Infiltrated, glandular vestiges	22	
	Obliterated	19	63
Submucosa			
	Intact	4	
	Infiltrated	40	
	Obliterated	19	63
Muscle layers			
	Intact	6	
	Laminated	39	
	Fragmented	8	
	Obliterated	10	63
Subserosal and mesenteric infiltration			
	Infiltrated	50	
	Non-infiltrated	13	63
Total Number of Histological Sections			63

Criteria for the Diagnosis of Lymphosarcoma on the Basis of Examination of Alimentary Lesions. The gastro-intestinal tract, together with lymph nodes and spleen, are the sources of tissue for the histological diagnosis of lymphosarcoma in the living animal, and in this survey an attempt was made to establish diagnostic markers in alimentary lesions. The two main characteristics of the alimentary lesions were the predominant tumour cell and the destructive nature of the process. Diffuse destructive infiltration of more than one of the layers of the wall of the canal by streams or sheets of immature, mature, or mixed lymphoid cells was accepted as positively diagnostic of lymphosarcoma. However, where only the mucous membrane was involved, some care was necessary to differentiate lymphosarcoma from an inflammatory mononuclear reaction or an immunogenic focus. A high frequency of mitotic figures and active destruction of the glandular tissue also appeared to be essential criteria.

THE KIDNEYS.

In forty-two cases the kidneys were involved and the distribution of renal lesions amongst the three forms of the disease is shown in Table 1.

Morbid anatomy. Adhesions between the capsule and the cortex were not found, although subcapsular lesions were noted in 3 cases. In almost all of the dogs, lesions of lymphosarcoma were observed bilaterally.

The most severely affected kidneys were characterised by a multiplicity of discrete, greyish-white tumour nodules which measured up to 1 cm. in diameter./

diameter. They were scattered throughout a thickened cortex and were slightly raised on the surface of the organ, Figure 19. This type of lesion was found in approximately 16% of the cases. In a further 33%. small cream coloured foci up to 0.25 cm. in diameter and also larger wedge-shaped foci were found throughout the cortices. The base of a wedge-shaped lesion lay along the surface of the cortex and its apex pointed towards the juxta-medullary area. In many instances, these lesions were outlined by a narrow zone of intense hyperaemia which gave them the appearance of typical renal infarcts.

In the remaining 50% of the kidneys which proved to be infiltrated, there was a fine cream coloured stippled network over the surface of the kidneys and this was also found in the depth of cortices. Visible lesions in the medulla were noted in one or two organs.

In general, the kidneys which were affected with lymphosarcoma were enlarged, but this was never to a very marked degree.

Histological findings. Sections from 39 of the 42 positive cases were available for study and the results are summarised in Table 11.

Subcapsular infiltration was marked in 3 cases and in six others there was interstitial infiltration in the medulla. All the remaining lymphosarcoma lesions were confined to the cortex, or the juxta-medullary area.

In the cortex, three degrees of tumour involvement were noted. Firstly, there were small, widely scattered foci lying in the interstitium, often around the small blood vessels. These tended to be centrally destructive, but/

TABLE 11

Summary of the Renal Lesions in Lymphosarcoma of the Dog.

Total number of cases		39
Subcapsular infiltration		3
Cortical infiltration		37
Interstitial foci	17	
Small foci and wedge-shaped lesions	13	
Tumour nodules	6	
Discrete tumours	1	
Juxta-medullary infiltration		12
Medullary infiltration		6
Other features		5
Infarction	3	
Vascular mural lesions	1	
Widespread tubular casts	1	

but had infiltrated peripherally into the interstitial spaces without much apparent damage to adjacent tubules or glomeruli, Figure 21. This pattern was found in roughly half of the kidneys. Secondly, larger foci and radial wedges were observed. These were also peripherally infiltrative, but were associated with localised destruction of the tubules. Adjacent glomeruli were less affected and remained prominent and well defined even when the surrounding tubules had been totally obliterated. This pattern was observed in approximately 1/3 of the sections. Thirdly, in 6 cases, there was widespread invasion of areas of the cortex with active destruction of tubules and to a lesser extent, the glomeruli, Figure 20. All three formations could be observed in some of these sections. In some larger secondary tumours, the only proof in a given field that the tissue was actually renal was the presence of one or two small glomeruli with pyknotic nuclei which were isolated in uniform sheets of lymphoid tumour cells.

Casts within tubules were a common feature, but were only seen in large numbers in one section where widespread tubular atrophy had developed. Small recent infarcts were a feature of three of the sections and in one section this was clearly due to tumour invasion and necrosis of the adjacent blood vessels.

Foci in the juxta-medullary area were observed in 12 sections. These lesions were especially prominent around the larger blood vessels which, however, showed no severe mural lesions.

Case no. 19424, of the aberrant type, was exceptional in that it presented/

presented unusual kidney lesions. These were large expanding and well defined lymphoid masses which were neither peripherally infiltrative nor encapsulated, but which had grown to cause distortion of the adjacent renal tissue.

Chronic interstitial nephritis. Since this disease is common in the adult dog in Glasgow and its environs, every available kidney section was examined for evidence of the condition. In several sections, small foci of lymphoid cells were found in the cortex and it was not possible to tell whether these were neoplastic or inflammatory in origin. Such lesions were ignored, for they were too small and sparse to be of any clinical significance.

None of the 39 cases in which lymphosarcoma was observed showed additional evidence of severe lesions of chronic interstitial nephritis, but many of the largest tumour foci had destroyed almost all evidence of the former renal tissues. More than a dozen other cases were found to have lesions of chronic interstitial nephritis. Most were mild to moderate in extent, and comprised fine cortical lympho-fibrotic foci and radial scars, some of which extended to, and had pitted, the otherwise smooth cortical profile. At such points, adhesions between the capsule and the parenchyma were observed. Tubular casts were common in such sections and areas of marked tubular dilatation were observed. It was not considered likely that chronic interstitial nephritis would cause diagnostic confusion.

Pyelo-nephritis. One case of pyelo-nephritis was found. This was associated with a marked mononuclear reaction below the renal pelvic mucosa and this cellular infiltration had continued upwards between some of the tubules in the medulla, but no lesions were seen in the cortex.

THE TONSILS.

Twenty-six dogs were found at autopsy to have macroscopically affected tonsils. Of these, 25 were of the multicentric form, 1 of the alimentary form and none of the aberrant type, Table 1.

Morbid anatomy. Affected tonsils were pale in colour and had smooth surfaces; they were usually enlarged bilaterally and protruded from their crypts. In a few cases, they were so grossly swollen that they partially occluded the entrance to the pharynx, but even in such cases, there was never any evidence of surface erosion or extensions into the adjacent tissues.

Histological findings. Twenty-one sections were available from the 26 cases and a summary of the histological examinations is given in Table 12.

The epithelium was stretched and thinner than normal, but continuity was preserved in 20 cases. In 3 of these, erosion of the basal layers by active lymphoid tumour cells had taken place, but the superficial cells remained intact. In one section, a breach in epithelial integrity had occurred without any resulting inflammatory reaction; this was considered artefactual.

In 15 of the sections, the typical follicular arrangement was replaced by uniform sheets of tumour cells. Vestigial follicles, which were noted in certain affected lymph nodes, were also observed in 4 tonsillar sections. Expanding tumour follicles were an outstanding feature in one instance.

The mucous glands, which lie deep to the lymphoid tissue in the tonsil of the dog, were infiltrated in half of the cases. This was quite varied, although/

TABLE 12

Summary of the Histological Findings In Tonsils Affected with Lymphosarcoma.

Mucous membrane			
	Intact	17	
	Partially eroded	3	
	Torn	1	21
Follicular pattern			
	Preserved	1	
	Vestiges	4	
	Tumour follicles	1	
	Absent	15	21
Mucous glands			
	No infiltration	9	
	Slight infiltration	8	
	Moderate infiltration	2	
	Marked infiltration	1	
	Absent	1	21
Other features			
	Diffuse necrosis	2	
	Neutrophils	1	

although mainly of a mild interstitial nature, there being only 3 cases where moderate or severe invasion was observed. In nearly ½ of the sections, the mucous glands were well defined and completely free from tumour cell infiltration. Diffuse necrosis or infiltration with myeloid elements were rare.

Lymphosarcoma of the canine tonsil was characterised by marked distension of the tissue without any loss of surface integrity and only nominal infiltration into associated tissues. This was in contrast to the depressed, ulcerative, hard, intensively invasive and destructive lesions of squamous cell carcinoma which is the other major malignant disease of the tonsil of the dog.

THE HEART.

The heart was affected in 12 dogs, all but one of which were in the multicentric group, Table 1.

Macroscopically, there were no predilection sites and most of the lesions were small whitish foci up to 0.5 cm. in diameter within the myocardium and appeared as unraised spots on the surface. In 5 of the dogs much larger foci were observed. In one case, there were many well defined, distinctly raised tumours which were up to 3 cms. in diameter in the myocardium. They were present in the walls of the auricles and ventricles on both sides of the heart. Another case had brownish-white tumour foci, approximately 0.5 - 1.0 cm. in diameter, in the walls and septum of the left ventricle. A much larger focus was found traversing the wall of the right side. In 2 cases, a single discrete lesion was reported. In one other dog, much of the wall of the right ventricle was completely replaced by lymphoid tumour tissue, Figure.24.

Histological findings. The nodules and larger foci of tumour tissue in the myocardium comprised sheets of lymphoid tumour cells which had destroyed the myocardial muscle fibres at the site of the lesion and were also expanding, stretching and distorting adjacent unaffected musculature. Many of the lesions were infiltrative and lamination of the muscle fibres was a feature, Figures 25 and 26. Some sections showeddsmall areas of subendocardial or sub-pericardial tumour infiltration.

THE LUNGS.

The lungs were affected with lymphosarcoma in 20 dogs. Fourteen of them were in the multicentric group and the other 6 were of the alimentary type, Table 1.

Morbid anatomy. The lungs were macroscopically normal in 25% of the cases which were later found to have histological evidence of the disease. In another 25%, the signs of tumour involvement were not particularly marked. On section of a lobe, there were either scattered areas of round greyish foci, which were not always visible on the surface, or else a diffuse greyish network over wide areas. In the other 50% of affected lungs, well defined, isolated,yellowish tumour nodules were observed within the lung parenchyma. These were between 0.5 and 2.5 cm. in diameter, Figure 23.

Histological findings. In a few sections, where there had been no macroscopical evidence of tumour infiltration, some of the capillaries and venules were found to be grossly distended by masses of leucocytes, of both lymphoid and myeloid types. Where such vessels were sectioned longitudinally, they and their branches were tightly packed with those cells.

In several lung sections, lesions were noted particularly in the alveolar spaces around the bronchioles, Figure 22, and in one instance there was marked infiltration of the submucosa of a bronchiole. Such lesions were assumed to be part of the tumour process because their cell types corresponded to the modal cell type of the tumour. In other sections, tumour cell infiltration was concentrated around the smaller blood vessels.

Where tumour nodules were present in the lung tissue, whole areas had become partially or completely replaced by sheets of tumour cells. In some sections, surviving alveoli contained clumps of lymphoid cells which were more darkly stained than those elsewhere, Figure 22. Although these tumour nodules were macroscopically discrete, there was no evidence of encapsulation of the nodules and there was active infiltration of adjacent lung tissue at their peripheries.

THE BONE MARROW.

Infiltration of the bone marrow by the tumour process was observed in 15 dogs, 14 of which were of the multicentric type, and the other was an alimentary case, Table 1.

Morbid anatomy. Where the bone marrow was infiltrated, there was usually a relatively homogenous red or rust coloured, highly cellular tissue present throughout the medullary cavities of the long bones. However, this was not indicative of leukaemic infiltration, since myeloid hyperplasia, which occurred frequently, presented a very similar appearance.

Histological findings. In 3 of the cases, the marrow section was indistinguishable from any other lesions of lymphosarcoma, being composed of uniform sheets of lymphoid tumour cells. Five cases presented focal lesions. There was crowding out of hyperplastic erythroid and myeloid elements and megakaryocytes. There was little evidence in these sections of fat cells. A much more diffuse infiltration of the normal marrow elements by lymphoid cells was observed in the remaining 7 cases, but the marrow was again highly cellular, Figure 27.

A high number of sections revealed marked myeloid hyperplasia without evidence of an increase in the lymphoid cell population, Figure 28.

OTHER AFFECTED TISSUES.

A list of the other tissues in the body which were found to be affected by lymphosarcoma is given in Table 2.

Foci of tumour cells, which were locally destructive and peripherally infiltrative or invasive, were characteristic of most of the these other organs and tissues.

TUMOUR CELL MORPHOLOGY.

The cell morphology of canine lymphoid tissue. The cell types found in the lymphoid tissue of man which have been described by Ham (1961) were almost identical to those observed in the lymphoid tissue sections of 20 dogs which were clinically free from disease. Ham recognised only three cell types in the lymphoid cell line, the lymphoblast, the medium lymphocyte and/

and the small lymphocyte. In the dog, it was possible to identify 4 cell populations, lymphoblasts, large lymphocytes, medium lymphocytes and small lymphocytes. Identification of the modal cell in each group was relatively simple, but there was so much variation that many individual cells could not be classified with certainty.

The lymphoblast was the most readily identifiable of the modal cell types. It was one of the largest cells in the tissue, with a well defined round or angular cell membrane and a narrow rim of pale basophilic cytoplasm, occasionally revealing a fine perinuclear halo. The nucleus was usually round, but it varied from oval to angular, and its membrane was thin and defined by a narrow lining of condensed chromatin. Fine chromatin particles were scattered throughout the nuclear sap which remained relatively clear and unstained. A leptochromatin network radiated from a prominent central nucleolus to link up the chromatin particles. The nucleolus was either eosinophilic and translucent, or of a pale basophilic colour, depending on the degree of chromatin condensation on and around it. There were occasional blast cells whose almost naked nucleolus occupied over 50% of the nucleus. Others showed 2 or 3 nucleoli, some of which were marginated on the nuclear membrane.

The large lymphocyte was almost as large as the lymphoblast. Its nuclear cytoplasmic ratio was also similar, but condensation of the chromatin was more marked, and the nucleoli were small and quite inconspicuous. Large chromatin particles were observed in the lining chromatin of the nucleus and similar/

similar particles were present in the sap, tending to overly and obscure the nucleoli. The supporting chromatin network was also coarser than in the blast cell.

The medium lymphocyte was a smaller cell which showed even more marked differentiation. The nuclear cytoplasmic ratio was often low, but could be 1:1, and the cell membrane was well defined. The cytoplasm was pale and no perinuclear halo was visible. The nuclear membrane was obscured by large dense blocks of chromatin which were also found centrally, partly obscuring the nuclear sap which was somewhat basophilic.

The small lymphocyte had little or no visible cytoplasm. Its nucleus was round or slightly indented and almost entirely packed with chromatin blocks. Where the nuclear sap was still in evidence, it was distinctly basophilic.

Cells indistinguishable from those of the lymphoid cell series, but with a much more basophilic cytoplasm, were assumed to belong to the plasma cell line. The mature plasma cell had a nucleus identical to that of the small lymphocyte, but the nuclear cytoplasmic ratio was at least 1:1. The cytoplasm was quite basophilic often with a perinuclear halo.

The primitive reticular cell, probably better termed simply the stem cell, was the largest cell in lymphoid tissue. It was characterised by an oval nucleus in a round, apparently empty space, surrounded by lymphocytes. The nuclear cytoplasmic ratio was often less than 1:1 and the cytoplasm could scarcely be recognised because it was so pale. Its cell membrane was also difficult/

difficult to identify but could sometimes be appreciated as it lay against neighbouring cells. The stem cell nucleus was oval with a finely etched nuclear membrane lined by only a narrow, slightly particulate, chromatin film. A small nucleolus lay in the centre or just off-centre, apparently coated by a chromatin film from which fine chromatin strands radiated towards the chromatin lining of the nuclear membrane. The nuclear sap was unstained. Occasionally, a prominent pale nucleolus was found in a stem cell.

The reticulo-endothelial cell was very similar in appearance to the stem cell except that its cytoplasm was faintly basophilic and the cell membrane was well-defined. In the sinuses of the nodes, these cells were linked by cytoplasmic bridges into a loose network. On section, the reticulo-endothelial cells which were lining sinuses were thin and flattened and contained a nucleus which was compressed and dense.

Similar cells, which had rounded up and contained cell debris, carbon particles, haemosiderin or other material within their fairly extensive cytoplasm, were identified as macrophages.

The cell morphology of the tumour tissues. With the light microscope, sections of tissues affected with lymphosarcoma and stained with haemotoxylin and eosin contained tumour cells which, as individual cells, were indistinguishable from those found in the lymphoid tissue of dogs which were free from this disease. Tumour cells could only be recognised as such because they tended to form relatively uniform cell sheets and these cell formations were infiltrative and destructive.

The modal tumour cell type or types which were present in lymph node sections from 109 cases are shown in Table 13. From this, it can be observed that 60 of the 109 sections presented lesions in which the dominant cell type was a large lymphocyte or more immature cell, whilst 42 cases had a tumour cell population in which maturer lymphoid cells were well represented. In order to relate the modal cell type or types with the age and sex of the animal and also with the duration of the clinical illness, these cell groups were termed the "immature cell group" and the "mixed and mature cell group" respectively, Figures 29, 30, 31 and 32. In 7 sections the cytology was too poor to enable recognition of the modal cell. In 3 other sections, there was a high myeloid element and plasma cells were numerous in one other. There was no statistical difference in the distribution of modal tumour cells between the two major forms of the disease, $\chi^2 = 2.17$ with one degree of freedom.

A comparison was made between the tumour cell types in node sections and those found elsewhere throughout the body. In 47 cases there was definite evidence of internal variation, but this variation was less marked when the tumour cells were classified into the "immature cell group" and the "mixed or mature cell group." However, the differences in tumour cell types from organ to organ were still too wide for this form of classification in 17 cases, Table 14. Thus, apart from 1/6 of the total number of cases examined, the dominant tumour cell type in lymph nodes affected with lymphosarcoma in the dog was an indication of the tumour cell population in lesions in other parts of the body.

TABLE 13

The Modal Tumour Cells in the Lymph Node Sections.

Cell Types	Multicentric Group	Alimentary Group	Total
Stem Cells	2	0	2
Pleomorphic blast cells	3	0	3
Lymphoblasts	19	6	25
Lymphoblasts and large lymphocytes	10	12	22
Large lymphocytes	2	6	8
Blasts, large and medium lymphocytes	3	1	4
Medium lymphocytes & blasts	1	1	2
Large and medium lymphocytes	4	5	9
Large, medium and small lymphocytes	0	2	2
All cell types	6	12	18
Medium and small lymphocytes	2	1	3
Plasma cells	0	1	1
Myeloid elements	3	0	3
Unidentifiable	3	4	7
Total	58	51	109

TABLE 14

Tumour Cell Types in 47 Cases Where There was a Variation in the Modal Cell from Organ to Organ.

Immature cell types	23
Mixed or mature types	7
Wide variation	17
Total	47

Mitotic rates. The mitotic rate was estimated for each affected lymph node section at a magnification of x 500 and classified on the following basis. Where a mitotic figure was observed in every fifth field or more which was selected at random, the rate was "low." If one or more figures were found regularly in every fourth or fifth field, the term "moderate" was employed. When every second or third field presented signs of mitosis, this was classified as "high;" the term "very high" being reserved for those instances where almost every field contained more than one figure. This was an arbitrary method and precise estimates were not possible due to the variations in the rate of mitosis which could occur from area to area within a section. However, it was believed to give a reasonable indication of the differences in the rates of mitosis amongst the sections in this series.

The mitotic rates in lymph node sections from 109 cases are given in Table 15. In the "immature cell group," 48 of the 60 cases had a mitotic rate which was high or very high, whilst, in the "mixed or mature cell group," 29 out of 38 had rates which were moderate or low. Statistical analysis showed that there was a highly significant difference between the two groups, at the 0.1% level, $\chi^2 = 29.33$ with one degree of freedom. It could thus be concluded that the mitotic rate was closely related to the tumour cell type, and that higher rates were found in the more immature cell sheets.

Imprint films of affected lymph nodes. In 14 cases, lymph node imprint films were prepared and stained with Leishman's stain. Histological sections were prepared from the same nodes and stained with Haematoxylin and Eosin. The tumour cell types observed in each of the two methods are given in Table 16.

TABLE 15

Mitotic Rates of the Modal Cell Types in the Lymph Node Sections.

Cell Types	Estimated Mitotic Rates: Very High	High	Moderate	Low	Not recorded	Total
Stem cells	1	1	0	0		2
Pleomorphic blast cells	1	2	0	0		3
Lymphoblasts	2	21	2	0		25
Lymphoblasts and large lymphocytes	5	10	6	1		22
Large lymphocytes		5	1	2		8
Blasts, large and medium lymphocytes		1	1	2		4
Medium lymphocytes & blasts		1	1			2
Large & medium lymphocytes		2	2	5		9
Large, medium and small lymphocytes			1	1		2
All cell types		5	3	10		18
Medium & small lymphocytes				3		3
Plasma cells				1		1
Myeloid elements		2	1			3
Unidentifiable					7	7
Total	9	50	18	25	7	109

Six of the fifteen cases showed tumour cell types which were identical in the imprints and the sections; in six others, there was an apparent shift towards a more differentiated cell type in the imprints; in the 2 remaining cases, there was much less evidence of pleomorphism in the imprints as compared to the sections.

When classified into "immature" and "mixed and mature" groups, only one case, No. 25004, would have required re-classification.

TABLE 16.

Tumour Cells Identified in Imprint Films and Histological Sections from the Same Lymph Node Affected with Lymphosarcoma.

Case No.	Imprints.	Histology.
12998	Lymphoblasts & large lymphocytes	Lymphoblasts & large lymphocytes
17807	Large, medium & small lymphocytes	All types
23157	Lymphoblasts	Lymphoblasts & large lymphocytes
24477	Lymphoblasts	Lymphoblasts
24644	Large lymphocytes	Lymphoblasts
25358	Lymphoblasts & large lymphocytes	Lymphoblasts
25369	Lymphoblasts	Lymphoblasts
25394	Lymphoid and myeloid cells	Lymphoid and myeloid cells
25257	Large lymphocytes	Lymphoblasts
25004	Large lymphocytes	All types
25800	Large lymphocytes	Lymphoblasts
23913	Lymphoblasts & large lymphocytes	Lymphoblasts
25690	Lymphoblasts	Lymphoblasts
25784	Lymphoblasts	Lymphoblasts

SPECIAL EXAMINATIONS.

HAEMATOLOGICAL FINDINGS.

Venous blood samples were taken from 91 dogs in which lymphosarcoma was confirmed at autopsy. There were 50 cases of the multicentric form, 39 of the alimentary type and 2 of the aberrant form.

The Erythrocytes.

The erythrocyte sedimentation rate. (E.S.R.). A total of 224 examinations was carried out and the results are given in Table 17. In the multicentric form of the disease, more than half of the readings were less than 10 mm./hour, but 20% were higher than 30 mm./hour. In the alimentary form, readings greater than 10 mm./hour were observed in only 25% of the samples. The aberrant cases appeared to follow the pattern of the multicentric cases.

Packed cell volumes. (P.C.V.). The results of 258 examinations are presented in Table 18. In the multicentric form, 123 of the 182 readings were less than 40%, 56 were under 30% and 8 of these had fallen below 20%. A similar trend was observed in blood samples from the other two forms of the disease. However, 3 of the samples from the alimentary form gave readings which were higher than the normal range, which probably reflected the greater risk of haemoconcentration associated with the more persistent fluid loss in this form of the disease.

Haemoglobin estimations. Table 18 shows the result of 249 haemoglobin estimations. Over 50% of all samples from the multicentric cases gave readings which/

TABLE 17

Erythrocyte Sedimentation Rates in Dogs with Lymphosarcoma.

mm./hour	Multicentric Type	Alimentary Type	Aberrant Type	Total
0-	84	49	5	138
10-	18	3		21
20-	16	3		19
30-	7	4	1	12
40-	12		2	14
50-	9	3	1	13
60+	3	2	2	7
Samples	149	64	11	224
Dogs	44	36	2	82

TABLE 18

P.C.V., Haemoglobin Levels and Total Erythrocyte Counts.

Packed Cell Volumes					Haemoglobin Levels					Total Erythrocyte Counts				
%	MC.	Alim.	Aber.	Total	gm/100 ml.	MC.	Alim.	Aber.	Total	$\times 10^6$	MC.	Alim.	Aber.	Total
<20	6	3	2	13	<4	1	0	-	1	<2	3	1		4
20-	48	11	2	61	4-	18	8	3	29	2-	57	14	6	77
30-	67	15	7	89	8-	67	16	8	91	4-	53	31	5	89
40-	43	21		64	12-	62	32		94	6-	27	17		44
50-	16	12		28	16-	18	15		33	8-	1	4		5
60+		3		3	20+		1		1	10+	-	-		
Samples	182	65	11	258	Samples	166	72	11	249	Samples	141	67	11	219
Dogs	50	37	2	89	Dogs	48	39	2	89	Dogs	46	35	2	83

which were less than 12 gm./100 ml. of blood. Of the samples from the alimentary cases, approximately 33% were less than 12 gm./100 ml. of blood. Each test carried out on the blood samples from both cases of the aberrant group was in this low range.

Total erythrocyte counts. In Table 18 the results of 219 estimations are shown. Anaemia, as evinced by a total count of less than 4 million erythrocytes per cu.mm., was found in 60 out of a total of 141 samples from the multicentric cases. Only 15 out of a total of 67 samples from the alimentary cases showed readings in this low range and in this form of the disease, 4 results exceeded 8 millions per cu.mm.

M.C.V. and M.C.H.C. Table 19 shows the Mean Corpuscular Volumes and Table 20 gives the Mean Corpuscular Haemoglobin Concentrations of the erythrocytes.

These data indicated a relatively normal M.C.V. in the majority of samples, but there appeared to be some tendency towards microcytosis in some of the samples from cases of the alimentary type; 11 of the 65 samples gave results less than 60 cu.μ.

An abnormally high or low M.C.H.C. was only observed in a small number and this could well be due to technical variations alone.

The progress of anaemia in lymphosarcoma. There were 19 dogs from which more than 4 blood samples were examined during the course of the disease. Fourteen animals became progressively anaemic with a relatively steady decline in/

TABLE 19

Mean Corpuscular Volumes.

	Multicentric	Alimentary	Aberrant	Total
cu.μ				
< 50	3	-	1	4
50-	4	11	1	16
60-	22	12	1	35
70-	39	15	6	60
80-	38	17		55
90-	19	5	1	25
100-	7	5	1	13
Samples	132	65	11	208
Dogs	43	35	2	80

TABLE 20

Mean Corpuscular Haemoglobin Concentration.

	Multicentric	Alimentary	Aberrant	Total
(%)				
< 23%	4	1		5
23%-	152	62	10	224
46%+	1	2	1	4
Samples	157	65	11	233
Dogs	43	35	2	80

in the total erythrocyte count, the P.C.V. and the concentration of haemoglobin. In two instances, the dogs were frankly anaemic throughout the course of the clinical disease, but in two others there was no measurable anaemia. One additional case was anaemic to begin with, but this improved.

Circulating normoblasts. Immature erythrocytes were observed in many of the blood films. These were mainly eosinophilic normoblasts, but earlier forms were observed, especially when normoblasts were present in relatively large numbers. Table 21 shows the number of blood films containing normoblasts in anaemic and non-anaemic dogs with lymphosarcoma. Table 22 gives the concentration of normoblasts in three anatomical groups, expressed as a percentage of the circulating leucocytes. Table 23 presents the concentration of normoblasts according to the degree of anaemia in the multicentric group.

From these results, it was concluded that a high percentage of the blood films from dogs with the multicentric form of the disease contained normoblasts and, whilse their presence was not clearly related to anaemia, those which showed more than 10 normoblasts per 100 leucocytes were statistically more likely to occur in anaemic animals, $\chi^2 = 6.7$ for one degree of freedom.

Summary. From the foregoing results, it was concluded that progressive anaemia was common in lymphosarcoma of the dog, particularly in the multicentric form of the disease. The anaemia was normocytic and normochromic and circulating normoblasts were a recurring feature, especially in the multicentric cases where high numbers were often associated with frank anaemia.

TABLE 21

The Relationship of Normoblasts

And the Total Erythrocyte Count.

	Multicentric cases	Alimentary cases	Aberrant cases	All cases
Total number of examinations	141	67	11	219
Films with normoblasts	108	17	1	126
Erythrocyte counts:				
$< 4 \times 10^6$/cu.mm.	45	8	-	53
$4+ \times 10^6$/cu.mm.	48	7	1	56
Unrecorded	15	2	-	17

TABLE 22

The Numbers of Circulating Normoblasts.

Normoblasts /100 leucocytes	Multicentric cases	Alimentary cases	Aberrant cases
< 1	55	14	1
1-	39	3	
10-	9		
20-	1		
30-	2		
40-	2		
50+			
Totals	108	17	1

TABLE 23

The Numbers of Circulating Normoblasts Compared with Anaemia in Multicentric Cases of Lymphosarcoma.

Normoblasts /100 leucocytes	R.B.C. $< 4 \times 10^6$	R.B.C. $> 4 \times 10^6$	R.B.C. unrecorded	Total number of cases
1	17	28	10	55
1-	18	18	3	39
10-	6	1	2	9
20-	1			1
30-	1	1		2
40+	2			2
Totals	45	48	15	108

The Leucocytes.

Total leucocyte counts. The results of 261 examinations are shown in Table 24. Leucocytosis was accepted as a total white cell count in excess of 16000/cu.mm. This was observed in 65% of blood samples from 50 multicentric dogs and 67% of those from alimentary cases. All samples from the 2 aberrant cases showed leucocytosis.

Differential leucocyte counts. In the preliminary differential counts, three main cell types were recorded, viz. the neutrophils, lymphocytes and eosinophils. Repeated examinations failed to demonstrate that the monocyte, observed in human blood, did in fact occur in canine blood.

The absolute neutrophil, absolute lymphocyte and absolute eosinophil counts of 247 blood films are given in Tables 25, 26 and 27, respectively. Samples showing a neutrophilia alone, a lymphocytosis alone, or neutrophilia and lymphocytosis are listed in Table 28.

Neutrophilia was an outstanding feature, occurring in approximately 65% of the blood samples from the three groups.

Approximately 1 in every three blood samples revealed an absolute lymphocytosis in the multicentric and the alimentary cases. Lymphocytosis was also a feature of all the blood samples taken from the two aberrant cases.

An absolute lymphocytosis was frequently masked by the presence of neutrophilia and this type of leucocytosis was observed in 25% of the multicentric cases, 28% of those of the alimentary type and 7 samples from the aberrant cases.

TABLE 24

Total Leucocyte Counts.

	x 10^3/cu.mm.							
	0-	16-	32-	48-	64-	80+	Total counts	Number of dogs
Multicentric	63	66	22	9	3	14	177	50
Alimentary	24	26	16	3	3	1	73	39
Aberrant	-	4	2	4	-	1	11	2
Total	87	96	40	16	6	16	261	91

TABLE 25

Absolute Neutrophil Counts.

x 10^3/cu.mm.

	0-	4-	12-	24-	36-	48+	Total counts	Number of dogs
Multicentric	4	54	69	20	5	17	169	48
Alimentary	0	17	29	9	8	4	67	37
Aberrant	1	3	-	3	2	2	11	2
Total	5	74	98	32	15	23	247	87

TABLE 26

Absolute Lymphocyte Counts.

x 10^3/cu.mm.

	0-	5-	10-	15-	20-	25-	30+	Total counts	Number of dogs
Multicentric	114	26	11	6	2	1	9	169	48
Alimentary	42	18	5	1	-	1	-	67	37
Aberrant	-	5	4	1	1	-	-	11	2
Total	156	49	20	8	3	2	9	247	87

TABLE 27

Absolute Eosinophil Counts in Canine Lymphosarcoma.

	per cu. mm.				
	< 1600	1600-	2400+	Number of cases	Number of dogs
Multicentric	157	2	10	169	48
Alimentary	65	2	-	67	37
Aberrant	11	-	-	11	2
All cases	233	4	10	247	87

TABLE 28

The Incidence of Leucocytosis in Dogs with Lymphosarcoma.

	Multicentric Type	Alimentary Type	Aberrant Type	All cases
Number of dogs	48	37	2	87
Number of counts	169	67	11	247
Leucocytosis	113	56	11	180
Neutrophilia alone	58	31	-	89
Absolute lymphocytosis alone	12	6	4	22
Neutrophilia and lymphocytosis	43	19	7	69

Eosinophilia. A marked eosinophilia was present in 10 blood samples from the multicentric case, number 25394, which showed persistent absolute leucocytosis.

The Identification of Lymphoid Cells in the Peripheral Blood of the Dog.

In thin well stained blood films from dogs, which were not suffering from lymphosarcoma, there was considerable variation in the morphology of cells of the lymphoid series.

The modal cell types. The small lymphocyte was a compact cell slightly greater in diameter than an erythrocyte. It had a round or indented dense nucleus in which chromatin condensation was marked. A fine rim of pale or deep blue cytoplasm was present and in it, there were often a few azurophilic granules particularly in that part of the cytoplasm which was adjacent to the nuclear indentation. The medium lymphocyte was approximately 1½ times the diameter of the red cell. Its nucleus was less dense than the small lymphocyte and the cytoplasm was more abundant. The nucleus was round, indented, or angular and the chromatin was present in thick clumps. The cytoplasm varied in colour from deep blue to colourless. The large lymphocyte was a cell with a round or ovoid profile. It measured up to 3 times the diameter of an erythrocyte, and its nuclear cytoplasmic ratio was up to 1:1. The cytoplasm was pale blue, sometimes with a slightly greyish appearance and vacuoles were occasionally present. Fine azurophil granules were observed, but these were not a constant feature. The nucleus was round or oval/

oval and the nuclear membrane was distinct. The pachychromatin formed fairly well defined strands on a paler background and this chromatin partially obscured any small nucleoli which were present. Other cells similar in size to the large lymphocytes were also found. Their cytoplasm was indistinguishable from that of the large lymphocytes, but there was marked lobulation of the nuclei. These large lobulated lymphocytes had nuclei which were U-shaped and bilobed. Many of them had a cloverleaf pattern. In some there was evidence of nuclear and cytoplasmic vacuolation, but there were sufficient numbers of those cells with good morphology to accept them as representative cells, and not as artefacts. Their nuclear chromatin was usually finer in texture than the non-lobulated large lymphocytes and nucleoli were rare. Fig.33.

Intermediate cell types. In a good blood film it was possible to identify the modal cells without difficulty, but there was so much variation in cell morphology that every possible intermediate form could be observed. Many small and medium lymphocytes were found to have marked nuclear indentation which gave the appearance of lobulation and the cytoplasm of these cells was often deep blue. The cytoplasmic nuclear ratio of the medium lymphocyte varied widely. Some of these cells were characterised by a nuclear cytoplasmic ratio of approximately 1:1. They had a deep basophilic cytoplasm with a perinuclear halo and were assumed to be circulating plasma cells. Finally, there were cells present with a narrow rim of cytoplasm, but with a nucleus indistinguishable from that of the large lymphocyte.

The term "atypical lymphocyte" should not be used to describe every lymphoid cell which deviates from the modal types.

Effete lymphoid cells. In films where the cytology was otherwise excellent, cells were observed which appeared to be senile or disrupted lymphocytes. Three forms were encountered. The first type was approximately 1½ - 2 times the red cell diameter. Its nucleus was compressed centrally leaving a pale area surrounded by nuclear remnants which were marginated on the cell membrane. The second type was a cell devoid of cytoplasm or a nuclear membrane. This gave the edge of the naked nucleus a ragged outline. It was oval or round, pale pink in colour, with no distinct chromatin pattern. In cells of this type a prominent nucleolus was observed in some instances. The third type was 3 - 4 times the red cell diameter. It was found most often in the posterior section of the body of the film and was composed of a loosely arranged skein of pink or basophilic fibrils.

The lymphoblast. This cell was only very occasionally observed in non-leukaemic films and so to establish criteria for its identification, imprint smears from lymph nodes were prepared and examined. Nodes from healthy dogs and those affected with lymphosarcoma were used.

Lymphoblasts were large cells at least twice the diameter of an erythrocyte. They had round, indented, or angular nuclei which occupied most of the cell leaving a narrow rim of blue cytoplasm which was devoid of granulation. A perinuclear halo was present in a minority of these cells. The nuclear membrane was well defined and there was early condensation of the chromatin to form a fine stippled network. This was much paler in colour than in lymphocytes of the same size. One or more nucleoli were constantly found. These were large, prominent and outlined, but never obscured by chromatin.

Cytological variations due to film depth. The morphology of cells in a film was governed to some extent, by differences in film thickness. In a thick, crowded area of a film, the nuclei of mature neutrophils were dense, compressed and dark blue to black in colour. Similar cells in a thin part were much larger in diameter; their nuclei were much paler and displayed chromatin clumps. Differences were also observed amongst lymphocytes in thick and thin areas of such films.

These observations underline the need to standardise the methods of preparing blood films for comparative studies.

Lymphoid cell distribution in a blood film. A differential lymphocyte count was not attempted until the film was first scanned to ensure that cell distribution was not grossly abnormal. There was often a tendency for the large lobulated and the effete cells to congregate near the edges and tails of the film. Failure to check this could result in quite inaccurate counts.

The incidence of leukaemia. The term leukaemia was defined as an absolute lymphocytosis greater than 30,000/cu.mm., irrespective of the types of circulating lymphoid cells, i.e. both the so-called acute and chronic forms of frank leukaemia, or as the presence of circulating lymphoblasts with an absolute lymphocyte count of less than 30,000/cu.mm., i.e. subleukaemia.

Leukaemia was observed in 13 dogs, but clear haematological evidence was found only in 16 of the total of 56 blood samples from these animals, Table 29. The absolute lymphoblast count ranged from 300/cu.mm. to just over 5,000/cu.mm.

TABLE 29

The "Type" of Leukaemia Observed in Dogs with Lymphosarcoma.

	Multicentric	Alimentary
Subleukaemia:		
Number of dogs	4	2
Leukaemic blood samples	5	2
Total blood samples	10	8
Leukaemia, "Acute" :		
Number of dogs	1	-
Leukaemic blood samples	1	-
Total blood samples	1	-
Leukaemic, "Chronic" :		
Number of dogs	6	-
Leukaemic blood samples	8	-
Total blood samples	37	-

Thus, approximately 88% of the dogs which were subjected to haematological examinations were never leukaemic according to our criteria. Leukaemia was neither constant nor progressive and in 6 dogs from which three or more blood samples were examined, only 2 of them were leukaemic during the terminal stages of the disease.

The distribution of lymphoid cells in cases of lymphocytosis. An aleukaemic absolute lymphocytosis was frequently encountered in dogs with lymphosarcoma and a comparison was made of the lymphoid cell types in dogs suffering from the multicentric form of the disease, the alimentary form and also a few dogs which presented lymphocytosis, but were affected with diseases other than lymphosarcoma, including pyometra, chronic interstitial nephritis and carcinoma of the pancreas. The lymphoid cells in the blood films were classified as 1) small and medium lymphocytes, 2) large non-lobulated lymphocytes, 3) large lobulated lymphocytes and 4) unclassified lymphoid cells.

A total of 43 films was examined, including 21 films from multicentric cases, 16 films from alimentary cases and 6 from dogs with other diseases. The results are shown in Table 30.

There were no statistical differences in the distribution of circulating lymphoid cells amongst the three groups, and it was concluded that no characteristic changes occur in the peripheral lymphoid blood cells of dogs with lymphosarcoma which have aleukaemic absolute lymphocytosis.

TABLE 30

The Distribution of Circulating Lymphoid Cells in Cases of Aleukaemic Absolute Lymphocytosis.

		Multicentric Lymphosarcoma	Alimentary Lymphosarcoma	Other Diseases
Numbers of Films		21	16	6
$\times 10^3$/ cubic millimetre	Absolute Lymphocytosis	10.86 ± 6.7	9.67 ± 6.19	8.7 ± 3.21
	Small and medium Lymphocytes	6.7 ± 5.92	6.0 ± 5.44	3.9 ± 1.41
	Large Non-lobulated Lymphocytes	1.73 ± 7.74	1.41 ± 1.10	1.5 ± 1.77
	Large Lobulated Lymphocytes	1.87 ± 1.79	1.79 ± 0.99	2.5 ± 1.11
	Unclassified Lymphoid Cells	0.55 ± 1.62	0.46 ± 0.495	0.8 ± 0.53

BIOCHEMICAL EXAMINATIONS OF THE BLOOD.

Blood Urea Estimations.

The results given in Table 31 show that 54 out of a total of 182 samples gave readings of 60 mg. of urea per 100 ml. of blood or more. The elevated results were from 28 dogs. Table 33 presents the anatomical types involved, together with the gross appearance of the kidneys at the post-mortem examination. Sixteen of these dogs were found to have kidneys which were not macroscopically abnormal and three animals in this group had blood urea levels greater than 200 mg./100 ml.

These results may indicate that extra-renal uraemia was present in certain cases of lymphosarcoma of the dog.

Total Plasma Protein Estimations.

The results of 129 examinations of blood from dogs with lymphosarcoma are given in Table 32. Approximately 40% of the estimations were lower than normal and 5 samples from dogs with the alimentary form of the disease gave readings of less than 2 gm./100 ml.

In almost every case where multiple samples were taken during the course of the disease, the decline in the plasma protein levels was progressive.

The A/G Ratios.

The distribution of the A/G ratios in dogs with lymphosarcoma is presented in Table 34. The normal range was taken as 0.6 to 1.2 and this was observed in 49 of the 104 estimations. Eight were higher than 1.2 and the other 47 results were lower than normal.

TABLE 31

Blood Urea Estimations.

Blood urea in Mgs/100 ml. blood

	<10	10-	30-	60-	90-	120-	150-	180-	210+	Total	No. of dogs
Multicentric		43	25	13	6	7	1	2	2	99	43
Alimentary	1	26	28	10	9				2	76	43
Aberrant		4	1	1	1					7	3
Total	1	73	54	24	16	7	1	2	4	182	89

TABLE 32

Total Plasma Protein Estimations.

Protein in G/100ml. of plasma

	2-	3-	4-	5-	6-	7-	8-	9+	Total	No. of dogs
Multicentric	0	6	14	29	11	7			67	39
Alimentary	5	9	13	19	7	3	1		57	36
Aberrant type		2	3						5	3
Total	5	17	30	48	18	10	1		129	78

TABLE 33

The Macroscopical Appearance of Kidneys from 28 Dogs with Lymphosarcoma whose Blood Urea Levels Rose Above 60mg/100 ml.

	Lesions of lymphosarcoma	Lesions of nephritis	No gross abnormality observed	Total
Multicentric	8	1	7	16
Alimentary	1	2	8	11
Aberrant type			1	1
Total	9	3	16	28

TABLE 34

The Albumen/Globulin Ratios.

A/G ratio.

	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	Total	No. of dogs.
Multicentric	1	5	3	3	9	9	12	2	2	3	3	2	2	2		3	61	37
Alimentary		2	5	8	9	4	5	2	4	1			1				41	31
Aberrant type		1			1												2	2
Total	1	8	8	11	19	13	17	4	6	4	3	2	3	2		3	104	70

Plasma Albumen and Plasma Globulin Levels.

Tables 35 and 36 show the total albumen and total globulin levels respectively of the plasma or serum which were derived from the A/G ratio and total plasma protein estimation of 104 blood samples from 70 dogs with lymphosarcoma. The fibrinogen, which is associated with the globulin fraction in the plasma, is small enough to be ignored.

Nearly 50% of the albumen levels were lower than the range accepted as normal at this hospital, 2 - 3.9 G/100ml. 93% of the globulin estimations were within the normal range of 2 - 4.9 G/100ml.

From this, it was concluded that the decrease in the total protein of the blood was due to a fall in albumen concentration whilst the globulin fraction remained relatively stable.

Alkaline Phosphatase Estimations.

The results of the alkaline phosphate estimations are presented in Table 37. The upper limits of normal for the dog were taken as 15 to 20 King Armstrong units.

Almost 34% of all the blood samples gave an abnormally high result, including nearly 41% of those from multicentric cases and 21% of the samples from dogs with the alimentary form of the disease.

The Serum Transaminases.

Tables 38 and 39 show the S.G.P.T. and S.G.O.T. readings respectively from 24 dogs with lymphosarcoma. The normal values were taken as < 40 S.F. units for S.G.P.T. and < 40 S.F. units for S.G.O.T.

TABLE 35

Serum Albumen Levels.

	gms./100 ml.								Samples	Dogs
	<1	1-	2-	3-	4-	5-	6-	7+		
Multicentric	5	16	31	7	2				61	37
Alimentary	5	20	13	3					41	31
Aberrant type	1	1							2	2
Total	11	37	44	10	2				104	70

TABLE 36

Serum Globulin Levels.

	gms./100 ml.								Samples	Dogs
	<1	1-	2-	3-	4-	5-	6-	7+		
Multicentric		1	18	32	9	1			61	37
Alimentary		4	11	20	5		1		41	31
Aberrant type			2						2	2
Total		5	31	52	14	1	1		104	70

TABLE 37

Alkaline Phosphatase Levels.

	K.A. Units							
	< 20	20-	40-	60-	80-	100+	Total	No. of dogs
Multicentric	36	9	4	3	5	4	61	37
Alimentary	42	4	2	2	1	2	53	32
Aberrant type		1		1		2	4	3
Total	78	14	6	6	6	8	118	72

TABLE 38

S.G.P.T. Levels in Dogs Suffering from Lymphosarcoma.

S.F.

	< 40	40-	50-	60-	70-	80-	90-	100+	Total	No.of dogs
Multicentric	17	2	1		1				21	14
Alimentary	13	1	1					1	16	10
Aberrant type									0	
Total	30	3	2		1			1	37	24

TABLE 39

S.G.O.T. Levels in Dogs Suffering from Lymphosarcoma.

S.F.

	< 40	40-	50-	60-	70-	80-	90-	100-	Total	No.of dogs
Multicentric	13	3	2		1	1	1		21	14
Alimentary	13			1	1			1	16	10
Aberrant type									0	
Total	26	3	2	1	2	1	1	1	37	24

Blood Bilirubin.

The results of 105 estimations of the total bilirubin in the blood of 65 dogs with lymphosarcoma are presented in Table 40. Readings which were 0.7 or less were found in 80% of the samples. The rest were derived from 13 dogs, three of which were visibly affected with jaundice. The bilirubin levels in these 3 dogs were 2.6 mgm./100 ml. or greater in 6 of the 9 samples examined. Of the 10 dogs with raised total bilirubin levels, but free from clinical jaundice, 2 showed extensive infiltration of the liver by tumour cells, 4 showed moderate infiltration, 2 were slightly infiltrated and 2 others were unaffected.

Summary.

Abnormal results in routine biochemical tests of the blood of dogs with lymphosarcoma were probably a reflection of the progressive organ failure which is associated with this disease, but none of the tests had diagnostic significance.

RADIOLOGICAL REPORTS.

Radiological examinations were carried out on 72 dogs which were shown to have been suffering from lymphosarcoma at post-mortem examination. A summary of these reports is given in Table 41.

The X-ray data reflected the later morbid anatomical findings rather well. In the multicentric form of the disease, increases in size of the spleen and liver were readily observed on plates and involvement of the thoracic/

TABLE 40

Bilirubin Levels in the Blood of Dogs with Lymphosarcoma.

mgm./100 ml.

	< 0.2	0.2-	0.8-	1.4-	2.0-	2.6-	3.2-	3.8-	4.2+	Total samples	Total dogs
Multicentric	29	19	5		1	1	1		2	58	34
Alimentary	22	13	2	1	1	1			2	42	28
Aberrant	1		2	1	1					5	3
Total	52	32	9	2	3	2	1		4	105	65

TABLE 41

Summary of the Radiological Reports.

	Multicentric	Alimentary	Aberrant	Total
Lung	4	1		5
Bronchial nodes	8	2		10
Anterior sternals	6			6
Anterior mediastinum	3	4		7
Hydrothorax	1	3		4
Spleen	17	4	2	23
Liver	16	7	1	24
Mid-abdominal mass	4	7	1	12
Sublumbar nodes	7			7
Prostate	1			1
Ascites		1		1
Barium meal				
+ ve		1		1
0		1		1
- ve		2		2
Gastric F.B.	1			1
N.A.D.	8	9		17
Number of dogs examined	37	32	3	72

thoracic and intra-abdominal lymph nodes was often quite obvious. This was also true of the other two forms of the disease, except for one interesting discrepancy in the reports on the alimentary type. Out of 32 dogs in this group, only 7 cases were reported as having masses in the mid-abdomen or anterior abdomen, although it later transpired that tumour masses, either of the gastro-intestinal tract or drainage lymph nodes, were a constant feature. Such masses were readily palpable in 27 of the 53 dogs in the alimentary group which were examined clinically.

There were not enough radiological examinations performed following a barium meal to determine if lesions of the alimentary system could be regularly seen.

THE CLINICAL FINDINGS.

Of the 130 dogs suffering from lymphosarcoma which were autopsied, 115 had been examined before death and clinical data were available.

THE PREVALENCE OF LYMPHOSARCOMA IN DOGS ADMITTED TO THE HOSPITAL.

Approximately 10,000 dogs were admitted to the hospital during the interval when cases of lymphosarcoma were collected for this study. Out of this total, rather more than 150 dogs were reported to be suffering from the disease, although not every case was confirmed histologically. This represented an incidence of 1.5% of admissions. However, the condition occurred in adult dogs usually over 2 years of age and around 70% of dogs admitted to this hospital were in this age group, Table 44. Thus, if the incidence of lymphosarcoma was expressed as a percentage of the adult dogs admitted, a conservative estimate would be 2%.

BREED INCIDENCE.

Table 42 presents the distribution of 115 cases of the disease in the dog in 22 breeds. The term mongrel was employed to describe terrier type dogs with no recogniseable pedigree. Labrador crosses are popular in this area and were included as a separate group since 2 cases of lymphosarcoma were found in such dogs.

Neither Boxers nor Scottish Terriers were over-represented in this series, although the Scottish Terrier was approaching the 5% level of significance $\chi^2 = 3.58$ with one degree of freedom. The Wire Haired Fox Terrier was the sole breed which was over-represented at the 5% probability level, $\chi^2 = 4.41$ with one degree of freedom.

Of those breeds which were prominent in the list of admissions, the poodle was under-represented at the 1% probability level, $\chi^2 = 7.36$ with one degree of freedom.

THE SEX INCIDENCE.

The numbers of males and females in the group with lymphosarcoma are given in Table 43 together with the sex incidence of a sample of dogs admitted to the hospital. It was assumed, for the purposes of statistical analysis, that those dogs which had been admitted without their sex being recorded were in a similar sex ratio to those where the sex was noted.

In comparing the sex ratios of the lymphosarcoma group with the admission group, no significant differences could be established between the two samples, $\chi^2 = 0.6502$ with one degree of freedom.

TABLE 42

Breed Incidence.

	Canine Lymphosarcoma Series				Admissions to Hospital		
	MC	Alim.	Aberynant	Total	Jan-June 1957	July-Dec 1961	Total
Alsatian	5	11	-	16	37	55	92
Boxer	7	1	-	8	30	36	66
Bull terrier	2	-	1	3	7	17	24
Cairn terrier	1	-	-	1	15	25	40
Border collie	9	13	1	23	58	94	152
Corgi	1	-	-	1	6	13	19
Fox Terr. S.	-	1	1	2	4	6	10
Fox Terr. W.H.	1	5	-	6	9	11	20
Golden Retr.	-	-	1	1	2	7	9
Greyhound	2	-	-	2	15	23	38
Irish Setter	-	1	-	1	0	3	3
Labrador	8	3	-	11	26	44	70
Labrador x	-	1	1	2	6	20	26
Min.Pin.	1	-	-	1	0	0	0
Poodle	-	1	1	2	26	63	89
St. Bernard	-	1	-	1	0	0	0
Scottish Terr.	2	4	-	6	7	15	22
Sealyham	-	-	1	1	2	1	3
Shetland Col.	1	-	-	1	4	9	13

	Canine Lymphosarcoma Series				Admissions to Hospital		
	MC	Alim.	Aggrant	Total	Jan-June 1957	July-Dec 1961	Total
Spaniel	2	4	-	6	33	43	76
West Highland	4	-	1	5	7	16	23
Other breeds	-	-	-	-	38	123	161
Mongrel	8	7	-	15	53	125	178
Total	54	53	8	115	385	749	1134

TABLE 43

Sex Incidence.

	Lymphosarcoma Survey	Admissions Jan-Jun 1957	Admissions Jul-Dec 1961	Total. 12 month Admission sample
Males	72	197	356	553
Females	40	122	248	370
Neutered males	0	1	2	3
Neutered females	1	3	1	4
Sex not recorded	2	62	142	204
Total	115	385	749	1,134

From this, it could be concluded that the incidence of the disease was similar in both sexes.

THE AGE INCIDENCE.

The age distribution of dogs with lymphosarcoma was compared with that of a sample of dogs which had been admitted to the hospital and the results are given in Table 44.

There was clearly a highly significant difference between both samples in dogs under one year of age. In three other age groups, significant differences were found between the observed and expected incidences in the lymphosarcoma group. In dogs from 1 - 4 years of age $\chi^2 = 29.8^{**}$ with one degree of freedom; in those from 5 - 11 years, $\chi^2 = 20.8^{**}$ with one degree of freedom; in dogs 12 years of age and upwards $\chi^2 = 3.4$ n.s. with one degree of freedom.

From these results, it would appear that the incidence of lymphosarcoma in the dog at this hospital, when compared with a sample of admissions, is very low under one year old, low between 1 - 4 years, rises very steeply in the 5 - 11 year group and then declines in the older group. It may well be that this will prove to be a normal distribution which would be different from the assumed age distribution of most malignant tumours.

THE DURATION OF THE DISEASE.

The periods of survival following the onset of the clinical disease are given in Table 45 and this information is summarised in Table 46.

TABLE 44

Age Incidence.

	Lymphosarcoma survey -				Admissions half year		Total
Age in years	MC	Al.	Ab.	total	1957	1961	
Less than 1					82	170	252
1-	1			1	27	65	92
2-	4	2		6	30	52	82
3-	4	1		5	24	54	78
4-	4	4		8	17	30	47
5-	6	2	2	10	19	31	50
6-	8	8		16	21	36	57
7-	7	6	1	14	16	43	59
8-	3	9	3	15	26	30	56
9-	4	4		8	21	28	49
10-	1	6		7	12	22	34
11-	2	8	1	11	8	12	20
12-	4	2	1	7	10	8	18
13-	1	1		2	2	4	6
14-	0	0			2	2	4
15+	1	0		1		3	3
Not recorded or unknown	4	0		4	68	159	227
Total	54	53	8	115	385	749	1,134

TABLE 45

The Duration of the Overt Disease in Weeks.

Weeks	Multicentric	Alimentary	Abeyrant	Total
1-	3	3		6
2-	3	4	2	9
3-	8	7	1	16
4-	6	4	2	12
5-	2	12	1	15
6-	3	3		6
7-	2	1		3
8-	5	2	1	8
9-	3	2		5
10-	1	2		3
11-				0
12-	4		1	5
13-				
14-	2			2
15-				
16-	1	3		4
17-				
18-		1		1
19-				
20-		2		2

Weeks	Multicentric	Alimentary	Aberrant	Total
21-				
22-				
23-				
24-	2	2		4
25-				
26-				
27-				
28-	1	1		2
30-		1		1
32-	1			1
52-	1			1
62-	1			1
Unknown	5	3		8
Totals	54	53	8	115

TABLE 46

Summary of the Data on Duration of the Disease.

Duration in Weeks.

	Mean	Median	Modal	Range	Number of cases
Multicentric	10	30	3	1-62	49
Alimentary	8	15	5	1-30	50
Aberrant form	5	6	-	2-12	8
All cases	8	30	3	1-62	107

In the multicentric form, the expectancy of life after the onset of symptoms was 10 weeks on average, but more than 70% (35 dogs) were dead within a shorter interval. Just over 28% (14 dogs) were dead within 1 month after onset. Three dogs lived longer than 6 months and one of these survived for just over 1 year and 2 months.

In the alimentary type the average duration was 8 weeks, and 68% (34 dogs) succumbed within this interval after the initial symptoms developed. 28% (14 dogs) died within 4 weeks of onset. In the alimentary group only 2 animals survived for 6 months and none were alive 12 months after the onset of the clinical signs of the disease.

The aberrant form appeared to show a trend very similar to the alimentary type.

Taking the disease as an entity and again ignoring cases where the duration was unknown, 58 dogs (54% of the cases) were dead less than 6 weeks after the earliest clinical signs had been observed by the owners. Out of a total of 107 dogs, 82% had died by the end of the 12th week.

An estimation of the duration of this disease cannot be very accurate since it is based partly on an owner's opinion. However, if one accepts this difficulty, it is still obvious that lymphosarcoma in the dog is a disease of comparatively short duration in the majority of instances and the duration is not markedly different in the two main forms of the disease.

THE SYNDROMES OF LYMPHOSARCOMA IN THE DOG.

The clinical reports on 115 dogs with lymphosarcoma are summarised in Table 47/.

TABLE 47

Summary of the Clinical Features of the Disease.

	Multicentric	Alimentary	Aberrant	Total
Number of cases	54	53	8	115
Onset				
Sudden	18	26	5	49
Gradual	34	25	3	62
Not recorded	2	2	-	4
Bodily Condition				
Obese	3	1		4
Good	10	3	2	15
Fair	6	2	1	9
Emaciated	34	46	5	85
Not recorded	1	1	-	2
Demeanour				
Alert	14	7		21
Dull	38	45	8	91
Not recorded	2	1	-	3
Fever				
Present	6	2		8
Absent	44	51	8	103
Undulating	4	-		4

	Multicentric	Alimentary	Aberrant	Total
Appetite				
Good	16	5	1	22
Poor/absent	33	39	7	79
Variable	3	9	-	12
Not recorded	2	-		2
Thirst				
Normal	33	22	2	57
Excessive +	12	13	3	28
2+	9	11	3	17
3+	5	7	0	12
Not recorded	1	-	-	1
Emesis				
None	36	14	3	53
Occasional	18	28	2	48
Frequent	0	7	3	10
Repeated	0	4	0	4
Not recorded	0	0	0	0
Faeces				
Constipated	5	2	1	8
Normal	23	10	4	37
Diarrhoea :				
Fluid faeces	24	35	2	61
Haemorrhagic faeces	2	6	1	9
Not recorded	0	0	0	0

	Multicentric	Alimentary	Aberrant	Total
Abdominal Palpation				
Mid-abdominal mass	11	27	1	39
Hepatomegaly	16	6	1	23
Splenomegaly	23	1	2	26
N.A.D.	18	20	6	44
Tachypnoea	15	5	0	20
Dyspnoea	1	5	1	7
Cough	7	1	2	10
Hydrothorax	2	2		4
Palpable superficial lymph nodes grossly enlarged 49) moderately enlarged 5)	54	0	Rt.prescap only	55
Ascites	1	2	0	3
Oedema	9	1	0	10
Pale Mucosae	6	6	1	13
Jaundice	5	3	1	9
Tonsils - grossly enlarged	11			11
Gross Thymic mass	1	2		3
Eyes				
Ocular discharge	2	5		7
Infiltration	3			3
Bilateral Glaucoma	1			1
Skin Lesions	2			2

Table 47. Only those symptoms which had emerged before there was any evidence of impending death were tabulated. This was to ensure that the clinical descriptions were a reasonable reflection of the actual disease and did not include the terminal phases, which tend to be common to many diseases.

Clinically, the recurring feature of the multicentric form of the disease was the enlargement of the main sites of lymphoid tissue throughout the body. Superficial lymphadenopathy was constantly present, affected lymph nodes being readily palpable and varying from 3 up to 10 times their usual size. They tended to be bilaterally symmetrical, smooth and painless and were well defined, firm, rubbery in texture, relatively mobile and very rarely adherent to the overlying skin or adjacent tissues. An enlarged spleen was palpable in 23 of the 54 dogs in this group. This was frequently associated with a heavily infiltrated liver, the edges of which could be palpated well behind the costal arch. Tonsillar involvement, as judged by non-ulcerated, pale, grossly enlarged tonsils protruding from their crypts, was a feature in only 20% of the dogs.

Of the non-specific symptoms, the modal syndrome was as follows:- depression, emaciation and alimentary disturbances including poor appetite and diarrhoea which became persistent and occasionally haemorrhagic. Thirst was abnormal in a few cases, but this may have been compensatory to the emesis which was never severe. Tachypnoea was observed in 15 dogs.

A highly pathognomonic symptom, which was seen in 9 dogs, was subcutaneous oedema. This was found on one or more of the following sites:- the hind limbs, the external genitalia, under the lower jaw and the ventral aspect of the sternal region.

Pale mucous membranes were observed on a few occasions, jaundice was noted in less than 10% of the dogs and a gross "thymic mass" was found in one case only. Infiltration of the eyes, skin lesions of lymphosarcoma, hydrothorax and ascites were also rare developments.

The full syndrome of the alimentary type was much less specific than that of the generalised form. Typical findings were marked depression, emaciation and absence of fever. These were associated with alimentary disturbances, especially with persistent diarrhoea which was present in 41 of the 53 dogs. In 6 of the diarrhoeic group, the faeces were blood-stained. Thirst was also related to the degree of vomiting which was much more marked in this form of the disease. A finding of considerable importance was a firm, well defined and painless mass in the mid-abdomen. This palpable mass was usually non-radio-opaque.

Jaundice, hydrothorax, ascites, "thymic masses" and subcutaneous oedema were each observed in less than 5% of the dogs. Neither tonsillar nor skin lesions were found.

The distribution of lesions in the alimentary-mesenteric area of cases of the alimentary form of lymphosarcoma is shown in Table 48. Symptoms which were associated with those lesions are presented in Table 49. A comparison was made between the degrees of vomiting and the type of diarrhoea observed where the mesenteric node alone was affected and also where tumours were found along the canal. These results are given in Table 50. No distinct relationships between symptoms and the sites of the lesions were found in this/

TABLE 48

The Distribution of Lesions in the Alimentary-mesenteric Area of 53 Clinical Cases of Alimentary Lymphosarcoma in the Dog.

Total cases	Mesenteric lymph node	Stomach	Duodenum	Jejunum	Ileum	Colon & Rectum	Ascites
53	45	8	34	23	25	9	2
100%	85%	15%	64%	43%	47%	17%	3.7%

TABLE 49

Alimentary Symptoms in Relation to the Alimentary Lesions.

Lesion Complex	Poor Appetite	Thirst	Emesis	Diarrhoea	Abdominal Mass Palpable	No. of dogs
Stomach only	1	1	1		1	1
Small Intestine	6	4	5	4	3	6
Colon, rectum & lymph nodes	1	1	1	1		1
Mesenteric L.N. only	10	4	7	9	6	11
Alimentary system and mesenteric L.N.	30	21	25	27	17	34
Total	48	31	39	41	27	53
Percentage	90.5	58.5	73.5	77	51	100

TABLE 50

Emesis and Diarrhoea Associated with Intestinal-mesenteric Lesions in Cases of the Alimentary Form of Lymphosarcoma.

	Sites of the Lesions.	
	Mesentoric lymph node alone	Intestinal tumours
Number of Dogs	11	40
Emesis:		
Occasional	7	19
Frequent	-	7
Persistent	-	4
Faeces:		
Diarrhoea	8	26
Haemorrhagic diarrhoea	1	5

this investigation. However, in a small number of cases where vomiting was severe prior to the initial clinical examination, the syndrome was not unlike that which is associated with a foreign body in the small intestine. Stenosing or perforating tumours of the small intestine were usually found in such dogs.

The syndrome of the aberrant form of the disease was similar in many respects to that of the alimentary form, although 2 of the 8 dogs were found to have palpable enlargement of the spleen which is an unusual finding in the alimentary type. In one case, No. 10513, the prescapular lymph node on one side only was enlarged and tumified, whilst all the other superficial lymph nodes were macroscopically unaffected. In the rest of the group, neither superficial lymphadenopathy nor tumours of the gastro-intestinal tract or mesenteric node were found before or after death.

In none of the three forms of the disease was there any sign which could be clearly attributed to tumour infiltration of the central nervous system.

CLINICAL PHASES DURING THE DISEASE.

In the multicentric type, three stages were noted in the clinical progress of the disease, a primary Phase I, a secondary Phase II and a tertiary or terminal Phase III.

In Phase I a dog presented no symptoms other than enlargement of the superficial lymph nodes with, in certain cases, hepatosplenomegaly and tonsillar enlargement. The dog remained alert, fully active and retained its appetite during/

during this period. Indeed, the general condition of the animal belied the very serious nature of the disease. It was understandable that an owner would not readily accept an opinion that the life of the dog at that moment was already measureable in weeks, or the data which showed that nearly 5 out of 6 animals die or have to be destroyed within 3 months of the initial clinical examination and that almost 40% of dogs die 4 weeks or less after the disease is first recognised.

Phase II emerged progressively as a syndrome which was characterised by steady loss in bodily condition associated with mild alimentary disturbances.

The terminal Phase III was usually sudden in onset and was accompanied by marked depression, emaciation, inappetence, which was followed by dehydration, signs of toxaemia, circulatory failure and death.

Irrespective of the stage at which the disease was first diagnosed there was seldom any appreciable increase in size of affected nodes or spleen throughout the disease. In three cases, however, there was undoubtedly extension of the tumour process during the period over which clinical observations were made. In case number 12998 an enlarged superficial inguinal lymph node was found at the time of a surgical intervention for pyometra. This node was removed and on histological examination proved to be lymphosarcomatous. Regular negative clinical examinations were carried out subsequently on this patient for some months, after which, superficial lymphadenopathy developed. This became a typical case of the multicentric type. In case number 23913, splenomegaly was the initial diagnosis and there was, at/

at that time, no enlargement of the superficial lymph nodes. These became grossly involved approximately 3 - 4 weeks after splenectomy. In case number 25394, gross enlargement of the prescapular lymph nodes and moderate enlargement of the other superficial nodes developed suddenly about 4 months following splenectomy. Frequent clinical examinations were carried out in the intervening period and these were negative.

From the clinical reports, it appeared that the duration of the multicentric type of the disease was related to the length of time that Phase I persisted. Once Phase II supervened, the terminal phase was imminent and this final stage was usually fatal within 7 days of onset. These distinct phases were not characteristic of every case, for the first signs of disease observed by the owner might co-incide with the onset of Phase II or Phase III.

Unlike the multicentric form, no definite clinical phases were found in the alimentary type. In many instances the full syndrome ushered in the first clinical manifestation of the disease. However, other cases were much more gradual in development and they presented a pattern of persistent alimentary disturbances with progressive loss in bodily condition, the cause of which was obscure at first. Eventually, the loss of condition, the persistent diarrhoea and the presence of a palpable mid-abdominal mass lead to a tentative diagnosis of malignant disease.

DIAGNOSTIC MARKERS.

In the multicentric type, the tentative diagnostic markers were bilaterally symmetrical, painless, superficial lymphadenopathy plus hepatosplenomegaly with, occasionally,/

occasionally, gross non-ulcerative pale tonsils and subcutaneous oedema in an adult dog. This diagnosis could be confirmed by histological examination of an enlarged superficial node.

In reaching a tentative diagnosis of alimentary lymphosarcoma two factors were important. Firstly, the association of progressive loss in bodily condition, persistent diarrhoea and a firm mass palpable in the mid-abdomen with lymphosarcoma of this particular type and secondly, the relatively very high incidence of lymphosarcoma as opposed to other malignant conditions affecting the gastro-intestinal tract of the dog. Exploratory laparotomy was the sole means of identifying the disease and a biopsy of a mural tumour or affected mesenteric node permitted definite histological confirmation.

Diagnosis of the aberrant form was comparatively simple where gross splenomegaly was present and typical lymphosarcoma changes of the spleen could be observed at laparotomy. However, a final positive diagnosis had to await post-mortem examination where this feature was absent.

CLINICAL AND PATHOLOGICAL RELATIONSHIPS.

CLINICAL AND PATHOLOGICAL RELATIONSHIPS.

The duration of the overt disease in 115 dogs was compared with the proposed anatomical forms of lymphosarcoma, with the dominant tumour cell types and with sex. The results are given in Table 51. Similarly, the ages of the dogs were compared with the anatomical forms, the dominant tumour cell types and the sex, Table 52. Finally, the ages of the dogs were compared with the duration of the clinical disease and these results are shown in Table 53.

TABLE 51

The Duration of Overt Lymphosarcoma Compared to the Forms of the Disease, Tumour Cell Types and Sex.

	Duration in Months.														
	<1	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-	11-	12+	Unknown duration	No. of dogs
Multicentric	14	13	9	6	1	-	2	1	1	-	-	-	2	5	54
Alimentary	14	20	6	-	4	2	2	2						3	53
Aberrant	3	3	1	1											8
Immature cell types	20	20	11	4	-	2	3	2	1	-	-	-	2	3	68
Mixed or mature cell types	8	11	4	2	3	-	-	1	-					3	32
Variable	2	5	1	1	1	-	1							2	13
Unidentifiable	1	-	-	-	1										2
Males	19	26	11	3	3	2	1	3	1				1	2	72
Females	12	10	5	3	2	-	3						1	5	41
Sex not recorded				1										1	2
Nos. of dogs	31	36	16	7	5	2	4	3	1	-			2	8	115

TABLE 52

The Ages of 115 Dogs with Lymphosarcoma Compared to the Forms of the Disease, Tumour Cell Types and Sex.

	Ages in Years.																
	<1	1-	2-	3	4	5	6	7	8	9	10	11	12	13	14	15+	Age Unknown	No. of dogs
Multicentric	1	4	4	4	6	8	7	3	4	1	2	4	1		1	4		54
Alimentary		2	1	4	2	8	6	9	4	6	8	2	1					53
Aberrant					2		1	3			1	1						8
Immature cell types	1	2	5	4	7	8	9	8	6	2	6	4	2		1	3		68
Mixed Mature cells		2	-	2	2	7	2	4	2	3	4	3				1		32
Variable		2	-	2	1	1	3	2	-	1	1							13
Unidentified								1	-	1								2
Males	1	3	3	7	7	10	11	8	2	5	8	4	1			2		72
Females		3	2	1	2	6	3	7	6	2	3	3	1		1	1		41
Sex not recorded					1											1		2
Nos.of dogs	1	6	5	8	10	16	14	15	8	7	11	7	2		1	4		115

TABLE 53

The Relationship between Age of the Dog and the Duration of Overt Lymphosarcoma in 115 Dogs.

Duration in Months	Ages in Years. <1	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	15+	Age Unknown	No. of dogs
< 1	1	2	1	2	1	5	4	6	3		3	2	1					31
1-		1	2	2	3	5	6	5	3	2	4	2					1	36
2-		1	1	1	2	3	2	1			3	1	1					16
3-			1		2	1	1					1					1	7
4-				2						3								5
5-									1	1								2
6-					1				1	1						1		4
7-				1		1			1									3
8-					1													1
9-																		
10-																		
11-																		
12+										1		1						2
Duration unknown		2					1	1			1		1				2	8
No. of dogs	1	6	5	8	10	16	14	15	8	7	11	7	2		1	4		115

No correlations could be established which might have proved of value in assessing the outcome of individual cases of lymphosarcoma in the whole group. In particular, there was no correlation between the dominant tumour cell type and the duration of the clinical disease.

Thirteen dogs were leukaemic at some stage in the course of the disease, Table 29. Of the 6 animals which presented a peripheral blood picture similar to that of chronic leukaemia in man, 3 died within one month after onset of the disease, 2 survived for between 1 and 2 months, and one lived for over 12 months. In the case of the 6 subleukaemic dogs, 2 died within one month, 2 survived one month, but died soon afterwards and the other 2 lived for nearly 6 months. The only animal which showed a peripheral blood picture comparable to the acute leukaemia of man succumbed within 4 weeks of the start of the illness.

The relatively low incidence of truelleukaemia in dogs suffering from lymphosarcoma, approximately 13% of the entire group and the variation in the duration of the disease irrespective of the peripheral blood picture indicated that terms such as acute, sub-acute and chronic had no useful clinical meaning in lymphosarcoma in this species.

DISCUSSION.

The aims of this survey were to describe and classify the lymphoid tumours which have been commonly observed at this hospital. These appeared to form a relatively distinct single disease entity which was in contrast to the complex as observed in man. Other haemopoietic tumours, although found in the dog, were comparatively rare.

In 130 confirmed cases in dogs, it was possible to distinguish three forms of the disease which were based on the distribution of the gross lesions in the body. They comprised a multicentric type, an alimentary type and a so-called aberrant type.

The first two were equally represented in the present series and accounted for approximately 94% of the cases.

There were strong clinical reasons for adopting such a classification, for each of the two major forms of the disease was associated with a relatively distinct clinical syndrome. These syndromes were related to two quite different groups of conditions which were of importance in differential diagnosis. On the one hand, there were those diseases which were accompanied by enlargement of superficial lymph nodes. On the other hand, there were conditions of the abdomen which could result in a syndrome similar to that of alimentary lymphosarcoma. The confirmatory diagnostic methods were also based on this classification. For example, in the multicentric type, where bilateral and symmetrical enlargement of the superficial lymph nodes was frequently encountered, diagnosis could be confirmed from a biopsy of an enlarged superficial lymph node. In the alimentary form of the disease, the clinical signs often indicated the presence of a possible abdominal tumour. Exploratory laparotomy was necessary to establish a diagnosis in this type. An observation in this study of interest to surgeons, was that in 87% of dogs with alimentary lymphosarcoma, there was gross tumour involvement of the mesenteric lymph node. Single or multiple tumours of the stomach or intestines were found in the remainder./

remainder. Thus, one of two simple diagnostic techniques could have lead to a diagnosis of all cases of multicentric and alimentary forms of this disease in the present series.

In the rare form of lymphosarcoma, the so-called aberrant type, there were lesions in the liver in 7 of the 8 dogs. Three of the cases also presented typical lymphosarcomatous changes in the spleen, and the kidneys were heavily infiltrated in 4 of the animals. Although this particular form of the disease is much more difficult to diagnose from the symptoms shown, an exploratory laparotomy would have proved valuable in diagnosis.

The results of the study are in contrast to earlier clinico-pathological reports by Bloom and Meyer (1945); Innes et al (1946); Jennings (1953); Irfan (1961) and Backgren (1965), where the generalised form of the disease alone was described. Both Irfan (1961b) and Meier (1957) recognised that there were other lymphoid tumours in this species which could not be included in the generalised form, but they refused to accept that these other forms were definitely part of a single complex. It may well have been that the criteria which were used in earlier studies to select cases included superficial lymphadenopathy. This would have lead to the exclusion of the alimentary and aberrant forms which have been recognised in the present survey. Support for this opinion is gained from the essentially pathological surveys of tumours by Cotchin (1954) and Smith (1963). Both observed a more varied lesion distribution than has been reported in the clinical papers on lymphatic tumours in the dog.

From the strictly clinical viewpoint, the multicentric type as described in this thesis was identical to the disease found by Bloom and Meyer (1945); Innes et al (1946); Jennings (1953); Irfan (1961b); Meier (1957) and Bäckgren (1965). In this study, gross enlargement of most of the superficial lymph nodes was very commonly encountered and we would agree with Hutyra et al (1949) that there is no other disease of the dog which results in bilaterally symmetrical enlargement of the superficial lymph nodes. In some dogs, the gross enlargement of nodes was confined to one or two groups which were again bilaterally symmetrical. In a small number with the multicentric form of the disease, the nodes were all just moderately enlarged. In such cases, it was observed that nodes which are not ordinarily readily palpable, such as the axillary and superficial inguinals, were enlarged, palpable and bilaterally symmetrical. If this was found in association with an enlarged spleen, a tentative diagnosis of lymphosarcoma was justified.

The three phases of the disease, as recently reported by Bäckgren (1965) were also observed. A preclinical phase must occur and it is this phase of the disease which presently defies diagnosis.

Lymphosarcoma of the gastro-intestinal-lymphatic complex is one of the most important causes of a non-radio-opaque mass in the mid-abdomen of an adult or aged dog. It would also appear to be the most common malignant tumour of the intestines found in dogs which are admitted to this hospital; an observation which is in agreement with Cotchin's survey (1954). In 50% of the dogs with the alimentary form of the disease, a mass was readily palpable in the mid-abdomen during routine clinical examinations and this is believed to be a significant diagnostic marker.

Histological features were common to all three types of the disease in the present study and rare exceptions were assumed to be biological variations. The formation of discrete tumours as well as widespread infiltration of tissues by tumour cells was observed simultaneously. Therefore, the histological distinction between lymphatic leukaemia and lymphosarcoma, as reported in man by Robbins (1962) and in the dog by Squire (1965), was not observed. The major lesions of lymphosarcoma of the dog, particularly on the former sites of lymphoid tissue, were composed of sheets of lymphoid cells which were highly invasive and infiltrative in character. Tumour cell types varied from very immature stem cells to mature lymphocytes and they were not related to any particular anatomical form of the disease. More than one tumour cell type was often observed in lesions and it was not always possible to determine a single predominant tumour cell type as claimed by Squire (1965). Tumour cell types also varied from one region of the body to another. However, if two main cell types were recognised, an "immature cell group" and a "mixed or mature cell group," then the tumour cell population of affected lymph nodes did reflect the general tumour cell population in the great majority of cases. A close relationship existed between the rate of mitosis and the degree of differentiation of the tumour cells; the more immature cell sheets demonstrated a higher rate of mitosis when compared to more mature cell sheets.

Too much emphasis on tumour cell morphology may well be misplaced. Squire (1965) had little support for his speculation that the precise identification/

identification of a tumour cell type might be of value because each cytological form might be found to have a specific causal agent. In experimental oncology in mice, it is now well established that some viruses may cause tumours of quite different histological character. The outstanding example is the polyoma virus, Gross (1961).

It is highly doubtful if imprint smears, as advocated by Bloom and Meyer (1945) and later by Squire (1965), have any major advantages over well made histological sections. Even if the identification of the dominant tumour cell can be more readily made by this technique, imprints cannot be used as a substitute for histological sections.

In the current survey, it was seldom found possible to differentiate individual lymphoid tumour cells from normal lymphoid cells and the identification of tumour cells was based on the histological features of the tumour tissue, as well as other cellular characteristics such as invasiveness, destructive tendencies and the rate of mitosis.

Positive diagnosis of the disease was made from histological findings. Up to the present time, detailed studies of affected tissues have not been published. Therefore, criteria were established for the histological diagnosis of lymphosarcoma in lymph nodes and alimentary system. The histological sections were derived from cases post-mortem in most instances, but there were no reasons to suspect that the conclusions could not be applied to biopsy sections.

Despite the facts that the histological characteristics of this disease are outstanding and a large number of detailed post-mortem examinations were carried out, very little information was gained from the study on the pathogenesis of the disease. Whether this condition arises on a single site and then spreads by metastases or else develops simultaneously in many centres remains speculative. There is no point in adding to speculation at the present time, since it is unlikely that the pathogenesis will be fully understood until the condition can be reproduced experimentally, or techniques are devised to enable its recognition at an early sub-clinical phase.

Blood examinations had little diagnostic value in lymphosarcoma of the dog. True leukaemia was seldom an outstanding feature and the leucocyte picture of the peripheral blood varied enormously. Neutrophilia, an absolute lymphocytosis, an absolute leucocytosis and a perfectly normal blood leucocyte picture were observed. This is the pattern which has been well documented over the years. Although lymphocytosis was common, the distribution of the lymphoid cell types in blood films was not sufficiently different from other disease states to be of value in haematological investigations. The anaemia which was regularly observed was normocytic and normochromic and high numbers of circulating normoblasts were observed, particularly in the multicentric forms of the disease when anaemia was present. Again, this phenomenon has been observed by earlier workers.

None of the biochemical tests on the blood were pathognomonic, but low plasma protein concentrations, due to a decrease in the albumen fraction, were found in a high number of samples.

Radiological methods were useful to confirm the presence of an enlarged spleen or liver. Tumour masses in the anterior mediastinum, enlarged anterior sternal lymph nodes and tumours in the pre-pelvic region of the abdomen were also readily observed on x-ray plates. However, grossly tumified mesenteric lymph nodes were often impossible to find radiologically.

The duration of lymphosarcoma of the dog was relatively short in this series, averaging 8 weeks, with a range of 1 - 62 weeks.

Wire Haired Fox Terriers were over-represented and Poodles were under-represented in this study when compared with a sample of dogs which were admitted to the hospital. Boxers were not over-represented and Scottish Terriers were almost, but not quite, over-represented statistically. These two breeds have recently been reported as over-represented in a study by Bäckgren of dogs with lymphosarcoma in Sweden. The Scottish Terrier has for long been assumed to show a high incidence of the disease in Great Britain, but no statistical evidence has been given for this assumption. Meier (1957) observed a high incidence of lymphosarcoma in some breeds, but regarded this as an indication of the normal dog population in his area.

No sex incidence was established in dogs with lymphosarcoma which were admitted to this hospital.

The age incidence may well prove to have a normal distribution with a peak somewhere between the ages of 5 and 11 years. This has been observed also by Smith (1965) and is not in accord with the age distribution in other malignant diseases where the incidence increases with increasing age.

Comparisons were made in this study amongst the clinical, pathological and haematological data to try to establish correlations which might prove of value in prognosis in the individual animal, as has been the case in man. No such relationships could be established, except that the onset of Phase III in the multicentric cases indicated that death would follow within a few days.

By definition, the disease in the dog appeared to be closely related to the lymphosarcoma of Lumb (1954) and to the lymphoblastoma and lymphocytoma of Gall and Mallory (1942). The term maliguant lymphoma was introduced by the latter workers to embrace a wide group of lymphoid tumours in man including Hodgkin's disease. Because of the essential simplicity of the disease in the dog, this term, which was first used for lymphoid tumours in the dog by Bloom and Meyer (1945), has added very little to our appreciation of this disease.

In this study, a few cases were observed which had a histological resemblance to giant follicular lymphoma as found in man. This was probably a pre-lymphosarcomatous lesion in the dog and a strict comparison between this manifestation and its relatively benign counterpart in man is probably not justified.

Terms which have been used to describe lymphosarcoma of the dog such as lymphatic leukosis, lymphadenosis, Hodgkin's disease, pseudo-Hodgkin's disease and reticulosis ought now to be regarded as obsolete. They are difficult to define and are thus open to mis-interpretation.

SECTION II.

LYMPHOSARCOMA OF THE CAT.

INTRODUCTION.

Tumours of the lymphoid tissue are a common form of malignant disease which is encountered in cats in this hospital. Detailed post-mortem examinations have been carried out on 48 animals which were suffering from such tumours and these formed the basis of the following pathological and clinical study.

There have been no reports of the field incidence of spontaneous lymphosarcoma, but Cotchin (1952) listed, according to anatomical systems, 226 tumours of the cat which had been observed at the Royal Veterinary College, London. Lymphoid tumours represented 15% of the total. There were 6 cases of lymphatic leukosis, 8 cases of lymphosarcoma of the mesenteric lymph nodes, 10 intestinal lymphosarcomas, 4 renal lymphosarcomas, 1 uterine and 4 thymic lymphosarcomas. One other case in the thymus was described as a thymoma. In this series, lymphatic tumours were encountered more frequently than the cutaneous carcinomas. Later , in an extended study, Cotchin (1957) classified 426 tumours of the cat, three-quarters of which were malignant. Again, 15% of the total were lymphoid tumours which were widely distributed throughout the body systems.

Mulligan (1951) reported 5 cases of lymphosarcoma in a series of 70 tumours of the cat.

The most detailed investigations of lymphoid tumours of the cat have been carried out at the Angell Memorial Animal Hospital in Boston and these studies have been reported by Nelson and Holmworth (1953); Holmworth and Nelson (1955) and Holmworth (1960a, 1960b). The diagnosis was confirmed by biopsy or at post-mortem examination in the majority of cases, but the actual numbers autopsied in detail were not published. The most important sites of gross tumour formation were the kidneys and intestines, followed by the anterior mediastinum, the mesenteric lymph nodes and the spleen. Other sites which were often simultaneously affected included the liver, stomach, heart, skin and salivary glands. Lesions of lymphosarcoma in the nasal passages were specifically mentioned, since these were often mis-diagnosed initially. Although virtually every site in the body could become infiltrated with lymphoid tumour cells, the central nervous system appeared to be least frequently affected. In their initial report, Nelson and Holmworth (1953) described the condition as "a locally malignant tumour with diffuse infiltrative type of growth and an apparent preference for the abdominal organs, particularly the small intestine, the kidney and the liver." Fourteen cases were reported and each had been studied histologically except one. In their second report on a further series of 15 cats, Holmworth and Nelson (1955) acknowledged that tumours of the anterior mediastinum seemed "to constitute as much of an entity in the thoracic pathology of cats as do the intestinal and kidney tumours in abdominal pathology."

In the clinical report by Holmworth (1960a), the incidence of haemopoietic tumours in cats in the Boston Hospital was given as 16% and 197 cases were observed/

observed in 1425 routine post-mortem examinations. These tumours were almost all of the lymphoid type. In 140 clinical cases, the ages ranged from 0.5 - 17 years, but 50% of the animals were aged from 1 - 5 years. There were 2 male or neutered male cats affected for every female or neutered female affected.

Blood changes were pathognomonic in only 15% of the cases. An attempt was made to distinguish frank lymphatic leukaemia, which was characterised by generalised lymphadenopathy, hepatosplenomegaly and true leukaemia, from the disease termed visceral lymphosarcoma, but no histological criteria were given to support this distinction. Progressive anaemia and normoblasts in the peripheral blood were a frequent observation. In the earlier reports, the condition was graded according to the degree of malignancy of the tumour cells in the tissues, Grades I - IV. Grade IV types were actively dividing lymphoblasts which were regarded as highly malignant. The degree of malignancy was claimed to be related to the extent of the tumour process at the time of death, but this grading was not employed in the extended study by Holzworth (1960a). Instead, an attempt was made to correlate the duration of the disease with the dominant cell types in the peripheral blood in the cases of frank leukaemia. Acute forms of the disease were associated with high numbers of circulating lymphoblasts, the sub-acute forms with lymphoblasts and atypical lymphocytes and the chronic type with high numbers of lymphocytes. A relationship was claimed between the acute form and a fulminating syndrome. A single case was observed which could not readily be diagnosed either as a lymphoid tumour or an erythroleukaemia.

In the English language, other convincing reports of lymphosarcoma of the cat are sparse. Murray (1908) described a round cell sarcoma of the small intestine in an 8 or 9 year old castrated male cat. Kirk (1931) reported a case of lymphosarcoma of the ileum with metastatic lesions in the kidneys; the diagnosis was confirmed histologically. Bloom (1937) described a case of generalised lymphosarcoma in a cat in which the superficial lymph nodes were 6 - 12 times their usual sizes. The total leucocyte count was aleukaemic, 15,150/cu.mm. An early clinical feature of this case, which had attracted attention, was a unilateral exophthalmus. This later proved to be part of the lymphosarcoma tumour process. Locke (1948) described a lymphoid tumour of the anterior mediastinum which he termed thymoma, a term also used by Loveday (1959) to describe a carcinoma of the thymus in a cat. Patterson and Meier (1955) reported 2 cats with alimentary lymphosarcoma, one of which survived surgery for 23 months. Lymphosarcoma of the thymus in an 8 month old kitten was reported by Morgan (1959). Histologically, the criteria necessary for a diagnosis of lymphosarcoma were fulfilled. Wilson and Gilmore (1962) described a case involving both of the kidneys and the right lung; they stressed that this was one of the main patterns seen at Boston.

From reports in the French veterinary literature it is clear that lymphosarcoma of the cat was recognised as a distinct entity in the early years of the present century. Thus Petit (1902) recognised a multicentric case which was confirmed histologically and later he reported lymphadenomas of the intestines/

intestines in 2 cats, Petit (1908). Ball and Auger (1925) described a cahectic 18 month old cat which had presented bilaterally symmetrical tumours in the lymph nodes of the head and neck. This was diagnosed as lymphosarcoma or lymphoblastoma on histological examination. Other carcase nodes were unaffected and leukaemic changes were not found in the liver and spleen. Ball and Collet (1931) also described a round cell sarcoma which was associated with the small intestine in a six year old cat.

Examples of non-lymphoid malignant diseases of the haemopoietic system are uncommon. Myeloid leukaemia has been diagnosed by Eyestone (1951); Baker (1954) and Meier & Patterson (1956). Basophilic or mast cell leukaemia has been reported by Lillie (1931); Meier & Gourley (1957) and Head (1958). Holzworth (1960b) cited 2 cases of eosinophilic leukaemia from personal communications.

MATERIALS AND METHODS.

The cats which have been included in this study were admitted to this hospital between the years 1954 and 1964 and found to be suffering from lymphosarcoma.

The methods employed were similar to those described for the dog in Section I of this part of the thesis. The normal haematological values, which were accepted, were those given by Schalm (1961) and the normal values for the biochemical tests were those which were in use at the hospital and were similar to those given for the dog.

CLASSIFICATION OF THE DISEASE IN THE CAT.

As in the dog, the classification, which was proposed, was based on the distribution of the major lesions of the disease throughout the body. Four forms of lymphosarcoma were recognised, a multicentric form, a "thymic" form, an alimentary form and an aberrant form in which were placed those cases which did not conform to one of the other three groups.

THE PATHOLOGY OF THE DISEASE.

The macroscopical and microscopical appearances of lymphosarcoma in the organs and tissues which were affected with lymphosarcoma were studied. These included the lymph nodes, liver, spleen, alimentary system, kidneys, lungs, heart, bone marrow and pancreas.

THE DISTRIBUTION OF THE LESIONS.

The distribution of the lesions in 48 confirmed cases of lymphosarcoma of the cat is shown in Table 54. The patterns appeared to evolve into 4 main types, a multicentric form, a "thymic" form, an alimentary form and an aberrant type. In the multicentric form there was enlargement and tumour infiltration of the superficial lymph nodes, the mesenteric and other abdominal nodes and, less commonly, the liver and spleen. In 1/3 of these cases the kidneys were also involved, but the lungs, heart, tonsils and alimentary system were less frequently affected. In the so-called "thymic" form of the disease, the main tumour mass was found in the anterior mediastinum at the site of the former thymus. Metastatic lesions were observed in/

TABLE 54

The Distribution of the Lesions in 48 Cases of Lymphosarcoma in the Cat.

	Multicentric type	Thymic type	Alimentary type	Aberrant type	Total
Lymph nodes.					
Superficial	14			1	15
Thoracic	9				9
Anterior sternal	1		5	1	7
Mesenteric	13		18		31
Other abdominal	12		5	1	18
Thymic mass	5	5			10
Liver	7	2	15	4	28
Spleen	8	1	5		14
Alimentary tract	2	1	18		21
Kidney	5	2	9	1	17
Lung	3	1	2	2	8
Heart	2	1			3
Tonsil	3				3
Marrow			1	1	2
Pancreas			2		2
Total cases	14	5	24	5	48

in other parts of the body, but the superficial lymph nodes were not involved. The most common type was the alimentary form which was associated with lesions of the mesenteric lymph node and the intestines. The liver was frequently involved, but the kidneys, spleen and lungs were affected to a lesser extent. A fourth small group of cases was found in which the liver was the principal organ affected, but this was accompanied by tumour infiltration in widely distributed areas of the body such as the marrow, kidneys and the anterior sternal lymph nodes. In one case, the forelimb, together with the submaxillary and prescapular lymph nodes on one side, were involved with the liver. This group of cases was termed the aberrant form.

THE LYMPH NODES.

The Nodes of Healthy Cats.

Lymph node sections from 20 cats, which were showing no signs of clinical disease, were examined both macroscopically and microscopically and compared with the description given for lymph nodes in man by Ham and Leeson (1961). Macroscopically, after section of a node, it was often difficult to distinguish cortex from medulla. Even where there were colour changes to differentiate cortex from medulla, their junctions were often difficult to determine. Microscopically, this greater cellularity of the medulla was the main differentiating feature between cat lymph nodes and the lymph nodes of man and the dog. The medullary pegs tended to be very fine and lace-like in the cat, but the medullary sinuses were sometimes packed with reticulo-endothelial cells. This did not appear to be sinus catarrh, because the associated subcapsular and cortical sinuses were usually less heavily populated with reticulo-endothelial cells.

Typical germinal centres were bordered by a ring of mature or medium lymphocytes and the central portion was composed of larger cells which were always rather loosely arranged and of a variable nature. For example, it was usual to find lymphoblasts, large lymphocytes, medium lymphocytes, stem cells, reticulo-endothelial cells and active macrophages within these centres. Occasionally, a plasma cell or plasma cell precursor could be identified. The degree of activity of the germinal centre appeared to be related to its diameter, the presence of interstitial cell debris, mitotic figures and the presence of a few very active immature cells with bold, pale nucleoli, which contrasted sharply with the more mature cells of the centre. Reactive secondary centres were no more heavily populated with cells than inactive ones.

Lymph Nodes Affected with Lymphosarcoma.

The anatomical distribution of affected lymph nodes is given in Table 54. The distribution of these nodes had a marked influence on the classification of the disease into anatomical and clinical types.

Morbid Anatomy.

A typically affected node was enlarged, discrete, yellowish or light tan in colour and encapsulated. On section, it was filled with a yellowish highly cellular tissue. Occasionally, congestion, infarction and necrosis were observed. Extension from the node to the surrounding tissues was uncommon, except for those tumour masses encountered in the anterior mediastinum, which had a tendency to invade the adjacent lung or pericardial tissues and those in the posterior part of the para-aortic lymph node chain which/

in at least one cat, which had invaded the associated sub-lumbar voluntary muscles. In the cat, lymph nodes were not increased in size to the same extent as they were in the dog. It was thus necessary to examine all superficial lymph nodes where one or two groups were moderately enlarged and infiltrated. Where there is bilateral, moderate lymphadenopathy of all superficial lymph nodes including those less accessible ones, such as the axillary and superficial inguinal lymph nodes, then lymphosarcoma must be considered as a possible cause of the disease process.

In marked contrast to the moderate increases in size of the superficial lymph nodes, affected mesenteric nodes tended to be grossly enlarged, firm and somewhat lobulated. They were readily palpable and easily identified in the mid-abdomen.

Microscopical Findings.

38 sections were available from 33 cats and a summary of the findings is given in Table 55. In nine cases, no sections of nodes were prepared, because the main sites of the lesions were in other organs. In three instances, the sections were so severely infected that only histological changes typical of severe infection were observed, which may have obscured any previous lesions of lymphosarcoma. In another three cases, no sections were available for study.

A typically affected lymph node was composed of uniform sheets of lymphoid cells which had destroyed the general architecture and abolished the cortical medullary differentiation. There was marked flooding of all the sinuses and, in many instances, these could no longer be identified. In eight sections, there/

there was evidence of condensed small follicles of mature lymphocytes within the tumour cell sheet, or else marginated. These were assumed to be vestigial follicles. In two cases lymph nodes were not entirely affected, only one area presenting clear evidence of lymphosarcoma whilst the rest of the node, including the medulla, was indistinguishable from the node of a healthy cat. In five cases, there was no clear cortico-medullary differentiation, but the central portion of the node was composed of large round tumour foci without adjacent trabeculae. These were assumed to be grossly tumified medullary pegs.

There was evidence of multiple tumour foci in both the cortex and the medulla in four cases. Sections were composed of tightly packed foci of fairly uniform blast or stem cells. These were expanding and encroaching upon adjacent sinuses which were packed with more mature cell types, Figures 34, 35, and 36. The tumour foci had expanded so that they were almost uniform cell sheets in two sections, Figure 37. This resembled giant follicular lymphoma.

The integrity of the capsule was maintained in only four sections. In the remainder, there was evidence of infiltration leading to destruction of the capsule. Infiltration of the peri-nodal area was clearly evident in 26 sections and in eight others, could be observed either as shallow streams of cells lying just outside the capsule, or as small masses of tumour cells lying in the depression at the capsular-trabecular junctions. In two sections, there was clear evidence of an accessory capsule around the node, but in the majority/

TABLE 55

Summary of the Histological Findings of Lymph Node Sections of Cats with Lymphosarcoma.

Number of cases	33	
Number of sections	38	
General architecture		
Preserved	3	
Loss of	35	38
Cortico-medullary differentiation		
Visible	3	
None	35	38
Peri-node areas		
Negative	4	
Slight infiltration	8	
Moderate infiltration	12	
Marked infiltration	14	38
Capsule		
Intact	4	
Laminated	21	
Fragmented or Obliterated	13	38
Subcapsular sinus		
Patent in parts	0	
Flooded, but visible	9	
Obliterated	29	38
Cortical trabeculae		
Intact	3	
Laminated	9	
Fragmented or Obliterated	26	38
Follicles		
Well defined in parts	2	
Vestigial	8	
Replaced by cell sheets	24	
Tumour follicles	4	38
Cortical sinus		
Patent in parts	1	
Flooded, but visible	2	
Obliterated	35	38
Medullary trabeculae		
Intact	0	
Laminated	5	
Fragmented or Obliterated	33	38
Medullary pegs		
Tumified	5	
Indistinguishable from cortex	31	
Unaffected	2	38

Medullary sinus

Patent in parts	1	
Flooded, but visible	1	
Obliterated	36	
		38

Other features

Necrotic foci	4
Diffuse cytopathic changes	2
Diffuse haemorrhages	1
Accessory capsule	2
Fibrosis	0
Foci of eosinophils	0
Multinucleated cells	0
Foci of neutrophils	2
Foci of plasma cells	0

majority of sections, as in the dog, it comprised loosely arranged stroma which appeared to be condensations of loose connective tissue. Foci of necrosis were not encountered frequently, nor were diffuse cytopathic changes. At post-mortem examination, nodes were selected which did not show signs of obvious necrosis, so that this observation is not necessarily a reflection of the true incidence of necrosis in cat lymph nodes. More informative was the absence of marked fibrosis, multinucleated cells of the Reed-Sternberg type eosinophils and plasma cells. Foci of neutrophils were observed in only two lymph node sections.

THE LIVER.

In 28 cats, the liver was affected with lymphosarcoma, Table 54. Again, as in the dog, the liver was one of the main sites of the disease and affected livers were found in each of the four forms of the disease in the cat.

Morbid Anatomy.

Affected livers were often enlarged and were usually pale. Occasionally, white or cream coloured and fairly well defined spots, up to 0.5 cm. in diameter were visible on the surface and were scattered throughout the parenchyma. The cut surfaces were characterised by exaggeration of the portal triads to form a distinct greyish network or mottling effect.

Histological Findings.

A summary of the histological findings is presented in Table 56. Sections were studied from each of the 28 affected cats. In every section which was examined,/

TABLE 56

Summary of the Histological Findings in Lesions of Lymphosarcoma of the Liver of the cat.

Number of cases	28		
Number of sections	28		
General architecture		**Infiltration around central veins**	
Preserved	27	Occasional	4
Lost	1	Absent	24
	28		28
Portal triad infiltration		**Degenerative changes in Hepatic cells**	
Occasional triads	7		
Majority infiltrated:		Central zone	10
slightly	8	Central and mid-zone	2
moderately	9	Peripheral and mid-zone	0
markedly	4		
		Entire lobule	0
Absent	0	Absent	16
	28		28
Infiltration of Sinusoids		**Other lesions**	
Small foci	7	Subscapsular infiltration	2
Moderate foci	2		
		Haemosiderosis	3
Large foci	2		
		Megakaryocytes	2
Streams of cells	1		
		Multinucleated liver cells	1
Absent	16		
	28		
		Fibrosis of portal triads	1
		Multiple small abscesses	1

examined, there was some evidence of infiltration of the portal triads. In 21 cases, almost all the triads were infiltrated to some extent and in 13 of these, the infiltrations were quite distinct even with the low power of the microscope. However, in seven cases, only occasional triads were affected. Such triads were slightly involved and in one section, there was moderate infiltration of a single triad with small abscesses which formed satellites in the sinusoids of the surrounding lobules.

In more than half of the sections, the sinusoids were free of tumour cells. The main sign of infiltration in the sinusoids was small scattered foci causing slight distortion of the hepatic cell lines. In four cases, these foci were much larger and in two of them, the effect was one of multiple tumours throughout the lobules. In only one section were fine streams of tumour cells within the sinusoids observed. This was a very common observation in the dog.

Collections of tumour cells around the central veins were never noted throughout an entire section.

Fatty changes of the hepatic cells were only observed in the central and mid-zonal areas of the lobules, whilst the peripheral zone cord cells were not usually abnormal.

Haemosiderosis was seen in three cases and megakaryocytes within the sinusoids in two others. Fibrosis of portal triads and multiple small abscesses were observed on individual sections. Multinucleated liver cells were seen in one section which was heavily infiltrated, Figures 38 and 39.

THE SPLEEN.

The spleen was affected with lymphosarcoma in 14 cases and affected spleens were observed in each of the forms of the disease except the aberrant type, Table 54.

Morbid Anatomy.

Eight of the affected spleens were either enlarged or grossly enlarged, but five were judged not to be increased in size. However, the usual splenic profile was preserved with rounding of the edges, even where there was gross increase in size.

An affected spleen was usually bluish-pink or of a red slate colour and on section, there was preservation of the capsule. Loss of central definition, with the pulp assuming a reddish and uniformly cellular appearance, was common, but in 6 cases, there was marked hyperplasia of the Malpighian corpuscles.

Histological Findings.

The histological data are summarised in Table 57. In every case, the capsule of the spleen was completely intact and no signs of tumour cell infiltration was found. The ratios of trabeculae to splenic pulp were low in 5 sections and moderate in the rest. Trabecular infiltration with tumour cells was observed in more than half of the sections.

In eight of the sections, the entire splenic pulp had been replaced by lymphoid tumour cells. Marked hyperplasia of the Malpighian corpuscles was noted in 6 other sections. In three of these, this was associated with marked infiltration/

TABLE 57

The Histological Findings in Lesions of Lymphosarcoma of the Spleen of the cat.

Total number of cases	14	
Number of sections	14	14
Capsule		
Intact	14	
Infiltrated	0	
Not on the section	0	14
Trabecular pulp ratios		
Low	5	
Moderate	9	14
Trabecular infiltration		
Present	8	
Absent	6	14
Splenic pulp		
Diffuse infiltration	8	
Hyperplasia and transformation of the Malpighian corpuscles	3	
Malpighian hyperplasia and transformation plus infiltration of the red pulp	3	14
Megakaryocytes		
Not observed	8	
Occasional	2	
Several	2	
Numerous	2	14

infiltration of tumour cells from the tumified corpuscles into the red pulp, but not to the extent where there was total uniformity of the cell sheet. In the other three cases, the tumified corpuscles were tightly packed with uniform blast cells and, although some blast cells were present in the sinuses of the red pulp, the hyperplastic Malpighian corpuscles had remained discrete. In the red pulp of one case, number 24908, there was marked evidence of erythropoiesis which accentuated the tumified bodies, Figures 40 and 41. Where the tumified Malpighian bodies had remained discrete, case number 25522, traces of the former corpuscles could sometimes be found, Figures 42, 43 and 44. Intermediate stages, when the tumour cells appeared to be budding from the edges of condensed corpuscles, were also observed.

Megakaryocytes were not observed in eight of the sections. In 2 sections they were seen occasionally, in two sections several of these cells were observed and in two others, they were found in great numbers. In three of the cases, the megakaryocytes were immature, with pale vesiculated multiple nuclei each one of which had a prominent pale eosinophilic nucleolus.

THE ALIMENTARY SYSTEM.

Twenty-one cats had tumours of the alimentary system and these lesions were found mainly in the alimentary form of the disease, Table 54.

Morbid Anatomy.

Lymphosarcoma was typified by discrete, spherical or ovoid tumours of the stomach or intestines, which were often multiple. They were usually pale/

pale yellow or off-white in colour, smooth surfaced and occasionally adherent to adjacent loops of intestine or areas of the omentum. On section, the masses were composed of uniform, firm, yellow or white tissue which had involved all layers of the tract and had caused ulceration of the mucosae. Occasionally, there were central necrosis and signs of impending perforation.

A more diffuse form of the disease along the intestine was associated with a regular and marked thickening of the canal walls. In a few cases, the mucosa appeared hypertrophic and in parts, had corrugations resembling Johne's disease in the bovine animal.

It should be borne in mind that 18 of the 21 cases, in which lesions of the alimentary system were observed at the post-mortem examination, belonged to a group which has been termed the alimentary form of the disease in the cat and is characterised by gross lesions in the alimentary-mesenteric-lymphatic region. The distribution of such lesions is shown in Table 58. From this table, it will be noted that only 6 cases had lesions of the alimentary system without involvement of the mesenteric lymph node. Other lesions accompanying these alimentary tumours in the 6 cases are shown in Table 59. There were only 3 cats in which lesions were discrete and confined to the alimentary tract and one other animal where these lesions were diffused along the intestines.

Histological Findings.

Sections of the alimentary system were available from 15 of the 21 affected cats and a summary of the histological findings are given in Table 60.

Lesions/

TABLE 58

The Distribution of the Main Lesions in 25 Cats with the Alimentary Form of Lymphosarcoma.

Lesions both on the gastro-intestinal tract and the mesenteric lymph node	12
Lesions on the tract only	6
Mesenteric lymph node alone	6
Number of cases of the alimentary type	24

TABLE 59

The Distribution of Lesions of Lymphosarcoma in Six Cases of the Alimentary Type where the Mesenteric Lymph Node was not Affected.

Case Numbers	Liver	Spleen	Other abdominal lymph nodes	Lesions of the stomach or intestines
3184				+
7700	+		+	+
14699	+	+		+
18667				+ ("Johné's Type")
24026				+
25368				+

TABLE 60

A Summary of the Histological Findings in Lesions of Lymphosarcoma of the Alimentary System, of the Cat.

Total number of cases	21	
Number of sections	15	
Mucous membranes		
Intact	1	
Necrotic or fibrotic	3	
Infiltrated	5	
Vestigial glands	4	
Obliterated	2	15
Submucosae		
Intact	1	
Moderate infiltration	3	
Marked infiltration	11	15
Muscle layers		
Intact	2	
Laminated	5	
Fragmented	7	
Obliterated	1	15
Subserosal or mesenteric areas		
Infiltrated	11	
Not infiltrated	4	15

lesions were characterised by streams of tumour cells which were infiltrating into the original structures and then increasing in magnitude until the structures were destroyed and eventually replaced by the invading cell sheets, Figure 45 . Necrosis, with some associated fibrosis, was noted in the mucosal area in three sections. In one case, an intact and apparently uninfiltrated mucous membrane was noted. Adjacent to this, there was marked tumour infiltration of the musculature and mesentery. Infiltration of the mucosae was moderate in five cases and this had resulted in tumour cell infiltration between the glands causing distortion of the profiles of the glands, but only minor destruction of these elements. In four sections, only vestiges of those glands remained amongst the intensely destructive sheets of lymphoid tumour cells.

Infiltration of the submucosa was related to the degree of involvement of the mucous membrane and in that section where the mucous membrane was unaffected, the submucosa was also free of tumour cells.

In two of the fifteen sections, the muscle layers were intact, but there was infiltration of the adjacent mucosa. In the other 13 sections, infiltration was observed; lamination in five sections, fragmentation in seven and obliteration in one, Figure 46.

Marked infiltration was a feature of the subserosal or mesenteric regions in 11 of the sections.

Serial sections were prepared from an alimentary tumour of case number 25368. The core of this mass was composed almost entirely of lymphoid cells with/

with small glandular vestiges just below the surface of the ulcerated mucous membrane whereas, at its junction with the ileum, only mild lamination of the muscular layers and slight mucosal infiltration were observed histologically. The ileum above and below this mural tumour was not microscopically abnormal.

THE KIDNEYS.

Metastatic lesions of lymphosarcoma were observed in the kidneys of 17 cats; usually both organs were affected. Lesions occurred in each of the proposed forms of the disease, Table 54.

Morbid Anatomy.

Affected kidneys were moderately to markedly enlarged depending on the degree of tumour cell infiltration. In 9 cases, there was a fine speckling over the surface of the kidney and on section, the cortex presented a fine mottled appearance due to tumour infiltration. In 2 cases, round pale yellow foci, up to 1 cm. in diameter and bounded by a narrow ring of hyperæmia, were observed on the surface of the kidney. On section these were found to represent pale yellow wedge shaped lesions of the cortex outlined by intense hyperaemia. The base of each lesion lay on or close to the capsule and its apex pointed towards the juxta-medullary area.

Tumour masses, which occupied large areas of the cortex, were found in 6 cases. In one of them, case number 25522, both kidneys were markedly enlarged and on section, the cortex was found to be grossly thickened and almost completely replaced by cream coloured tumour tissue.

Histological Findings.

Sections were available from each affected cat and the histological data are summarised in Table 61.

The smallest tumour foci were poorly defined and were composed of short streams of tumour cells infiltrating amongst the renal tubules without much resulting evidence of severe tubular damage. In some of these sections, the infiltrations appeared to have an affinity for the perivascular areas of the smaller blood vessels of the cortex. Wedge-shaped lesions in the cortex were associated with marked tubular destruction and a reduction in the number of visible glomeruli. The more massive lesions which occupied much greater areas of the cortex, were also associated with elimination of many renal tubules and glomeruli, Figure 47. Surviving tubules usually presented signs of nephrosis with pyknosis of nuclei, but in some cases, the nuclei had disappeared. Glomerular tufts in infiltrated areas usually showed pyknosis of cell nuclei and appeared somewhat shrunken.

Small subcapsular foci of tumour cells were noted in four sections and in five sections, there were lesions in the juxta-medullary areas, particularly around the blood vessels. Medullary tumour foci were only observed in one section.

None of the histological changes observed in the kidney sections could be attributed to an inflammatory process.

TABLE 61

A Summary of the Histological Findings in Lesions of Lymphosarcoma of the Kidneys of the Cat.

Number of cases	17	
Number of sections	17	
Cortical lesions		
Focal, infiltrative only	9	
Focal, destructive	2	
Massive, destructive	6	17
Other features		
Subcapsular infiltration	4	
Juxta-medullary infiltration	5	
Medullary infiltration	1	
Peri-vascular infiltrations	5	
Fibrosis	0	

THE LUNGS.

Evidence of lymphosarcoma was found in the lungs of 8 cats and pulmonary lesions were observed in each of the anatomical forms of the disease, Table 54.

In most cases, there were few macroscopical signs of the disease apart from scattered greyish pin-head sized nodules in the lung substance. In 2 cases, well defined foci up to 0.5 cm. in diameter were distributed sparsely in the parenchyma.

Histologically, most of the lesions were arranged around the smaller bronchioles and veins, but in three lungs the main lesions were present in the alveolar fields. The alveoli were packed with tumour cells and there was septal infiltration.

THE HEART.

Areas of lamination of the muscle bundles of the myocardium were found in three cats.

THE BONE MARROW.

The femoral bone marrow of 2 cats was found to be clearly infiltrated with lymphoid tumour cells. In one case, this infiltration had completely replaced all evidence of the former marrow activity, but in the other, large/

large foci of tumour blast cells were scattered in areas of the marrow which were otherwise hyperplastic. Large numbers of immature megakaryocytes were also observed in this section.

In several other sections of bone marrow which had been selected because of signs of marked cellularity throughout the medullary cavities of the femurs, hyperplasia of all the normal haemopoietic elements was featured.

THE TONSILS.

In three cats, the typical architecture of the tonsils had been completely replaced by sheets of lymphoid tumour cells.

THE PANCREAS.

This gland was infiltrated in two instances. It took the form of intra-lobular and intra-acinar infiltration by tumour cells with progressive destruction of the glandular epithelium.

SUMMARY.

Widespread infiltration of tissues and organs by tumour cells as well as discrete tumour masses characterised all forms of the disease in the cat and most of the tumour masses were highly invasive peripherally. Thus it was impossible to distinguish one form of lymphosarcoma from another on histological criteria in this species.

TUMOUR CELL MORPHOLOGY.

The Cell Morphology of Feline Lymphoid Tissue.

The cells found in the lymphoid tissues of the healthy cat were in almost every respect similar to the lymphoid cells of the dog. However, in the undifferentiated cells in the cat there seemed to be less chromatin condensation than similar cells in the dog. In addition, there was rather more variation in the nuclear cytoplasmic ratios in feline lymphoid cells as compared to those of the dog.

The Dominant Tumour Cell Types.

Malignant cells in lymphosarcoma were indistinguishable from normal lymphoid cells and they were recognisable as tumour cells only by their abnormal numbers and the formation of pathognomonic cell sheets.

In lesions of lymphosarcoma of the cat, it was relatively simple to determine the majority tumour cell type since there were only small degrees of variation in the cell type throughout an organ. There was also uniformity in the cell type throughout the body in a given case.

The dominant cell type, classified as "immature", Figure 48, or "mixed" or "mature," Figure 49, in 48 cats is shown in Table 62. It will be observed that in none of the 4 forms of the disease was there a predominance of one or other of the two tumour cell groups. From this, it was concluded that there is no more likelihood of one tumour cell type rather than the other occurring in the different anatomical forms of this disease in the cat.

There was a close relationship between the tumour cell type and the mitotic rate. The more undifferentiated cell sheets showed a higher rate of mitosis than the more mature cell sheets.

TABLE 62.

The Distribution of the Dominant Tumour Cell Type in the 4 Anatomical Forms of Lymphosarcoma of the Cat.

	Multicentric	Alimentary	Thymic	Aberrant	Total
Immature cell sheets	4	10	2	2	18
Mixed or mature cell sheets	7	13	3	2	25
Unidentifiable	3	1		1	5
Total cases	14	24	5	5	48

SPECIAL EXAMINATIONS.

The Haematological Findings.

Thirty seven blood samples were examined from 25 cats which were subsequently shown to have been suffering from lymphosarcoma. A summary of the haemograms is given in Tables 63 and 64. The normal values used were those given for the cat by Schalm (1961).

The erythrocyte sedimentation rates (E.S.R.) were very variable and all but one exceeded 10mm./hour. In well over 50% of the samples, readings higher than 30mm./hour were recorded. These readings showed a negative correlation with the total erythrocyte counts, Figure 50.

More than half of the readings of the packed cell volumes (P.C.V.) were below 30% and slightly over 1/5 gave readings of less than 20%. In 30 of the 34 samples, the haemoglobin estimations were below average for the cat and 10 of those were abnormally low. This trend was followed in the total erythrocyte counts, where over 50% of the counts were less than 6 million/cu.mm. and 33% had fallen below 4 million per cu.mm.

The Mean Corpuscular Haemoglobin Concentrations (M.C.H.C.) and the Mean Corpuscular Volumes (M.C.V.) were within the normal ranges for the cat in the majority of samples, although in 5 cases, the M.C.H.C. was lower than normal.

From these figures it was concluded that the E.S.R. was related to the degree/

degree of anaemia and the lower the total erythrocyte count fell, the higher the erythrocyte sedimentation rate rose. The type of anaemia which developed in the cat with lymphosarcoma was usually a normocytic and normochromic one.

The total leucocyte count was above the upper limit for the cat in 9 samples. In 10 cases, the absolute neutrophil counts were higher than 15,000/cu.mm. and in 7 samples, the absolute lymphocyte count was in excess of 7,500/cu.mm. However, in 14 out of 36 samples, the absolute lymphocyte counts were less than 2,500/cu.mm.

Lymphosarcoma in the cat was, therefore, associated with comparatively low leucocyte counts and lymphocytopenia was very frequently observed. A minority of cases showed neutropenia.

In 2 cats, 4 blood samples, a lymphocytosis in excess of 30,000/cu.mm. was observed. The circulating lymphoid cells in both animals included numbers of typical lymphoblasts. In one other cat, where the lymphocyte count was 4,900/cu.mm., lymphoblasts represented 17% of the lymphoid cells. In several other blood films, lymphoid cells were found which contained small nucleoli. These nucleoli were partially obscured by clumps of chromatin and it was thought that the chromatin in such cells was too condensed for them to be classified unconditionally as blast cells. The so-called "smudge" cells and "atypical" lobulated lymphocytes were also observed. Such cells are by no means uncommon even in the blood of cats which are clinically quite healthy and are certainly not pathognomonic of lymphosarcoma.

Eosinophilic normoblasts were observed in a minority of blood films.

TABLE 63

Data on the Circulating Erythrocytes of 25 Cats Suffering from Lymphosarcoma.

Erythrocyte Sedimentation Rates		Packed Cell Volumes		Haemoglobin Concentrations	
mm./hour	Samples	%	Samples	gms./100 ml.	Samples
0-	1	< 20	7	< 4	3
10-	6	20-	11	4-	7
20-	5	30-	11	8-	20
30-	4	40-	3	12-	4
40-	7	50-		16-	
50-	3	60+		20+	
60+	4				
	30		32		34

Total Erythrocyte counts		M.C.H.C.		M.C.V.	
mil./cu.mm.	Samples	%	Samples	cu.μ	Sample
< 2	3	< 20		< 40	1
2-	7	20-	5	40-	9
4-	8	30-	24	50-	14
6-	11	40-	2	60-	1
8-	1	50+		70-	1
10+				80-	1
				90+	
	30		31		27

TABLE 64

Circulating Leucocytes in 25 Cats with Lymphosarcoma.

Total Leucocyte counts		Absolute Neutrophil counts		Absolute Lymphocyte counts	
10^3/cu.mm.	Samples	10^3/cu.mm.	Samples	10^3/cu.mm.	Samples
< 5	5	< 5	5	< 2.5	14
5-	23	5-	21	2.5-	15
25-	4	15-	9	7.5-	2
50-	3	25-		15.0-	1
75-	2	35-		30.0-	1
100+		45-	1	60.0-	3
		55+		90.0+	
	37		36		36

TABLE 65

Blood Biochemical Tests Carried out on 12 Cats with Lymphosarcoma.

Blood urea		Plasma protein		A/G ratio	
mg./100 ml.	Samples	Gm./100 ml.	Samples		Samples
25	3	4		0.5	1
25-	9	4-	4	0.5-	10
50-	3	5-	5	1.0	1
75-	1	6-	4	1.5-	1
100-	1	7+		2.0+	
125-	1				
150+					
	18		13		13

The Blood Biochemistry.

There were technical difficulties in obtaining blood samples which were suitable for biochemical examinations from many of the cats, since they were critically ill at the time of admission. Because of this, relatively few samples were examined. The data are summarised in Table 65.

The serum protein levels were low in most cases, but the albumen/globulin ratios remained within normal range. The blood urea levels, in 12 of the 18 samples examined, were less than 50mg./100 ml.

THE CLINICAL FINDINGS.

THE DURATION OF THE DISEASE.

The duration of the disease could only be measured from the onset of the clinical signs until death. The durations of this phase of lymphosarcoma of the cat are shown in Table 66. Twenty-eight of the 39 cats were dead within 2 months after the onset and nearly 40% of the group died within 4 weeks of the onset of the illness. No evidence was found that there were differences in the duration of the disease amongst the anatomical forms.

THE SEX INCIDENCE.

The sexes of 39 cats affected with lymphosarcoma are given in Table 67A and a comparison between this distribution and that of a sample of cats, which were admitted successively to the hospital, is shown in Table 67B.

The ratio of males to females in the lymphosarcoma group was not statistically different from the ratio in the sample of admissions, $\chi^2 = < 3.84$ with one degree of freedom.

From this it was assumed that there was no sex incidence in cats with lymphosarcoma.

THE AGE DISTRIBUTION.

The age distribution of 39 cats with lymphosarcoma is given in the upper part of Table 68. From this, it will be clear that numbers are too small to justify a statistical analysis. Until more cases in each form of the disease are studied, it should be assumed that there is no age incidence in any of the anatomical forms of the disease. However, it will be observed that 3 of the/

TABLE 66

The Duration of Overt Lymphosarcoma in 39 Cats.

	Duration in weeks.					Not recorded	Total
	< 4	4-	8-	12-	16-		
Multicentric	2	5	1			3	11
Thymic	1	2		1			4
Alimentary	9	5		1	3	2	20
Aberrant	3	1					4
Total	15	13	1	2	3	5	39

TABLE 67A

The Sex Distribution of 39 Cats Affected with Lymphosarcoma.

Males		
Entire	15	
Neutered	9	24
Females		
Entire	6	
Neutered	8	14
Not recorded		1
Total		39

TABLE 67B.

A Comparison Between the Sex Incidence in a Group of Cats with Lymphosarcoma and a Group of Cats Admitted to this Hospital in Succession for Treatment for Diseases in General.

	Lymphosarcoma Group	Hospital Admission Group
Males	24	56
Females	14	50
Not recorded	1	-
Totals	39	106

TABLE 68

The Ages of 39 Cats Affected with Lymphosarcoma

Compared with the Ages of 95 Successive Admissions

to the Hospital.

	Ages in years																		
	< 1	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-	11-	12-	13-	14-	15-	16	Not recorded	Total
Multicentric			4	1	1	1			1		1							2	11
Thymic		3							1										4
Alimentary		1	2	1		2	2		1	2	3		3	1				2	20
Aberrant			1	1	1												1		4
Total		4	7	3	2	3	2		3	2	4		3	1			1	4	39
Admissions to Hospital	21	11	9	6	6	3	4	2	7	4	6	2	4	3	6	0	1		95

the 4 cats with the so-called thymic form of the disease were between 1 and 2 years of age, which is not surprising since the thymus atrophies early under normal circumstances.

The ages of 95 cats which were admitted to this hospital in succession are given in the lower part of Table 68. A statistical comparison was carried out between the numbers of cats in both the lymphosarcoma and the admission groups to determine if any differences could be established. Four age groups were taken, less than 1 year old, 1 to 5 years of age, 6 to 10 years of age and 11 years of age upwards. There was an obvious significant difference between the numbers in the youngest age group. In the older age groups, with one degree of freedom, χ^2 = 0.46 n.s. in the 1 - 5 year old group, χ^2 = 0.0013 n.s. in the 6 - 10 year old group and χ^2 = 0.82 n.s. in the oldest age group.

From this, it could be concluded that in cats with lymphosarcoma which are admitted to this hospital, there is a low incidence in animals under 12 months, but there is neither a higher nor a lower incidence of this disease of the cat at this hospital than would be expected from the ages of cats which have been admitted for disease conditions in general.

THE SYNDROMES ASSOCIATED WITH LYMPHOSARCOMA OF THE CAT.

A summary of the main clinical signs and symptoms of 38 cases of the disease is presented in Table 69. In 3 cases, although a post-mortem examination had been carried out, only a general pathological report was available, but death was due to lymphosarcoma.

The Wasting Syndrome.

A basic syndrome was observed in almost all of the cats where lymphosarcoma was allowed to reach an advanced stage. There was progressive loss of bodily condition until emaciation was so marked that the outlines of the bones of the head, spinal column and pelvis became prominent and readily palpable. Abdominal fat was also gradually lost. This disease was afebrile and accompanied by marked lethargy and depression.

The Additional Features in the Different Patterns of the Disease.

In the multicentric form, there was enlargement of the superficial lymph nodes, including the axillary and superficial inguinal nodes, in the majority of cases. There was seldom any obvious reason, such as infection, to account for the lymphadenopathy and the nodes were usually bilaterally symmetrical, firm, relatively mobile and painless. In some of the cats, the increase in lymph node sizes was only moderate. In 6 of the 11 cases the enlarged mesenteric lymph node could be palpated and readily identified as such. Respiratory signs were observed, particularly in those cases where a tumour mass was also present in the anterior mediastinum. This so-called thymic mass was palpable at the anterior inlet of the thorax in a few cases. In the multicentric type, the onset could be either sudden or gradual. Thirst, diarrhoea and vomiting were not common symptoms. In 2 cats, the mucous membranes were pale and the tonsils were found to be enlarged in 2 others. In one cat, the eyelids were swollen.

In 6 of the cats, the main tumour mass was centred in the anterior mediastinum. This was the "thymic form" and the presenting symptoms were respiratory. Dyspnoea was caused by the space occupying effect of the mass in the chest. This mass could be palpated at the base of the neck where it protruded through the thoracic inlet. The wasting syndrome was observed in three of these cats, but one presented respiratory symptoms which were so severe that it was destroyed whilst still in good bodily condition.

The alimentary form was characterised at post-mortem examination by gross infiltration of the mesenteric lymph node, the alimentary tract, or both the node and the tract. Tumours at these sites were not consistently associated with severe diarrhoea. In only 6 of the cases was persistent diarrhoea recorded whilst the animal was in the hospital, although a history of diarrhoea was given in most instances. In hospitalised cats, there was usually inappetence and very little evidence of faeces within the large intestine. Three of the animals showed marked thirst, but vomiting was never excessive and was noted in less than 50% of the cases. A firm, painless and mobile mass was palpable in 14 of the 19 clinical cases and an enlarged spleen was palpated in one cat. The onset was described as sudden in 12 cases.

The aberrant form of the disease was associated with infiltration of the liver in each cat, but the livers were never grossly enlarged. In one of the animals, the left kidney was markedly enlarged and in another, a forelimb and the submaxillary and prescapular nodes on the same side of the body were affected.

Where lesions of lymphosarcoma in the kidneys were massive, there were clinical signs of uraemia terminally.

Ascites was observed in 2 cases of the multicentric type and 4 cases of the alimentary form. Hydrothorax was found in 2 cases of the multicentric type, one in the "thymic" form and two in alimentary cases. Pale mucous membranes were found particularly in the alimentary form of the disease. Jaundice was not observed in any cat with the disease.

DIAGNOSTIC MARKERS.

Pathognomonic changes, both qualitative and quantitative, in the lymphoid cells of the peripheral blood did occur in this disease, but the incidence was too low for blood to be used as a diagnostic tool.

Confirmation of the diagnosis of lymphosarcoma depended almost entirely on histological criteria and accordingly, diagnostic methods must be designed to select the best tissues for this purpose. Because of this, the subdivision of the cases into their anatomical forms was justified.

Where there was bilateral enlargement of the superficial lymph nodes, a biopsy of an enlarged lymph node could establish a diagnosis. Unlike the dog, increase in size of all the superficial lymph nodes was not pathognomonic for lymphosarcoma in the cat. In this latter species, there were cases where gross hyperplasia of the lymph nodes was found, but the nodes were simply highly reactive histologically. This situation must be differentiated from that histological form of the disease which is associated with nodes packed with tumour follicles and which resembles giant follicle lymphoma in man.

In the case of the alimentary form of lymphosarcoma in the cat, the intestines or the mesenteric lymph nodes were constantly involved in the tumour process. Therefore, exploratory laparotomy and histological examination of suspected tumour tissuewere necessary to establish a diagnosis.

The "thymic" type could be diagnosed with reasonable confidence by radiological examination of the thorax of the standing cat after any fluid present in the pleural cavities had been drained. A tumour mass in the anterior mediastinum other than lymphosarcoma is rare in the cat.

Positive diagnosis of the aberrant form of the disease was possible if there were superficial lesions or if the kidneys were grossly affected and palpable. Otherwise, this condition was difficult to distinguish from any other destructive lesion of the liver.

TABLE 69

Summary of the Clinical Findings of 38 Cats with Lymphosarcoma.

	Multicentric	Thymic	Alimentary	Aberrant	Total
Number of cases	11	4	19	4	38
Onset					
Sudden	5	2	12		19
Gradual	5	1	6	4	16
Not recorded	1	1	1		3
Bodily condition					
Obese					
Good	2	1		1	4
Fair	1		2	1	4
Emaciated	7	3	17	2	29
Not recorded	1				1
Demeanour					
Alert	2	1	3		6
Dull	8	3	16	4	31
Not recorded	1				1
Fever					
Present			1		1
Absent	10	4	18	4	36
Undulating					
Not recorded	1				1

	Multicentric	Thymic	Alimentary	Aberrant	Total
Appetite					
Good	2	1	1	1	5
Poor or absent	8	3	16	3	30
Variable			2		2
Not recorded	1				1
Thirst					
Normal	9	3	10	4	26
Excessive +	1	1	6		8
Excessive 2+					
Excessive 3+			3		3
Not recorded	1				1
Emesis					
None	8	3	10	4	25
Occasional	2	1	9		12
Frequent					
Repeated					
Not recorded	1				1
Faeces					
Normal or absent	8	3	13	4	28
Diarrhoea	2	1	6		9
Haemorrhagic diarrhoea	1				1
Abdominal palpation					
Mid-abdominal mass	6	0	14	left kidney	21

	Multicentric	Thymic	Alimentary	Aberrant	Total
Hepatomegaly	4				4
Splenomegaly	5		1		6
No abnormality detected	3	4	5	3	15
Ascites	2		4		6
Respiratory system					
Tachypnoea	4		1		5
Dyspnoea	1	3	1	1	6
Cough	4		2		6
Hydrothorax	2	1	2		5
Cyanotic		1		1	2
Palpable superficial lymph nodes.	11				11
Pale mucosae	2	1	9	2	14
Jaundice					
Enlarged tonsils	2				2
Palpable thymic mass	3	3			6
Eyes					
Corneal ulceration				1	1
Swollen eyelids	1				1

CLINICAL AND PATHOLOGICAL RELATIONSHIPS.

THE RELATIONSHIP BETWEEN AGE AND DURATION.

Table 70 shows the relationship between the duration of the clinical phase of lymphosarcoma in 36 cats compared with their ages. No statistical difference was found between 2 age groups, less than 5 years and 5 years and over. $\chi^2 = < 1$ with one degree of freedom. Thus, there is no difference in the duration of the disease in younger as compared to older cats.

TABLE 70.

The Duration of Overt Lymphosarcoma in 36 Cats Compared with Age.

		Less than 5 years of age	5 years and over	Not recorded	Total
Duration in weeks	< 4	8	7		15
	4-	6	4	1	11
	8-	1			1
	12-		2		2
	16+		2		2
Duration not recorded		4	1		5
Total		19	16	1	36

THE RELATIONSHIP BETWEEN THE DURATION OF THE DISEASE AND THE DOMINANT TUMOUR CELL TYPE.

The duration of the clinical phase of lymphosarcoma in 36 cats was compared with the dominant tumour cell type and the results are presented in Table 71. Where the dominant tumour cell was "immature," 11 of the 15 cases died within 2 months after the onset of the symptoms of the disease. In those instances in which the tumour cell type was "mixed or mature," 14 of the 20 cats died within the same period. No statistical difference could be found between the two groups, $\chi^2 = 0.05$ with one degree of freedom and it can be concluded that a knowledge of the dominant tumour cell type is of no value in predicting the duration of the illness.

TABLE 71.

The Duration of Overt Lymphosarcoma in 36 Cats Compared with the Dominant Tumour Cell Type.

	Duration in weeks						
	less than 4	4-	8-	12-	16+	Not recorded	Total
Immature cells	6	5				4	15
Mixed or mature cells	8	6	1	2	2	1	20
Unidentified	1						1
Total	15	11	1	2	2	5	36

DISCUSSION.

From this study, it would appear that the disease of the cat, which has been termed lymphosarcoma, is characterised both by the formation of discrete tumours in various sites in the body and also widespread infiltration by tumour cells into organs and tissues. All forms of lymphoid cells were observed in the tumour lesions and it was not found possible to establish any relationship between the dominant tumour cell type, which tended to be relatively uniform throughout the body and the duration of the overt phase of the disease. In the majority of cases, the histological picture was one of uniform sheets of lymphoid cells, but in a few cases, features in the lymph nodes similar to those of giant follicular lymphoma were observed. In the cat, this may well prove to be a pre-lymphosarcomatous state as it often is in man.

Cases were subdivided on the basis of the patterns of the gross lesions in order to illustrate the differing manifestations of the disease. This is of some importance in differential diagnosis and in choosing the best tissue for biopsy. There was no evidence to suggest that the subdivisions revealed different entities and the condition was regarded simply as a single disease complex. Until the aetiology is clearly established and the pathogenesis worked out, there is no point in looking for subtle differences which might be thought to justify the use of the terms visceral lymphosarcoma, lymphatic leukaemia, or malignant lymphoma to describe one or other of the various manifestations of this disease.

The results of this investigation correspond closely with those published by the Boston workers. They are also in agreement with the obeervations of Cotchin.

As is the case in the dog, none of the diagnostic tests in current use permitsdiagnosis of lymphosarcoma at a stage when therapeutic or surgical measures could be expected to be of value in prolonging life. Once a palpable tumour develops or the characteristic symptoms begin, then this malignant disease has already reached a stage where it must inevitably prove fatal. Tests to determine the pre-sarcomatous or latent phase of the condition will have to be developed, but this will not be possible until much more is known of the pathogenesis of this disease.

PART TWO.

A PRELIMINARY REPORT ON TRANSMISSION EXPERIMENTS WITH LEUKAEMIA (LYMPHOSARCOMA) IN THE CAT.

INTRODUCTION.

As part of a long term research programme designed to test the hypothesis that lymphosarcoma in the domestic mammals is caused by an infective agent, cats, dogs, pigs and cattle have been used for transmission experiments.

One of the objectives has been to induce fast-growing tumours in susceptible animals of a given species by the inoculation of materials prepared from tumour tissues taken from spontaneous cases of the disease in the same species. Rapidly developing tumours would be harvested, processed and inoculated once more into susceptible animals. After one or more passages, the speed of growth of the tumours and the virulence of an infective agent might become enhanced whereby high titre filtrates could be obtained. When this stage was reached, controlled experiments could then be carried out to prove the hypothesis. In addition, the pathogenesis of the disease could be established in detail and serological and biochemical tests could be devised as early diagnostic markers.

During the course of such experiments, tumours would be examined in the electron microscope to ascertain if any virus, or virus-like particles, were present in the tumour cells which would also be grown artificially to find out if any cytological or cytopathic changes could be observed which might indicate that virus production or release was in progress.

Examinations would also be carried out to find any clinical or haematological markers which could indicate the onset of the experimental disease.

This is an interim report on a series of experiments in the cat which was begun in 1961 and is to be continued for several years.

MATERIALS AND METHODS.

Tumour tissues were harvested aseptically from cats with naturally occurring and experimental lymphosarcoma. Preparations from these tissues were inoculated into kittens during their first day of life. Such kittens were born at an isolated cattery, surrounded by double fencing, which was located some 12 miles north of Glasgow. They were housed and reared at these premises.

During the course of experiments, clinical and haematological examinations were carried out regularly. Haematological examinations were performed on free flowing blood from venepucture of one of the superficial veins on the external aspect of the pinna of the ear. Total erythrocyte and total leucocyte counts were carried out with an electronic particle counter as described in Part 3 and the initial 1:500 suspension of blood in phosphate buffered saline was prepared at the cattery from whole blood. The P.C.V. was estimated by the Hawksley microhaematocrit method, after Fisher (1962). Oxyhaemoglobin was measured by the method described by Bell et al (1945).

Thin blood films were made at the cattery and later stained with Leishman's stain in the laboratory. A differential leucocyte count was carried out after the method of Dacie (1963). A minimum of 400 white cells was counted on each film, but only on those where leucocyte morphology was excellent.

A group of adult male and female cats wwas collected in the early part of/

of the project and introduced into the cattery after a period of quarantine. These animals have been kept to provide successive litters of newborn kittens as free of disease as possible. No adult cat is now admitted to the cattery before undergoing a prolonged period of strict isolation and observation.

RESULTS.

At the time of writing, July, 1965, the preliminary results of two of the original experiments are available and certain results from subsequent experiments.

EXPERIMENT ONE.

This was an initiation experiment in which material was prepared from a field case of lymphosarcoma in a cat and subsequently inoculated into kittens less than 12 hours after they were born.

Materials and Methods.

The passage material was obtained from a female cat, aged 8.5 years, number 19377, which had a lesion: complex of a large anterior mediastinal mass in the position of the original thymus, multicentric lymph node involvement and splenic enlargement. This is one of the common patterns of the disease observed in this species. Lymphoblasts were the predominant cell type.

The mediastinal tumour mass was removed aseptically immediately after death and was stored for 5 days at -40°C. It was then divided into smaller pieces and stored for 66 days in 50% glycerol and 0.1 M phosphate buffered saline (pH = 6.8) at - 10°C. A 1/10 suspension in 0.1 M P.B.S. was prepared by/

by hand grinding in a mortar and this was centrifuged for 30 minutes at 2,000 x g. The supernatant was removed and stored for 24 hours at -40°C before use.

Each of 4 kittens in a litter was injected with 0.5 ml. of the supernatant subcutaneously over the ribs within 12 hours of birth. The animals were reared as a group.

Clinical Observations.

The kittens were numbered 19377/9, /10, /11 and /12. They grew well and were weaned at the age of seven weeks. When they were six months old, routine clinical and haematological examinations were begun and these were continued throughout the experiment.

Six months after inoculation, 9, 10 and 12 had readily palpable superficial lymph nodes and 9 and 12 had splenic enlargement. Shortly thereafter, occasional sneezing and coughing occurred in all four kittens and they developed diarrhoea; these signs persisted throughout the experiment. By the 7th month, kitten 9 had several pea-sized nodules in its abdomen and kitten 10 had an easily palpable spleen and a mass in the anterior abdomen which was estimated to measure 7 cm. long by 2 cm. in diameter. Number 11 had two small nodules in the position of the main mesenteric lymph nodes and 12 had a mass similar to, but more mobile than, that of kitten 10. These nodules continued to enlarge and the faeces remained loose. There were sporadic attacks of foetid diarrhoea.

In the ninth month, kitten 11 was found dead one morning without having previously/

previously shown any signs of acute disease. In the succeeding three months, the abdominal swellings and splenic enlargements in the remaining cats became more marked and again there were sporadic episodes of anorexia, severe diarrhoea and intermittent, transient pyrexia.

In the 12th month, kitten 9 was heard making crying sounds as if in pain; at this time a mass in the mesenteric lymph node area was clearly palpable. Within a few days, this animal was found dead. From then on, kittens 10 and 12 showed profuse diarrhoea. They were anorectic much of the time and lost weight.

In the 15th month, kitten 10 was considered to be in the terminal stage of lymphosarcoma. Diarrhoea, dehydration and anorexia had produced a thin animal. The main abdominal mass, the surrounding abdominal nodes and several of the superficial nodes could all be clearly delineated. Gross splenomegaly was present. In order to obtain fresh material for electron microscopy, further transmission experiments and tissue culture, kitten 10 was destroyed.

Over the following 3 months, the sole survivor, kitten 12, gradually lost bodily condition although it remained alert and interested in its surroundings. Its superficial lymph nodes were palpable and the mid-abdominal mass still persisted.

Eighteen months after the start of the experiment, kitten 12, which had by now become very thin, began to deteriorate rapidly. It refused food and fluids and was dying. It was destroyed.

Haematological Findings.

Normoblasts were found in varying numbers in many of the blood films, although there were no other indications of anaemia except for kitten 10 where a terminal anaemia was unaccompanied by a normoblast response. Anisocytosis was observed in some of the films, but there was no evidence of the presence of Eperythrozoan felis in the red cells.

Neutrophilia, > 15,000 neutrophils/cu.mm., was persistent in kitten 10, but was observed rarely in the others. None of the kittens showed a lymphocytosis, > 10,000 lymphocytes/cu.mm., or signs of subleukaemia. An occasional large lymphoid cell was identified which had some of the characteristics of a blast cell, but it was more differentiated than a modal lymphoblast. This was not indicative of leukaemia, since such cells can be found in small numbers in the peripheral blood of some healthy cats. Some of the blood cells of cat /10 are shown in Figures 51, 52, 53 and 54.

The Pathological Findings.

The Pathological Findings.

Post-mortem report on kitten 1937/9. This was the second member of the litter to die and it was 12 months old at the time of death. At the post-mortem examination, the mesenteric lymph nodes were enlarged, the main node being 8 cm. long and 1.5 cm. in diameter. The central part of this node was completely occupied by white tumour-like tissue and the medulla could only be discerned at its ends. The superficial, anterior mediastinal and para-aortic lymph nodes were moderately enlarged and were speckled red and white on their surfaces. In most nodes, the medulla could still be recognised/

recognised as a narrow central line. The spleen was enlarged, thickened, deep red in colour and had markedly hyperplastic Malpighian corpuscles protruding from its cut surface. The liver was pale, friable and showed intensive lobular delineation. The Peyer's Patches, especially in the lower part of the jejunum, were plaque-like, enlarged and soft.

The lesion which probably resulted in death was a severe necrotic pancreatitis with associated fat necrosis. Several small, yet enlarged, lymph nodes were clustered around the common bile duct. However, the duct was not occluded.

Histological examination confirmed the presence of necrotizing pancreatitis and there was also present a peculiar type of sub-acute cytolytic necrosis of the liver. This was characterised by a reduction in the numbers of hepatic cord cells with great increase in size of the surviving cells, many of which were multinucleated. In addition to this, there was marked infiltration of lymphoid cells into the portal triads and the sinusoids, Figure 55. The main mesenteric lymph node was similar in many respects to that of the donor cat from which the inoculum was derived. There was some replacement of the medulla by cords and sheets of cells extending from the cortex. In the cortex, several areas showed necrosis and cytolysis. Germinal follicles were only occasionally present and these were irregular and not surrounded by the usual rim of small lymphocytes. The spleen contained enlarged Malpighian bodies and the pulp was packed with cells, many of which were karyorrhectic. No abnormalities were found in either kidney.

The/

The plaque-like lesions taken from the small intestine consisted of a streaming out of lymphoid cells throughout the whole thickness of the mucosa. They had pushed aside and replaced the crypts. The lungs showed several perivascular aggregates of lymphoid cells.

Post-mortem report on kitten 1937/10. This animal was the third in the group to die and it was destroyed so that fresh tissues would be obtained for electron microscopy, transmission experiments and tissue culture work.

At the post-mortem examination this was found to be a typical case of advanced multicentric lymphosarcoma, Figures 56, 57, 58 and 59. All of the carcase lymph nodes were involved and were moderately enlarged, e.g. the submandibular and retropharyngeal lymph nodes each measured approximately 1.5 x 1.0 x 1.0 cm.

The main mass was a grossly enlarged and tumified mesenteric node which measured 12 cm. long and 3 cm. in diameter. On section, there was total loss of cortico-medullary differentiation and the entire node was filled with a highly cellular, pale yellow, homogenous tissue. Many other lymph nodes, which are not normally prominent in the mesentery, had become markedly enlarged. All the other abdominal lymph nodes were also enlarged and visibly involved in the tumour process. The spleen was grossly enlarged and extended to the mid-line behind the umbilicus, and was of that deep red-blue colour often observed in lymphosarcoma. It was 5 cm. wide and 0.9 cm. at its greatest width. On section the pulp was dominated by grossly enlarged, protruding, yet discrete, Malpighian bodies.

The Peyer's Patches throughout the small intestine were enlarged and prominent and 2 of them were developing into gross tumours; one lying 25 cm. and the other 40 cm. from the ileo-colonic junction. The liver was very pale in colour, but presented no macroscopical evidence of tumour infiltration.

There was a spherical mass, measuring 2.5 cm. in diameter, on the site of the original thymus in the anterior mediastinum and on section, this was found to be completely composed of tumour tissue. This may have been the thymus or one of the anterior mediastinal lymph nodes. The rest of the broncho-mediastinal nodes were also enlarged and tumified. Both tonsils were pale and translucent and they had emerged from their crypts. The marrow was pale pink, homogenous and it filled all the medullary cavities.

Histologically, all the lymph node sections revealed the presence of typical lymphosarcoma. The general architecture and cortico-medullary differentiation had disappeared in most cases and the nodes were packed with uniform sheets of extremely immature lymphoid cells, Figures 60 and 61. In a few sections, there was still evidence of medullary pegs in the centre of the nodes, but they were filled with typical neoplastic cells. Scattered here and there over the tumour cell sheets, were foci of medium and small lymphocytes which were thought to be vestigial follicles. Infiltration of the capsule and the tissue outwith the nodes was not a constant feature. Tumour foci were found scattered throughout the lungs, especially in association with the smaller blood vessels, Figure 62. The Peyer's Patches and the small intestinal tumours were characteristic of lesions of lymphosarcoma of the/

the alimentary walls, as observed in field cases. The trabeculae of the spleen were fairly well preserved in some areas, but in many others, tumour cells had diffused to the extent that the original red and white pulp could no longer be distinguished, Figure 63. In other parts, this was not so marked and grossly enlarged Malpighian bodies, moderately well defined, could be observed. Such foci were composed of tightly packed tumour cells with similar cells distributed lightly in the adjacent red pulp. The bone marrow was hyperplastic with numerous immature megakaryocytes as well has high numbers of cells which were in all respects identical to the tumour cells found in other sites, Figure 64.

The neoplastic cells showed some degree of pleomorphism and the mitotic rate was high. A typical tumour cell was round, oval, or polyhedral in outline, with a low cytoplasmic nuclear ratio. It had an openfaced nucleus and a very prominent pale nucleolus. The cell membrane was well defined and the cytoplasm was pale, somewhat polychromatophilic and faintly cloudy, with an occasional perinuclear halo. The cytoplasm varied in amount from scanty to moderate. The nucleus was round or oval with a sharply defined nuclear membrane. Chromatin particles were strung along a fine chromatin network throughout the pale nuclear sap and slightly larger chromatin blocks were marginated or else adherent to the nucleoli. These nucleoli were usually single, central or excentral in position, large, translucent, amphophilic and never obscured by a chromatin film, Figures 61 and 64.

Tissues from this animal were harvested aseptically as soon as the kitten

was/

was dead and were placed in sterile honey jars containing sterile N/10 P.B.S. These tissues were subsequently stored in 50% glycerol and N/10 P.B.S. and placed at either -40°C or else at - 70°C. Small amounts of the tissue were retained at + 4°C for 24 hours and then used for the preparation of tissue culture monolayers.

At the moment of death, tumour tissues were also prepared for examination in the electron microscope.

Post-mortem report on 19377/11. This was the first kitten in the litter to die and at the post-mortem examination, the mesenteric lymph node appeared as a chain some 7 cm. long and 2 cm. in diameter, Figure 65. It was greyish yellow in colour and only in one or two parts could the compressed medulla be discerned. Both anterior and posterior to this node, a chain of lymph nodes, which would probably not have been visible in a normal cat, were present. These were mottled pink and white and on section, were excessively cellular. The nodes of the internal iliac and para-aortic chain were also moderately enlarged and had the same appearance as the mesenteric node. The anterior sternal node was similar, but there was no large mass in the anterior mediastinum. The unusual lymph node features were also observed in the nodes of the head. The spleen was grossly enlarged and extended to the midline. It was thicker than usual and its surface was mottled with miliary dark red and white spots. On section, numerous dark red infarct-like areas could be seen, whilst the Malpighian bodies were excessively prominent.

Histologically, the mesenteric node showed much replacement of the normal architecture/

architecture with medium sized lymphocytes, Figure 66. Germinal centres were present, but they were scanty and irregular. Infiltration of the capsule and extension into the surrounding mesentery was observed. The medulla was considerably encroached upon by sheets of cells growing down from the cortex. The liver showed multiple small areas of lymphocyte accumulations within the portal triads. In the small intestine, the Peyer's Patches were abnormal and penetrated laterally and upwards into the mucosa, displacing and replacing the crypts. The lungs presented multiple small lymphocyte foci in the perivascular interstia in some fields.

Post-mortem report on 19377/12. This was the last of the 4 kittens to die, and it was in extremely poor bodily condition at the time of death.

All of the carcase lymph nodes were slightly enlarged and many of them were congested. Lymph node aggregates which are seldom observed, such as the cervical chain and those within the rectal mesentery, were clearly visible. Many of them were congested. The main mesenteric nodes measured 4 x 2 x 1.5 cm. It was only slightly congested and the follicles were visible from without.

Only remnants of the original thymus were found in the anterior mediastinum. The heart and lungs showed no gross abnormalities. The gastro-intestinal canal was empty and the walls of the small intestine were thickened and the mucous membrane was thrown into folds. There were small haemorrhages in the submucosa. The walls of the large intestine were also thickened and occasional petechial haemorrhages were observed throughout its entire length.

The/

The liver was of a pale bronze colour. It was firm on section and not unduly friable. The spleen was not enlarged, but it had that greyish red colour which is often associated with lymphosarcoma. On section, the Malpighian bodies were hyperplastic and could be clearly identified. The tonsils were not abnormal. The bone marrow was deeply haemorrhagic throughout all the marrow cavities. No other gross lesions were observed.

Histologically, there was some degree of variation in the lymph node sections. In the mesenteric node, the cortex and medulla remained quite separate and there was no sign of infiltration into the medulla. The most noteable feature was the outer border of the cortex which was largely replaced by cells of the large lymphocyte and lymphoblast types, Figure 67. These cells were pale and only faintly basophilic and many exhibited a prominent pale nucleolus. Germinal follicles were only apparent in some parts of the cortex. One of the submaxillary nodes was not abnormal, but the other showed areas of the cortex where anaplastic cells, not unlike those found in 1937/10, were observed. The retrophyngeal nodes presented many areas of lymphoblast sheets containing no lymphocytes. Such fields were indistinguishable from lymphosarcoma, but the anatomical arrangement of the cortex and medulla persisted. Erythrophagocytosis was very prominent as well as congestion of the medulla. One of the prescapular lymph nodes was highly suggestive in that many areas of the cortex were replaced almost entirely by sheets of lymphoblasts of varying sizes. These cells all had prominent, usually single, nucleoli. This pattern has not been observed in the/

the lymph nodes of healthy cats. However, the cortex and medulla were again clearly differentiated and there was no infiltration of the cells outwith the node. Marked congestion and erythrophagocytosis were the outstanding features of the nodes of the cervical chain.

Scattered foci of lymphoid cells were found in the anterior mediastinal fat and these were thought to be remnants of the former thymus.

The spleen presented a marked feature in the Malpighian bodies. Sheets and buds of lymphoblasts were found at the periphery of many of the follicles. These areas were quite well defined and in some follicles had almost replaced the former follicular architecture. Slight infiltration of blast cells into the red pulp was apparently under way, Figure 68. The tonsillar lymphoid tissue was not abnormal, but the overlying epithelium showed some degree of hyperplasia.

Throughout the entire length of the gastro-intestinal tract there were catarrhal changes present. Dead neutrophils and cellular debris had distended many of the glandular crypts. Some of the lining glandular epithelial cells were less highly differentiated than usual. These lesions were most marked along the terminal portion of the small intestine, along the large intestine and the rectum. In the latter, there were haemorrhages into the lamina propria, an increase in lymphoid cells and occasional focus of neutrophils. The epithelium of the crypts in this region contained many rather anaplastic cells. However, there were no changes which indicated that lymphosarcoma was developing here. In the marrow, there were many cells resembling those found in the marrow

of/

of 19377/10, including multinucleated immature megakaryocytes and also large single nucleated cells with very prominent nucleoli and chromatin particles condensed onto sharply outlined nuclear membranes. Cells of the erythroid and myeloid series were less prominent than in the bone marrow of healthy animals.

The Fine Structure of the Tumour Cells from 19377/10.

Small pieces of tissue from the mesenteric mass, the spleen and the "thymic" mass were prepared for examination in the electron microscope. Portions of tissue, approximately 2 mm. in diameter, were chopped up and fixed in a 1% solution of "Zetterquist" isotonic buffered osmium tetroxide (Glauert, 1961) at +4°C for 45 minutes. The tissues were then washed 3 times in distilled water before being dehydrated in ascending grades of alcohol. They were placed in 70% alcohol for 10 minutes, for a further 15 minutes in 90% alcohol and then placed in absolute alcohol. The absolute alcohol was changed after 15 minutes. After a further 15 minutes, the tissues were embedded in prepolymerised methacrylate (Borysko and Sapranauskas, 1954). Ultrathin sections were cut on a Cambridge Ultramicrotome and mounted on "Formvar" covered grids and stained with lead (Millonig, 1961) and uranyl acetate (Watson, 1958).

In low powered electron photomicrographs, the tumour cells bore a marked resemblance to those observed in the light microscope under oil, Figure 69. The main cell type in the mesenteric lymph node had a large nucleus with marginated chromatin and a large distinct dense nucleolus. The nucleus filled most/

most of the cell and in the cytoplasm of many cells, there was a highly vesiculated area. This region was only observed in a proportion of cells in any one section and it was within such vesicles, that virus-like particles were found. These particles were enclosed within the vesicles, often in groups and were not apparently free in the cytoplasm or in the intercellular spaces.

The particles consisted of an electron dense nucleoid enclosed in a densely stained double membrane. The outer diameter of the nucleoid measured approximately 60mμ and the full particle measured around 100mμ, Figure 70.

Virus-like Particles in Monolayers from 19377/10.

Tissue culture monolayers were established by trypsinising chopped up portions of the fresh mesenteric tumour mass until the cells were sufficiently dispersed for them to be made into a suspension, centrifuged and then re-suspended in a nutrient medium; Eagle's medium plus 10% calf serum and 10% tryptose phosphate broth was used. The cell suspension was then transferred into 4 oz. sterile bottles, gassed with CO_2 and placed in an incubator. The cells were examined daily with an inverted microscope to determine if they were settling onto the glass and multiplying. When a confluent cell sheet was almost established, the cells were again trypsinised and resuspended in nutrient medium. The suspension was divided and used to seed fresh sterile 4 oz. bottles.

At the second passage, when the cells had been cultured for approximately one month, they were scraped from the glass, centrifuged lightly and prepared for/

for the electron microscope as described above. Large numbers of virus-like particles, identical to those found in sections from the actual tumour mass, were seen in the cultured cells. The cell membranes were not intact and the cells appeared to be disrupted.

The monolayers soon began to show some evidence of cell death and shortly afterwards, the monolayers in all of the bottles became fibroblastic and died off.

Subsequent and repeated attempts to recover viable tumour cells from the tumour tissues which had been stored at -40°C and -70°C were made, but it was not found possible to grow monolayers from the stored material.

EXPERIMENT TWO.

This was a further initiation experiment in which material from a different field case of lymphosarcoma in a cat was inoculated into newborn kittens.

Materials and Methods.

The inoculum was prepared from tumour tissue derived aseptically from cat number 17457, which was a multicentric and thymic case of spontaneous lymphosarcoma. Part of an affected para-aortic lymph node was processed exactly as in the previous experiment, except that the supernatant was thawed at 37°C and then re-frozen at -40°C one week before the inoculum was first used.

Seven kittens from 3 litters were injected with 0.5 ml. of the supernatant subcutaneously over the ribs. Routine clinical and haematological examinations were carried out as in experiment one.

The Clinical Findings.

The kittens were numbered 17457/1, /2, /3, /4, /5, /6 and /7. Up to the time they were 18 months of age, the kittens were in good health and condition. At this time, kitten 2 developed palpable enlargements of its superficial lymph nodes and a biopsy of the right prescapular lymph node was carried out. The node measured 2.0 x 0.75 x 0.75 cm. Four weeks after this operation, a similar mass had reformed at the site of the biopsy, although there had been no post-operative sequelae. Around the 22nd month of life, diarrhoea developed and thereafter this cat lost condition. One month later, euthanasia was carried out because the animal was dying. It was 23 months old.

The superficial lymph nodes of cat 1 were found to be prominent soon after this. There was evidence of a palpable mass in the mid-abdomen and the spleen was also palpable. During the following weeks, vomiting started and increased in frequency. The animal stopped eating and became dull and dehydrated. Antibiotic and fluid therapy failed to arrest the decline and the animal died at the age of 29 months.

Cat 5 had been in good health until shortly before cat 1 died when it developed mild upper respiratory symptoms. These were relieved following a course of anti-biotics. However, this cat gradually lost condition and at the age of 43 months, became dull and anorexic with a palpable mass, which could be readily identified, in the mid-abdomen. The cat was destroyed at this stage.

The remaining cats, 17457/3, /4, /6 and /7, are still fit and well in July/

July, 1965, although they show a mild disease of the upper respiratory system which is cyclical in nature. This does not interfere with appetite or bodily condition.

The Haematological Findings.

In none of the cats in this experiment was anaemia found as indicated by the P.C.V. and total erythrocyte counts. Normoblasts were found in the peripheral blood of cat 1 on two occasions during the final 5 months of life. These cells were also observed in blood films from cat 2 on 4 occasions, in two of which they represented more than 3% of the nucleated cells of the film. Normoblasts have been absent from the blood films of the other animals.

True leukaemia has not developed and significant rises in the total leucocyte counts have not been common. However, in cat 2 there was an absolute lymphocytosis on 5 occasions, but this occurred in an early phase of the experiment. The final leucocyte count in this cat showed a neutrophilia. Although cat 1 did not ever have an absolute leucocytosis, it did register an absolute lymphocytosis of 22,000/cu.mm. on one occasion about 22 months before it died. Cat 5 never had an absolute lymphocytosis, but there was neutrophilia on the day of death. The rest of the animals in the experiment have not shown gross changes in their leucocyte pictures.

The Pathological Findings.

The post-mortem report on cat 17457/1. This cat was in poor bodily condition at the time of death. There was no food present in the stomach. Apart from some thickening of the walls of the intestines no other gross macroscopical lesions were observed in the carcase. The marrow cavities were completely filled with a red cellular tissue.

Histologically, the only lesion of importance was a well developed chronic enteritis. Many of the glands of the mucous membrane showed thinning of the epithelium and blocking of the crypts by debris and dead cells. Other glands showed bizarre hyperplasia. The ratio between the lamina propria.and glands was disturbed, there being many more cells than normal in the lamina propria. These comprised active macrophages and cells of both the lymphoid and plasma cell series. There were, in addition, lines of infiltration of lymphocytes into the submucosa. This did not appear to be lymphosarcomatous. The associated lymph nodes showed no encroachment by the cortex into the medulla. However, the cortical follicular pattern was obscured and the predominant cell was a mature lymphocyte. No abnormality of the spleen was found. The bone marrow in sections was lace-like with well developed foci of erythroid and myeloid activity. A few moderately well differentiated megakaryocytes were present.

Lymph node biopsy from 17457/2. This node measured 2.0 x 0.75 x 0.75 cm. On section it was white, cellular and bulging. There was a trace of pink cortex ventrally and a small central area of medulla was still apparent.

Histologically, there was only slight loss in typical architecture and the cortico-medullary differentiation was preserved, although the junctions between cortex and medulla were poorly defined and the cortico-medullary ratio was much higher than in a normal superficial lymph node of the cat. The capsule, trabeculae and extra-capsular areas were well defined and intact. In the cortex, therewere moderate condensation of the follicles and an absence/

absence of germinal centres. The follicles were surrounded by masses of relatively uniform lymphoid cells of a mature type, but less differentiated than those within the follicles. These cell streams had flooded the sub-capsular and cortical sinuses and towards one pole, this was particularly obvious. Here, the cortical sinuses were packed and distended with cells which were streaming down into the medulla to flood the medullary sinuses. However, the medullary pegs were intact and they contained a typical cell population. The rate of mitosis varied.

Here and there within the cell sheets, were quite large blast-like cells with very bold nucleoli. Such cells can be observed on occasions in the nodes of healthy cats, but they were much more numerous in this section, especially towards the cortico-medullary junctions.

Post-mortem report on cat 17457/2. This cat was in very poor bodily condition at the time of death and the carcase was pale. In the thoracic cavity, there was a small amount of clear fluid and discrete, small, glistening, round or stellate, slightly raised nodules up to 0.6 cm. in diameter were found over the entire pleura, both the visceral and parietal parts. The lung fields, heart muscle and thoracic lymph nodes were not affected macroscopically.

Similar, although smaller, nodules were observed on the parietal and visceral peritoneum, especially on the mesentery and the omentum. The liver was light brown with rounded edges. It too was covered with multiple small nodules./

nodules. The kidneys presented a few subcapsular cream-coloured foci which were slightly raised, but not adherent to the capsules. The spleen measured 10.0 x 3.0 x 0.5 cm. It was pale pink and on section, there was hyperplasia of the Malpighian bodies. The alimentary tract was not grossly altered except in the region of the ileo-colonic junction, where there was a marked increase in the thickness of the wall of the intestine to form a well defined mass, measuring approximately 5.0 cm. long and 2.5 cm. in diameter. On section, the lumen of the intestine was found to be patent, but much narrower in diameter than usual.

The superficial lymph nodes were prominent and enlarged to rather more than twice their normal size. The mesenteric node was moderately enlarged, being 5.5 x 1.5 x 1.5 cm. The cervical lymph node chain was prominent, although only the posterior cervical nodes would have been palpable in life. Sections of these nodes revealed an increase in the relative widths of the cortices which were highly cellular, pale straw coloured and bulging.

The femoral marrow was pale red, homogenous and it filled the medullary cavities.

Histologically, the intestinal mass was composed of sheets of blast-like lymphoid cells which had grossly distended the submucosa, Figure 71 and spread into the mucous membrane, causing marked distortion and destruction of the glandular elements. In one or two areas there were sheets of cells indistinguishable from reticulo-endothelial cells. The rates of mitosis were moderate. The adjacent muscle layers of the mass were not infiltrated to any extent.

The small nodules on the pleura, peritoneum and liver were composed of foci of mesenchymal cells showing some degree of pleomorphism and a low mitotic rate. The superficial foci on the kidneys were lymphoid. The liver and kidneys, apart from those superficial foci, were not otherwise affected.

Sections of the lymph nodes showed fairly marked reticulo-endothelial hyperplasia, especially within the sinuses. There was reduction in the follicular pattern and only a few germinal centres were noted. These contained higher amounts of reticulo-endothelial and blast cells than usual. The nodes were somewhat blurred in appearance by streams of lymphoid cells, often with a high reticulo-endothelial component, which were flooding the subcapsular and cortical sinuses and flowing into the medullary sinuses, Figure 72. The medullary pegs were still fairly intact. The Malpighian bodies in the spleen were distended and comprised of uniform immature lymphoid cells. The corpuscles were well defined, but there was evidence of infiltration of the immature lymphoid cells into the red pulp. The bone marrow showed signs of hyperplasia and there were large numbers of immature megakaryocytes present, some of which contained phagocytosed neutrophils.

Samples of tissue from the pleura, peritoneum, lymph nodes and alimentary tract were submitted to the Department of Bacteriology at Ruchill Hospital, Glasgow. A detailed investigation was carried out there, but this material failed to reveal the presence of acid-fast bacilli.

Post-mortem examination of 17457/5. This cat was in poor bodily condition at the time of death. There was no food in the stomach or small intestine, but there was some evidence of scanty faeces in the terminal portion of the large intestine.

On opening the abdominal cavity, a small quantity of intestinal contents was found. This had escaped from a penetrating ulcer in the wall of the large intestine close to the ileo-colonic valve. The terminal 2 cm. of the ileum and the first 6 cm. of the colon were ballooned and rather thin-walled and especially in the immediate area of the ileo-colonic junction, there were several frank ulcers of the mucous membrane. The associated lymph nodes were enlarged and appeared reactive. However, the main mesenteric lymph node was moderately enlarged and appeared to be tumified. It was removed as aseptically as possible under the circumstances and after being well washed in sterile P.B.S., was sectioned. The tissue within was typical of lymphosarcoma.

No gross lesions were found elsewhere throughout the body, but the marrow was distinctly hyperplastic.

The ulcerative lesions of the mucous membrane of the colon were caused by streams of destructive, partially undifferentiated lymphoid cells, which were streaming from the submucosal lymphoid centres to disrupt the lamina propria and to flood and distend the inter-glandular spaces and then destroy the superficial epithelium and glandular elements. These lesions were relatively discrete and the mucous membrane and submucosa on either side was free from undue cellularity. This highly abnormal picture was indicative of lymphosarcoma.

SUBSEQUENT EXPERIMENTS.

A further large number of newborn kittens have now been inoculated with preparations of tumour tissues from several field cases of feline lymphosarcoma during/

during the last 24 months. The majority of these animals are less than 18 months of age and none has shown evidence of clinical lymphosarcoma to date. One of the kittens, which was injected with a preparation similar to the one used in experiment 1 and from the same source, showed some evidences of changes in its lymph nodes at death which indicated an early stage of the disease, Figures 73 and 74.

Four other kittens from 2 litters, which were inoculated with preparations from the tumour tissues of experimental cat 19377/10, have died and shown histological evidence which is not incompatible with a diagnosis of lymphosarcoma. One of the kittens died at the age of 2 months with extreme anaemia. All of its marrow cavities were filled with a pale straw coloured and highly cellular tissue which was composed of sheets of cells containing large numbers of lymphoblasts and stem cells, Figures 75 and 76. Another member of the same litter died at the age of 16 months from multiple miliary acute abscesses in the liver. The spleen was free from signs of infection, but the splenic follicles were pale and hyperplastic. Histologically, they contained large numbers of relatively uniform blast-like cells, Figures 77, 78 and 79. This follicular pattern is in striking contrast to ordinary reactive Malpighian corpuscles which often have secondary centres containing all types of lymphoid cells together with macrophages and stem cells. Particles similar to those observed in the tissues of 19377/10, the donor cat, were found in the spleen of this kitten when cells were examined in the electron microscope.

From another litter in the same experiment, one kitten died at the age of/

of 2 months with generalised moderate lymphadenopathy and marked hyperplasia of the bone marrow. In imprint smears of the marrow, large numbers of immature cells with large, pale, prominent and single nucleoli were observed. These cells were similar to lymphoblasts. There was little evidence of erythropoiesis and neutrophils and their precursors were virtually absent. A further kitten from this litter, which died at the age of 16 months, was found to have hyperplasia of the Malpighian corpuscles, Figure 80.

A feature of the animals in those experiments has been the presence of normoblasts in the peripheral blood. These cells have been observed in high numbers, but it is still too early to establish a correlation between this phenomenon and the development of experimental lymphosarcoma.

No signs of Eperythrozoan felis have been found in the blood films of any of the cats in the experimental cattery. Pooled blood from two groups of experimental cats, which was injected subcutaneously into disease free splenectomised cats approximately 14 days after removal of the spleen, failed to induce this form of parasitic anaemia.

DISCUSSION.

The findings in experiment 1, may indicate the transmission of a spontaneous multicentric lymphosarcoma, though it is impossible to be completely sure that the inoculum did not contain a few living cells which survived freezing and homogenisation. Filtration and high speed centrifugation were not used in this initial experiment as it was thought that they might decrease the titre of any infective agent which might be present. No tumours developed at/

at the sites of inoculation and the regional lymph nodes did not differ either in speed of enlargement or in size from the other nodes. No relationship was known or likely between the donor cat and the recipient kittens. The pattern of the disease produced was identical to that seen in many field cases, i.e. multicentric lymphoid neoplasia with the largest tumour mass present in the main mesenteric node. It will be recalled that 2 common lesion complexes in the cat are one in which the largest tumour mass is in the mesenteric lymph node and one in which the largest mass lies in the anterior mediastinum, possibly originating in the thymus. It is interesting that the source material in the experiment was derived from a case with a "thymic" mass, but the main lesions in the recipients were mesenteric.

It was unfortunate that the first two cases, 19377/11 and /9, died during the course of the experiment before they developed an obviously fatal lymphosarcoma. However, the lesions indicated that lymphosarcoma was moderately well developed at the time of death. The erythrophagocytosis and hepatocellular hyperplasia which was observed may be part of the syndrome or may represent some other disease process as yet unrecognised in the cat and which does not correspond to any known condition.

Cat 19377/10 which showed the advanced lesions was clinically similar to the others except in the final month before death, when it developed a marked enlargement of the mesenteric lymph node.

The neoplastic cell was of the stem cell type and it was possible that the/

the final rapid deterioration in cat 19377/10 was caused by the sudden proliferation of this cell type in which the virus-like particles were found. There was, of course, no evidence of any aetiological relationship between the observed particles and the tumour. However, it can be accepted that these particles were associated with the neoplastic cells and that the similarity in fine structure between these particles and the viruses of the murine leukaemias is striking (Dalton, 1962). No particles were observed in the lymph nodes of 14 apparently normal cats which were examined in the electron microscope.

The presence of normoblasts in the peripheral blood of a non-anaemic cat is not unusual where there is evidence of marked hyperplasia of the bone marrow associated with a leucocytosis, but it is exceptional to find these cells in blood films where the haemogram is otherwise normal.

There was considerable variation in the morphology of the lymphoid cells in the films, but no indications that leukaemia was present or was developing.

In experiment 2, the cause of death in cat 17457/1 was presumably chronic enteritis. The biopsy of the prescapular lymph nodes of 17457/2 presented certain features which were highly suspicious of the presence of early lymphosarcoma, but a firm diagnosis was not possible. In this cat, the disease which was discovered at the post-mortem examination was not one which has been recorded in the cat under field conditions. It appeared to be a tumour composed of foci and sheets of lymphoblasts as well as foci of mesenchymal cells.

It has been observed in experimental fowl and murine leukaemias that the causal agents are capable of producing strikingly different types of tumour according to the experimental conditions imposed, e.g. osteopetrosis in the fowl is accompanied in many experiments by a high incidence of nephroblastomas (Wilm's tumours) which appear to be inspired by the same virus which results in osteopetrosis in most cases. Again, thymectomised day old mice develop myeloid leukaemia when they are inoculated with a virus which causes typical lymphatic leukaemia in non-thymectomised and sham-thymectomised day-old mice of the same isogenic strain, Gross (1961).

Initiation experiments with preparations from spontaneous tumour tissues from field cases of lymphosarcoma in the cat which lead to malignant diseases of quite unexpected character are possibilities which must be recognised.

The intestinal ulcers in cat 17457/5 were typical of early lymphosarcoma. It is interesting to note that these lesions were developing on the same site as the intestinal tumour mass in cat 2.

The latent period of cat 5 makes it quite possible that other cats in this experiment may yet develop lymphosarcoma and so this experiment will continue, if necessary, for several more years.

It is, as yet, too early in the project to be certain that the histological changes in the spleen are the primary stages in the development of the disease in the passage experiments, using preparations from the experimental case, 19377/10. However, these changes are abnormal and they do resemble, to some/

some extent, lesions in this organ which are known to be associated with lymphosarcoma in naturally occurring cases in cats. In one of the kittens, the presence of particles which were similar to those found in association with 19377/10, may well prove to be another important observation in this research programme. There is also little doubt that the kitten whose marrow contained high numbers of blast and stem cells did die from a disease which is indistinguishable from lymphosarcoma.

The results of the experiments are certainly far from discouraging for there is now some evidence that the disease has been transmitted from spontaneous cases to experimental animals on two occasions. There is also some evidence that tissues recovered from one of the experimental cats have been used to induce early lymphosarcoma in other experiments. A virus-like particle has been observed in association with tumour cells in 2 cats and the particle was identical to one known to cause leukaemia in mice.

It took around 25 years of intensive studies and the production of isogenic strains of mice before the viral hypothesis of the aetiology of leukaemia in mice was proved beyond doubt by Gross (1951). It would, therefore, be surprising if it was found to be a simple matter to transmit lymphosarcoma from one cat to another, since the problems are probably far greater. For one thing, it is not possible to establish isogenic strains of cats at present.

Further progress in the cat studies will continue to be slow until reasonable quantities of tumour tissue can be induced to grow under experimental conditions. Modern techniques in Virology are at a stage when, once this was possible, the problems of leukaemia in the cat might well be solved within a very short time.

In those cats in which lymphosarcoma was found or suspected, there was an unusual haematological picture. Circulating normoblasts were observed in varying numbers, but not in association with a frank anaemia. It may well be that this was a well compensated haemolytic anaemia, but no marked deposits of haemosiderin in the tissues was found at the post-mortem examinations. On the other hand, circulating normoblasts are frequently found in the non-anaemic dog suffering from multicentric lymphosarcoma. This might prove to be a mild leukaemic response in the erythroid cell series in both species.

There were two valuable clinical observations made in these experiments. Firstly, there was the sudden development of a large tumour mass in the abdomen of kitten 19377/10 together with the rapid deterioration in the animal's general condition. This was a dramatic change over a period of 4 weeks, and indicated that the observations by many owners that this disease is sudden in onset may prove to be accurate. Secondly, a most characteristic syndrome was observed in some of the later kittens. They lost condition slowly over an interval of some weeks to become markedly emaciated. In the United Kingdom, this is a most rare syndrome to find in young cats which are free from a very heavy burden of internal parasites.

PART THREE.

THE CALIBRATION OF AN ELECTRONIC PARTICLE COUNTER FOR TOTAL ERYTHROCYTE AND TOTAL LEUCOCYTE COUNTS OF CAT BLOOD.

INTRODUCTION.

During the last decade, electronic particle counters have been devised which are capable of counting small particles suspended in a fluid medium. In comparison to the traditional methods of estimating the concentration of particles per unit volume, the electronic method offers certain advantages. It is rapid and accurate and demands the minimum of technical skill. In addition, since very large numbers of particles can be counted per estimation, it leads to a very marked reduction in the field error.

Electronic counters have been described and calibrated for human erythrocytes by Brecher et al (1956), for human leucocytes by Richer & Breakell (1959) and for the erythrocytes of the pig, sheep and cattle by Weide et al (1962). No reports have been published on the blood cells of the cat.

An electronic particle counter was evaluated as part of a programme to ascertain if haematological markers were present in the blood of cases of experimental lymphosarcoma in the cat.

THE PRINCIPLES OF AN ELECTRONIC PARTICLE COUNTER.

An electronic particle counter is an instrument designed to record changes in resistance to a steady electric current which passes between two platinum electrodes immersed in an electrolyte solution. The sole pathway for this current is through a small opening placed in a solid glass barrier between the electrodes. As a suspension of poor conducting particles, such as blood cells, in a good conducting fluid medium passes at a steady rate through/

through the orifice, an intermittent change in resistance to the current develops whenever one of the particles negotiates the aperture. The voltage pulse which is produced is directly proportional both to the volume of the particle and the current. A particle which is twice the volume of its neighbour will cause twice the voltage change, and halving the current will lead to a 50% fall in the voltage pulse for the same particle. All particles, including small particulate material in the suspension, produce voltage changes of varying sizes as they pass through the orifice, and thus a threshhold has to be determined to distinguish those particles which are to be included in the actual count from background debris which has to be screened off.

THE COULTER COUNTER, MODEL "D".

This instrument was designed specifically for total erythrocyte and total leucocyte counts of human blood. The diameter of the aperture is 100 μ. This must be not less than 4 - 5 times the maximum diameter of the particles to be counted. In this instrument it is possible to pre-set a threshold for both the red cells and the white cells of the blood. The counts are registered on a decade counter and the voltage pulses can be observed on an oscilloscope screen. Cell suspensions in a beaker are mounted on a spring platform below the orifice tube. The actual orifice and the external electrode must both be immersed completely in the suspension of particles at every count.

During operations, there are certain observations which enable a trained technician to judge the accuracy of a given count. Probably the most important indication of accuracy is given by the characteristic cadence of the instrument.

If/

If this alters in tone or speed then the count is being interrupted for some reason. Another indicator is the pattern of voltage pulses on the oscilloscope screen. Interruptions of the count or abnormal particles in the suspensions are readily recognised on this screen. Blockages of the orifice may be total or incomplete and this can be checked directly by means of a microscope which can be focused on to the aperture. The mean interval of a count is roughly 20 seconds. If this period lengthens, the accuracy of the count is questionable. Finally, since it takes only a matter of seconds for an individual count, replicate counts from the same suspension may be employed to estimate accuracy.

MANUFACTURERS INSTRUCTIONS.

An electrolyte solution, either saline or phosphate buffered saline (P.B.S.), is filtered through a 5" diameter sintered glass filter to ensure a background count which is not much greater than 200-250. Any substantially higher count must be subtracted from the total blood cell count.

Two samples of 20 ml. filtered P.B.S. are placed into two suitable beakers. 0.04 ml. of well mixed blood is drawn into a pipette which is then carefully wiped clean. The tip of the pipette is placed into the solution in the first beaker and the blood is slowly blown towards the bottom. The pipette is then gently washed out with the clear solution at the top. The beaker is stoppered and the suspension thoroughly mixed by gentle agitation to produce a 1:500 dilution of the original blood (Strictly 1:501). 0.2 ml. of this suspension is withdrawn and transferred to the second beaker and a similar technique is employed to create a 1:50,000 dilution.

To carry out a total leucocyte count, 0.2 ml. of a 2% saponin solution is added to the 1:500 suspension. Specially purified and tested saponin is supplied for this purpose by the manufacturer and this results in haemolysis of the erythrocytes in the suspension without immediate damage to the white cells. After approximately 10 minutes, the red cell debris has become very finely divided and the count can be carried out. The beaker is now gently agitated to ensure complete uniformity of the suspension, but care must be taken to ensure that undue froth does not develop which would produce false high counts. The count can now be carried out at the appropriate threshold setting. The actual result on the decade counter is the equivalent of the number of leucocytes per cubic millimetre, since the instrument counts 0.5 ml. = 500 cu.mm. of a 1:500 dilution. The background electrolyte count must be subtracted from this result if it is high enough to influence the total count.

Erythrocyte counts are carried out at the appropriate instrument settings with the 1:50,000 dilution. Both red and white cells are present in this suspension, but the white cells can be ignored unless the total leucocyte count is high and will influence the total erythrocyte count. Again, the background count must be determined to ensure that it does not also influence the cell count. Any subtractions for these reasons should be carried out after the red cell count has been adjusted for coincidence loss (see below), but before the result is multiplied by 100 to correct for the factor of dilution.

COINCIDENCE LOSS.

As the number of particles traversing the orifice per 0.5 ml. aliquot rises,/

rises, a point is reached where the instrument occasionally records only one cell when two or more pass through the orifice simultaneously. This results in a decade counter reading which is lower than the true number of cells which passed through. These cell groups occur in samples of 2, 3, 4 and so on in a Poisson distribution. Charts are provided by the manufacturers giving corrected counts above 10,000 on the decade counter. This means that almost every total erythrocyte count must be corrected as well as all leucocyte counts over 10,000.

THE FACTORS OF IMPORTANCE IN CALIBRATING AN ELECTRONIC COUNTER.

The makers' instructions are based on studies of human blood cells and it is necessary to ensure that the instrument is also accurate for blood cell counts on species whose blood cells are not identical in volume.

Five factors have to be considered when calibrating the instrument, 1) the apparatus itself should be precise enough to ensure repeatability of counts from the same suspension, 2) optimal aperture currents and thresholds must be ascertained for the blood cells of the species under study, 3) the electronic method must be compared with an established method, 4) the instrument must be shown to be reliable and 5) the apparatus should be accurate over a predicted range.

CALIBRATION EXPERIMENTS FOR TOTAL ERYTHROCYTE COUNTS.

EXPERIMENT I.

The Repeatability of the Instrument.

This was a preliminary test to find out if the instrument was able to record consistent counts from the same suspension of particles employing the maker's/

maker's estimations regarding aperture current and threshold settings for feline blood cells.

Materials and methods. The site of venepuncture was one of the superficial ear veins which lies along the external border of the pinna of the ear of the cat. The hair over a vein was carefully shaved with a dry razor blade and the site was thoroughly cleaned with absolute alcohol and allowed to dry, before a small transverse section was made through the raised vein. Once the blood was flowing freely and this might have taken 30-60 seconds, the vein was compressed again to ensure a continuing flow. Thirteen cats were bled in this fashion and in each case, a 1:500 dilution of blood in F.B.S. was prepared with a 0.04 ml. pipette and 20 ml. F.B.S. with a known low background count. From every 1:500 suspension, 0.2 ml. was transferred into 20 ml. F.B.S. to make a final dilution of 1:50,000.

Each of the 13 suspensions was counted in the instrument 6 times, a total of 78 separate counts. The first 3 counts from each sample were carried out in succession and in a series from cat 1 to cat 13. The remaining counts, 39 in all, were performed in random order.

Results and conclusions.

Table 72 gives the 6 counts per sample, the means and the coefficients of variation. The V varied from 0.61% to 2.04%, indicating that the instrument showed enough precision to merit a more detailed calibration. From these results there was no evidence that marked differences were found between successive counts and those which were carried out in random order.

TABLE 72.

Experiment 1: Replicate Total Erythrocyte Counts of Blood from Different Cats to Illustrate the Repeatability of the Instrument.

Replicate counts, x 10^6

		1st	2nd	3rd	4th	5th	6th	Mean	S.D.	C.V.
Cat	1	8.69	8.66	8.71	8.60	8.58	8.59	8.64	± 0.0557	0.64
	2	7.39	7.36	7.36	7.27	7.23	7.31	7.32	± 0.0781	1.07
	3	8.19	8.29	8.25	8.10	8.05	8.00	8.15	± 0.1149	1.41
	4	7.81	7.81	7.81	7.77	7.62	7.67	7.75	± 0.0831	1.07
	5	9.87	9.99	9.90	9.77	9.74	9.86	9.86	± 0.0906	0.92
	6	8.49	8.62	8.52	8.49	8.48	8.39	8.50	± 0.0742	0.87
	7	7.23	7.18	7.19	7.10	7.08	7.15	7.16	± 0.05745	0.80
	8	8.66	8.77	8.79	8.44	8.36	8.66	8.61	± 0.1758	2.04
	9	6.99	6.96	6.96	6.88	6.68	6.91	6.90	± 0.1131	1.64
	10	5.68	5.59	5.64	5.63	5.68	5.66	5.65	± 0.0346	0.61
	12	5.21	5.26	5.25	5.23	5.25	5.35	5.26	± 0.04796	0.91
	13	8.67	8.60	8.63	8.52	8.37	8.52	8.55	± 0.1072	1.25

Background count 250.

EXPERIMENT 2.

The Optimal Aperture Current and Threshold Setting for Total Erythrocyte Counts.

Materials and methods. Accurate initial dilutions of blood were not necessary in this experiment since the volumes of the cells were important rather than their total numbers. The technique used was such that the concentration of the blood cells would be within the normal range.

Approximately 2 ml. samples of blood were withdrawn from the right cephalic veins of 12 cats which were free from signs of clinical disease. Sterile disposable clear plastic syringes were used with 25G x 5/8th inch sterile disposable hypodermic needles. These syringes and needles were washed out immediately beforehand with a solution of 3% W/V of EDTA in 0.7% NaCl solution to prevent coagulation of the blood. 100 ml. of a 1:50,000 dilution of blood in P.B.S. (low background count) was prepared by a two-stage dilution method. In a volumentric flask, 1 ml. of well-mixed blood was made up to 500 ml. with P.B.S. and then 1 ml. of this suspension was diluted 100 times in a second volumetric flask. Each final suspension was gently agitated throughout the experiment to preserve uniformity in the suspension.

Approximately 20 ml. sub-samples were employed for the actual counts. There were three reasons for this, 1) the beakers used on the instrument were too small to hold the volume required to complete the experiment, 2) small sub-samples could be used briefly and then discarded before the suspension had time to settle to any extent and 3) the effect of the electric current on the blood cells would be minimal.

The background count of the filtered P.B.S. which was used for each sample was read at aperture current 3 and threshold 5.

At aperture current II, each of the 12 samples was counted in a threshold series beginning at 0 and rising to threshold 45 in units of 5. At the completion of this series, four further readings were taken at intermediate settings of 7, 9, 12 and 17.

This procedure was repeated as before, but at aperture current III. In addition, two further readings were made at the end at thresholds 19 and 3.

Results and conclusions. The results employing aperture currents II and III respectively are shown in Tables 73 and 74. With aperture current II, readings became stable from threshold 7 to threshold 10. If plotted as a cumulative frequency line on a graph, these readings create quite short plateaux preceded by irregular and inaccurate counts at lower threshold, e.g. in 7 out of the 12 samples, the counts at threshold 5 were "interrupted." There was also a sudden drop in counts over threshold 10 which indicated that a substantial part of the cell population lay between threshold 5 and threshold 10. With an aperture current of II, therefore, it would be necessary to select a threshold around 7 which would then be rather close to thresholds which repeatedly gave false readings due to a combination of cells and debris. In addition, the typical oscilloscope patterns at this aperture current were too short to distinguish cell spikes from those of debris along the baseline.

An aperture current of III was preferred for routine counts. The results showed that the plateaux were much wider, roughly over thresholds 7 to 12.

Counts/

TABLE 73

Threshold Counts* of 1:50,000 Dilutions

Sample Counts x 10^4

Threshold Setting	1	2	3	4	5	6
0	-	(4.87)	(3.52)	-	-	(3.06)
5	6.85	8.29	(5.39)	(4.86)	(4.59)	5.07
10	6.79	8.52	6.63	6.25	6.18	5.53
15	3.43	5.81	3.24	4.45	4.45	3.53
20	1.37	2.26	1.36	1.56	1.73	1.31
25	0.64	1.17	0.63	0.73	0.73	0.55
30	0.31	0.61	0.28	0.39	0.38	0.28
35	0.14	0.29	0.13	0.17	0.17	0.12
40	0.07	0.12	0.07	0.07	0.50	0.06
45	0.05	0.06	0.05	0.03	0.37	0.03
7	7.07	8.51	6.85	6.15	5.97	5.38
9	6.96	8.55	6.83	6.19	6.15	5.46
12	5.65	7.7	5.37	5.84	5.76	4.99
17	2.09	3.84	2.01	2.98	2.86	2.35
		Actual Background Counts of F.B.S.				
5	30	70	50	100	100	100

* Counts greater than 10,000 have been corrected for coincidence loss.

"Interrupted" counts are in brackets.

of Cat Blood at Aperture Current II.

7	8	9	10	11	12
-	(5.33)	(3.20)	(5.57)	(3.35)	(2.51)
(4.93)	8.63	(4.92)	7.58	(4.87)	(4.07)
6.69	9.37	6.00	8.77	6.42	5.4
5.52	7.88	2.96	4.97	4.36	3.92
2.85	4.12	1.03	2.00	1.79	1.50
1.25	2.09	0.51	1.09	0.83	1.26
0.65	1.19	0.23	0.57	0.45	0.33
0.37	0.65	0.10	0.25	0.21	0.17
0.20	0.34	0.06	0.13	0.13	0.08
0.10	0.16	0.05	0.07	0.07	0.04
6.74	9.23	6.35	9.07	6.31	5.63
6.88	9.36	6.45	8.96	6.48	5.74
6.67	9.19	5.36	7.48	5.84	5.29
4.80	6.0	1.75	3.46	3.16	2.76
50	70	85	85	100	100

TABLE 74

Threshold counts* of 1:50,000 Dilutions

Sample Counts x 10^4

Threshold Setting	1	2	3	4	5	6
0	-	(5.09)	(4.06)	-	-	(3.41)
5	(5.92)	8.25	6.68	(5.56)	6.12	5.58
10	7.18	8.67	7.52	6.39	6.32	5.63
15	7.14	8.81	7.51	6.38	6.39	5.59
20	6.52	8.56	6.74	6.27	6.28	5.27
25	4.44	7.14	4.57	5.47	5.46	4.26
30	2.43	4.57	2.44	3.55	3.48	2.51
35	1.60	2.60	1.57	1.87	1.86	1.39
40	1.07	1.66	0.98	1.14	1.11	0.84
45	0.72	1.24	0.67	0.75	0.73	0.56
7	7.24	8.39	7.49	6.30	6.22	5.58
9	7.32	8.55	7.56	6.35	6.33	5.63
12	7.31	8.73	7.53	6.35	6.46	5.56
17	7.04	8.71	7.44	6.30	6.30	5.63
19	6.68	8.53	6.85	6.25	6.27	5.40
3	6.95	7.73	6.73	5.65	5.70	5.25
		Actual Background Counts of P.B.S.				
5	30	70	50	100	100	100

* Counts greater than 10,000 have been corrected for coincidence loss.

"Interrupted" counts are in brackets.

of Cat Blood at Aperture Current III.

7	8	9	10	11	12
-	(5.61)	(3.84)	(5.12)	(4.17)	(3.27)
(5.66)	8.7	(5.67)	(7.28)	(5.56)	5.66
6.89	9.18	6.94	9.18	6.71	5.82
7.02	9.37	6.80	9.21	6.67	5.87
7.00	9.53	6.15	8.91	6.39	5.66
6.64	9.00	4.39	6.85	5.01	4.92
5.71	7.02	2.33	4.53	3.66	3.34
4.93	4.71	1.24	2.77	2.19	1.95
4.29	3.02	0.80	1.78	1.31	1.12
3.05	2.00	0.53	1.21	0.87	0.70
6.99	9.12	6.85	8.93	6.61	5.75
7.20	9.22	6.91	9.02	6.61	5.80
6.91	9.33	6.87	9.14	6.67	5.76
7.03	9.53	6.69	9.25	6.64	5.75
7.04	9.48	6.32	9.10	6.48	5.60
(5.33)	7.9	(5.47)	7.8	(5.11)	5.16
50	70	85	85	100	100

Counts tended to fall off less abruptly as the thresholds rose. Although "interrupted" counts were just as common at threshold 5 with this current, an optimal threshold could be selected well away from this source of error. Threshold 10 was considered to be suitable.

With an aperture current of III, repeat readings from the same sample at threshold 10 and 15 and also at 7, 9, 12 and 17, were very similar, although the latter four counts were made in succession much later in the experiment than the readings at thresholds 10 and 15 and from a different aliquot of the 1:50,000 suspension. At thresholds 19 and 20 the results were also consistent in each sample.

EXPERIMENT 3.

A Comparison of Total Erythrocyte Counts by Means of the Electronic and the Visual Methods.

Materials and methods. Approximately 2 ml. samples of blood were collected, as described in Experiment 2, from 16 clinically healthy cats. 0.04 ml. of blood from each sample was mixed with 20 ml. P.B.S. to give a suspension of 1:500 and then 0.2 ml. of this dilution was mixed with another 20 ml. P.B.S. to create a second suspension of 1:50,000. The 1:500 dilution was used to fill counting chambers of Improved Neubauer Haemocytometers, so that a direct comparison could be made between the electronic counts and the traditional chamber counts by means of a common 1:500 dilution. Red cell Thoma pipettes were employed to transfer the 1:500 suspensions to the haemocyometers. Whenever possible, 6 chambers were counted in the manner recommended by Dacie/

Dacie (1963). Filled haemocytometers were stored temporarily in a clear plastic box containing moistened gauze to reduce evaporation from the counting chambers. Any which showed signs of evaporation or flooding were not counted.

Each of the 1:50,000 suspensions was counted electronically 6 times, if possible, at aperture current III and threshold 10. The filtered P.B.S. background count was also recorded. The remains of each 1:500 suspension was used to estimate the total leucocyte count to ensure that it did not exceed a level which would introduce an unnecessarily high additional error into the red cell count.

Results and conclusions. The data are presented in Table 75. The coefficient of variation with the visual method ranged from 2.0% to 9.45%. The range with the electronic method, employing the same blood samples, was from 0.07% to 1.21%.

The results were subjected to statistical analysis. Using the method of least squares, where x was equivalent to the haemocytometer counts and y was equivalent to the electronic counts, the line of regression of y on x was $y = 0.86158x + 1.138981$. The correlation coefficient was $r = +0.9429$. The standard deviation from the regression was $s_E = 25.088$. Expressed as the coefficient of variation from the regression, when $x = \bar{x}$, this equalled

$$\frac{s_E \times 100}{\bar{y}}\ \% = \frac{25.088 \times 100}{759.0625}\ \% = 3.3\% \text{ approximately.}$$

The results indicated that there was a close relationship between the two methods.

TABLE 75

Experiment 3: Total Erythrocyte Counts

Sample Number	Haemocytometer counts					Estimated Erythrocyte counts /cu.mm. x 10^6
	Number of chamber counts	Mean of chamber counts	Variance	S.D.	C.V.	
1	6	313.3	39.46	± 6.82	2.00	7.83
2	6	330.8	228.16	±15.108	4.57	8.28
3	6	285.3	726.66	±26.959	9.45	7.13
4	5	276.8	120.95	±11.00	3.97	6.92
5	6	360.8	260.76	±16.15	4.48	8.53
6	6	285.5	216.16	±14.702	5.15	7.10
7	6	313.5	115.10	±10.728	3.42	7.85
8	6	296.2	41.36	± 6.431	2.17	7.40
9	5	273.4	252.24	±15.881	5.81	6.83
10	5	259.6	114.80	±10.714	4.13	6.46
11	6	248.8	102.96	±10.15	4.08	6.23
12	6	318.2	857.36	±29.280	9.2	7.95
13	6	323.0	646.00	±25.417	7.87	8.08
14	6	352.2	189.76	±13.777	3.91	9.00
15	6	308.8	63.76	± 7.985	2.59	7.72
16	5	255.6	180.80	±13.445	5.26	6.48

Using Visual and Electronic Methods.

Sample Number	Electronic Counts (Corrected)					Estimated Erythrocyte counts /cu.mm. x 10^6
	Number of Replicate counts	Mean of Replicate counts	Variance	S.D.	C.V.	
1	6	80,300	780,000	±883	1.1	8.03
2	6	83,030	14,000	±118	0.14	8.30
3	6	76,330	142,000	±377	0.49	7.55
4	6	68,950	334,000	±578	0.84	6.89
5	4	81,200	960,000	±980	1.21	8.12
6	6	71,620	438,000	±662	0.92	7.16
7	6	77,800	428,000	±654	0.84	7.78
8	6	80,020	134,000	±366	0.46	7.85
9	6	71,220	402,000	±634	0.89	7.12
10	3	65,300	-	-	-	6.53
11	4	66,950	57,000	±239	0.36	6.69
12	4	83,850	170,000	±412	0.49	8.39
13	6	77,150	3,000	± 54	0.07	7.71
14	4	90,600	160,000	±400	0.44	9.06
15	5	77,700	235,000	±485	0.62	7.77
16	3	65,000	-	-	-	6.50

EXPERIMENT 4.

Erythrocyte Dilution Experiment.

This experiment was designed to ascertain if the counter was accurate throughout its predicted range.

Materials and methods. 2 ml. samples of venous blood were withdrawn from 6 different cats. From each blood sample, 5 x 20 ml. of a 1:50,000 suspension of blood in P.B.S., were prepared and then mixed to give roughly 100 ml. of a 1:50,000 suspension. From each 100 ml. suspension, the following dilutions were made, 20 ml. of the suspension was withdrawn to be used as a 1:1 dilution; 10 ml. was added to 10 ml. of P.B.S. to produce a 1:2 dilution; 5 ml. was added to 10 ml. P.B.S. to make a 1:3 dilution; 5 ml. was added to 15 ml. P.B.S. to make a 1:4 dilution; 5 ml. was added to 20 ml. P.B.S. to give a 1:5 dilution and finally, 5 ml. was added to 25 ml. P.B.S. to produce a dilution of 1:6. 0.04 ml. and 0.2 ml. pipettes were used to make the initial 1:50,000 suspensions and 5 ml. and 10 ml. Gold Line E-Mil pipettes were employed to prepare the higher dilutions.

Each of the 6 dilutions from each blood sample was counted 6 times in succession at threshold 10 and Aperture Current III.

Results and conclusions. The data of this experiment are presented in Table 76. It will be noted from column 2 and column 3 in this table, that the relationships between the mean % of observed readings and those which were expected were close enough for the assumption that this instrument was accurate over its normal working range of 1 to 9 million red cells per cu.mm.

TABLE 76

Experiment 4: Erythrocyte

Dilution	Expected %	Mean observed %	Sample 1. Mean 6 counts & S.D.	Sample 1. Mean %	Sample 2. Mean 6 counts & S.D.	Sample 2. Mean %
1: 50,000	100	100	83,100 ± 502	100	90,600 "	100
1:100,000	50	53	43,900 ± 270	52.8	48,500 ± 255	53.5
1:150,000	33.3	36.1	29,500 ± 173	35.5	34,500 ± 224	38.1
1:200,000	25	27.1	22,600 ± 81	27.2	25,200 ± 89	27.8
1:250,000	20	21.9	17,900 ± 100	21.5	20,200 ± 276	22.3
1:300,000	16.6	17.4	14,400 ± 190	17.3	15,700 ± 179	17.3

Counts at Different Dilutions.

Sample 3.		Sample 4.		Sample 5.		Sample 6.	
Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %
82,400 ± 608	100	75,600 ± 482	100	78,300 ± 729	100	57,500 ± 322	100
43,500 ± 265	52.8	40,100 ± 141	53	41,800 ± 214	53.4	30,200 ± 167	52.5
28,800 ± 313	34.9	27,300 ± 161	36.1	28,200 ± 335	36.0	20,700 ± 100	36.0
22,000 ± 184	26.7	20,700 ± 161	27.4	21,200 ± 77	27.1	15,500 ± 214	26.8
17,600 ± 77	21.4	17,000 ± 89	22.5	17,100 ± 228	21.8	12,600 ± 141	21.9
14,200 ± 77	17.2	13,300 ± 118	17.6	13,800 ± 110	17.6	10,100 ± 261	17.5

ERRORS OF THE TOTAL ERYTHROCYTE COUNTS.

EXPERIMENT 5.

The Errors of the Electronic Method with a Single Sample of Blood.

This experiment was designed to demonstrate the combined technical, instrument and field errors of the method.

Materials and methods. From a single well mixed sample of cat blood, mixed with EDTA to prevent coagulation, 6 separate suspensions of 1:50,000 were prepared as described above. Different pipettes were used for each step. Each suspension was counted 6 times giving a series of 36 counts. Individual counts were made in random order.

Results and conclusions. The results are given in Table 77. The mean of the 36 counts was 70,600 and the standard deviation of ± 700 was ± 0.99% when expressed as the coefficient of variation. Coefficients of variation of counts from the same suspension demonstrated machine and field errors and they ranged from ± 0.54% to ± 1.13%. The coefficient of variation of counts from each individual dilution, which were selected at random, gave an indication of the machine, field and pipetting errors, which ranged from ± 0.65% to ± 1.26%.

From this, it was concluded that the electronic method of estimating the total erythrocyte count of a single blood sample was extremely accurate and the accuracy was not markedly altered by pipetting errors.

TABLE 77

Results of Experiment 5: The Total Erythrocyte Count: Errors of the Electronic Method with a Single Sample of Blood.

1:50,000 dilutions	Successive corrected counts						Mean & S.D.	C.V.
	a	b	c	d	e	f		
1	71,900	71,800	71,500	71,100	70,300	70,700	71,200 ±630	0.85
2	70,300	70,400	*	70,400	69,500	70,300	70,200 ±380	0.54
3	71,000	71,400	70,200	71,000	70,200	70,400	70,700 ±503	0.71
4	70,700	69,900	69,900	69,900	69,800	69,200	69,900 ±480	0.69
5	70,500	71,500	70,200	70,300	70,800	70,400	70,600 ±480	0.68
6	72,600	70,800	70,500	70,400	71,100	71,000	71,100 ±800	1.13
Mean & S.D.	71,200 ± 900	71,000 ± 730	70,500 ± 620	70,500 ± 460	70,300 ± 600	70,300 ± 620		
C.V.	1.26	1.03	0.88	0.65	0.85	0.88		

* An interrupted count which was not included in the statistical analysis.

EXPERIMENT 6.

The Errors of the Electronic Method in Blood Samples from Different Cats.

Materials and methods. Five separate dilutions of 1:500 were prepared from a well mixed venous blood sample. Each dilution was counted in the electronic counter at aperture current III and threshold 10 and the mean of two successive counts, corrected for coincidence loss, was recorded.

This was carried out on 10 individual samples of blood from different cats.

Results and conclusions. The results are presented in Table 78. An analysis of variance was carried out and this showed that the standard error of a single observation was 60,100 and the coefficient of variation = $\frac{60,100}{7,059,800} \times 100 = 0.85\%$.

From these results it was concluded that this method of estimating the total erythrocyte count of cat blood was highly accurate irrespective of the donor cat.

EXPERIMENT 7.

The Between Technician Discrepancies in the Electronic Method of Estimating the Total Erythrocyte Count with Cat Blood.

This experiment was designed to determine the statistical differences which might be found when three technicians were employed to carry out total erythrocyte counts on identical samples of cat blood.

TABLE 78

Results of Experiment 6: the Total Erythrocyte Counts and Errors of the Electronic Method in Samples of Blood from Different Cats.

Sample	Corrected mean counts of separate dilutions 1:50,000					Mean	S.D.	C.V.
	a	b	c	d	e			
1	52,100	52,600	52,100	51,900	52,400	52,200 ±	278.4	0.53
2	75,900	77,200	77,200	77,300	76,700	76,900 ±	587.0	0.76
3	68,500	67,700	68,500	68,800	68,800	68,500 ±	453.0	0.66
4	70,300	71,500	71,500	72,300	72,600	71,600 ±	894.5	1.25
5	82,700	*	83,700	83,700	83,800	83,500 ±	519.6	0.62
6	70,700	69,300	69,700	70,600	69,300	69,900 ±	687.4	0.98
7	71,300	70,900	70,100	69,900	70,400	70,500 ±	576.6	0.81
8	69,200	70,000	70,300	69,800	70,700	70,000 ±	561.3	0.80
9	70,700	71,400	72,000	71,200	71,700	71,400 ±	495.0	0.69
10	72,300	72,000	72,000	71,500	70,800	71,700 ±	589.5	0.82

* An interrupted count which was not included in the statistical analysis.

Materials and methods. Each of three technicians prepared two separate 1:50,000 suspensions of blood in P.B.S. with a known low background count. At aperture current III and threshold 10, a total erythrocyte count was estimated for each suspension by the person who had prepared it. The recorded result was the mean of two successive counts. A total leucocyte count was carried out on the preliminary 1:500 dilution in each case to ensure that no leucocytosis was present which would interfere with the accuracy of the erythrocyte count.

This procedure was carried out on 10 blood samples from different cats.

Results and conclusions. The results are presented in Table 79A. Note that the actual count, corrected for coincidence loss, is given. Such figures must be multiplied by 100 to give the estimated number of cells per cu.mm. No leucocytosis was observed in any of the samples and the background counts of the filtered P.B.S. were 250 or less.

The actual between technician discrepancies and the percentage between technician discrepancies were determined, Table 79B and the Student's t test was applied.

The mean percentage discrepancy between Technician 1 and Technician 2 was 2.22% with a standard deviation of ± 19.59% t = 0.49, which is not statistically significant. The mean percentage discrepancy between Technician 1 and Technician 3 was found to be 2.86% with a standard deviation of ± 4.07% t = 3.06, which is statistically significant at the 1% level.

TABLE 79A.

Results of Experiment 7: The Total Erythrocyte Counts of Cat Blood with the Electronic Method Employing Three Technicians.

Actual Erythrocyte Counts (Corrected)

Technician 1		Technician 2		Technician 3	
Dilution A	Dilution B	Dilution C	Dilution D	Dilution E	Dilution F
25,800	25,000	23,100	22,700	24,300	24,500
57,500	57,500	54,600	54,600	55,600	55,300
55,200	54,700	53,700	51,900	55,600	58,400
54,200	54,900	52,600	54,700	54,000	54,400
54,400	54,900	51,400	53,600	53,800	55,900
59,700	59,600	55,900	54,700	57,200	57,400
47,800	47,600	77,000	75,900	41,800	43,400
40,600	40,600	38,000	38,700	37,400	38,300
69,200	68,400	68,900	69,100	68,700	68,700
27,900	27,600	27,000	27,600	27,000	26,800

TABLE 79B.

Experiment 7: Between Technician Discrepancies in the Total Erythrocyte Counts of Cat Blood with the Electronic Method.

Blood Sample	Between Technician Discrepancies Technician 1 minus Technician 2				Between Technician Discrepancies Technician 1 minus Technician 3			
	A minus C		B minus D		A minus E		B minus F	
	Actual	%	Actual	%	Actual	%	Actual	%
1	2,700	10.5%	2,300	9.2%	1,500	5.8%	500	2%
2	2,900	5.0%	2,900	5.0%	1,900	3.3%	2,200	3.8%
3	1,500	2.7%	2,800	5.1%	- 400	- 0.7%	-3,700	-6.8%
4	1,600	2.9%	200	0.4%	200	0.4%	500	0.90%
5	3,000	5.5%	1,300	2.4%	600	1.1%	-1,000	-1.8%
6	3,800	6.4%	4,900	8.2%	2,500	4.2%	2,200	3.7%
7	-29,200	-61.1%	-28,300	-59.5%	6,000	12.6%	4,200	8.8%
8	2,600	6.4%	1,900	4.7%	3,200	7.9%	2,300	5.7%
9	300	-0.4%	- 700	-1.0%	500	0.7%	- 300	-0.4%
10	900	3.2%	0	0	900	3.2%	800	2.9%

From these results, it was observed that, in this highly accurate technique, differences could be found when one technician was compared with another. This indicated that each operator should be tested to determine his or her technical errors before it can be assumed that 2 or more technicians can be interchanged in a series of measurements during the course of an experiment.

CALIBRATION EXPERIMENTS FOR TOTAL LEUCOCYTE COUNTS.

EXPERIMENT 8.

Erythrocyte Haemolysis Experiment.

This experiment was designed to find out the optimal period when the total leucocyte counts remained stable following haemolysis of the erythrocytes in a diluted suspension of blood.

Materials and methods. From a blood sample from each of 6 cats, a 1:500 dilution in F.B.S. was prepared. To each suspension was added 0.2 ml. of a 2.0% solution of saponin in distilled water and this was gently agitated to mix well without creating too much froth. Each suspension was counted at an aperture current of II and a threshold of 18, immediately after the addition of saponin and then at the 5th, 10th, 20th, 30th, 40th, 50th and 60th minute.

Results and conclusions. Counts which were made immediately after the addition of the saponin solution were very high and erratic, since they would include erythrocytes and large erythrocytic debris. Such counts were not recorded. Table 80 shows the results of the subsequent counts. The five counts/

TABLE 80

Experiment 8: The Stability of the Total Leucocyte Count Following Haemolysis of the Erythrocytes.

Post-haemolysis interval in minutes	Corrected counts of 1:500 dilutions of blood in P.B.S.					
	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5	Cat 6
5th	30,800	34,000	28,400	23,300	21,000	18,500
10th	30,000	34,600	28,700	22,200	20,500	18,800
20th	30,300	33,400	28,400	22,700	20,400	19,400
30th	30,400	33,700	28,500	22,600	20,400	18,700
40th	29,900	33,800	28,200	22,300	20,100	18,500
50th	30,100	33,700	28,300	22,700	20,100	18,400
60th	30,100	33,800	29,000	23,000	20,900	19,300
Mean counts 10th - 50th minute	30,100	33,800	28,400	22,500	20,100	18,800
Variance	44,100	144,400	36,100	52,900	36,100	169,900
S.D.	± 210	± 380	± 190	± 230	± 190	± 400
V	0.70%	1.12%	0.67%	1.02%	0.94%	2.13%

counts between the 10th and 50th post-saponification minute in each blood sample were included in an analysis of variance which showed that the residual standard error of a single observation = 296 with a coefficient of variation of 1.15%

From these results, it was concluded that the total count, between the 10th and 50th minute after the addition of saponin to a 1:500 suspension of blood in P.B.S., remained stable.

EXPERIMENT 9.

The Optimal Aperture Current and Threshold Setting for Total Leucocyte Counts.

Materials and methods. As in Experiment 2, precise initial dilutions were not necessary for leucocyte plateaux, for it was cell volumes rather than cell numbers which were being investigated. However, the concentration of the leucocytes was kept within the predicted working range of the instrument.

Samples of blood were withdrawn from the right cephalic veins of 12 cats which showed no signs of clinical disease. EDTA solution was again employed to prevent coagulation of the blood. 1 ml. of each sample was mixed with P.B.S. in a 500 ml. volumetric flask and the total volume was made up to 500 ml. with P.B.S. to give a dilution of 1:500. A sub-sample of approximately 100 ml. was withdrawn and 1 ml. of a 2% saponin solution was introduced to the sub-sample. Ten minutes after its addition, when total haemolysis of the red cells should have been completed, 20 ml. aliquots of this treated suspension were withdrawn in succession and used on the instrument/

instrument for total leucocyte counts. The sub-sample was not used for more than 20 to 25 minutes to ensure stability in the total white cell counts throughout the experiment. Roughly 10 minutes before the first 100 ml sub-sample was discarded, a further sample was withdrawn from the volumetric flask and haemolysed as before. This could be repeated if necessary to maintain a supply of suspension under standard conditions. Throughout the entire experiment, both the main sample and sub-samples were gently agitated to keep the suspensions as uniformly mixed as possible.

At aperture current I, readings were taken from threshold 0 to threshold 80 in units of 5. Counts were continued at thresholds 90 and 100 and further readings were made at thresholds 7, 9, 12, 17, 22 and 27.

This was repeated at aperture current II and also at aperture current III, but in these cases, the final intermediate thresholds were omitted.

Results and conclusions. Data for the 12 samples are presented in Tables 81, 82 and 83.

Plateaux with aperture current I lay between threshold 7 and threshold 12. It would have been necessary to have selected threshold 7 or 9 at this current. Leucocytosis is common in the cat and it would prove impossible to carry out accurate counts at this threshold routinely, because of baseline interference.

Employing aperture current III, the optimal threshold setting was around 30 and plateaux were wide. Whilst the cell and debris populations were readily differentiated/

TABLE 81

Threshold Counts* of 1:500 Dilutions

Counts x 10^3

Threshold Setting	1	2	3	4	5	6
0	(25.0)	"	"	"	(10.9)	"
5	19.4	(9.6)	(9.3)	"	(4.9)	(9.9)
10	17.7	12.9	10.9	12.4	5.4	12.6
15	16.7	12.4	10.7	12.2	5.1	11.8
20	16.2	10.7	9.8	10.5	4.7	10.7
25	15.0	8.7	9.4	7.1	3.9	9.2
30	13.7	7.4	8.2	5.3	3.4	8.2
35	13.2	5.2	5.9	3.4	2.6	6.4
40	9.9	2.6	3.2	2.0	1.7	4.4
45	8.7	1.4	1.9	1.3	1.1	2.4
50	7.0	0.75	1.1	0.80	0.71	1.7
55	3.7	0.52	0.73	0.64	0.60	1.2
60	2.8	0.38	0.57	0.52	0.50	1.0

of Cat Blood at Aperture Current I.

7	8	9	10	11	12
-	(16.0)	(30.2)	(15.1)	(18.3)	(12.5)
(13.9)	(5.7)	(19.5)	(17.9)	(8.7)	(10.1)
19.1	7.6	19.1	22.2	12.2	14.6
18.7	7.3	18.4	21.9	11.7	14.0
18.6	6.7	16.7	21.0	11.1	13.1
16.9	5.1	15.6	19.8	10.3	11.3
14.0	4.2	14.6	18.7	9.1	9.8
8.8	3.4	12.6	17.1	8.1	7.7
3.9	2.3	8.3	12.9	5.6	6.0
2.0	1.6	5.0	8.1	3.9	4.1
1.3	1.0	3.3	5.4	2.9	2.8
1.0	0.75	2.2	3.5	2.1	2.2
0.80	0.59	1.6	2.4	1.8	1.8

Threshold Setting	1	2	3	4	5	6
65	2.2	0.30	0.40	0.47	0.40	0.77
70	1.9	0.24	0.40	0.37	0.40	0.67
75	1.7	0.25	0.30	0.37	0.33	0.62
80	1.7	0.24	0.28	0.29	0.32	0.55
90	1.5	0.20	0.26	0.28	0.32	0.44
100	1.2	0.19	0.27	0.24	0.27	0.41
7	18.3	12.3	10.9	11.9	5.4	11.7
9	18.0	13.0	10.9	12.5	5.4	11.6
12	17.2	12.6	10.6	12.2	5.1	11.6
17	16.2	11.5	9.6	11.8	4.8	10.5
22	15.4	9.2	8.7	8.6	4.4	9.1
27	13.7	7.7	7.7	6.1	3.5	7.7
		Actual Background Counts of P.B.S.				
5	30	70	50	100	100	100

* Counts greater than 10,000 have been corrected for coincidence loss.

"Interrupted" counts are in brackets.

7	8	9	10	11	12
0.72	0.51	1.2	1.7	1.5	1.5
0.61	0.39	1.0	1.3	1.2	1.3
0.52	0.37	0.80	1.0	1.1	-
0.50	0.31	0.70	0.9	1.0	1.0
0.50	0.24	0.60	0.7	0.86	0.8
0.38	0.21	0.50	0.6	0.70	0.7
19.1	7.6	18.1	(19.6)	(10.3)	14.4
19.4	7.4	18.0	(20.6)	11.6	14.3
18.8	7.2	17.5	21.9	11.8	14.1
18.6	7.0	16.6	21.1	11.4	13.4
17.1	6.0	15.8	20.1	10.5	12.3
12.6	4.6	14.1	18.7	9.3	9.9
50	70	85	85	100	100

TABLE 82

Threshold Counts* of 1:500 Dilutions

Counts x 10^3

Threshold Setting	1	2	3	4	5	6
0	"	"	"	"	"	"
5	(22.5)	16.6	15.0	"	"	(21.0)
10	21.8	15.5	13.1	15.0	6.5	18.5
15	19.9	14.7	11.5	13.2	5.8	13.3
20	19.5	13.8	11.3	12.6	5.5	12.2
25	18.5	13.4	11.1	12.4	5.2	11.7
30	18.0	13.1	10.5	12.3	5.1	11.1
35	17.7	12.1	10.3	11.3	4.9	10.8
40	17.2	10.9	9.7	9.5	4.5	9.8
45	16.2	9.5	8.9	7.7	4.2	9.2
50	15.2	9.1	8.5	6.4	3.8	8.5
55	14.4	8.1	7.9	5.3	3.6	8.0
60	13.4	7.4	7.3	4.6	3.4	7.2
65	12.5	6.2	6.4	3.9	3.3	6.1
70	11.2	5.3	5.6	3.5	3.1	5.6
75	9.8	4.6	5.1	3.2	2.9	4.8
80	8.9	4.2	4.4	2.9	2.7	4.4
90	8.1	3.6	3.9	2.6	2.6	3.9
100	7.4	3.4	3.5	2.4	2.4	3.6

* Counts greater than 10,000 have been corrected for coincidence loss.

"Interrupted" counts are in brackets.

of Cat Blood at Aperture Current II.

7	8	9	10	11	12
(31.8)	"	(39.8)	(29.4)	(33.8)	(28.7)
(21.1)	(10.2)	(33.2)	(22.0)	14.9	(19.8)
21.6	9.0	20.8	25.2	13.4	17.1
20.1	8.5	19.6	24.9	12.4	15.5
19.9	7.8	18.6	24.2	12.1	14.9
19.4	7.5	18.2	24.2	12.1	14.6
19.4	7.3	18.2	23.9	11.4	14.1
19.2	7.1	17.5	23.4	11.1	13.6
18.6	6.3	16.5	21.8	11.0	12.8
18.1	5.5	16.1	21.3	10.5	11.8
16.6	5.0	15.5	20.8	9.6	10.6
14.9	4.5	14.7	19.8	9.3	9.8
11.6	4.0	13.7	18.3	8.6	9.1
9.2	3.8	12.3	15.8	7.9	8.3
7.8	3.5	10.9	13.4	7.3	7.7
7.0	3.2	9.4	11.6	6.9	7.3
6.4	3.1	8.2	10.3	6.2	6.8
5.7	2.6	7.2	8.7	5.6	6.2
5.5	2.4	6.6	7.5	5.4	5.5

TABLE 83

Threshold Counts* of 1:500 Dilutions

Counts x 10^3

Threshold Setting	1	2	3	4	5	6
0		(30.6)	-	-	-	(27.8)
5		58.5	-	62.6	54.4	63.4
10		27.4	29.0	36.6	19.0	38.7
15		15.5	14.8	17.0	7.4	22.0
20		14.2	12.1	13.7	6.1	17.2
25		13.1	11.5	13.1	5.8	15.2
30		13.0	10.8	12.6	5.3	13.4
35		12.7	10.6	12.3	5.2	12.6
40		12.5	10.5	12.2	5.1	12.3
45		12.7	10.6	11.8	5.0	11.6
50		12.1	10.3	11.9	4.8	11.2
55		11.7	9.8	11.7	-	-
60		11.3	9.0	11.6	4.5	10.3
70		10.5	8.9	10.4	4.4	9.0
80		9.5	8.4	8.8	4.3	8.5
90		9.0	8.1	8.4	4.1	7.5
100		8.6	7.9	7.8	4.0	7.2

NOT CARRIED OUT

* Counts greater than 10,000 have been corrected for coincidence loss.

"Interrupted" counts are in brackets.

of Cat Blood at Aperture Current III.

7	8	9	10	11	12
	-	-	(29.1)	(28.9)	(41.0)
	(56.7)	(65.5)	(48.6)	(48.9)	(56.4)
	38.2	42.7	36.9	31.0	39.5
	13.6	24.0	24.8	15.2	20.9
	9.2	19.9	22.8	13.0	16.3
	8.0	18.8	22.0	12.5	14.8
	7.6	18.5	21.9	11.9	14.3
	7.4	18.0	21.2	11.6	13.8
	7.2	17.7	21.7	11.5	13.7
	7.4	17.7	21.1	11.5	13.7
	7.2	(18.1)	21.3	11.3	13.4
	7.1	17.5	21.2	11.4	13.4
	6.8	17.5	21.0	10.9	13.0
	6.5	16.4	20.0	10.6	12.3
	6.0	15.7	19.1	9.8	11.5
	5.8	-	18.7	9.3	11.1
	5.6	-	17.7	9.1	10.5

NOT CARRIED OUT

differentiated by this current, the oscilloscope cell pattern tended to be so tall that it exceeded the height of the screen. This did not influence the instrument counts, but it did prevent the oscilloscope screen from acting as a useful indicator of the accuracy of the count.

With aperture current II, the oscilloscope pattern was more easily interpreted. The cell and debris populations were quite distinct and a threshold value could almost be estimated visually. At this current, the optimal threshold was estimated as 20, or slightly less than this.

It was concluded that, for routine leucocyte counts, an aperture current of II and a threshold of 18 should prove satisfactory. These instrument settings were employed in all the subsequent calibration experiments.

EXPERIMENT 10.

A Comparison Between the Electronic and Visual Methods of Estimating Total Leucocyte Counts.

Materials and methods. 2 ml. samples of blood were withdrawn from the right cephalic veins of 12 cats which presented no signs of clinical disease. From each well mixed sample, a 1:500 dilution of blood in low background count,P.B.S. was prepared. To each sample was added 0.2 ml. of a 2% saponin solution (Coulter saponin) to cause haemolysis of the red cells. Six successive white cell counts were read from each suspension within the optimal interval after haemolysis. An aperture current of II and threshold of 18 were employed in the experiment.

The traditional method was that described by Dacie (1963) using white cell Thoma pipettes, white cell diluting fluid and Improved Neubauer Haemocyometers. A 1:20 dilution of blood was prepared from each of the 12 blood samples and six counting chambers were counted, whenever possible, with each dilution. Haemocytometers were housed in a clear plastic container with moistened gauze until used, to prevent evaporation.

Results and conclusions. The results are given in Table 84. Using the method of least squares, where x was equivalent to the haemocytometer counts and y was equivalent to the electronic counts, the line of regression was $y = 1.2972x - 1474$. The correlation coefficient between x and y was $r = + 0.9804$. The standard deviation from the regression was 1560. It could be shown that the above regression line could pass through the origin. This meant, in effect, that we could use $y = 1.2002x$. The standard deviation from this regression is 1630. The coefficient of variation from the regression when $x = \bar{x}$ is

$$\frac{s_E \times 100}{\bar{y}} \% = \frac{1630 \times 100}{15515} \% = 10.5\%$$

From this data it was concluded that the same particle population was counted with both the electronic and visual methods, but that the electronic counts were consistently and proportionately higher than those obtained by the visual method. This was not unexpected, since Berkson et al (1940) have noted that visual counts with a haemocytometer chamber are usually lower than the actual count, as confirmed by photographic methods.

Agreement between single counts with the two methods was less than that observed/

TABLE 84

Experiment 10: Total Leucocyte Counts

Sample Number	Haemocytometer Counts					Estimated Leucocyte counts / cu.mm.
	Number of Chamber counts	Mean of Chamber counts	Variance	S.D.	C.V.	
1	6	154.5	283.9	± 16.85	10.91	7,730
2	6	252.2	65.5	± 8.09	3.21	12,600
3	6	222.5	188.3	± 13.72	6.17	11,100
4	6	467.5	414.7	± 20.36	4.36	23,400
5	6	163.7	160.3	± 12.66	7.73	8,200
6	5	254.8	265.7	± 16.30	6.40	12,700
7	6	215.8	651.4	± 25.52	11.83	10,800
8	6	139.7	110.7	± 10.52	7.53	6,980
9	6	292.0	150.0	± 12.25	4.20	14,600
10	6	166.0	160.4	± 12.67	7.63	8,300
11	6	474.0	890.0	± 29.83	6.29	23,700
12	6	339.0	201.8	± 14.18	4.19	17,000

* Counts greater than 10,000 have been corrected for coincidence loss.

Using Visual and Electronic Methods.

Sample Number	Electronic Counts*					Estimated Leucocyte counts /cu.mm.
	Number of Replicate counts	Mean of Replicate counts	Variance	S.D.	C.V.	
1	6	8,350	49,000	± 221.4	2.65	8,350
2	6	18,300	56,000	± 236.6	1.29	18,300
3	6	14,000	4,000	± 63.25	0.45	14,000
4	6	28,800	12,000	± 109.54	0.38	28,800
5	6	9,150	11,000	± 104.88	1.15	9,150
6	6	12,400	12,000	± 109.54	0.88	12,400
7	6	12,720	29,680	± 172.28	1.35	12,720
8	6	6,530	6,600	± 81.24	1.24	6,530
9	6	18,630	7,400	± 86,02	0.46	18,600
10	6	8,270	102,600	± 320.29	3.87	8,300
11	6	28,330	170,600	± 413.03	1.46	28,300
12	6	20,650	227,000	± 476.45	2.31	20.650

observed in total erythrocyte counts. This may have been due to the higher field errors in both leucocyte counting methods and also to the errors involved in the less direct method of comparing the cell populations from the initial blood sample.

EXPERIMENT 11.

A Dilution Experiment to Ascertain the Effective Range of the Electronic Counter for Leucocyte Counts.

Materials and methods. The methods in this experiment were similar to that of Experiment 4, except that 100 ml. samples of a 1:500 suspension of blood in P.B.S. were employed instead of 1:50,000 suspension, the instrument settings were those which had been determined for leucocyte counts, but only 4 dilutions were used, 1:1, 1:2, 1:3, and 1:5.

Results and conclusions. The results are presented in Table 85. The expected and observed results can be noted in the second and third columns of the table, where the expected percentage of each dilution is compared to the observed mean percentage. The coefficient of variation of 6 replicate counts of each dilution was low enough for this comparison to be valid. It was found that the relationship between expected and observed results was close enough to assume that this instrument is accurate over a range between 2,600 and 46,400 leucocytes per cu.mm.

TABLE 85

Experiment 11: Leucocyte

Dilution	Expected %	Mean observed %	Sample 1. Mean 6 counts & S.D.	Sample 1. Mean %	Sample 2. Mean 6 counts & S.D.	Sample 2. Mean %
1: 500	100	100	12,700 ±118.3	100	18,000 ±118,3	100
1:1,000	50	50.2	6,300 ±77.5	49.6	8,700 ±173.2	48.3
1:1,500	33.3	34.5	4,300 ±63.2	33.9	6,600 ±167.3	36.7
1:2,500	20	20.5	2,600 ±63.2	20.5	3,400 ± 63.2	18.9

Counts at Different Dilutions.

Sample 3.		Sample 4.		Sample 5.		Sample 6.	
Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %	Mean 6 counts & S.D.	Mean %
21,300 ±173.2	100	16,700 ±148.3	100	46,400 ±244.9	100	24,800 ± 249	100
11,500 ±219.1	54.0	8,500 ±109.5	50.9	23,400 ±173.2	50.4	12,100 ±134.2	48.8
7,600 ±141.4	35.7	5,800 ±100	34.7	15,700 ±173.2	33.8	8,000 ±89.4	32.3
4,500 ±44.7	21.1	3,600 ±109.5	21.6	9,300 ±44.7	20.0	5,200 ±77.5	21

ERRORS OF THE TOTAL LEUCOCYTE COUNT

EXPERIMENT 12.

The Errors of the Electronic Method with a Single Sample of Cat Blood.

Materials and methods. From a single blood sample, 12 individual 1:500 dilutions of blood in P.B.S. were prepared with different 0.04 ml. pipettes. From each of these, a 1:50,000 suspension was also made. To the 1:500 dilutions was added 0.2 ml. of 2.0% saponin solution. After 10 minutes, the haemolysed suspensions were counted 6 times in succession at threshold 18 and aperture current II.

Erythrocyte counts were carried out on the high dilution samples at Threshold 10 and Aperture Current III and the mean of two successive counts was recorded.

Results and conclusions. The results are given in Table 86. The mean leucocyte count was 21,800 and the S.D. was ± 420, giving a coefficient of variation of ± 1.93%. From this, it was concluded that the total errors of this technique with a single blood sample were comparatively low.

The coefficient of variation of the 12 total erythrocyte counts was ± 1.02%.

EXPERIMENT 13.

The Errors of the Electronic Method in Blood Samples from Different Cats.

Materials and methods. Six separate 1:500 dilutions of blood in P.B.S. were prepared from each of 10 blood samples from different cats. The instrument was set at aperture current II and threshold 18 and each one of the individual suspensions was counted. The mean of two successive counts in each case was recorded after correction for coincidence loss, if this was indicated.

Results and conclusions. The results are presented in Table 87. An analysis/

TABLE 86

Results of Experiment 12: Total Leucocyte Counts; the Errors of the Electronic Method with a Single Sample of Blood.

1:500 dilutions	Successive counts (corrected)							1:50,000 dilutions
	a	b	c	d	e	f	x̄	
1	20,900	21,000	20,900	20,800	20,500	20,700	20,800	70,700
2	21,700	21,700	21,600	21,600	21,200	21,300	21,500	71,450
3	21,700	21,900	21,900	21,800	21,900	21,700	21,800	72,050
4	21,900	22,000	21,800	22,000	21,800	22,000	21,900	71,950
5	22,300	21,900	21,900	22,200	22,000	22,200	22,100	72,200
6	21,800	21,900	22,200	22,300	22,200	21,900	22,000	72,400
7	22,200	22,300	21,600	21,500	21,700	21,600	21,800	71,950
8	22,000	21,700	22,200	21,900	21,600	21,700	21,900	71,650
9	22,000	22,200	21,800	21,800	21,700	21,900	21,900	72,900
10	22,400	22,600	22,700	22,600	22,500	22,700	22,600	73,150
11	21,800	21,600	21,800	21,900	21,400	21,800	21,700	72,500
12	21,800	21,700	21,900	22,000	21,900	22,400	22,000	73,350

Leucocyte counts (72)

mean 21,800

S.D. $= \sqrt{476,300} = \pm 420$

V = ± 1.93%

Erythrocyte counts (12)

mean = 72,200 ($\times 10^2$)

S.D. = ± 740 ($\times 10^2$)

V = ± 1.02%

TABLE 87

Results of Experiment 13: Total Leucocyte Counts: the Errors of the Electronic Method in Samples from Different Cats.

Sample	Mean counts of separate dilutions of 1:500					Mean & S.D.	C.V.
	a	b	c	d	e		
1	20,800	21,900	21,500	21,800	22,000	21,600 ± 484.9	2.24
2	4,300	4,600	4,700	4,200	5,100	4,600 ± 360.4	7.83
3	19,400	19,300	19,900	20,900	19,800	19,900 ± 636.5	3.20
4	22,000	21,900	21,700	21,900	22,600	22,000 ± 346.4	1.57
5	21,000	21,600	21,300	21,700	21,200	21,400 ± 291.6	1.36
6	13,700	13,900	12,800	13,400	13,900	13,500 ± 463.6	3.43
7	16,300	16,300	16,500	*	16,600	16,400 ± 151.7	0.93
8	21,400	22,500	21,200	21,100	21,400	21,500 ± 565.7	2.63
9	23,000	22,400	22,400	21,700	22,400	22,400 ± 458.2	2.05
10	10,300	9,200	9,100	9,500	9,900	9,600 ± 499.9	5.21

Counts greater than 10,000 were corrected for co-incidence loss.

* A false count which was ignored in the statistical analysis.

analysis of variance showed that the standard error of a single observation was 548 with a coefficient of variation of 3.16%.

These results indicated that the electronic method was highly accurate, irrespective of the blood sample used.

EXPERIMENT 14.

Between Technician Discrepancies in the Electronic Method of Estimating the Total Leucocyte Count.

This experiment was designed to ascertain if there were significant differences in technical errors amongst three technicians.

Materials and methods. Each of three technicians prepared two separate 1:500 suspensions of well mixed blood plus EDTA in P.B.S. which had a known low background count. At aperture current II and threshold 18, each suspension was counted electronically 10 to 15 minutes after the addition of 0.2 ml. of a 2% solution of saponin in distilled water. The actual counts were supervised by the technician who had prepared the suspension and the mean of two successive counts was recorded. This procedure was carried out on 10 blood samples from different cats.

Results and conclusions. The results of each estimation are given in Table 88. The between technician discrepancies, which were derived from this data, are shown in Table 89.

The mean percentage discrepancy between Technician 1 and Technician 2 was 6.94% with a standard deviation of 4.69% ; t = 6.5, which is a statistically significant/

significant difference at the 0.1% level. Between Technician 1 and Technician 3, the mean percentage discrepancy was 0.53% with a standard deviation of 2.58% ; t = 0.89, which is not statistically significant.

It was, therefore, concluded that the errors of the method between Technicians were wide enough to justify an examination of individual technical performances for experimental purposes.

If these results are compared to those in Experiment 7, it will be observed that the differences in performances of Technicians 2 and 3 when compared to Technician 1 are now reversed.

TABLE 88

Experiment 14: The Total Leucocyte Counts of Cat Blood With the Electronic Method Employing Three Technicians.

Total Leucocyte Counts*.

Technician 1		Technician 2		Technician 3	
Dilution A	Dilution B	Dilution C	Dilution D	Dilution E	Dilution F
18,100	18,200	17,900	18,500	17,500	17,500
32,300	31,600	28,200	27,800	31,300	32,000
16,400	16,300	14,900	15,300	16,000	16,200
12,600	12,900	12,100	12,200	12,900	13,300
9,300	9,700	8,500	8,600	9,600	9,400
13,200	13,100	12,500	12,800	13,100	12,800
19,600	19,900	18,800	19,400	19,700	20,000
26,200	26,200	22,200	22,500	25,800	26,800
16,400	16,700	14,600	15,000	16,500	17,800
16,900	16,700	16,300	16,400	16,800	16,300

* Corrected for coincidence loss when necessary.

TABLE 89

Experiment 14: Between Technician Discrepancies in Total Leucocyte Counts on Cat Blood with the Electronic Method.

Blood Sample	Between Technician Discrepancies Technician 1 minus Technician 2				Between Technician Discrepancies Technician 1 minus Technician 3			
	A minus C		B minus D		A minus E		B minus F	
	Actual	%	Actual	%	Actual	%	Actual	%
1	200	1.1%	-300	-1.6%	600	3.3%	700	3.8%
2	4,100	12.7%	3,800	12%	1,000	+3.1%	-400	-1.3%
3	1,500	9.1%	1,000	6.1%	400	2.4%	100	0.61%
4	500	3.9%	700	5.4%	-300	-2.4%	-400	-3.1%
5	800	8.6%	1,100	11.3%	300	3.2%	300	3.1%
6	700	5.3%	300	2.3%	100	+0.75%	300	2.3%
7	800	4.1%	500	2.5%	-100	-0.51%	-100	-0.5 %
8	4,000	15.3%	3,700	14.1%	400	1.5%	-400	-1.5%
9	1,800	10.9%	1,700	10.2%	-100	-0.61%	-1,100	-6.6%
10	600	3.6%	300	1.8%	100	0.59%	400	2.4%

GENERAL DISCUSSION.

The traditional method of estimating blood cell counts has been shown to be associated with high errors in routine use by Berkson et al. (1940). However, provided that good technical skill is exercised and large numbers of cells are counted to reduce the field error, the method can be accurate. This has been fully discussed by Dacie (1963) who demonstrated that the errors in routine counts of the erythrocytes were so high that the technique was of little value in routine work. So far as the total leucocyte count is concerned, the high error, particularly the high field error, does not markedly affect its usefulness, since only quite wide variations in this count are recognised as being of value to the clinician.

In the traditional method, the risk of technicians introducing sub-conscious bias into the counts has been recognised and was the subject of a critical and statistical analysis of the data of earlier workers by Berkson et al. (1940).

Bias with the electronic method is negligible and the much greater number of estimations which can be attempted per hour makes it particularly desirable in laboratories where groups of experimental animals are being bled at the same time.

In calibrating the instrument for cat blood cells, it was found that the instrument settings were lower than those recommended by the manufacturer for human blood cells. This was probably due to the relative differences in sizes of blood cells in the cat and the human being.

The error of the total erythrocyte count was surprisingly low and electronic counts compared very well with those obtained with the haemocytometer. However, there was less agreement between the visual and electronic total leucocyte counts. Since there was good repeatability in electronic total leucocyte counts in suspensions from the same blood sample and also a low error in counts with different blood samples, even though it was necessary to introduce a chemical substance to cause haemolysis, it was assumed that the source of the error in the comparative counts was due to the known high technical and field errors with the haemocytometer and also the fact that the comparison was less direct than the one used with the erythrocytes.

Although the accuracy of the instrument is high, there are differences in performance between one technician and another. Therefore, to ensure that accuracy in the counting method is maintained during an experiment, only one technician should be employed throughout. In this series of experiments, technician 1 was responsible for all the calibration experiments and determination of technical errors of the technique.

The results of the experiments appeared to fulfil the conditions laid down for calibrating the instrument, and it can thus be concluded that for cat blood, the electronic method is accurate within the usual limitations of biological variation.

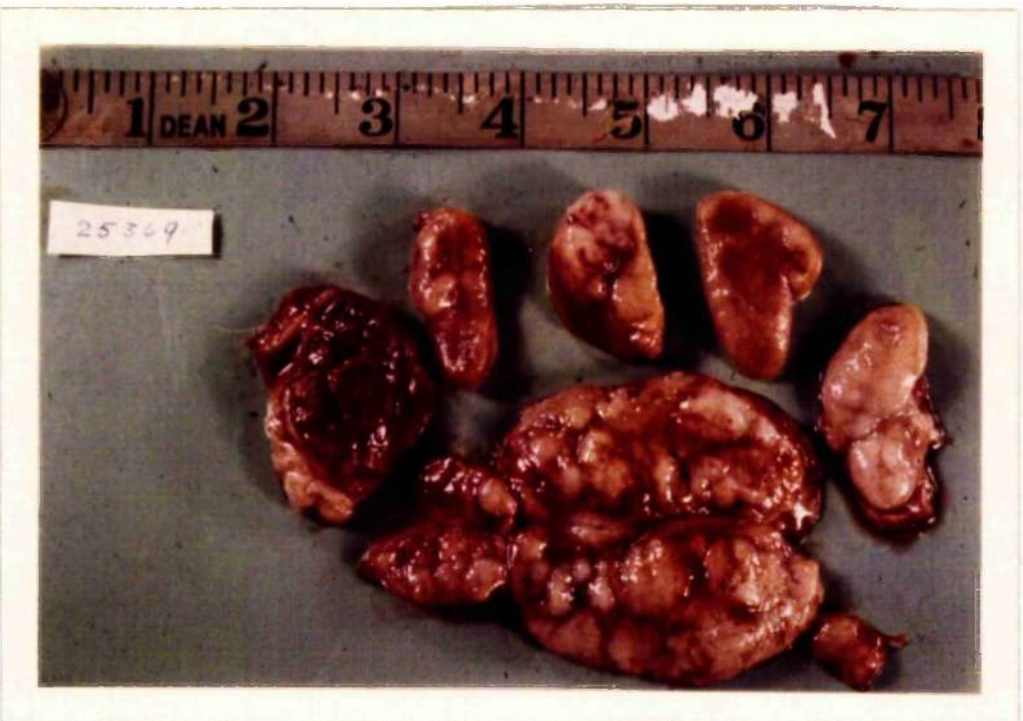

Figure 1. Canine lymph nodes. The cut surfaces of nodes affected with lymphosarcoma to illustrate the gross variations which may be found in the same dog.

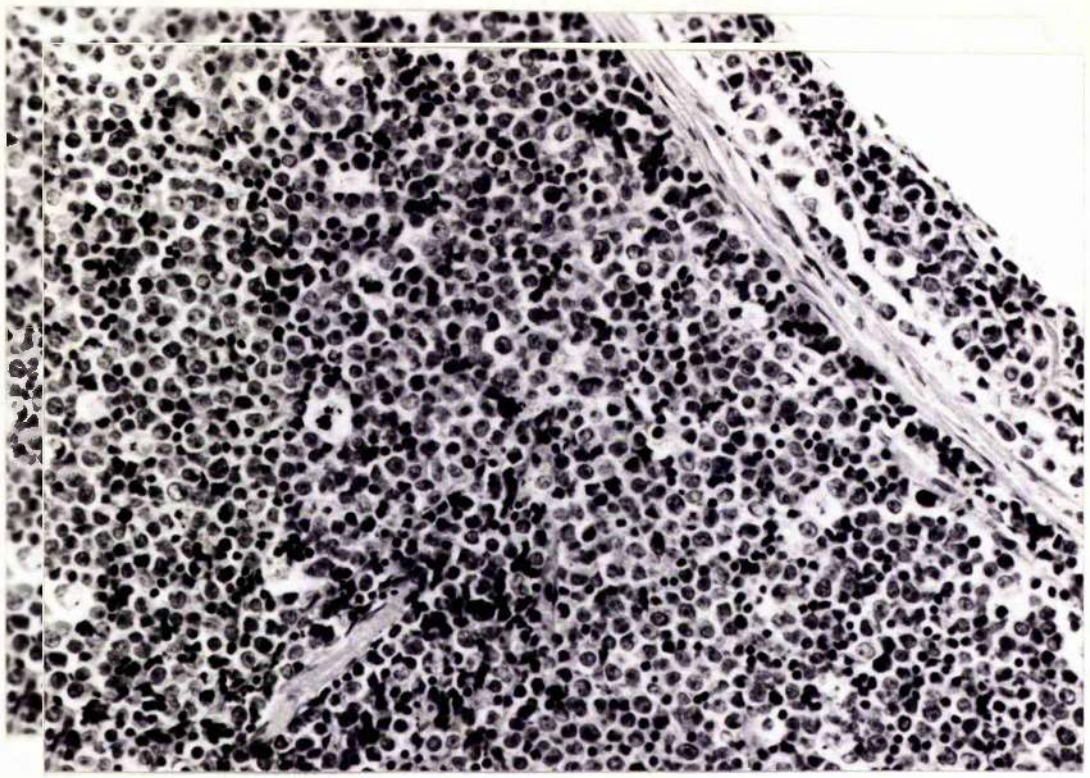

Figure 2. Canine lymph node. Slight infiltration beyond an intact capsule, upper right. H. & E. x 300.

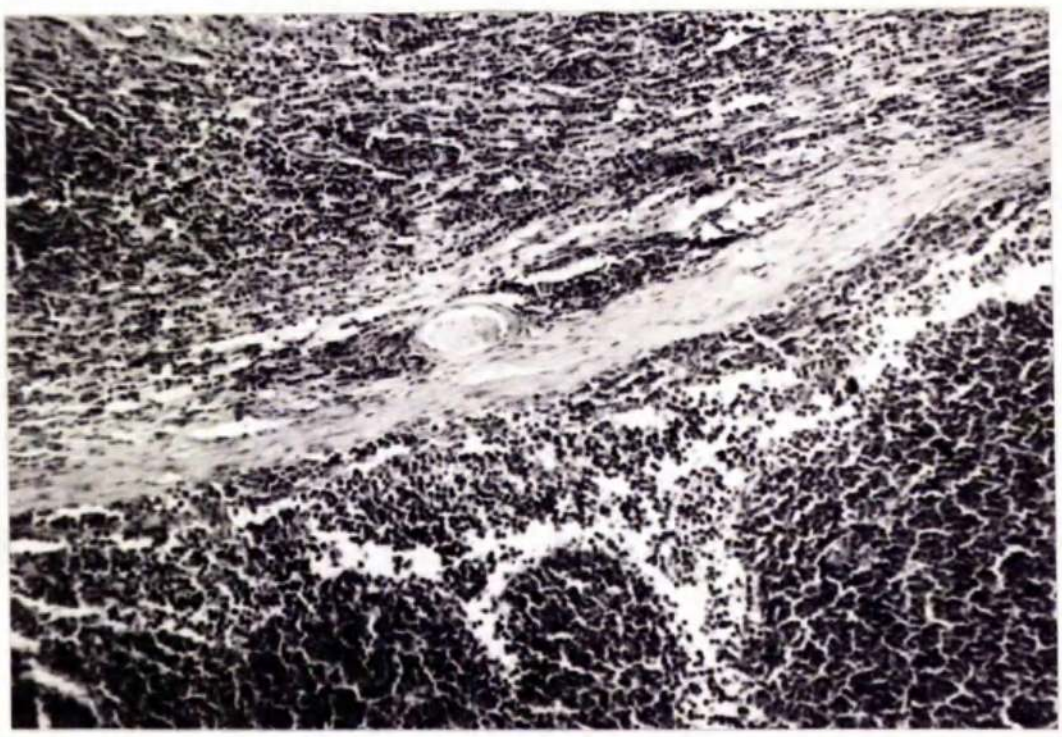

Figure 3. Canine lymph node. Severe infiltration into condensed connective tissue above. Note the relatively intact capsule and the heavy flooding of the subcapsular sinus. H. & E. x 150

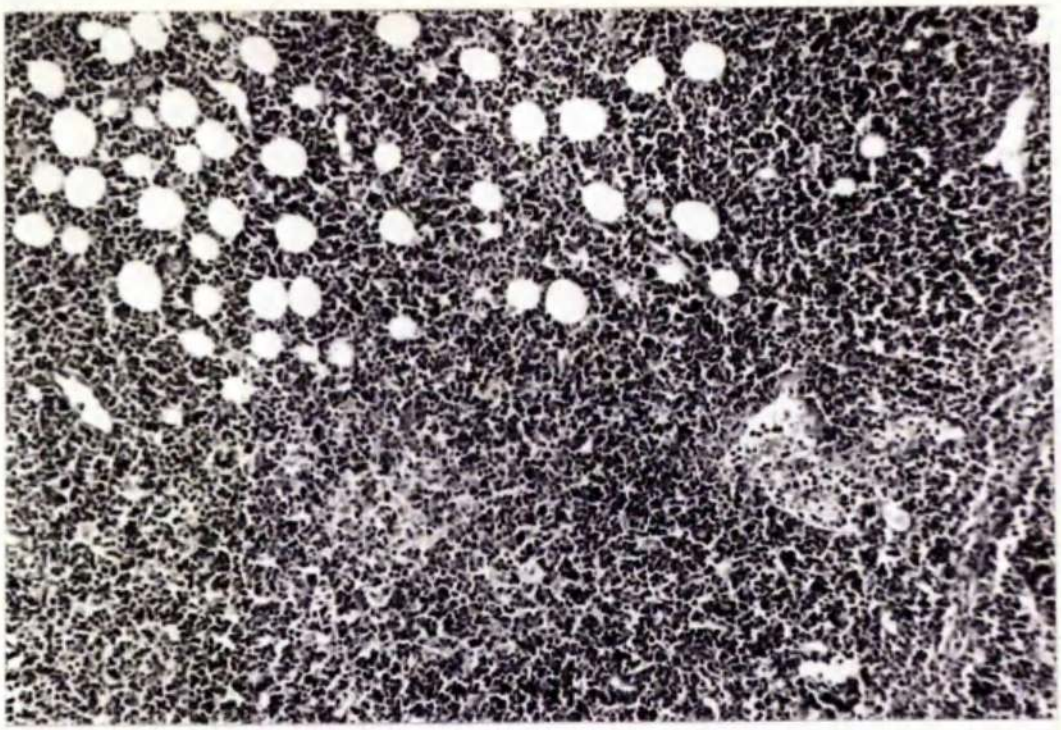

Figure 4. Canine lymph node. Massive invasion of the perinodal area. The clear spaces represent fat cells. A trace of the former capsule and cortex is present in the lower right corner. H.& E. x 150.

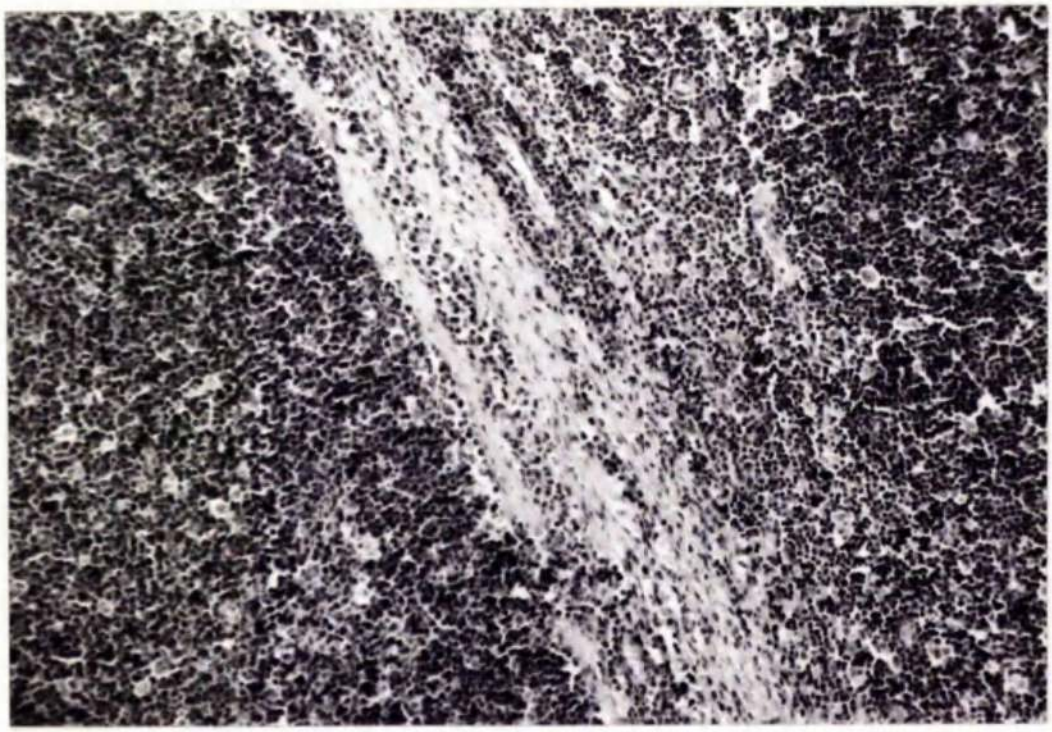

Figure 5. Canine lymph node. Massive invasion of the perinodal area from an affected node on the left, with lamination of the intervening capsule. H. & E. x 150.

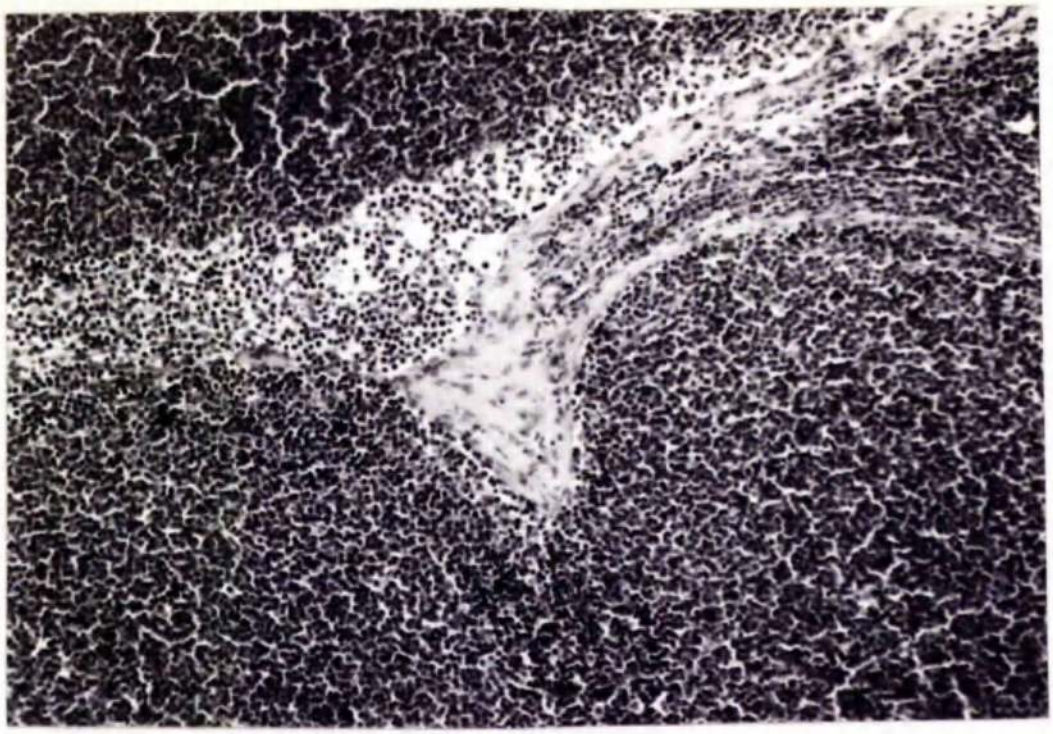

Figure 6. Canine lymph node. In the lower half, two tumour follicles are compressing the intervening sinus which contains more mature cells. Above the foci, a large sinus is being flooded with tumour cells which are also infiltrating a trabecula, upper right. H.& E. x 150.

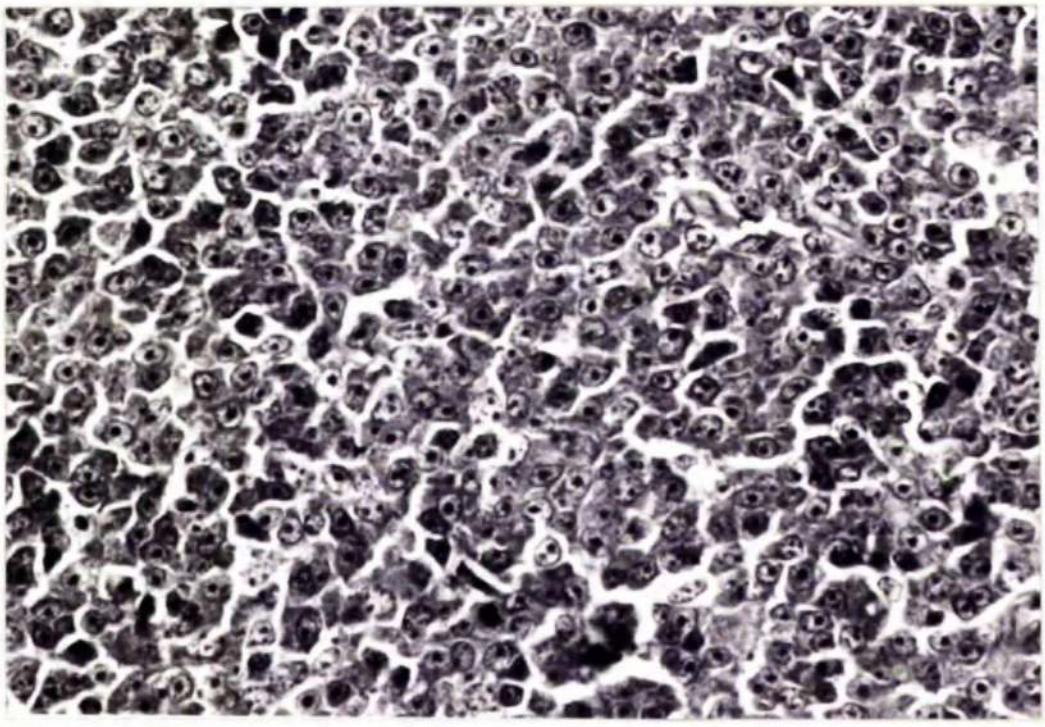

Figure 7. Canine lymph node. A higher magnification of a tumour follicle of Figure 6, to show the very large undifferentiated lymphoid tumour cells. H. & E. x 500.

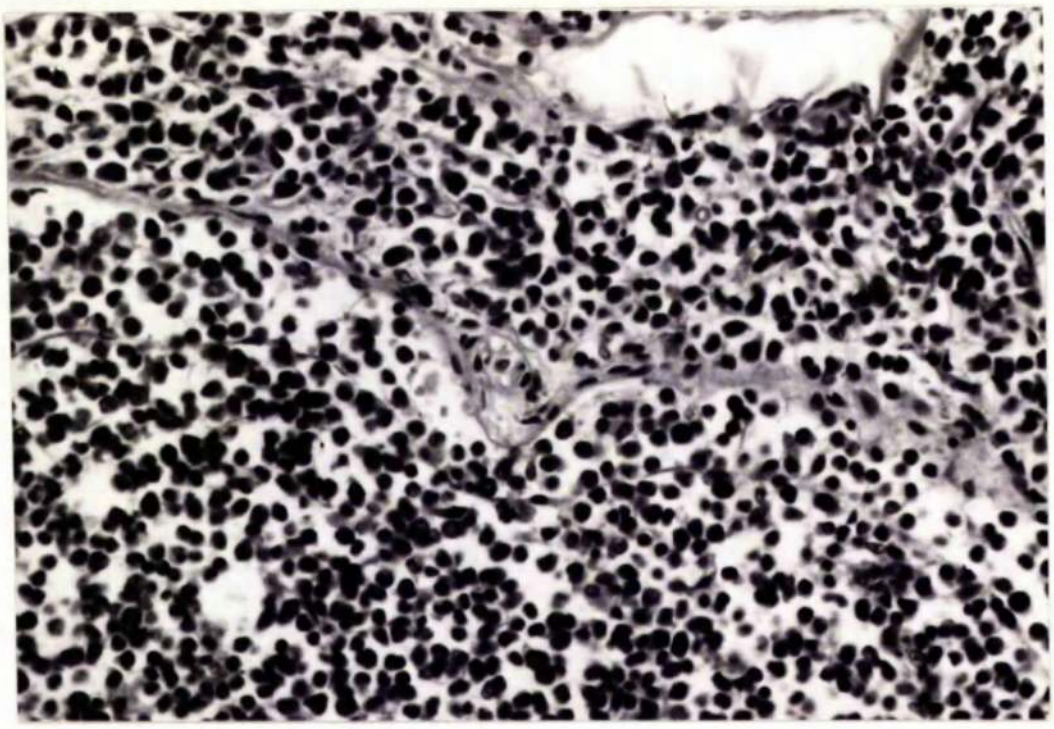

Figure 8. Canine lymph node. A cortical cell sheet is infiltrating upwards into a sinus; above the sinus, there is severe infiltration of a trabecula. H. & E. x 300.

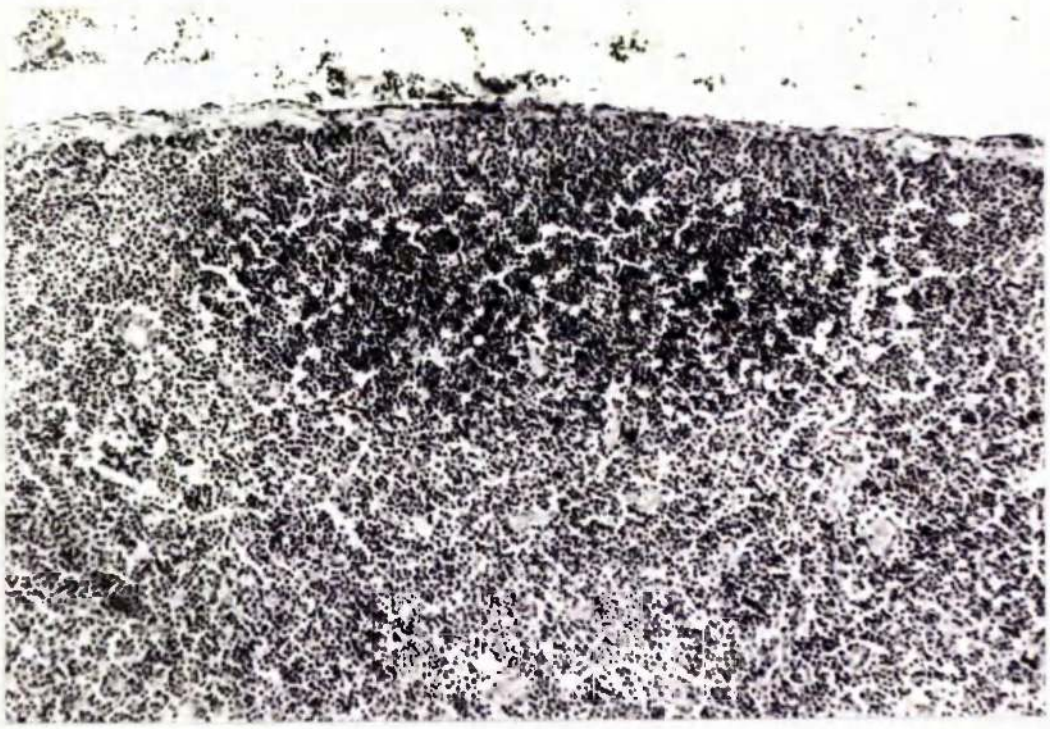

Figure 9. Canine lymph node. A compressed vestigial follicle can be observed below a thin intact capsule. H. & E. x 150.

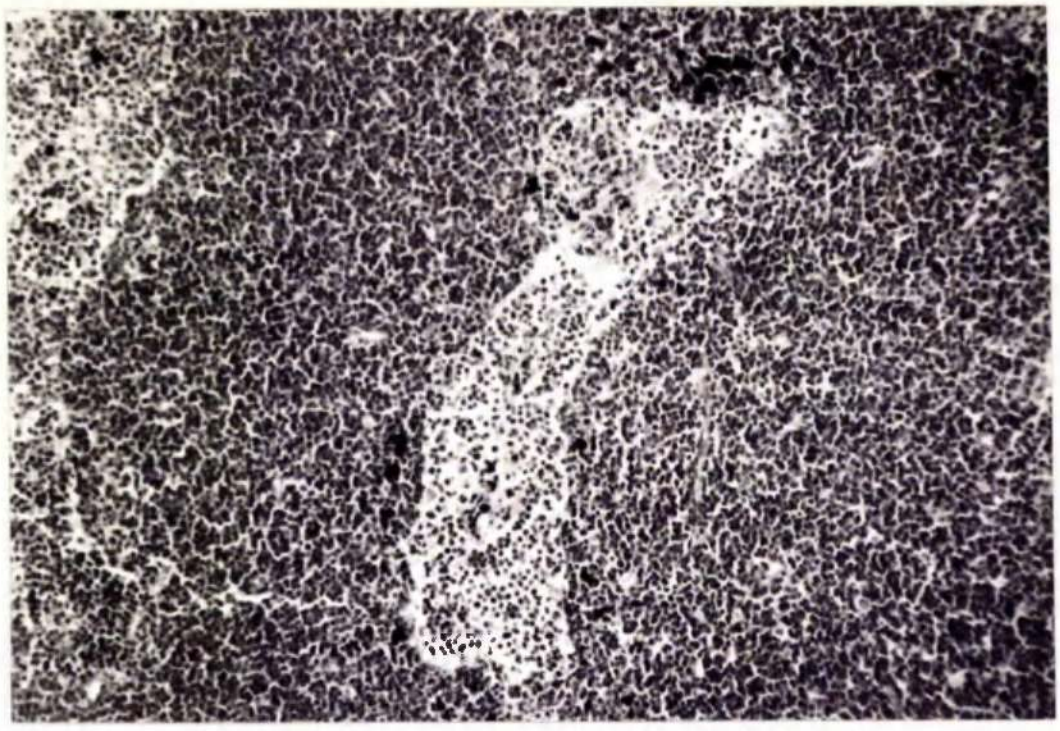

Figure 10. Canine lymph node. A cortical sinus heavily flooded with tumour cells, but still clearly visible. H. & E. x150.

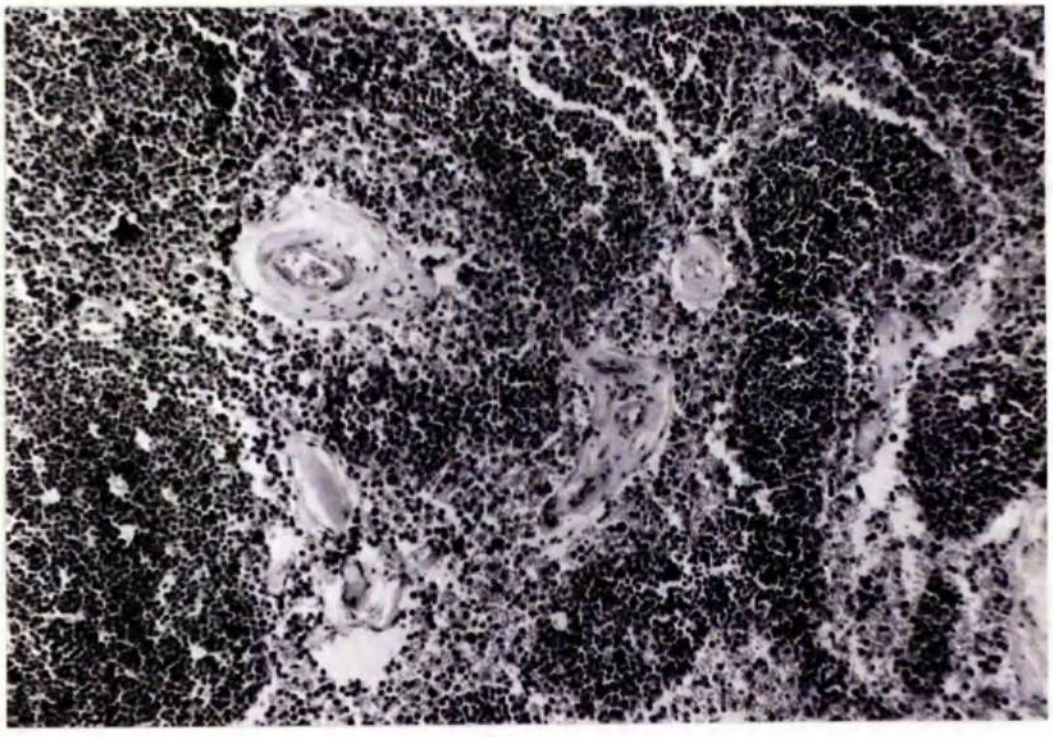

Figure 11. Canine lymph node. Tumour cell infiltration of the medullary pegs and sinuses. H. & E. x 150.

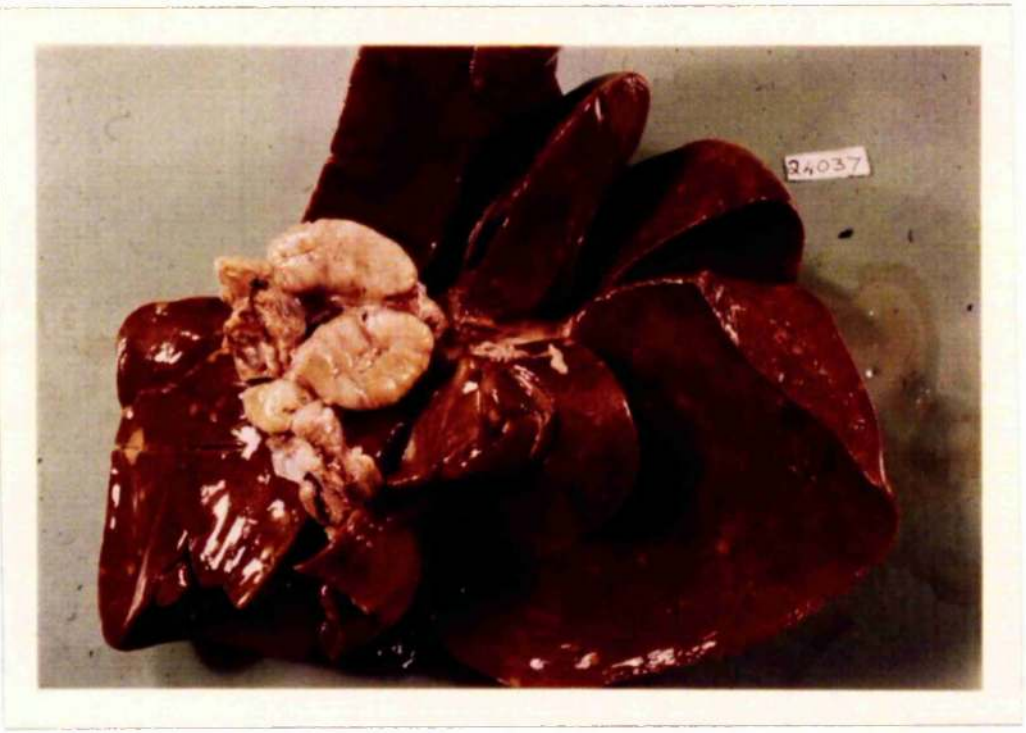

Figure 12. Canine liver. Small metastatic lesions of lymphosarcoma can be observed on the surfaces of all lobes. Part of the main lobe on the right has been sectioned to show the nodules in the parenchyma. The hepatic lymph node is grossly enlarged and has been cut to show the typical lymphosarcomatous tissue.

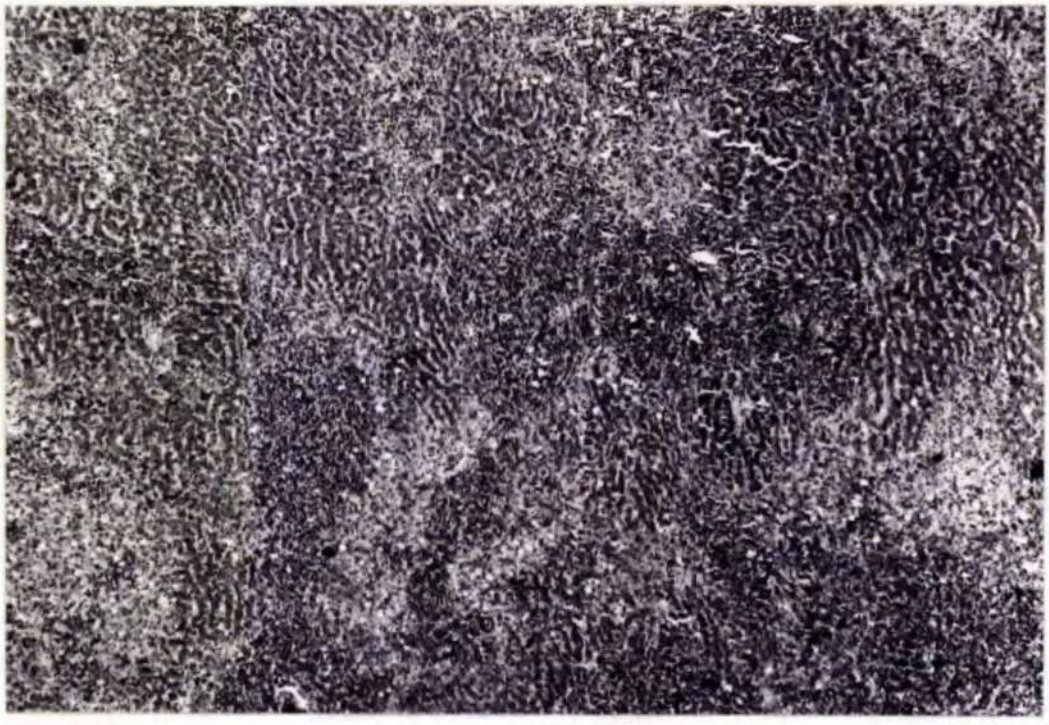

Figure 13. Canine liver. Tumour cell infiltration of the portal triads. H. & E. x 50.

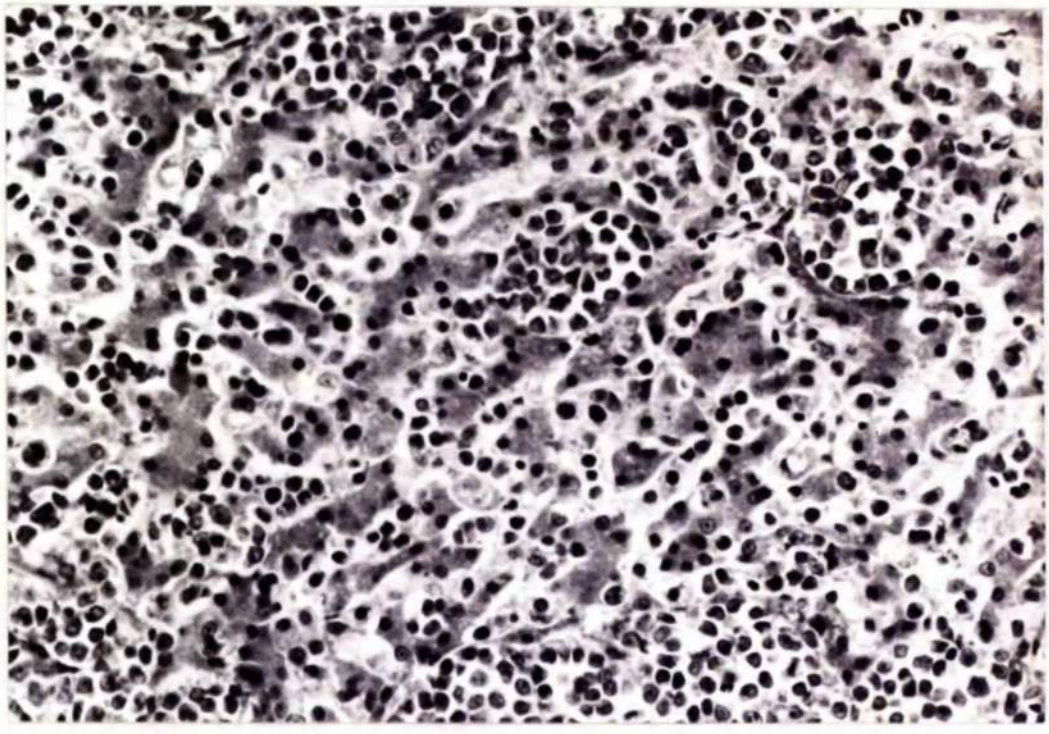

Figure 14. Canine liver. Higher magnification of Figure 13 to show the diffuse infiltration of sinusoids and clone formation. H. & E. x 300.

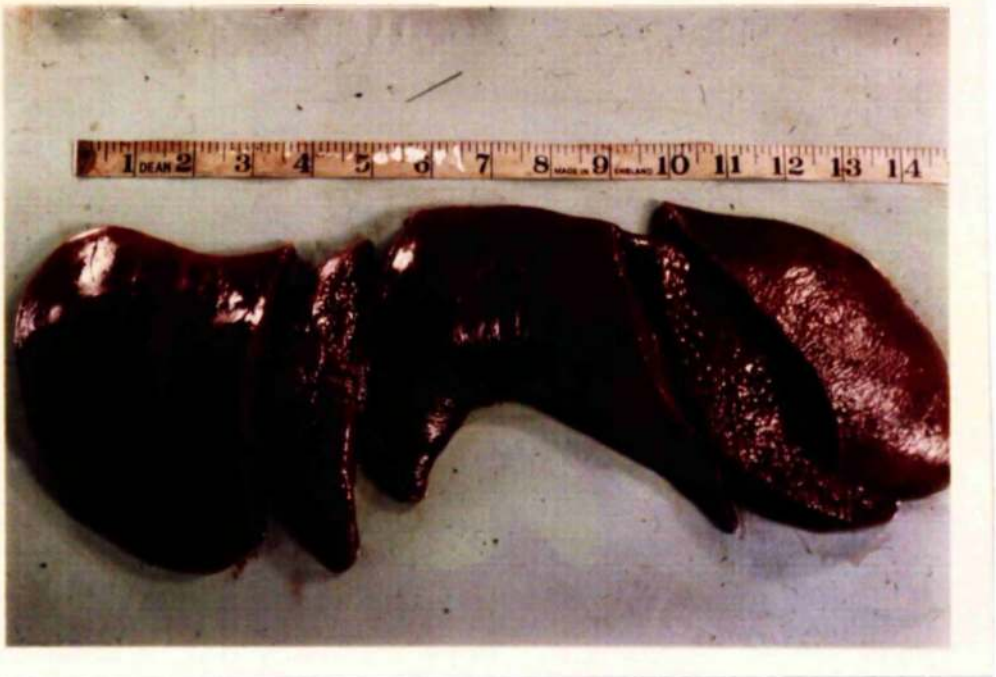

Figure 15. Canine spleen: affected with lymphosarcoma. Note that its edges are rounded, its profile is maintained, and the pulp shows signs of gross hyperplasia of the Malpighian bodies.

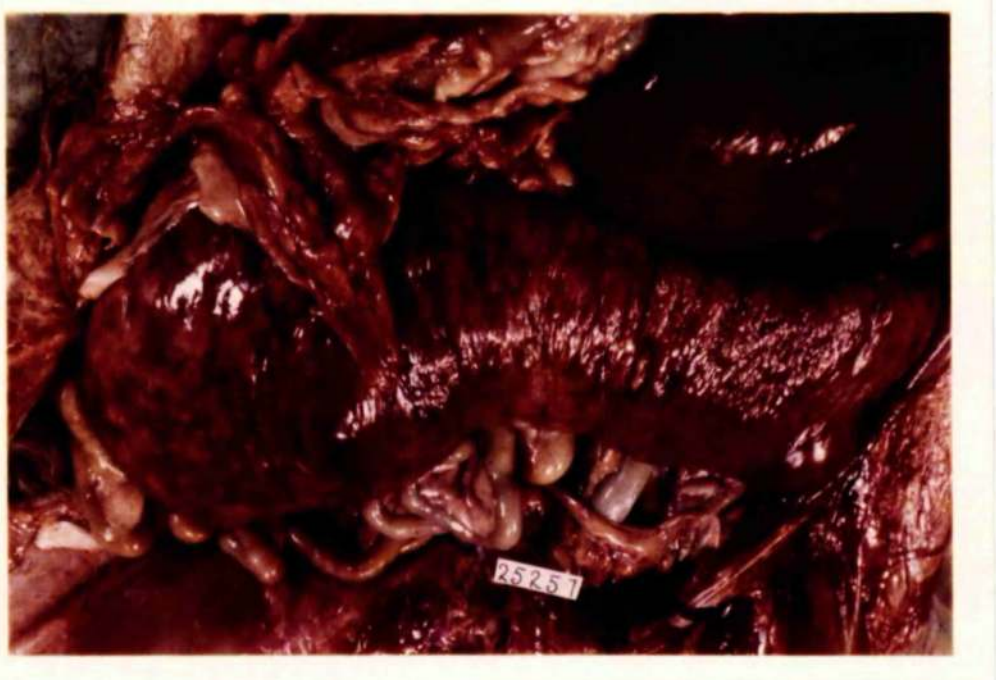

Figure 16. Canine spleen; affected with lymphosarcoma. There is a small yellow infarct in the centre of the middle third, and a raised tumour, probably a benign lymphoma, towards the right pole.

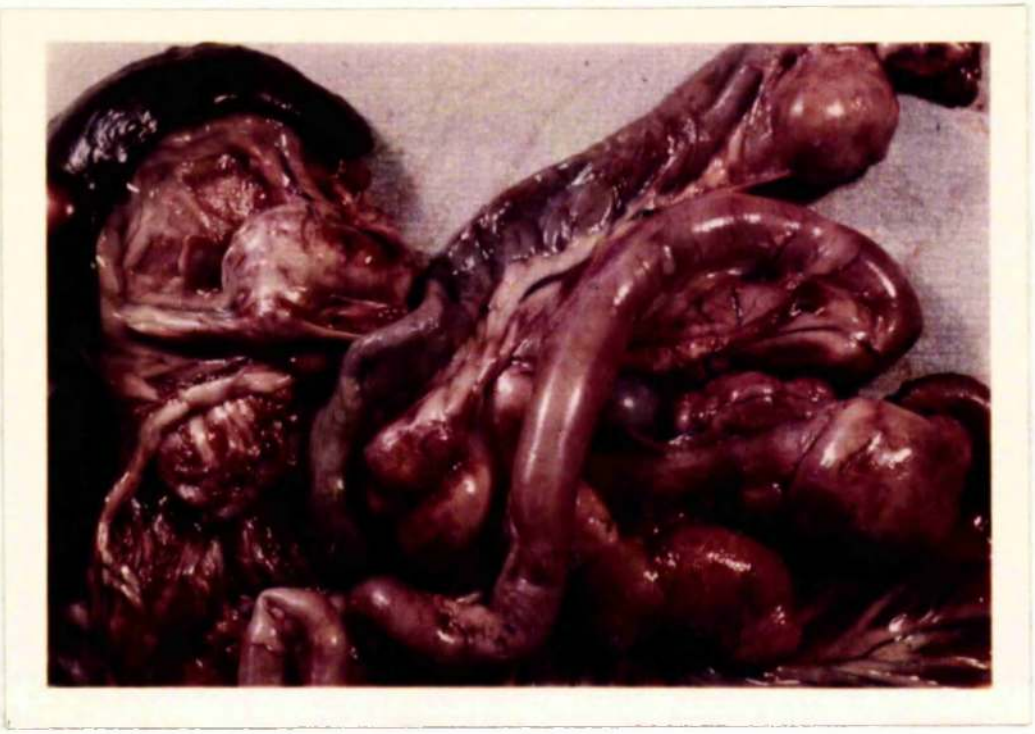

Figure 17. Canine Intestines. There are numbers of grossly enlarged and tumified lymph nodes in the mesentery. The spleen is not enlarged.

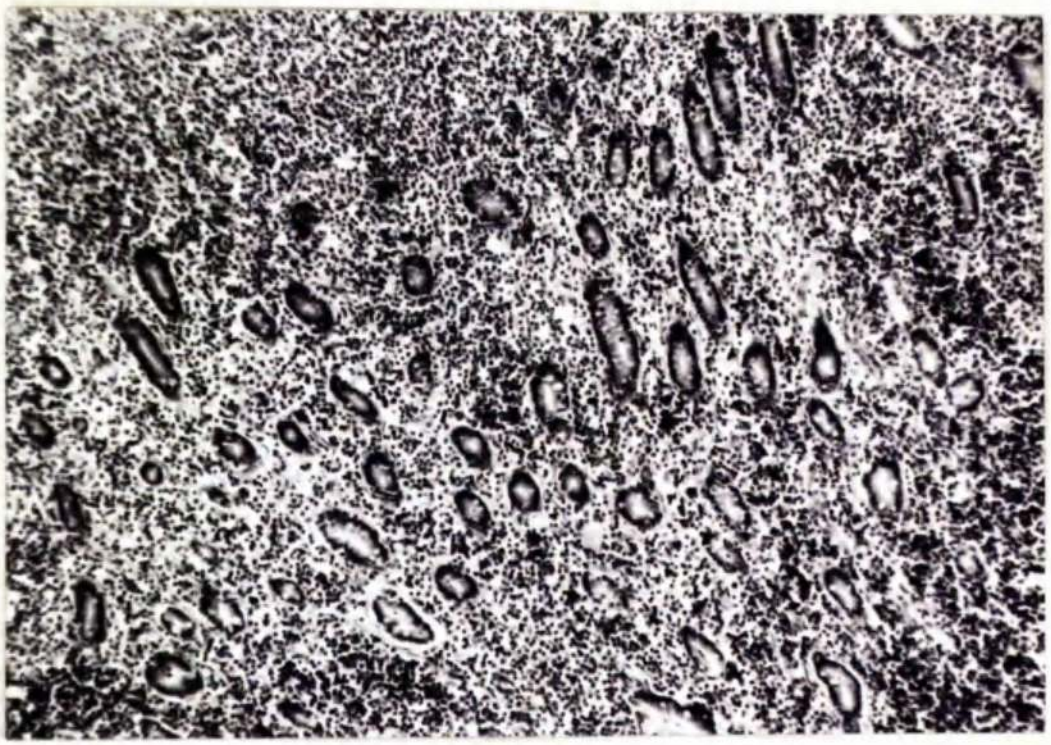

Figure 18. Canine small intestine. Massive invasion of the mucous membrane by tumour cells. H. & E. x 150.

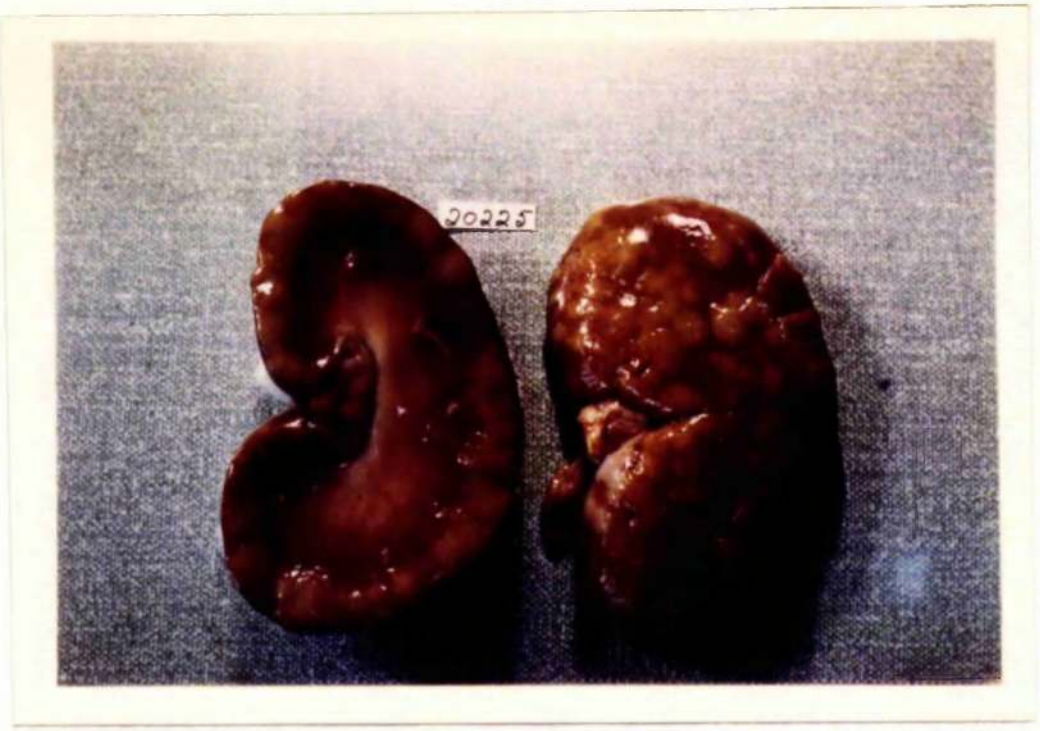

Figure 19. Canine kidney. The fairly discrete tumour masses lie almost entirely within the cortex.

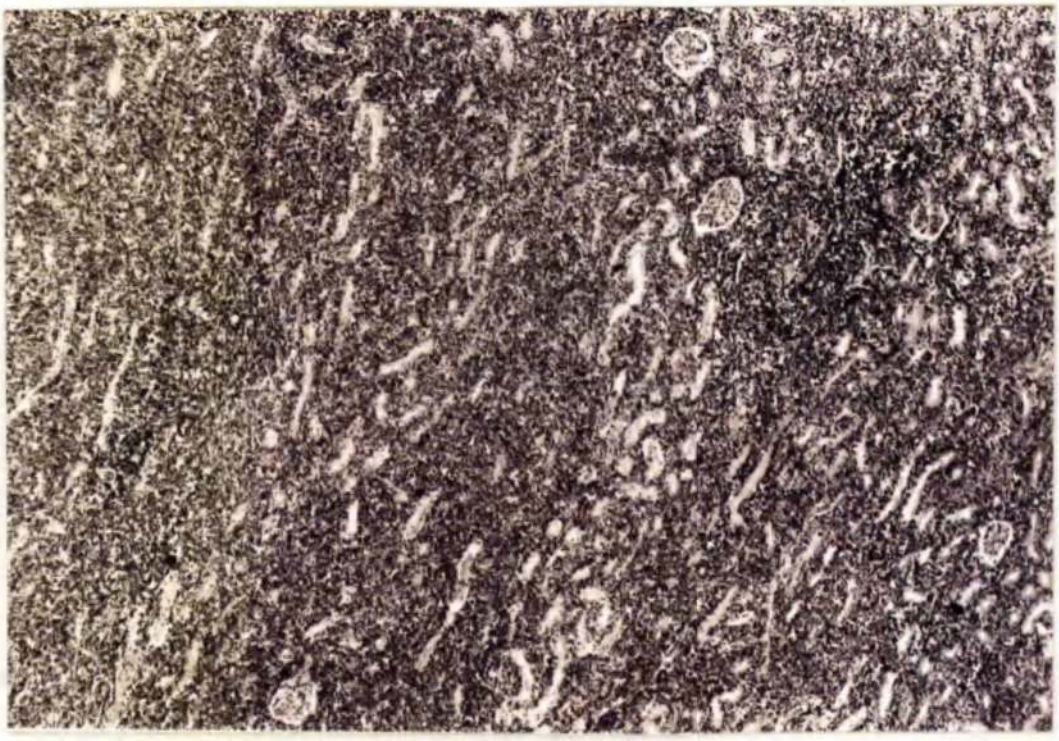

Figure 20. Canine kidney. A typical massive lesion with widespread tubular and glomerular destruction. H. & E. x 50.

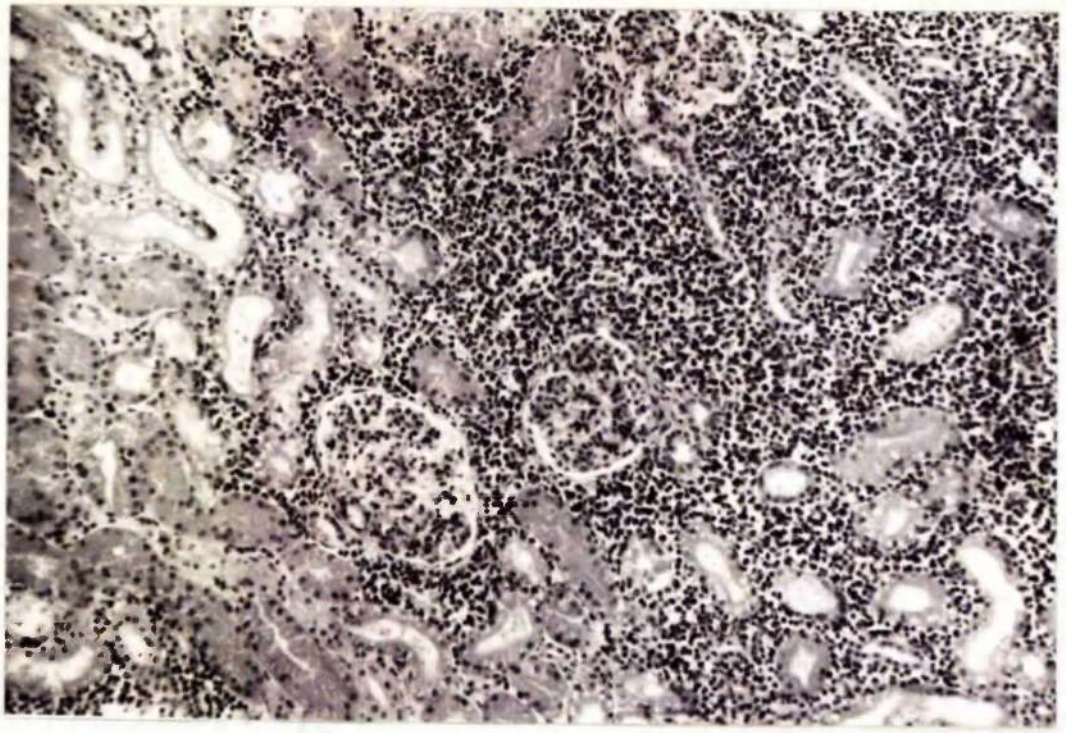

Figure 21. Canine kidney. A focal lesion with central destruction of tubules and peripheral interstitial infiltration. H.& E. x 150.

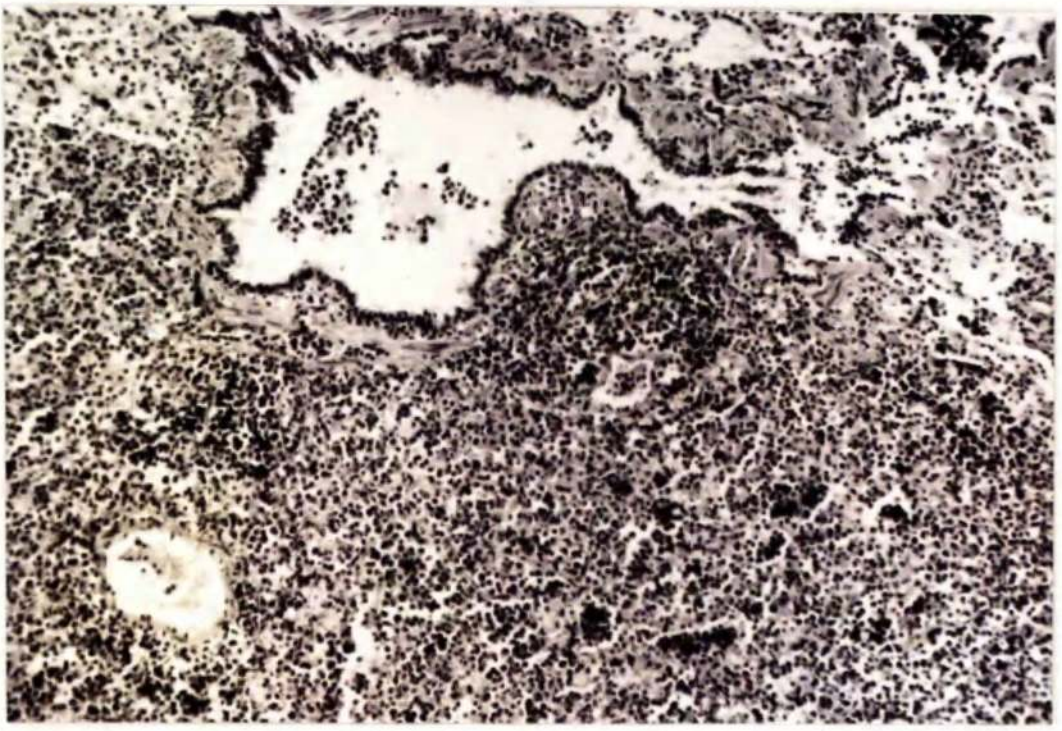

Figure 22. Canine lung. A lesion of lymphosarcoma adjacent to a small bronchiole. A few surviving alveoli are packed with darkly stained lymphoid cells. H. & E. x 150.

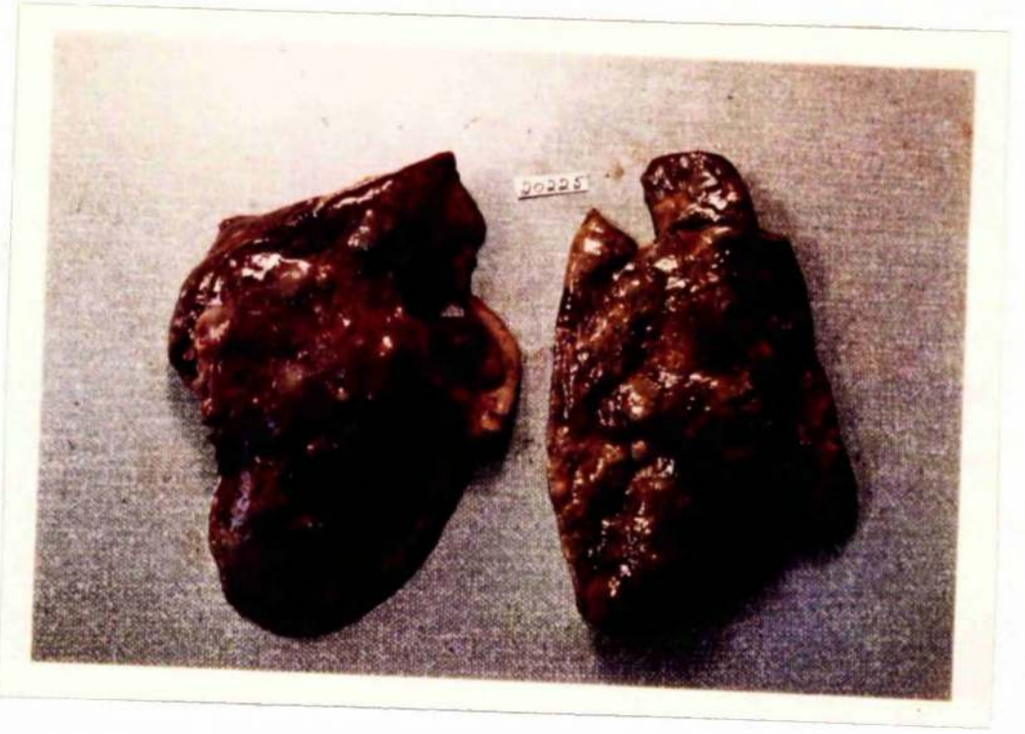

Figure 23. Canine lung. Parts of two lobes to show large discrete tumours of lymphosarcoma.

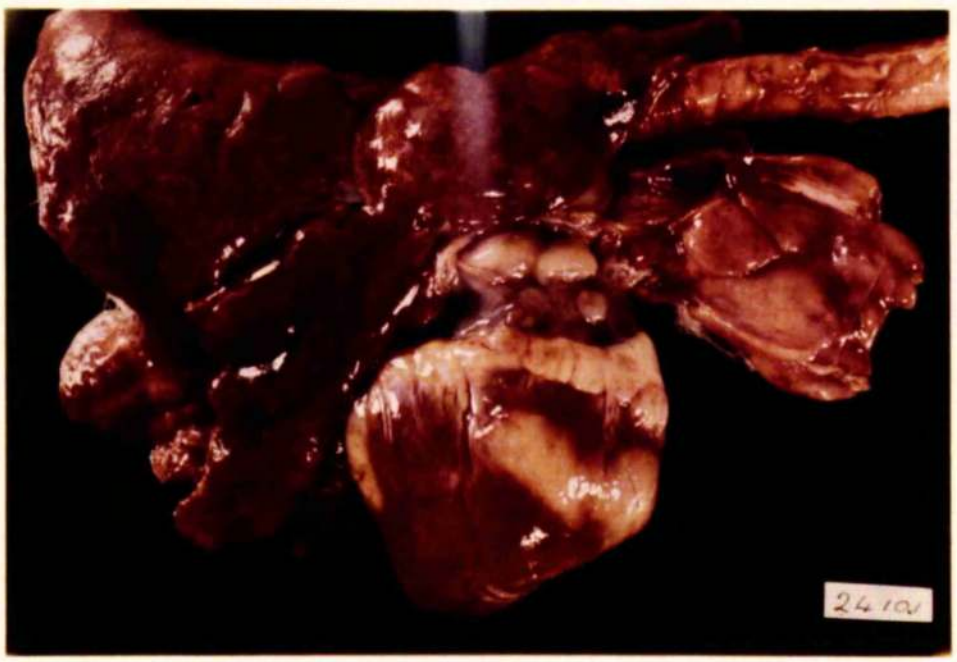

Figure 24. Canine heart and lungs. There is a massive lesion of lymphosarcoma in the wall of the right ventricle, and smaller masses in the right auricle.

Figure 25. Canine heart. The edge of a large ventricular tumour mass showing infiltration of the surviving muscle bundles at the periphery of the lesion. H. & E. x 150.

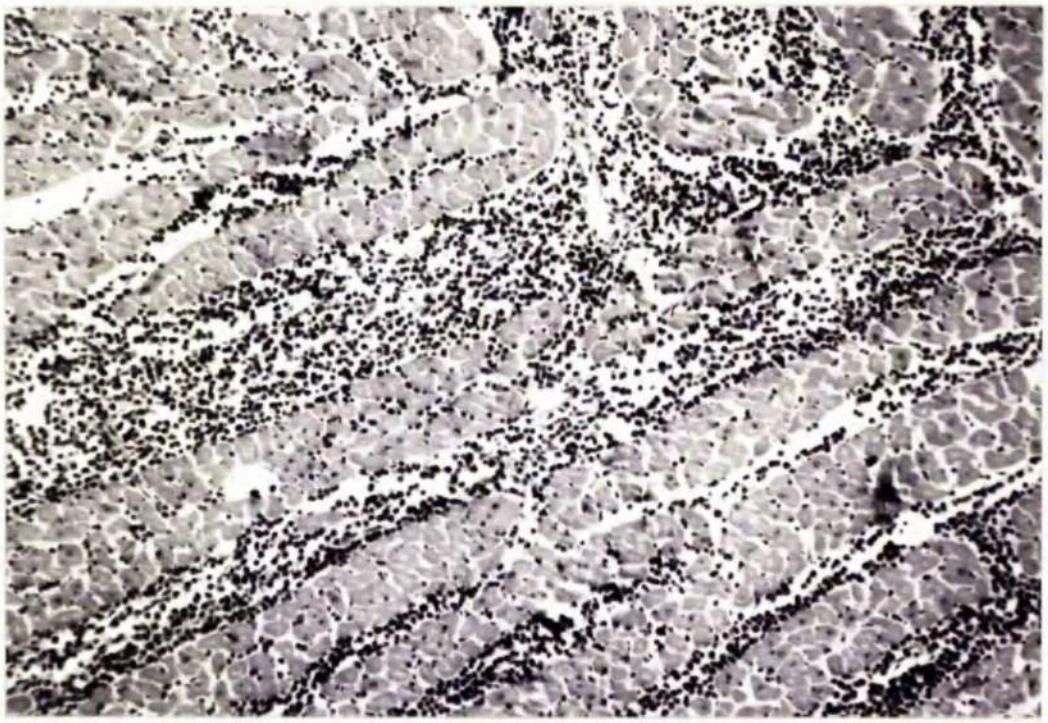

Figure 26. Canine heart. Widespread diffuse lamination of the muscle bundles by streams of lymphoid tumour cells. H. & E. x 150.

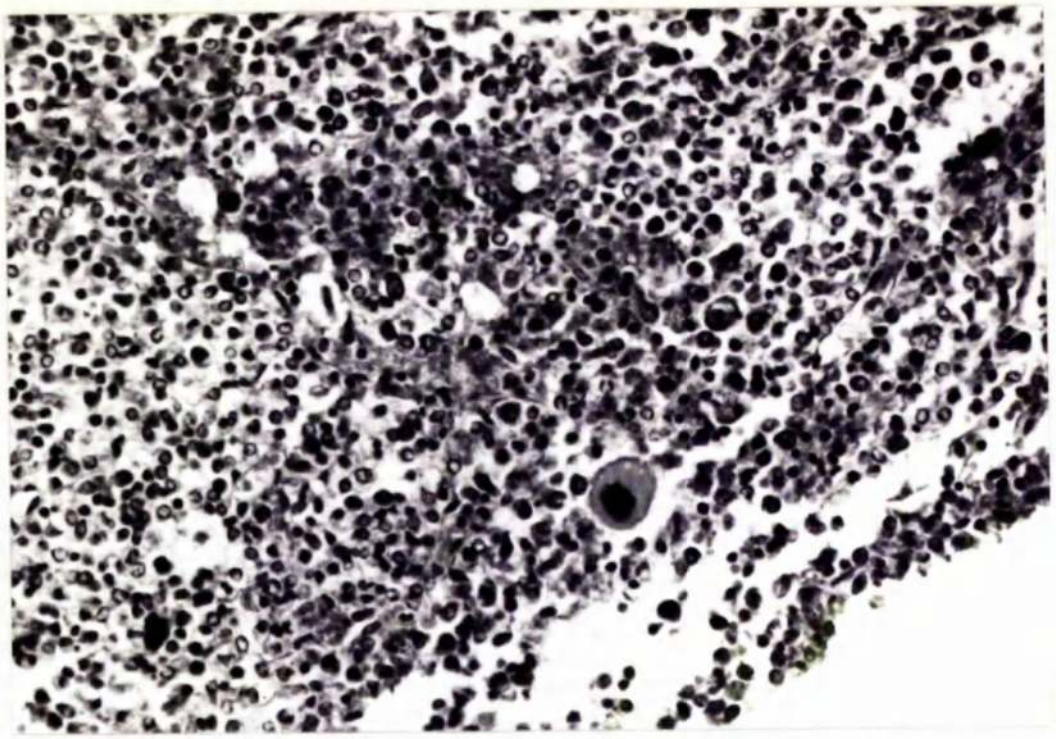

Figure 27. Canine bone marrow. Diffuse infiltration with lymphoid cells. H. & E. x 300.

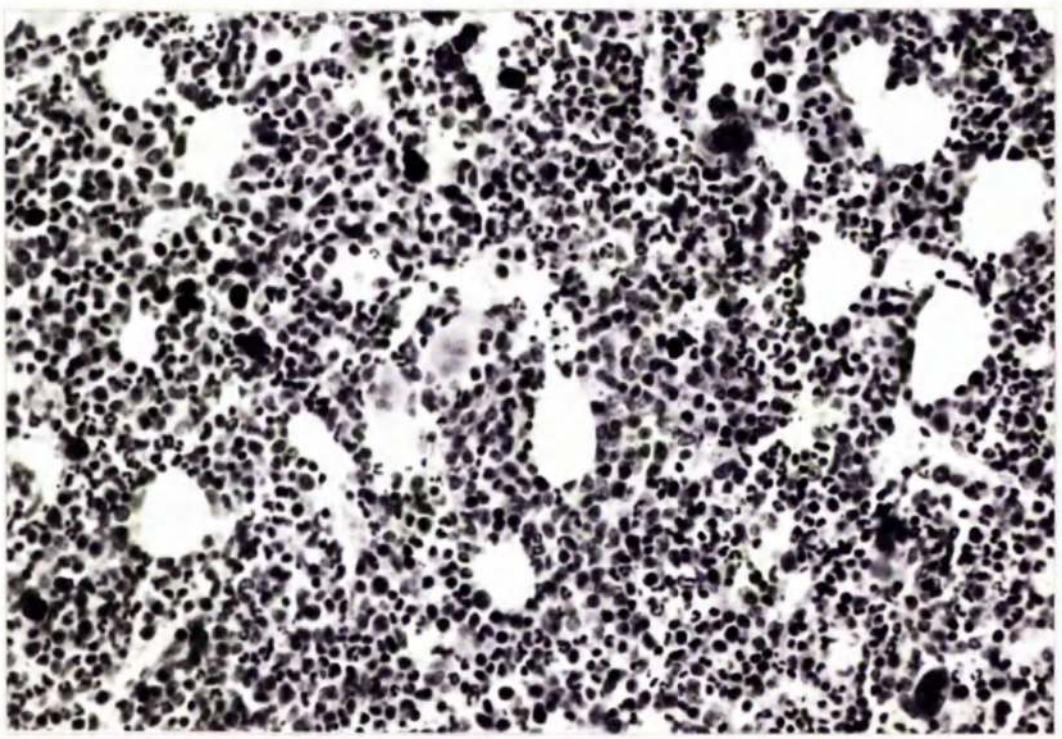

Figure 28. Canine bone marrow. Marked hyperplasia of the myeloid elements. H. & E. x 300.

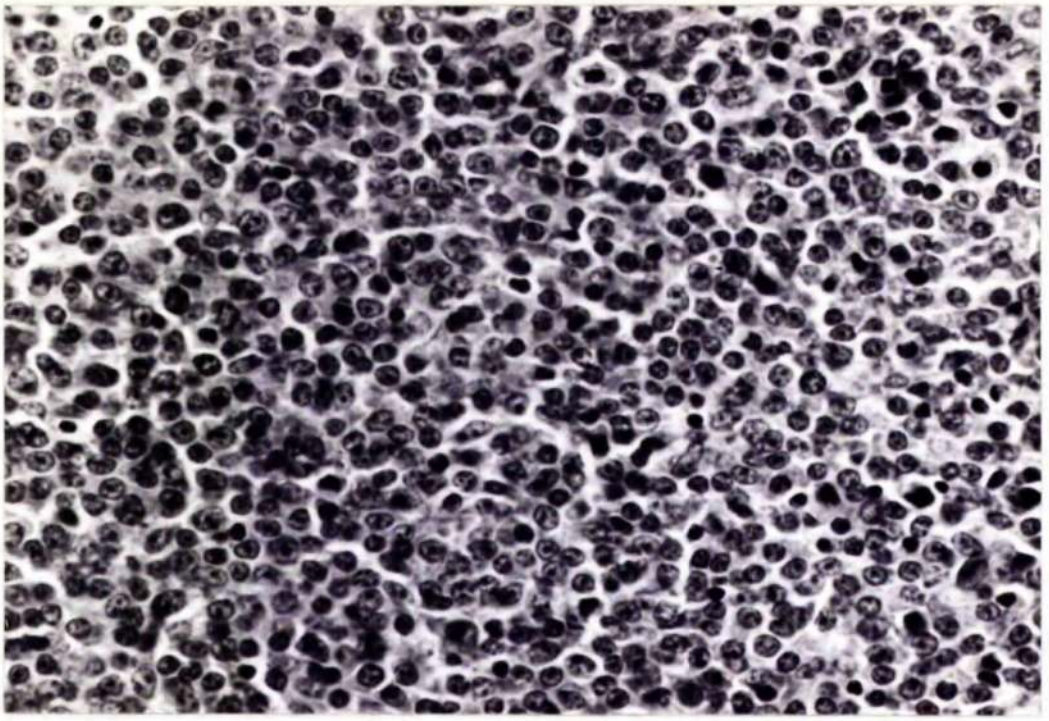

Figure 29. Canine lymph node. A typical "immature" cell sheet composed of lymphoblasts and occasional large lymphocytes. H.& E. x 500.

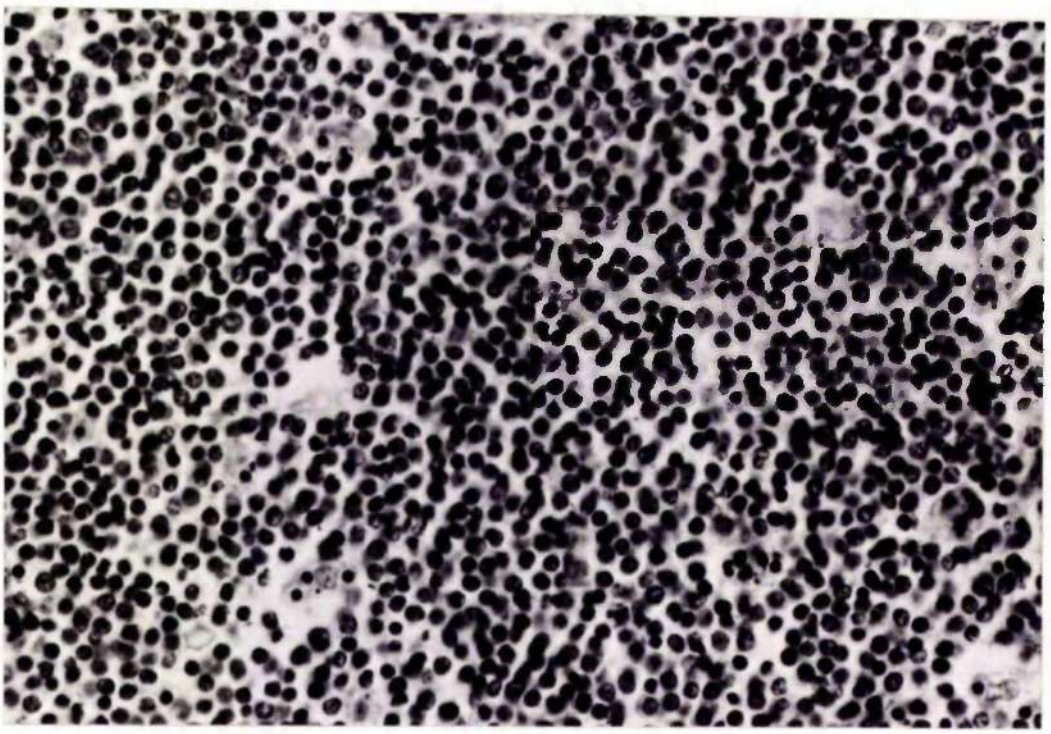

Figure 30. Canine lymph node. "Mature" tumour cell sheet composed mainly of medium and mature lymphocytes. H. & E. x 500.

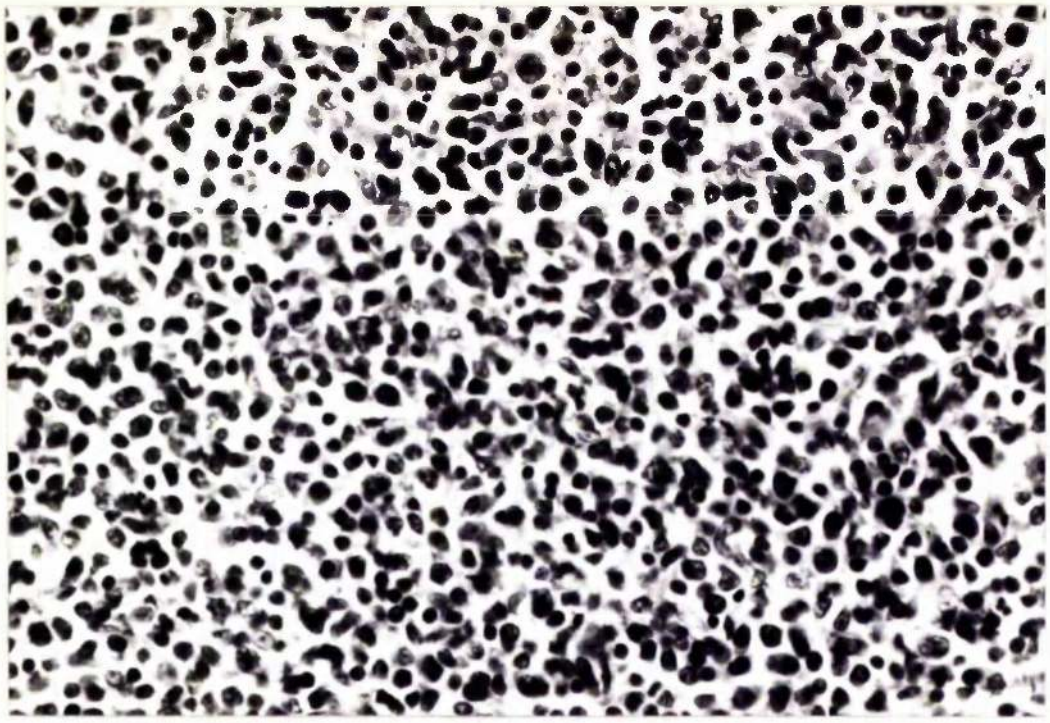

Figure 31. Canine lymph node. "Mixed" tumour cell sheet containing examples of all forms of lymphoid cells. H. & E. x 500.

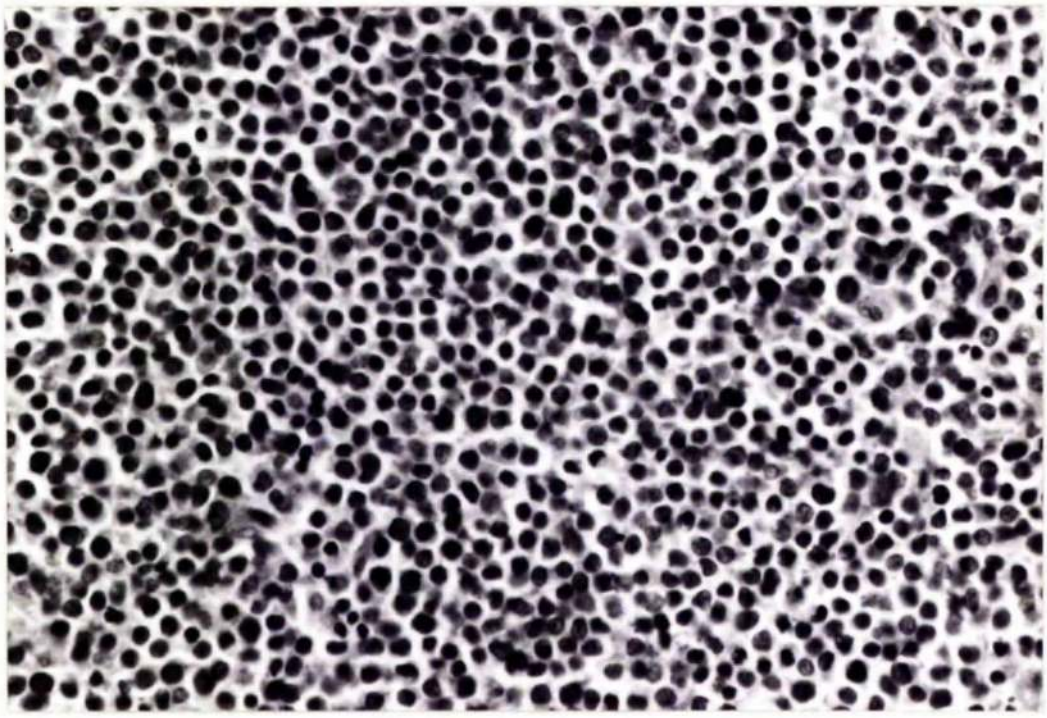

Figure 32. Canine lymph node. Tumour cell sheet composed mainly of large and medium lymphocytes. H. & E. x 500.

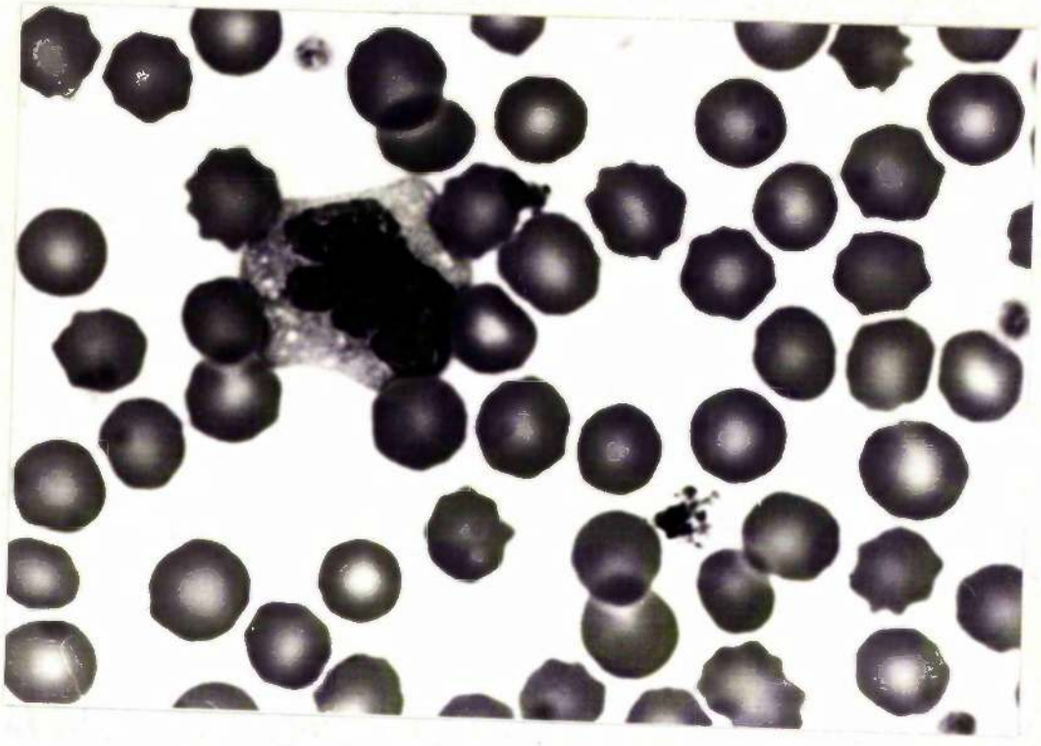

Figure 33. Canine blood film. A typical lobulated lymphocyte. Leishman's stain x 800.

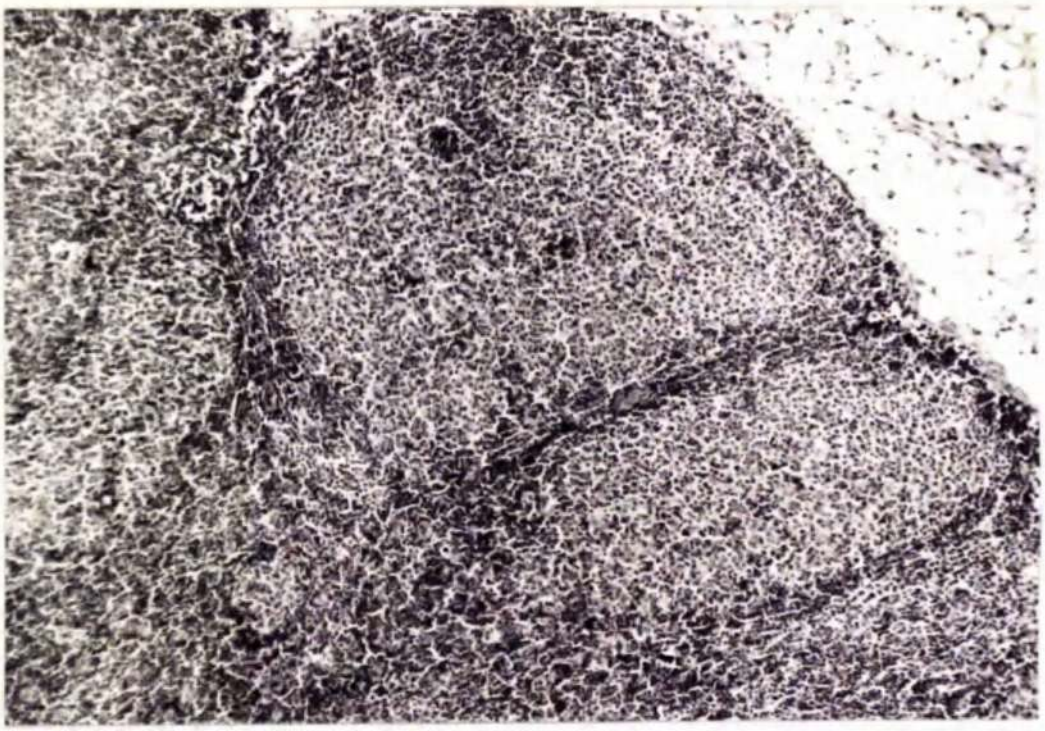

Figure 34. Feline lymph node. Large tumour follicles are compressing the cortical sinuses. They and the sub-capsular sinus are flooded with more mature lymphoid cells, but there is no infringement of the tissue outwith the node. H. & E. x 100.

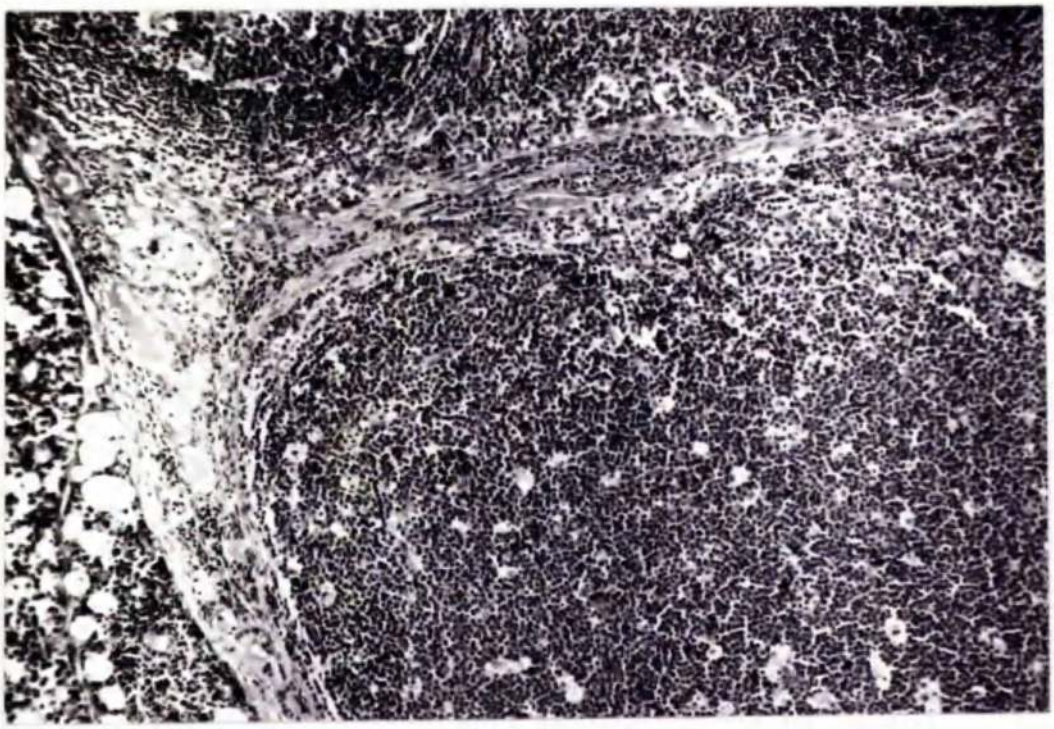

Figure 35. Feline lymph node. Tumour follicles are compressing the sub-capsular sinus and tumour cells are activ ely invading the capsule, cortical trabecula and the perinodal region. H.& E. x 100.

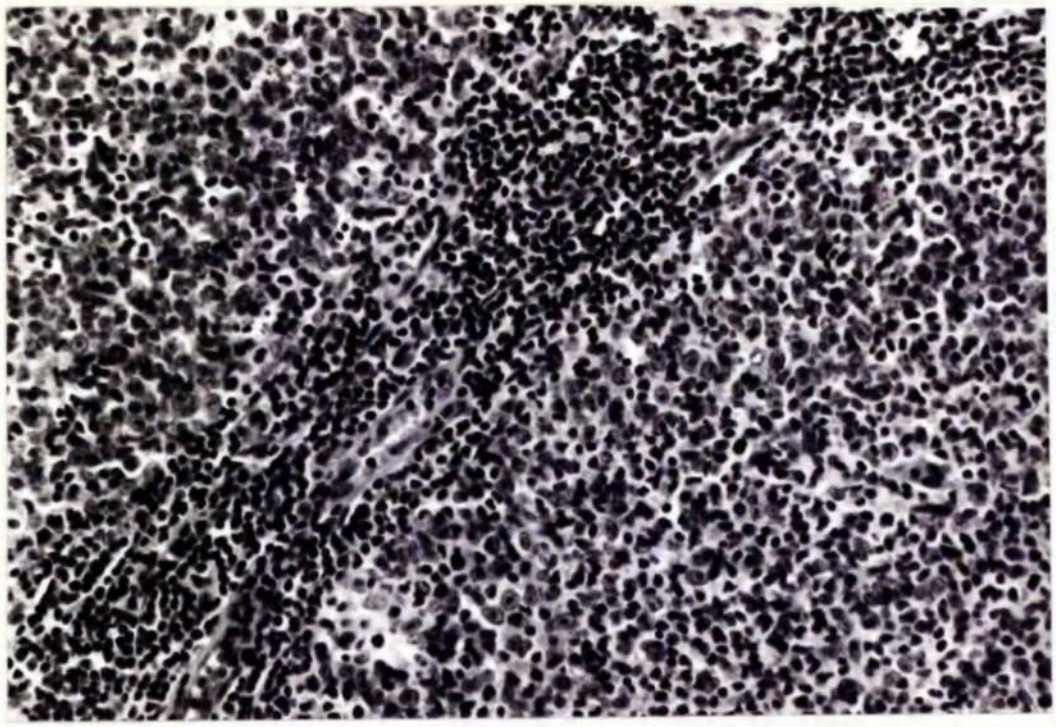

Figure 36. Feline lymph node. A cortical sinus containing mature lymphoid cells is being compressed between two tumour follicles. H. & E. x 250.

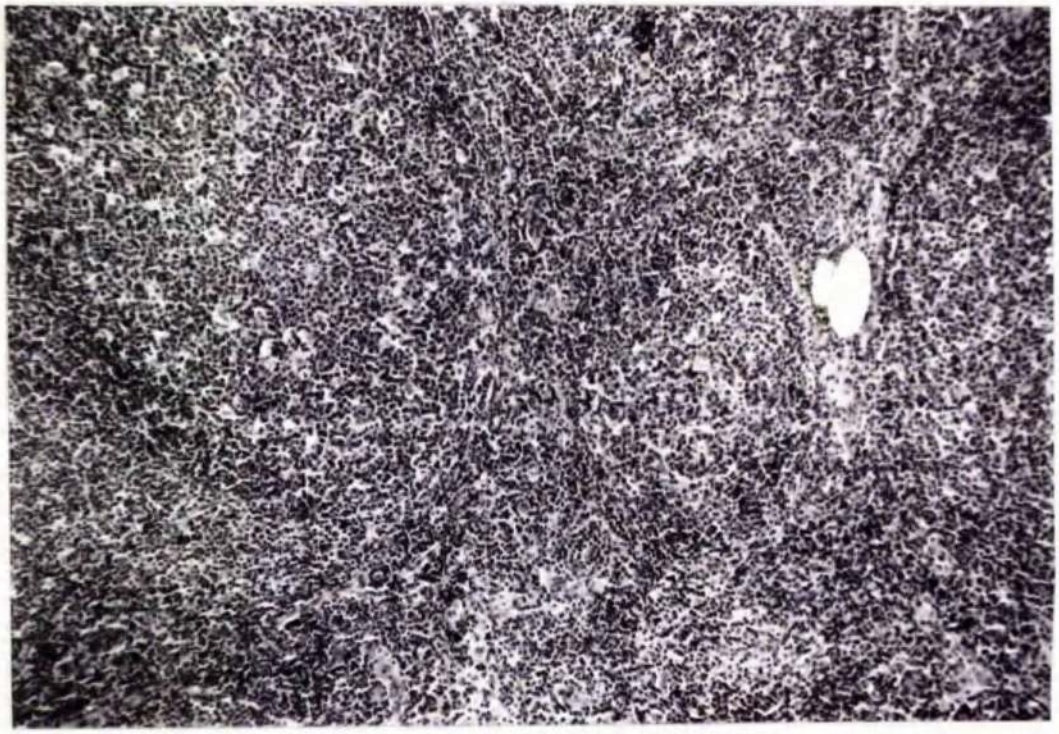

Figure 37. Feline lymph node. A tumour cell sheet which still retains the outlines of its former follicular pattern. H. & E. x 100.

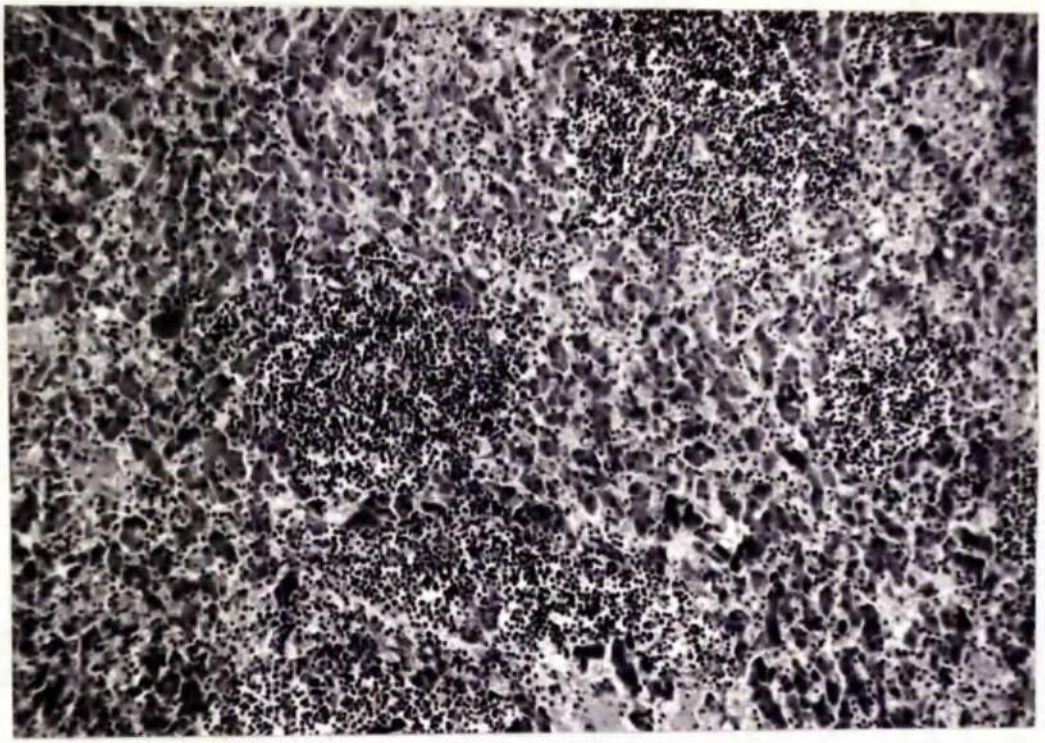

Figure 38. Feline liver. Marked invasion of the portal triads with diffuse tumour cell infiltration of the sinusoids. H. & E. x 100.

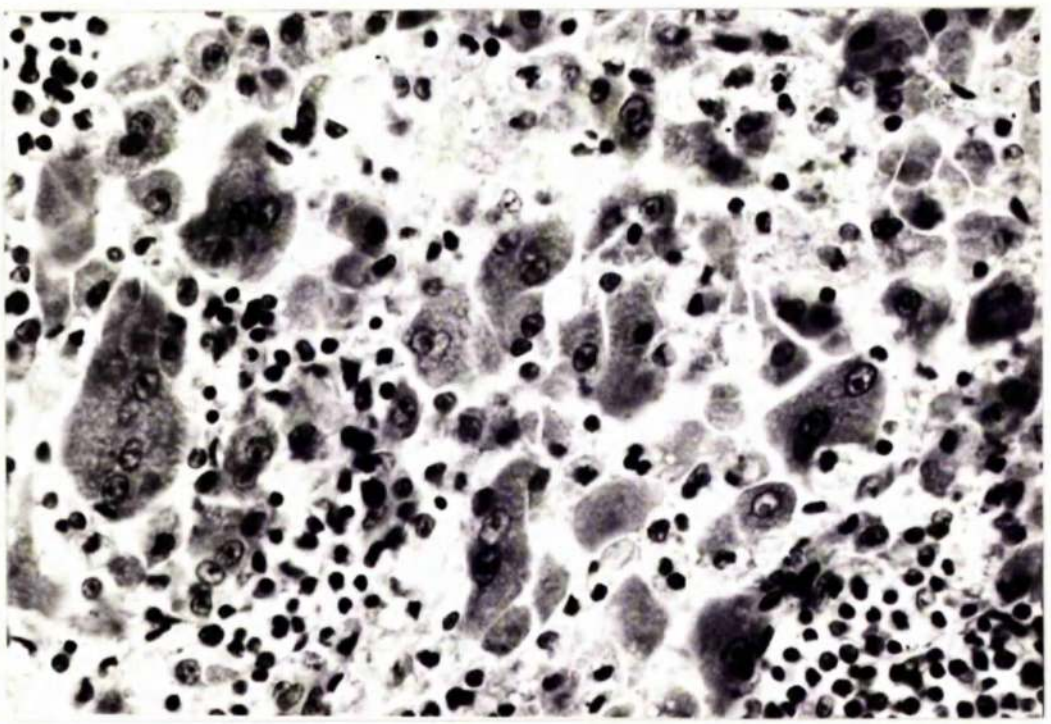

Figure 39. Feline liver. A higher magnification of the section above to illustrate multinucleated and degenerating cord cells. H.& E. x 400.

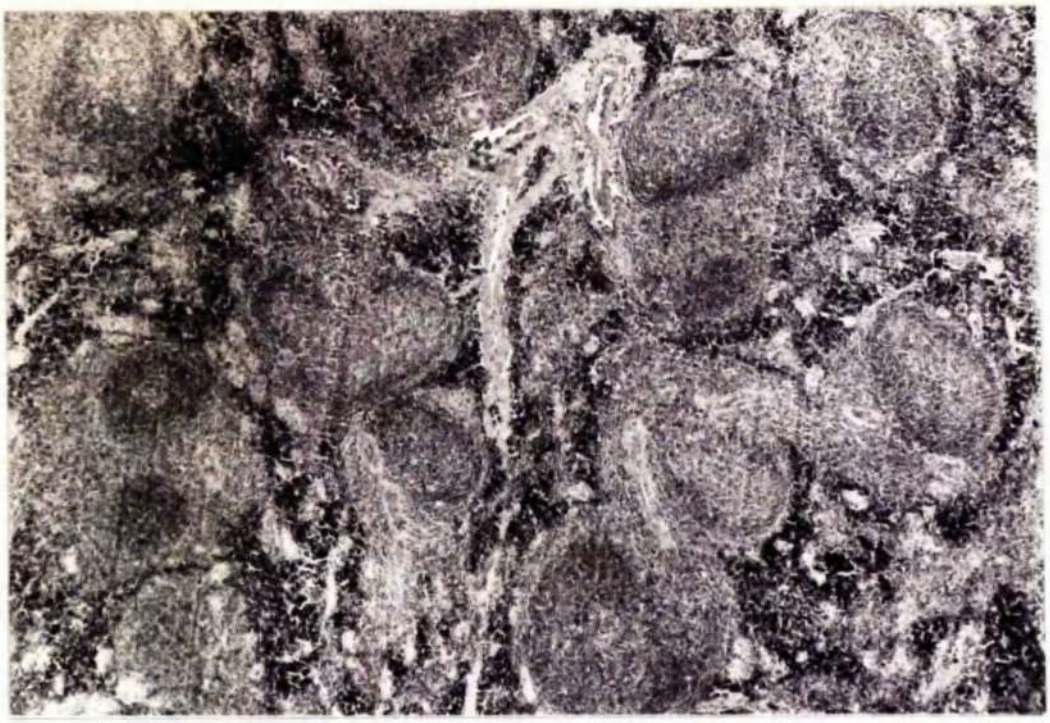

Figure 40. Feline spleen. Large tumified Malpighian bodies outlined by areas of erythropoiesis in the red pulp, case number 24908. H. & E. x 30.

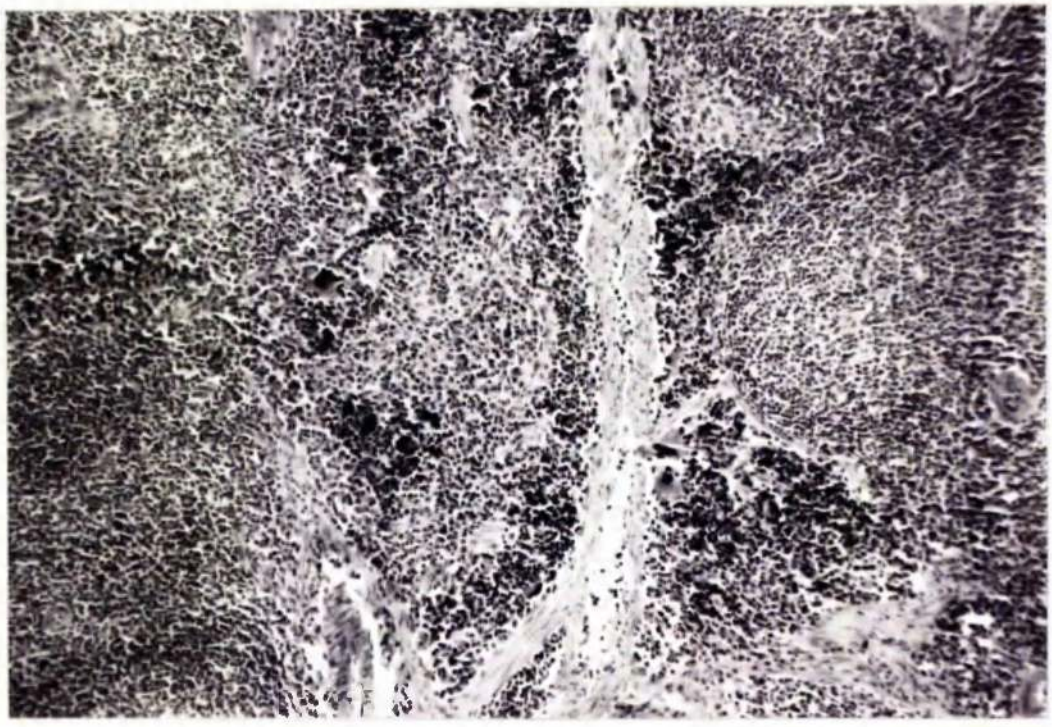

Figure 41. Feline spleen. A higher magnification of the section above to illustrate the expanding tumour follicles. H. & E. x 100.

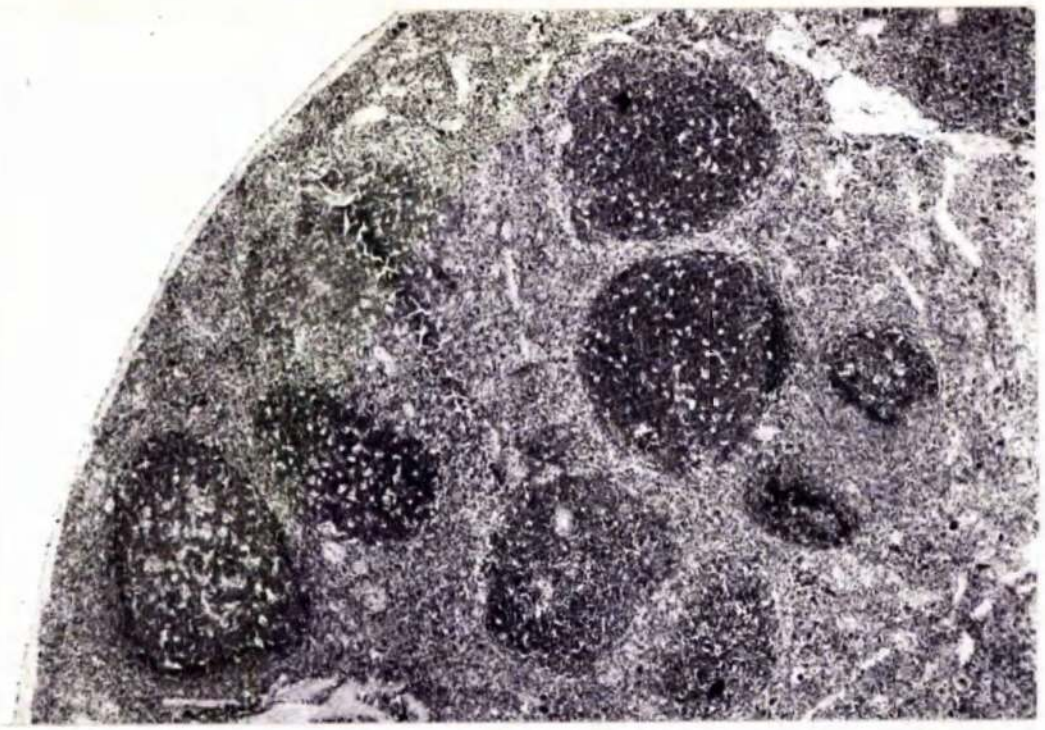

Figure 42. Feline spleen. Tumour hyperplasia of the Malpighian bodies, case number 25522. H. & E. x 30.

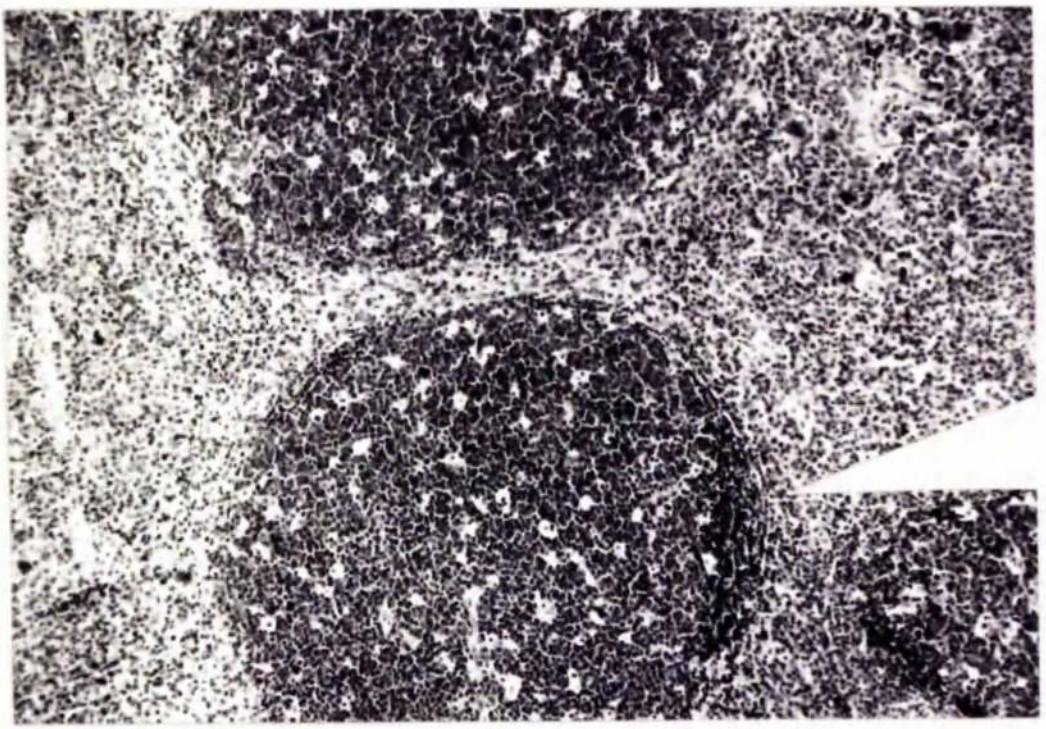

Figure 43. Feline spleen. A higher magnification of the section above to show the sharp definition of the follicles. Note the peripheral crescent of rather mature Lymphoid cells (arrowed). H.& E. x 100.

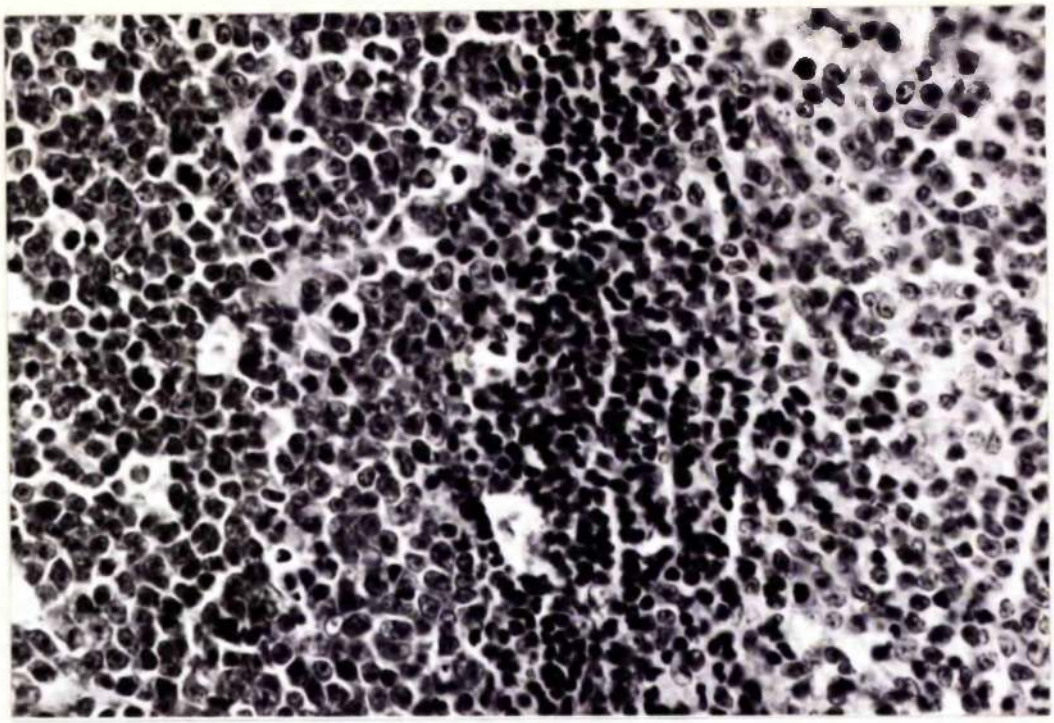

Figure 44. Feline spleen. A high magnification of the crescent arrowed in Figure 43 to show the heavy infiltration of tumour lymphoblasts into the red pulp on the right. H. & E. x 400.

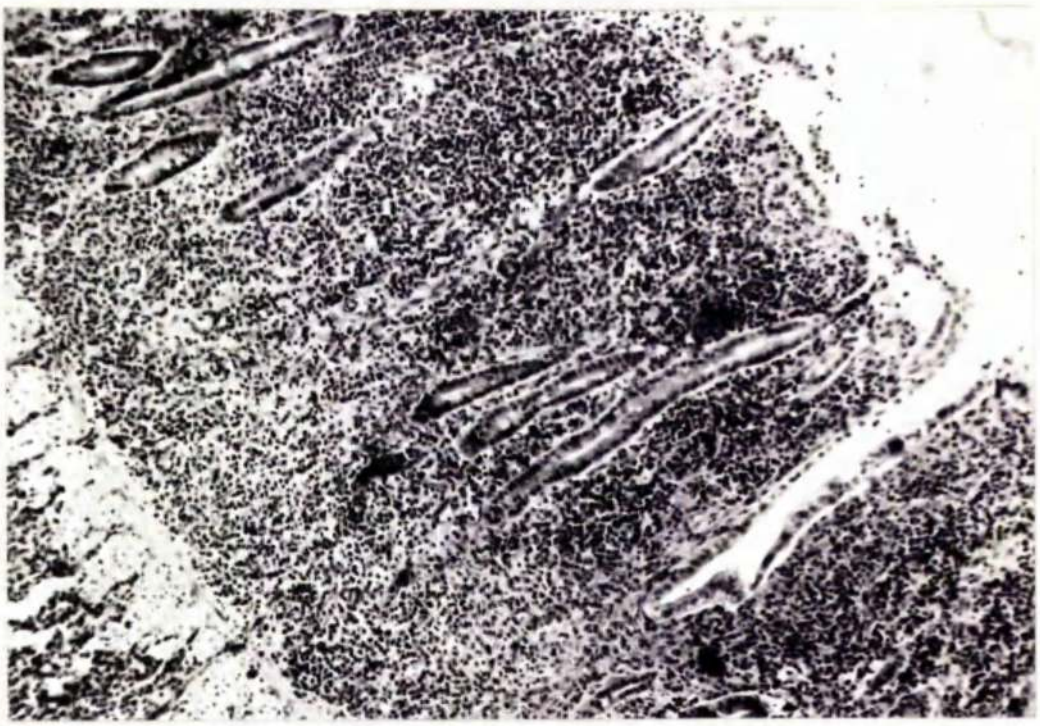

Figure 45. Feline small intestine. There is marked tumour cell invasion of the mucous membrane with distortion and destruction of the glandular elements. H. & E. x 100.

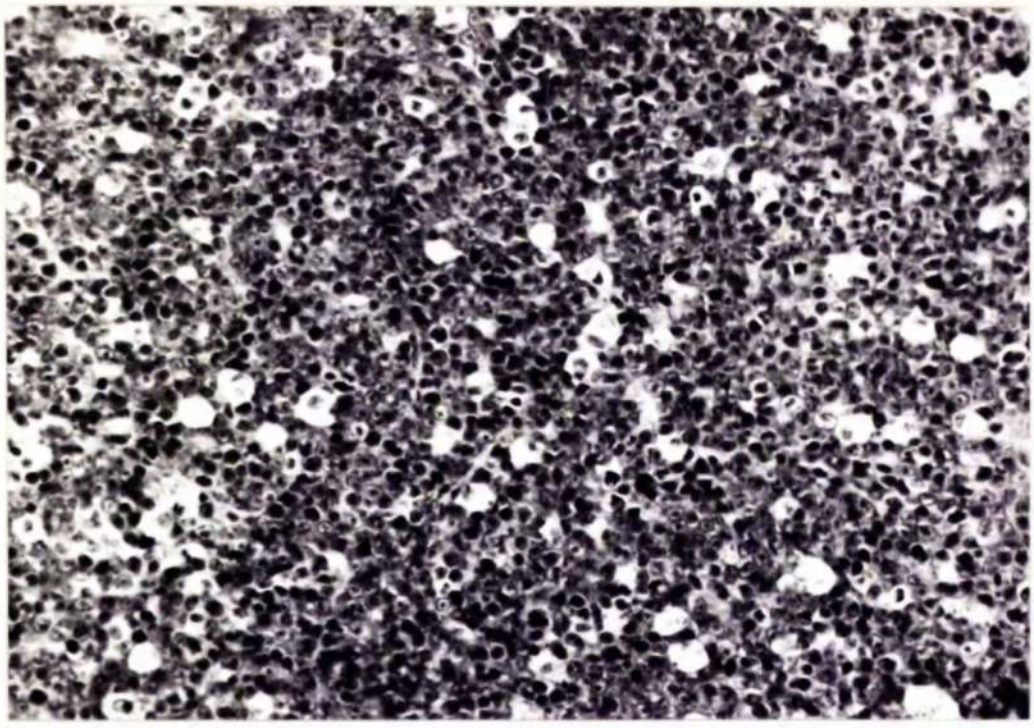

Figure 46. Feline small intestine. A typical cell sheet in a tumour mass showing many lymphoblasts, scattered reticulo-endothelial and dormant stem cells with a background of maturer lymphoid cells. H. & E. x 400.

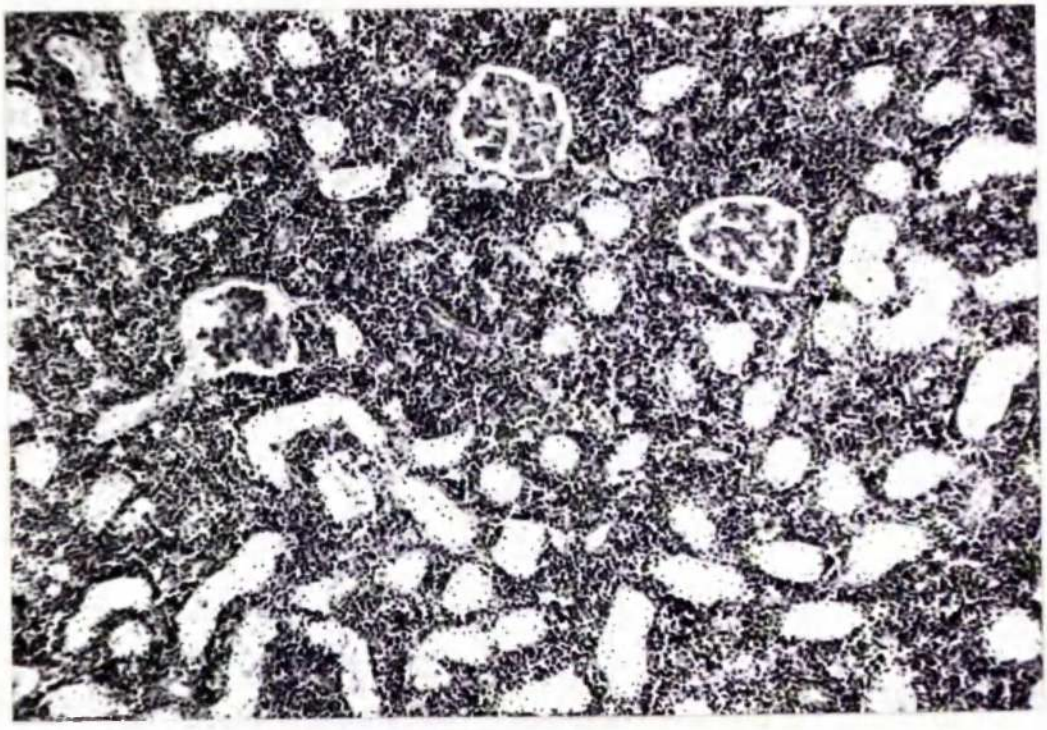

Figure 47. Feline kidney. Heavy tumour cell infiltration of the cortex with dissociation and destruction of tubules. H. & E. x 100.

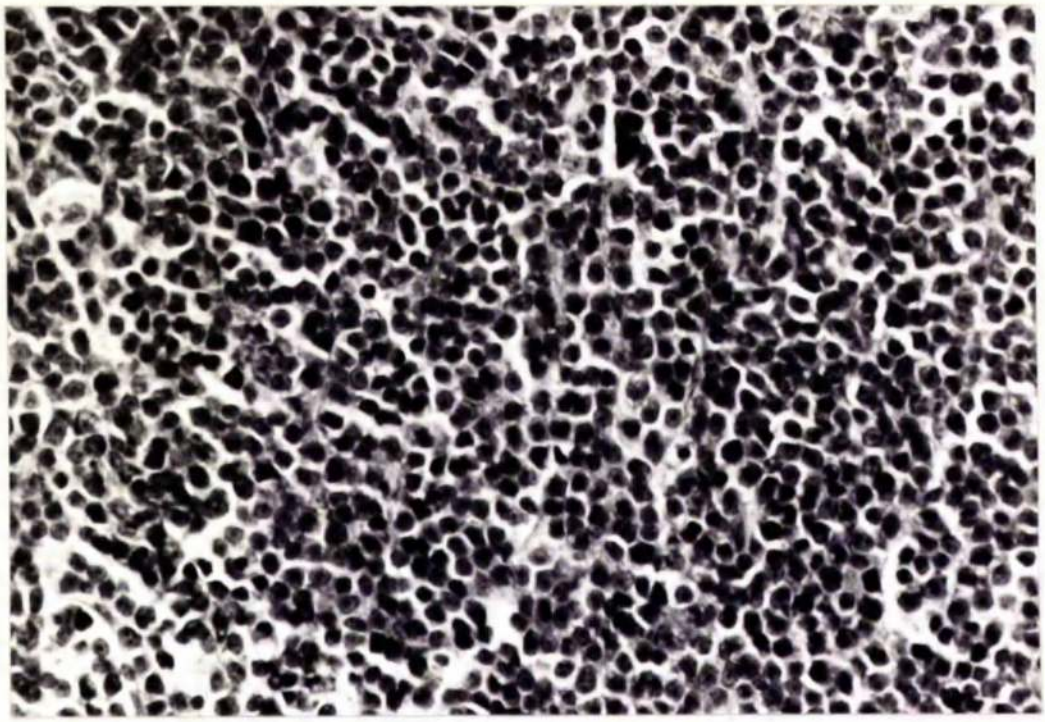

Figure 48. Feline lymph node. A tumour cell sheet in which the dominant cell is a lymphoblast. H. & E. x 400.

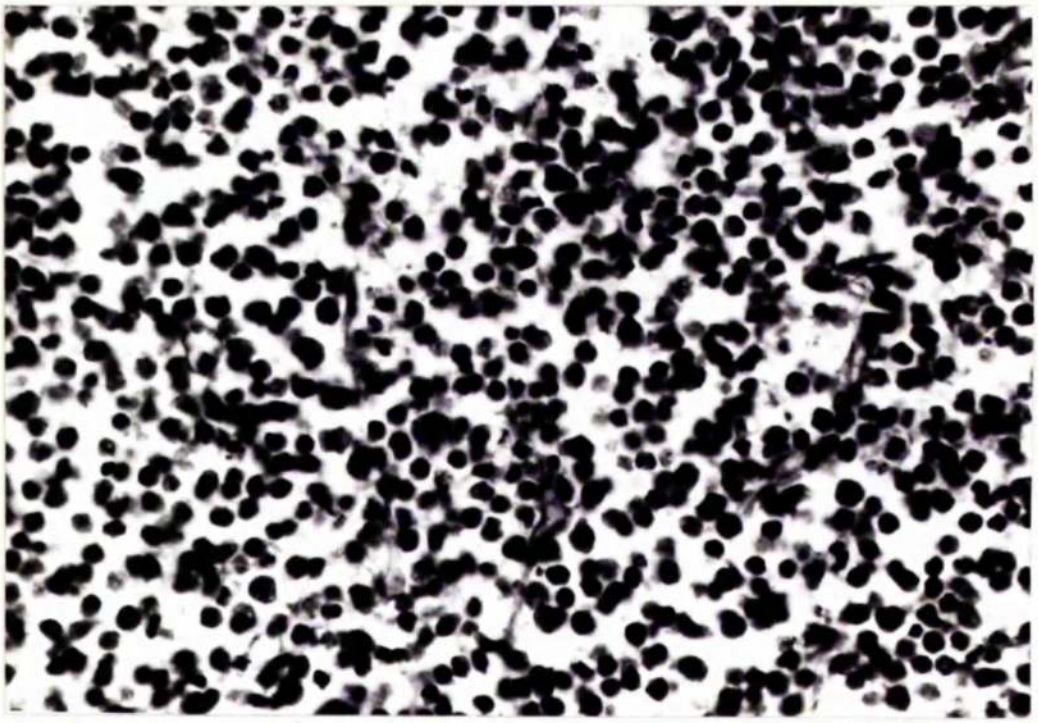

Figure 49. Feline lymph node. A tumour cell sheet composed of more mature lymphoid cells. H. & E. x 400.

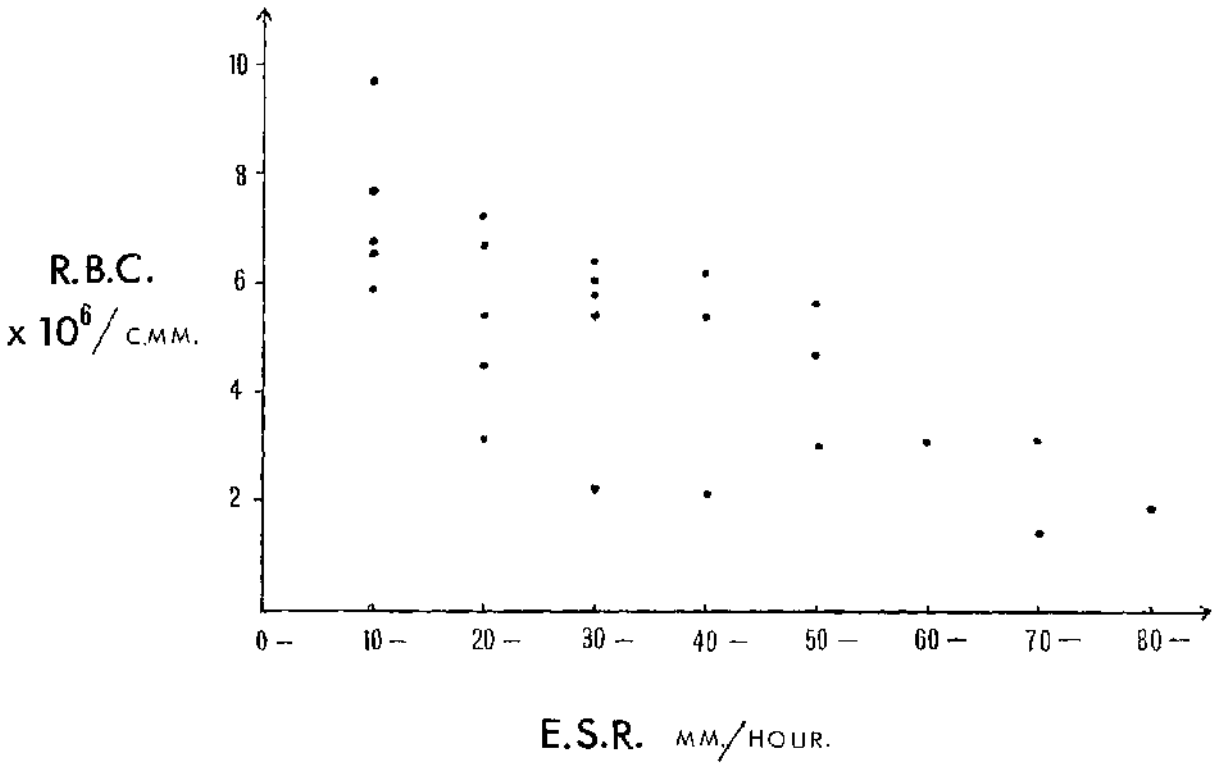

Figure 50. The correlation between total erythrocyte counts and the E.S.R. in cats with lymphosarcoma.

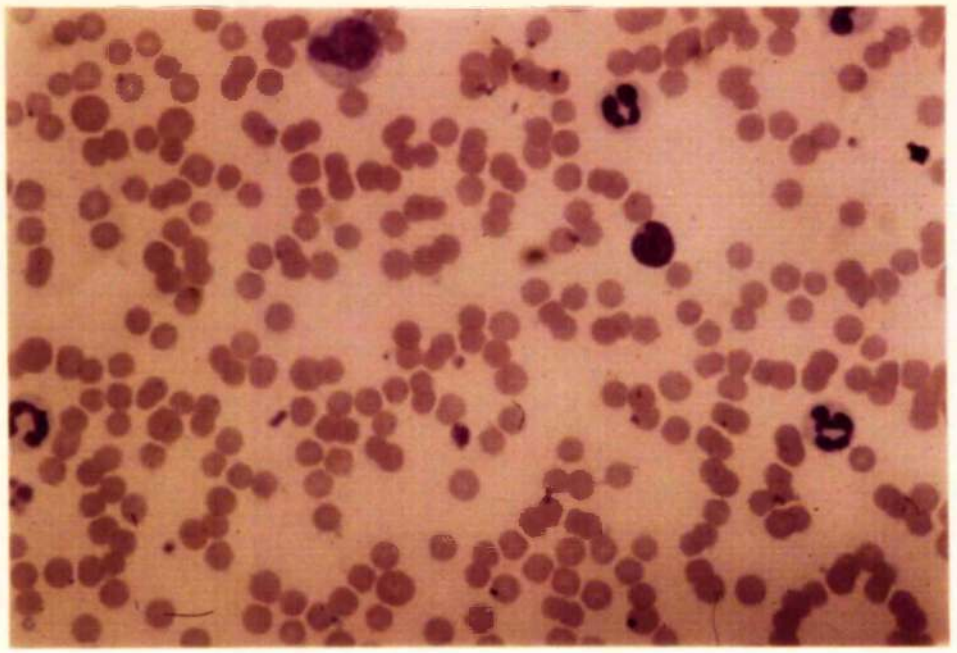

Figure 51. Blood film of cat 19377/10. There is a large lymphocyte, top left, a medium lymphocyte, centre right. The other leucocytes are neutrophils. Leishman's stain x 500.

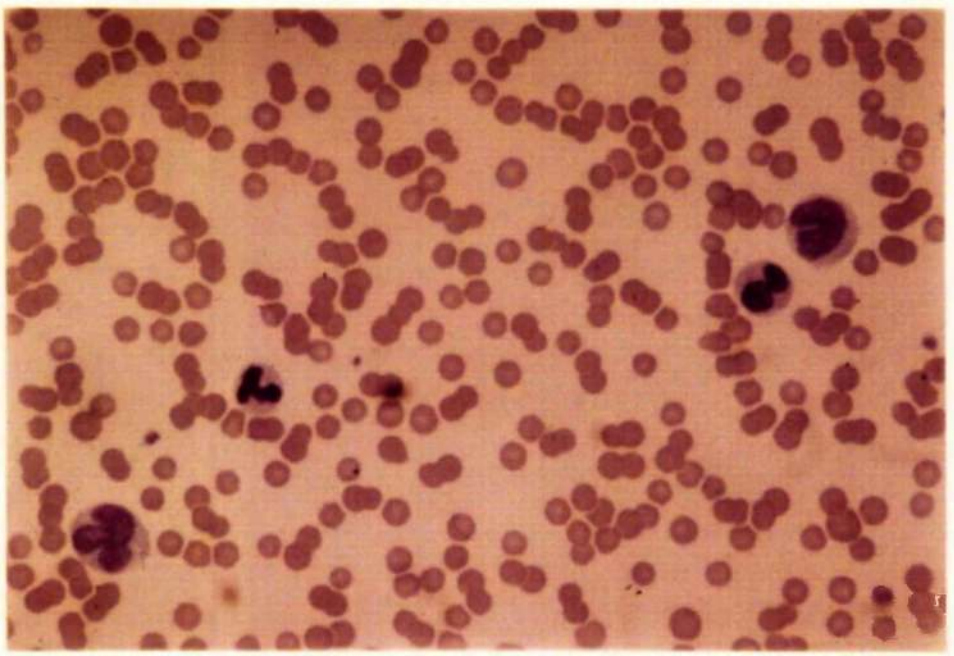

Figure 52. Blood film of cat 19377/10. Lobulated large lymphocytes are present, extreme left and extreme right, together with 2 neutrophils. Leishman's stain x 500.

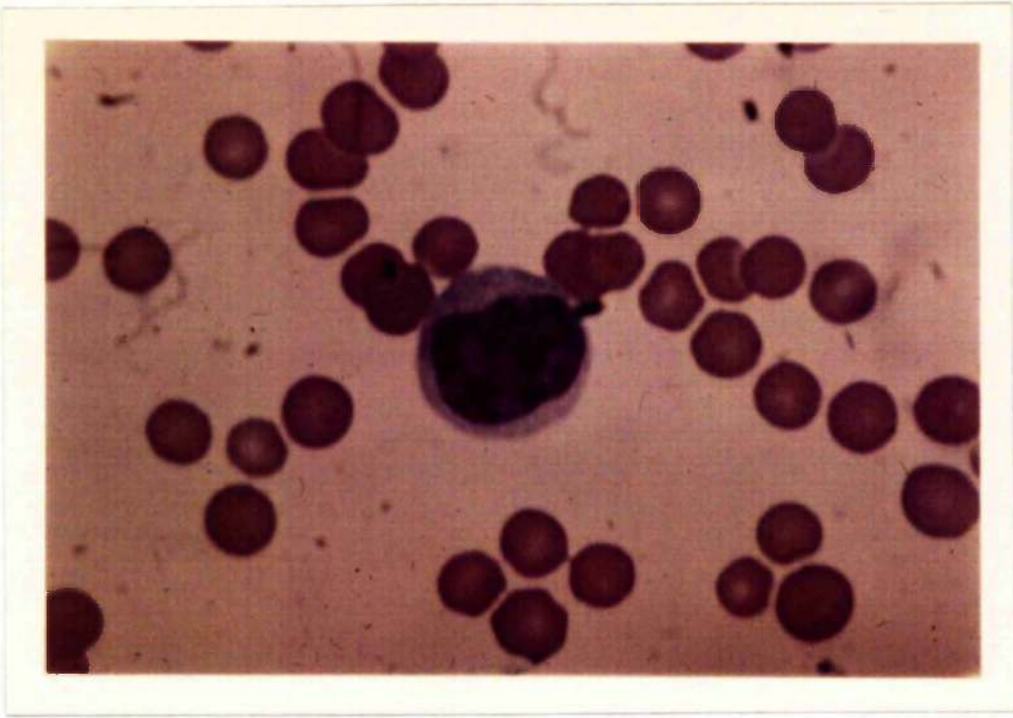

Figure 53. Blood film of cat 19377/10. A large lymphocyte. Leishman's stain x 1,000.

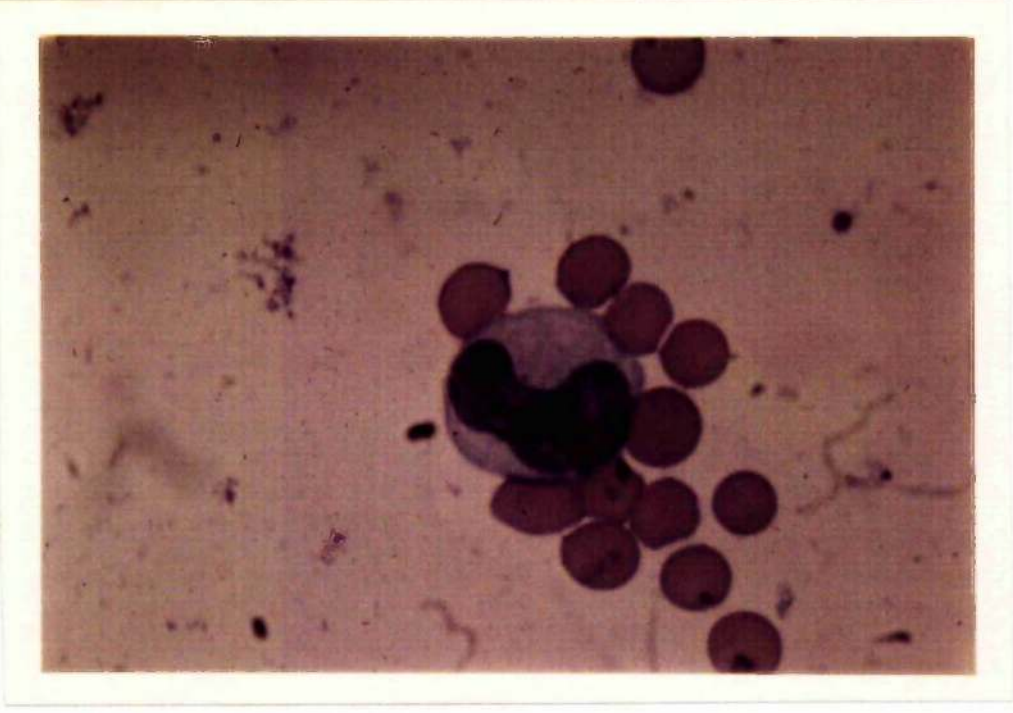

Figure 54. Blood film of cat 19377/10. A large lymphocyte. Leishman's stain x 1,000

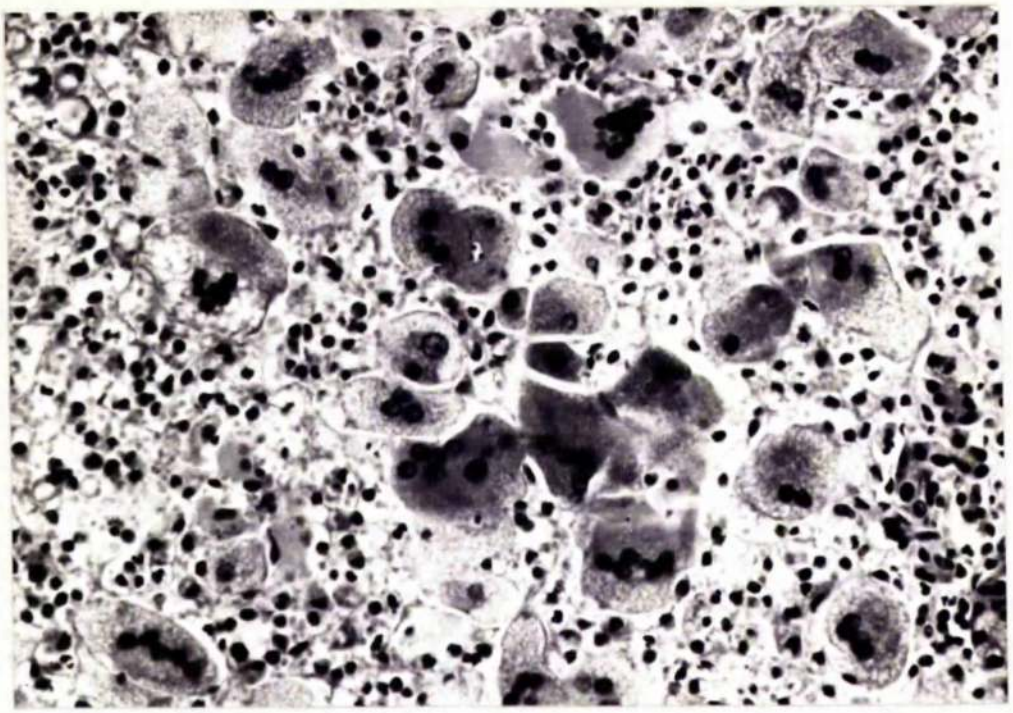

Figure 55. Liver of cat 19377/9. Note the large multinucleated hepatic cord cells and compare them with the cells of a field case in Figure 39. H. & E. x 500.

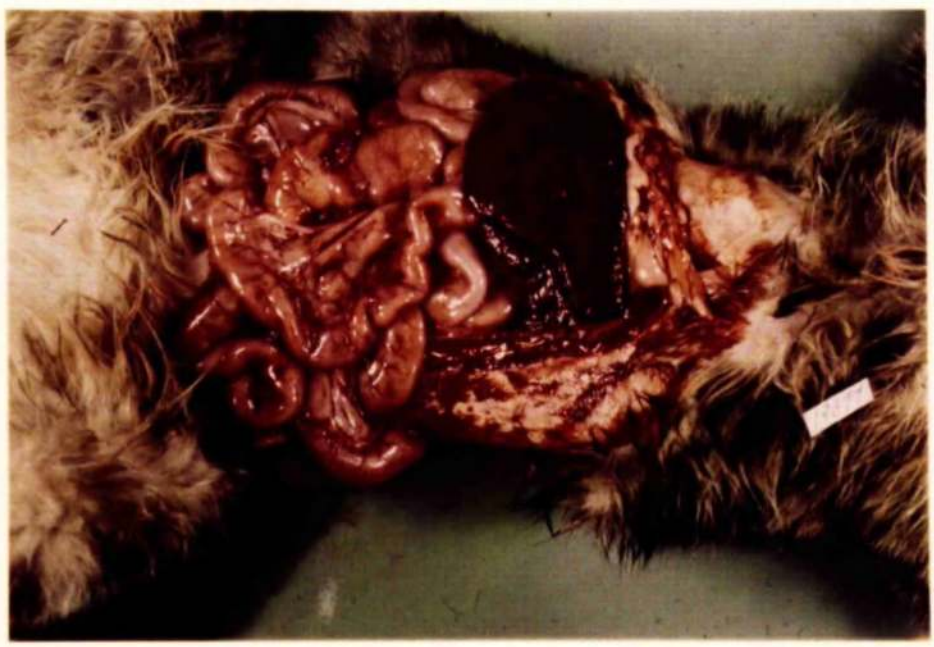

Figure 56. Cat 19377/10. Note the grossly enlarged spleen and the mesenteric lymph node mass.

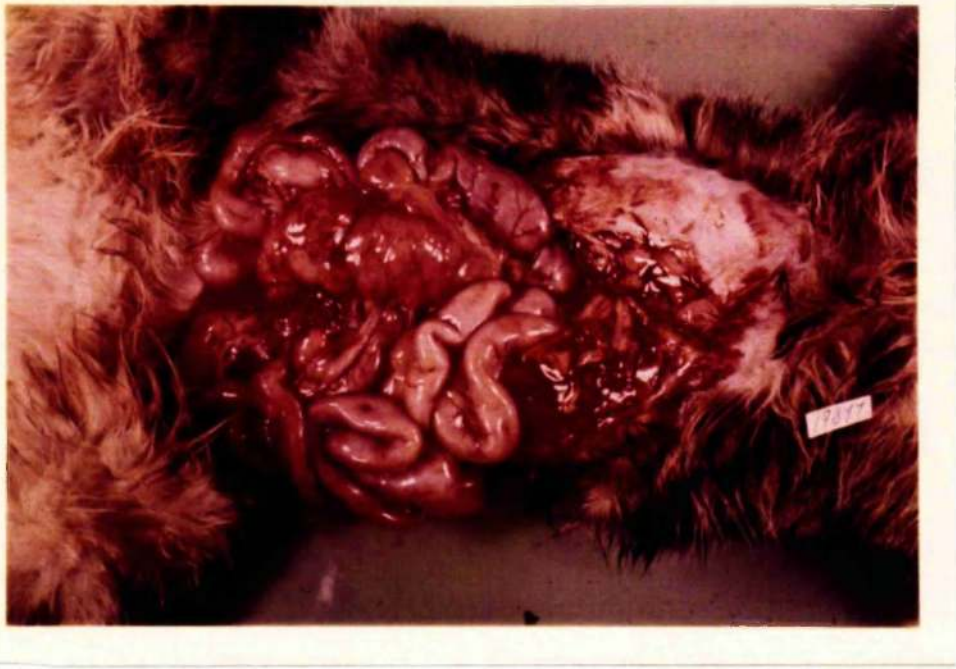

Figure 57. Cat 19377/10. Note the enlarged mesenteric lymph node.

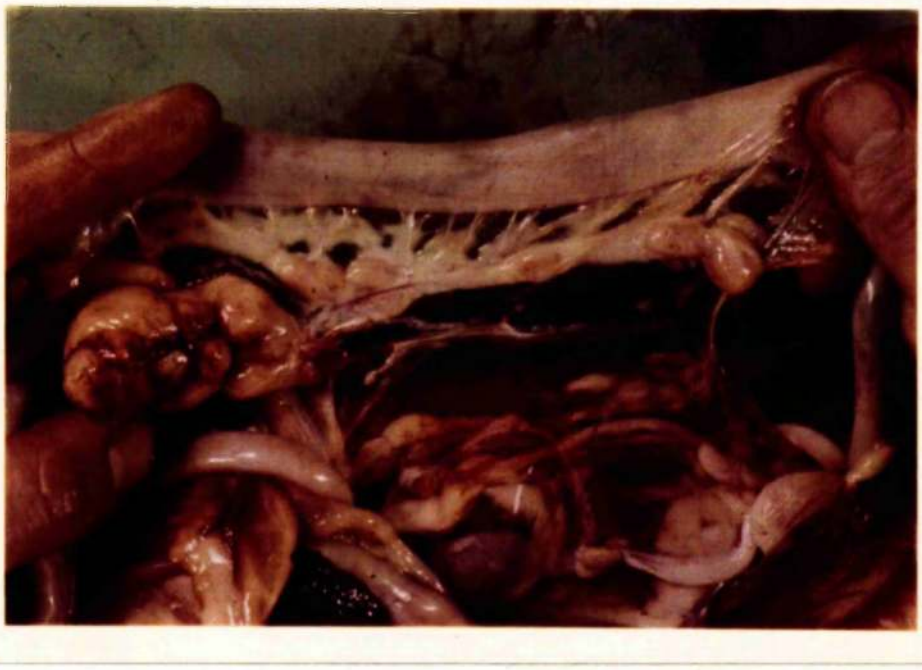

Figure 58. Cat 19377/10. Markedly enlarged and tumified lymph nodes in the mesentery of the large intestine.

Figure 59. Cat 19377/10. A cross section of the spleen to illustrate the hyperplastic Malpighian bodies.

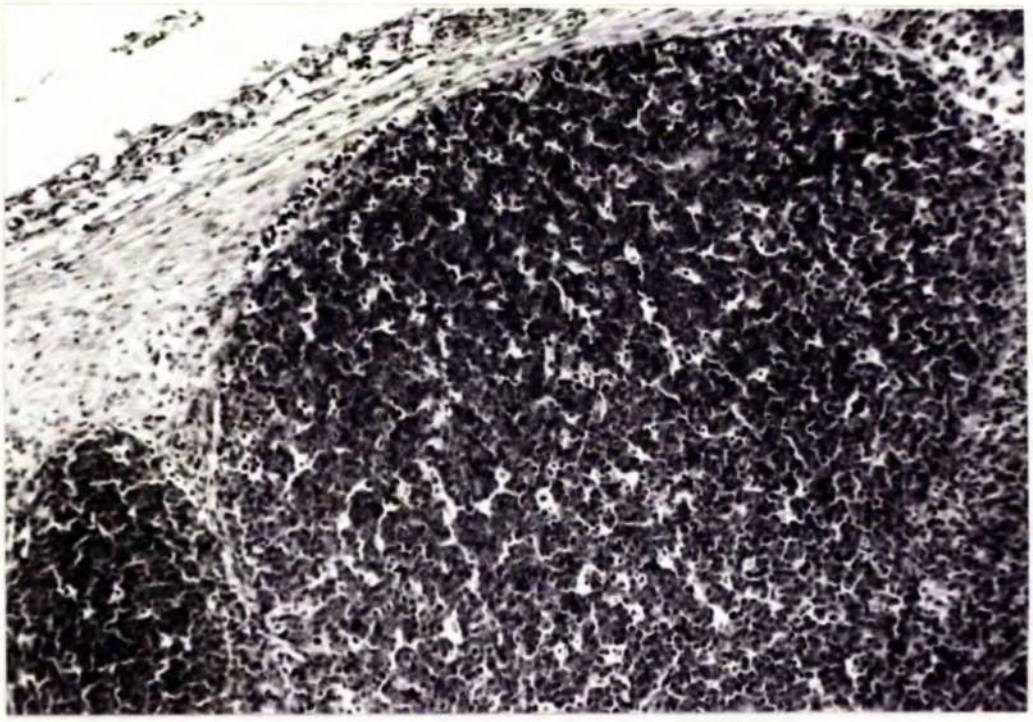

Figure 60. Cat 19377/10. The mesenteric lymph node to show the massive tumour follicles which are forming cell sheets and pressing against a relatively intact capsule. H. & E. x 150.

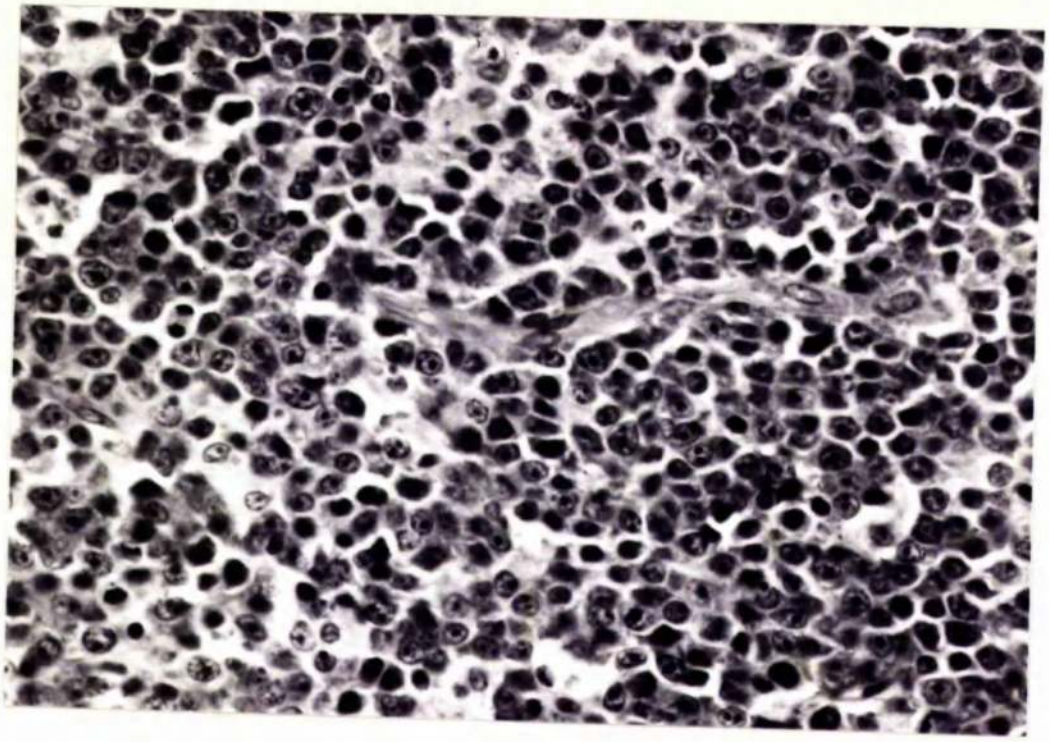

Figure 61. Cat 19377/10. The tumour cell sheet of the mesenteric lymph node. H.& E. x 500.

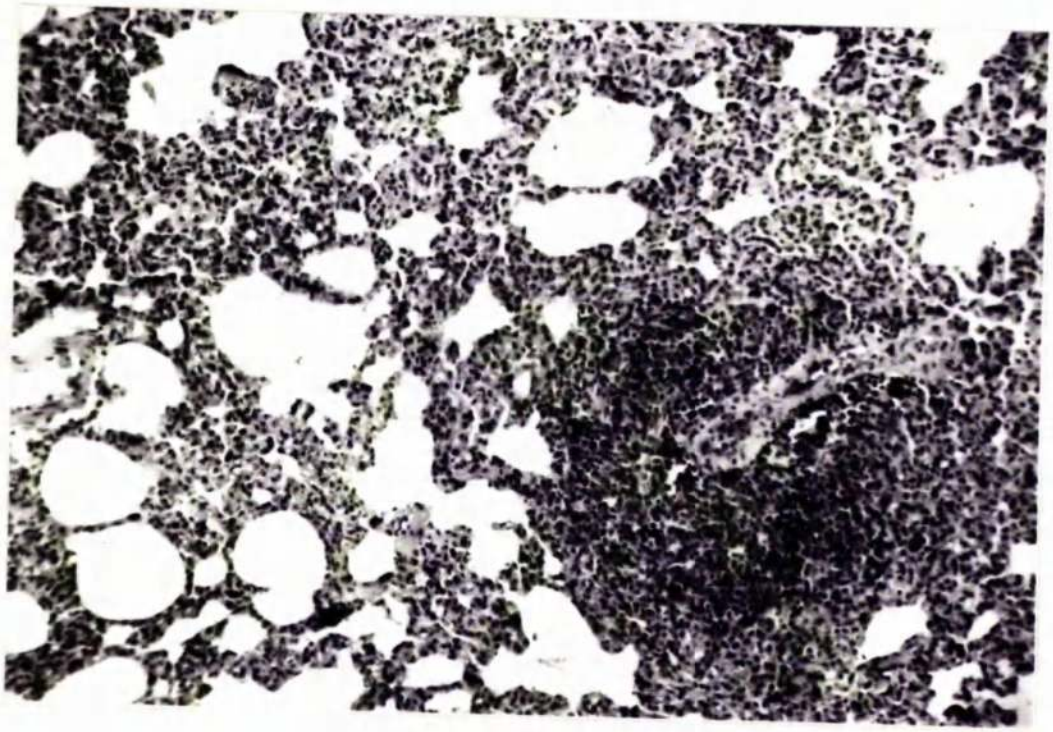

Figure 62. Cat 19377/10. A tumour nodule in the lung. H. & E. x 150.

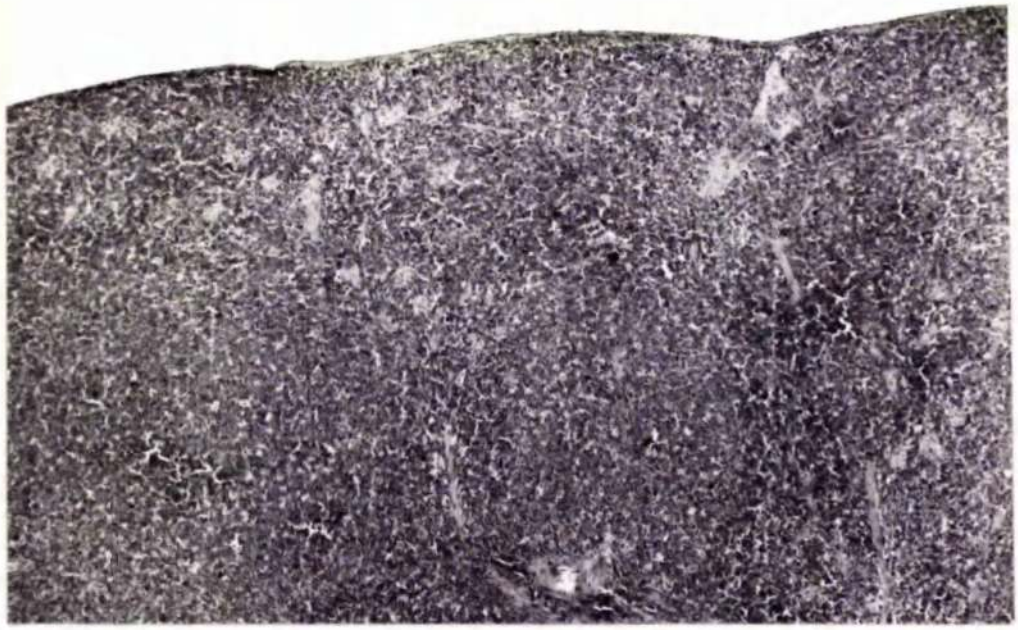

Figure 63. Cat 19377/10. A low power section of the grossly infiltrated spleen. H. & E. x 500.

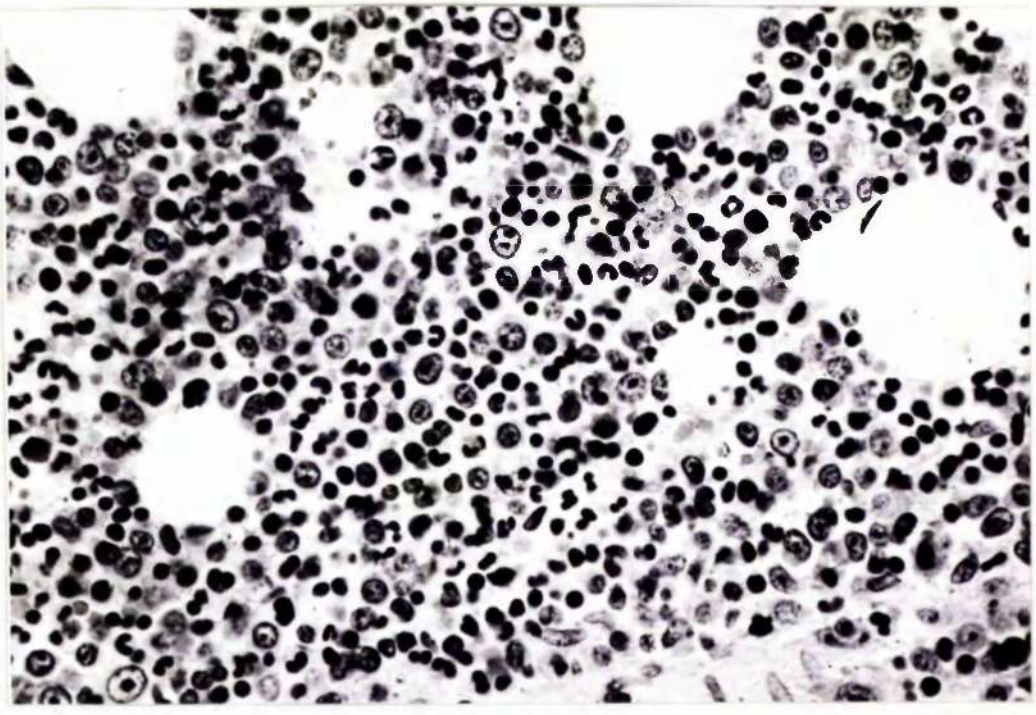

Figure 64. Cat 19377/10. The bone marrow showing cells similar to those in the lymph nodes. H. & E. x 500.

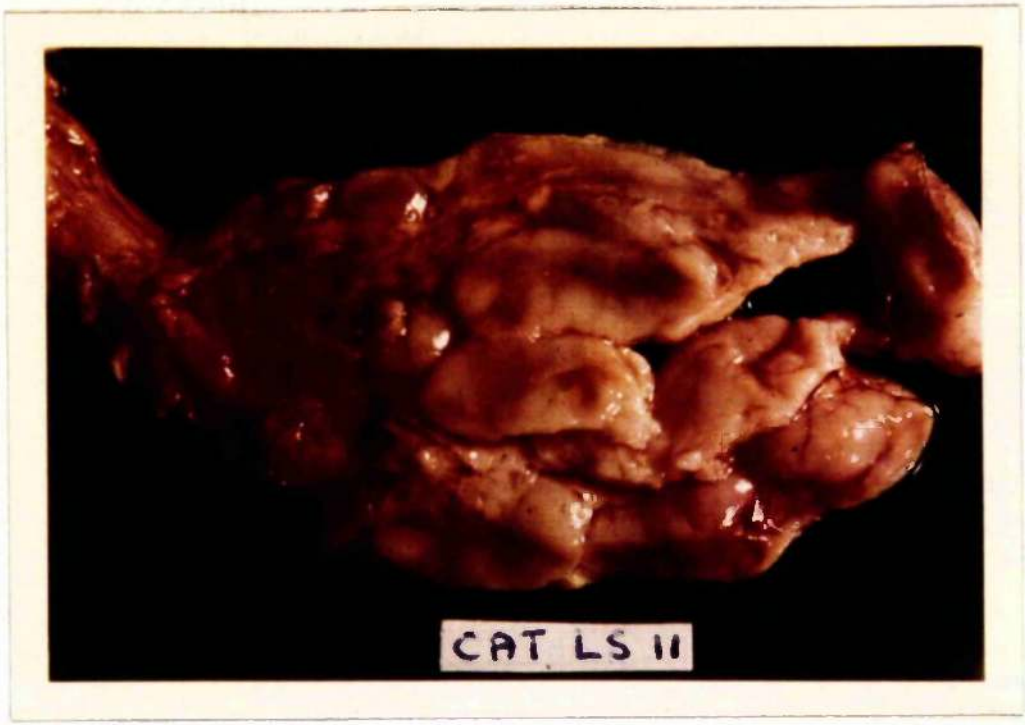

Figure 65. Cat 19377/11. Section of an enlarged lymph node.

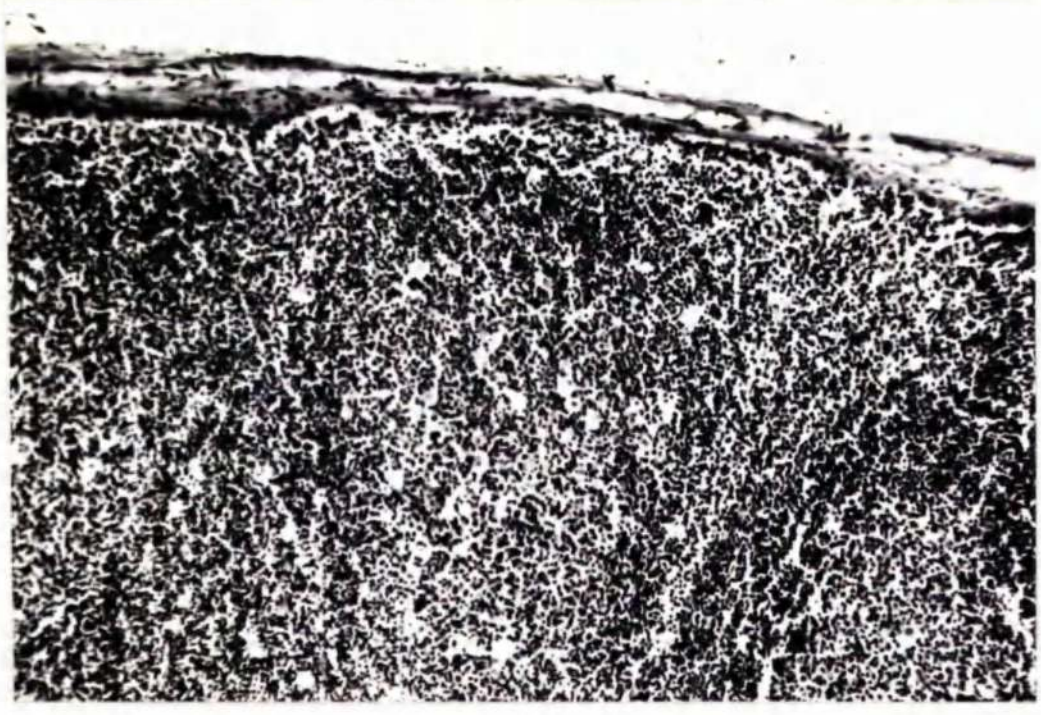

Figure 66. Cat 19377/11. The histological appearance of the mesenteric lymph node showing loss of follicular pattern, flooding of the subcapsular sinus and cell sheets streaming towards the medulla. H. & E. x 150.

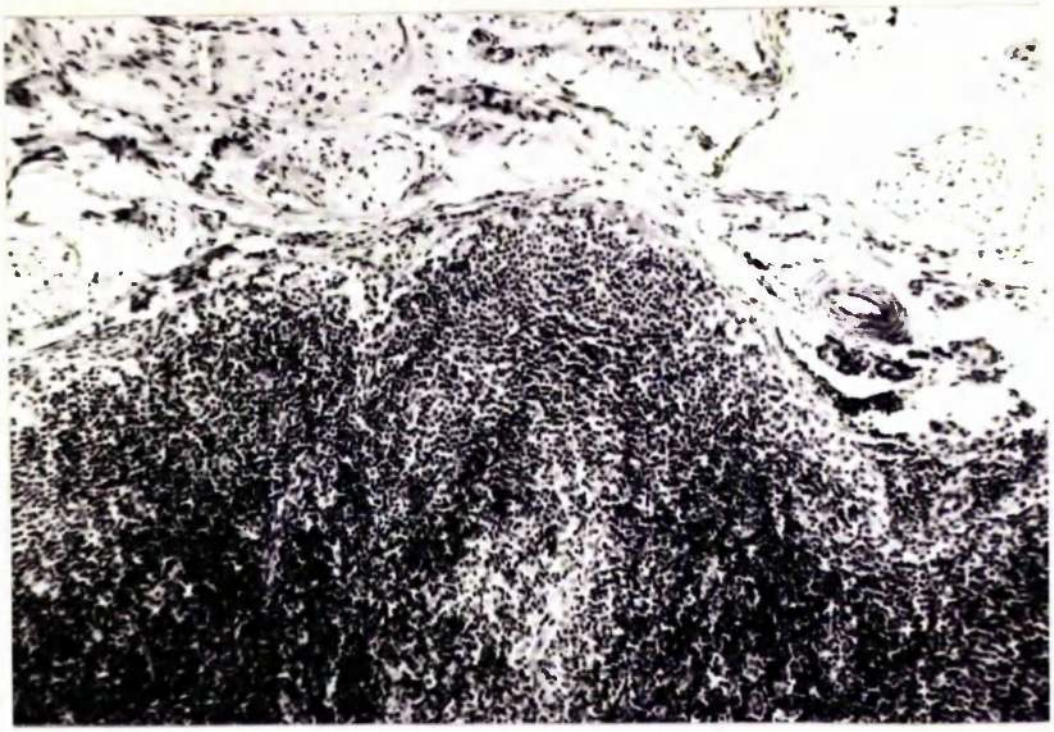

Figure 67. Cat 19377/12. Lymph node showing the breakdown of a germinal centre and flooding of the subcapsular sinus with pale immature cells. H. & E. x 150.

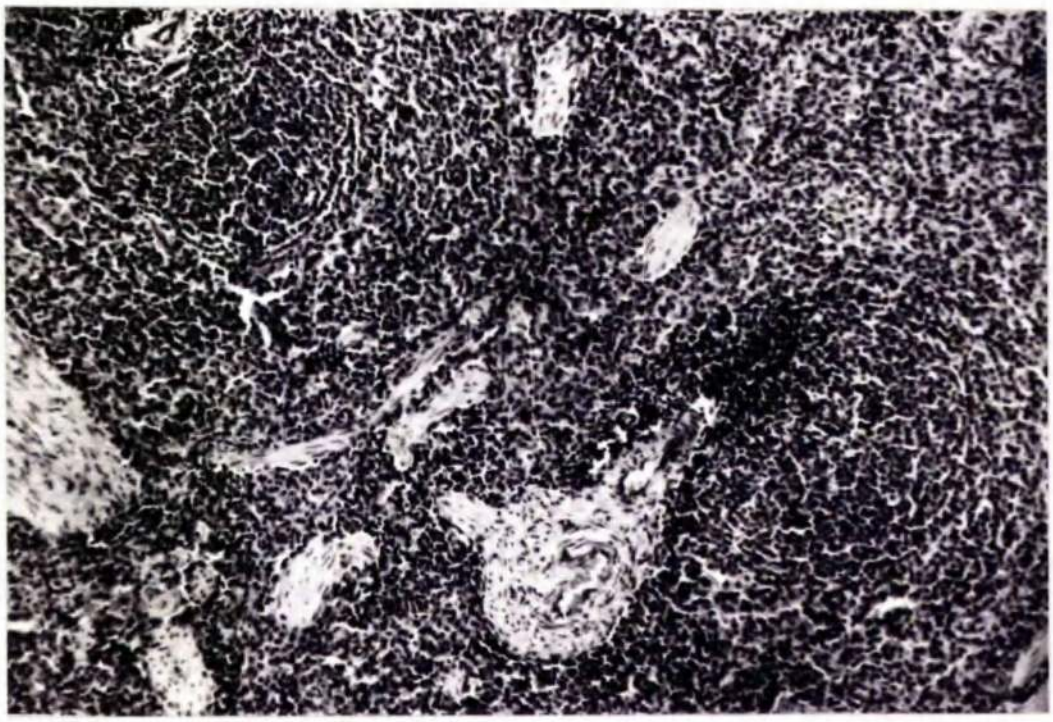

Figure 68. Cat 19377/12. Early changes in the Malpighian bodies. Note that the corpuscle on the right appears to be budding from a condensed vestige of a former corpuscle. H. & E. x 150.

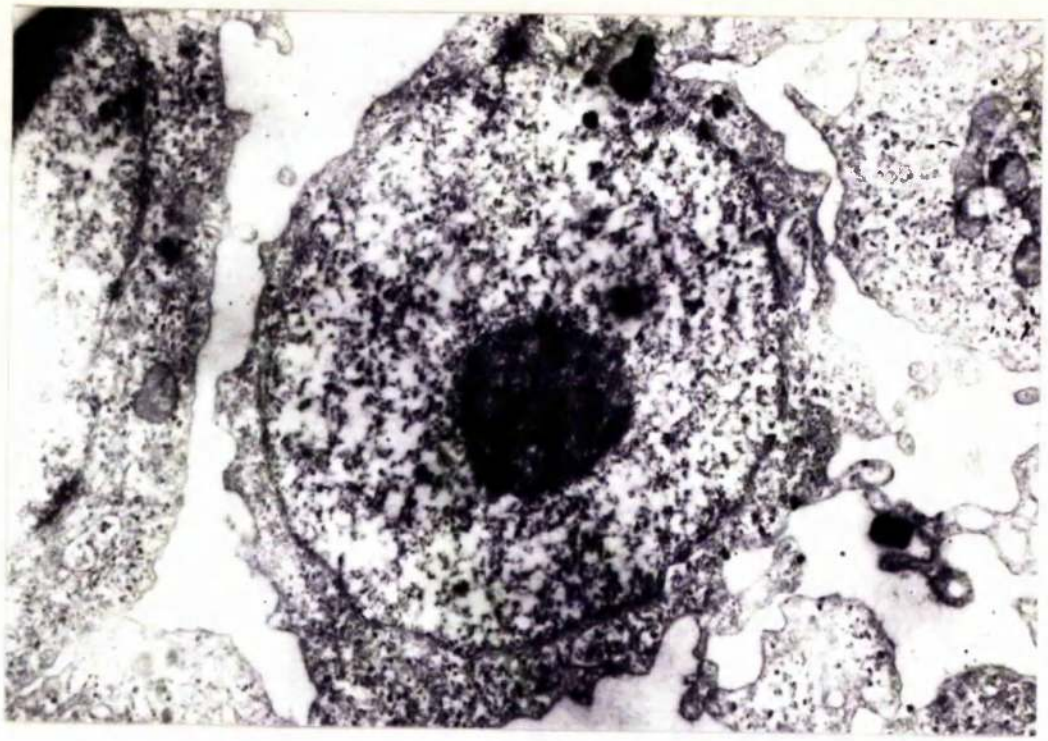

Figure 69. Cat 19377/10. A typical tumour cell as observed with the electron microscope.

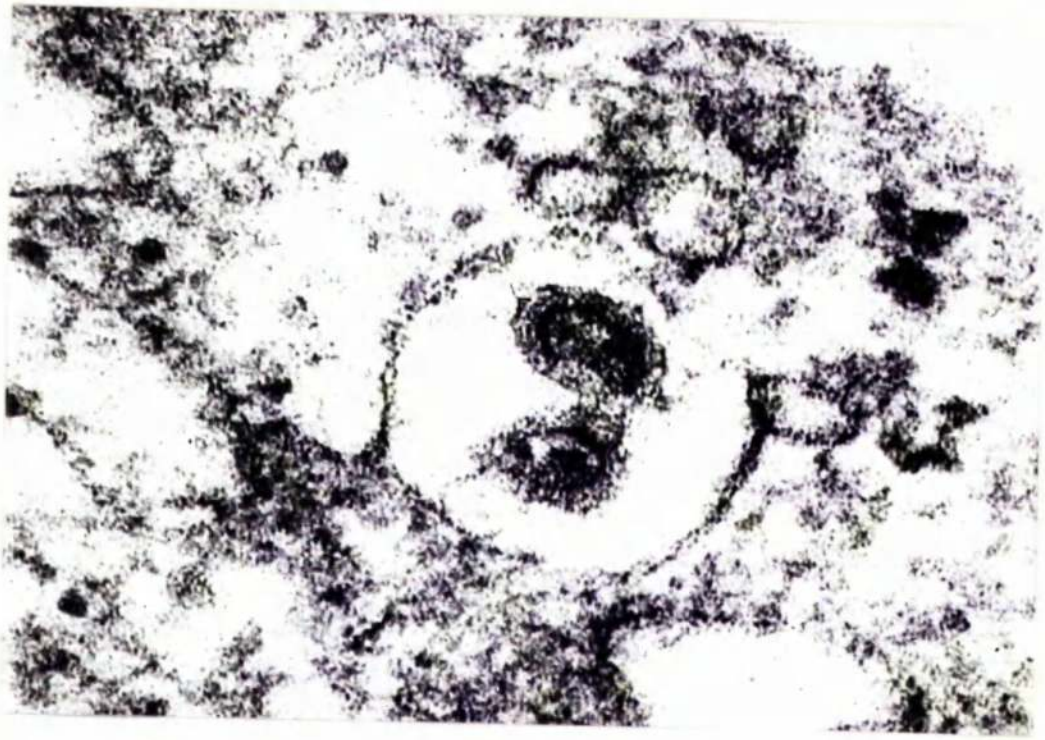

Figure 70. Cat 19377/10. The virus-like particle found in the tumour cells.

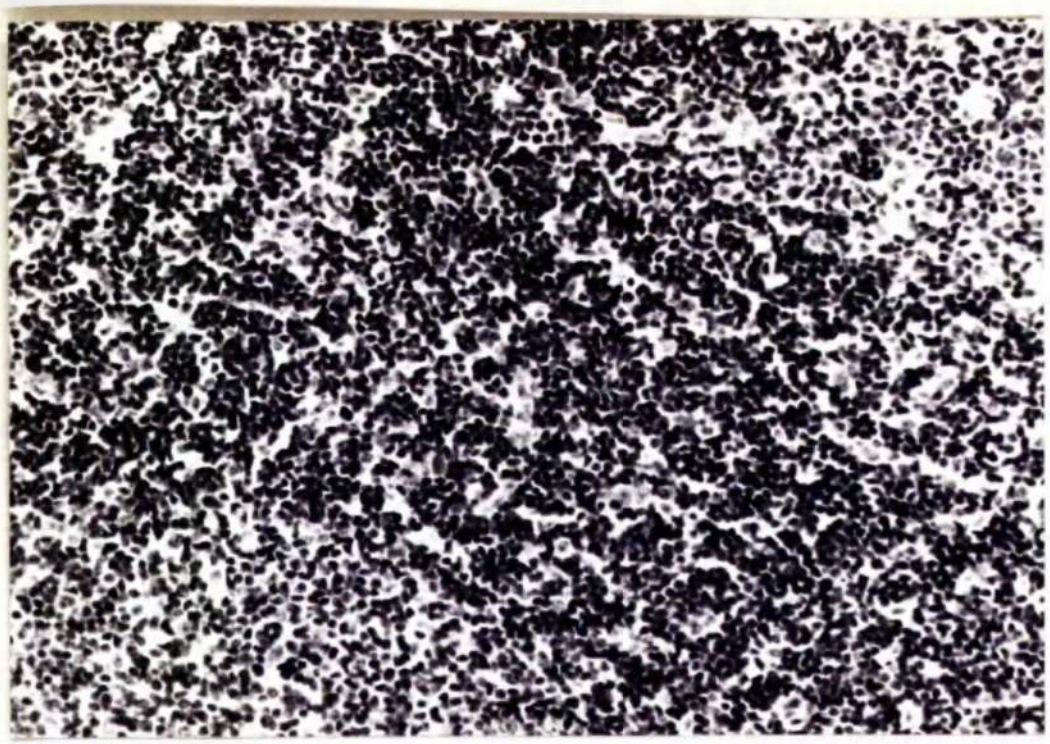

Figure 71. Cat 17457/2. Part of the submucosal tumour mass. H. & E. x 300.

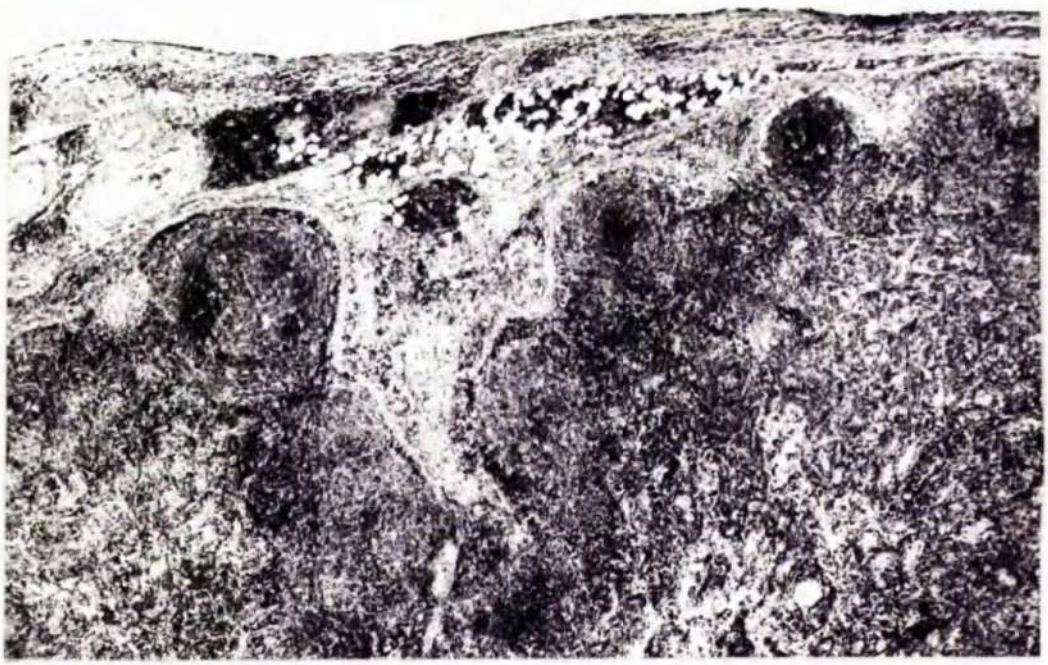

Figure 72. Cat 17457/2. The mesenteric lymph node showing capsular infiltration and early breakdown of follicles with streams of cells passing towards the medulla. H. & E. x 50.

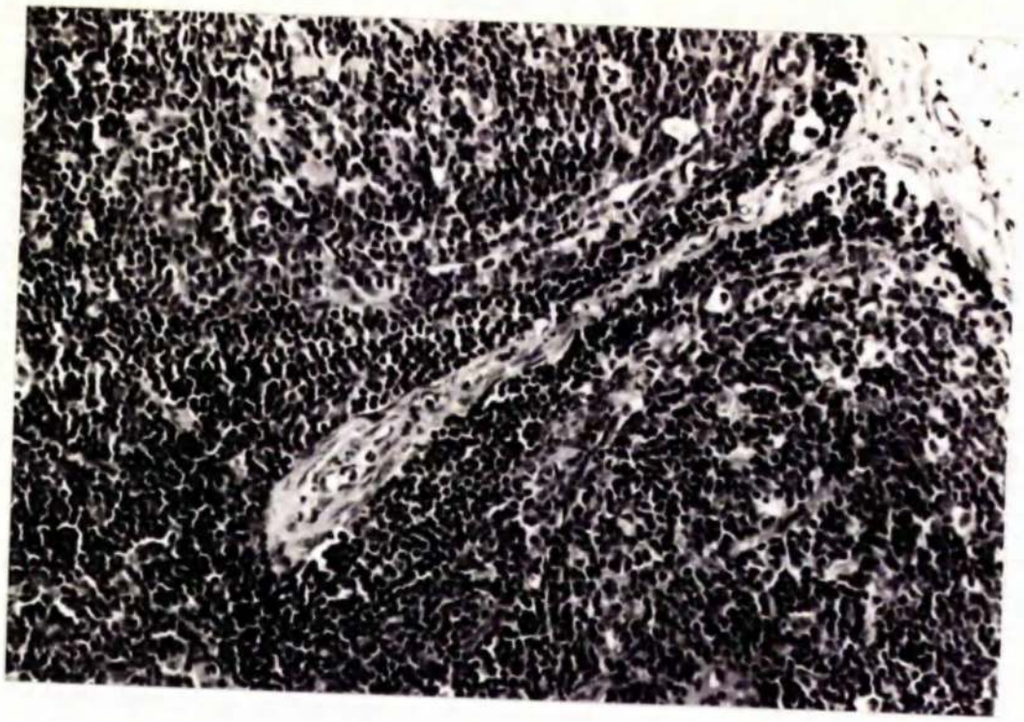

Figure 73. Cat 23510/54. A lymph node showing flooding of the sinuses. H. & E. x 300.

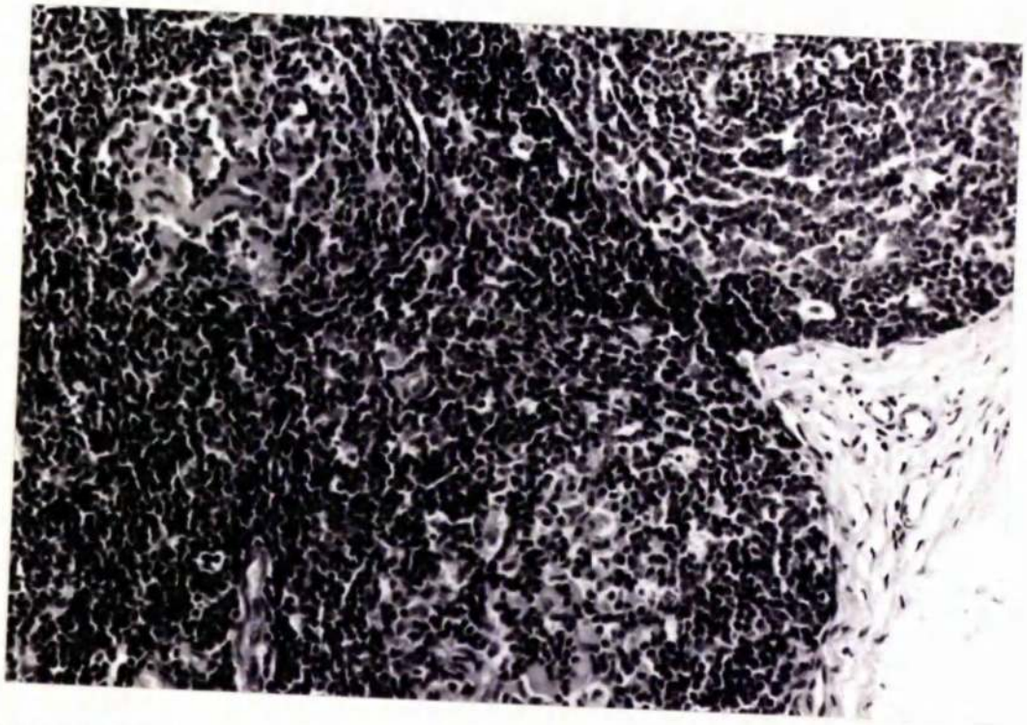

Figure 74. Cat 23510/54. Another lymph node showing flooding of the sinuses. H. & E. x 300.

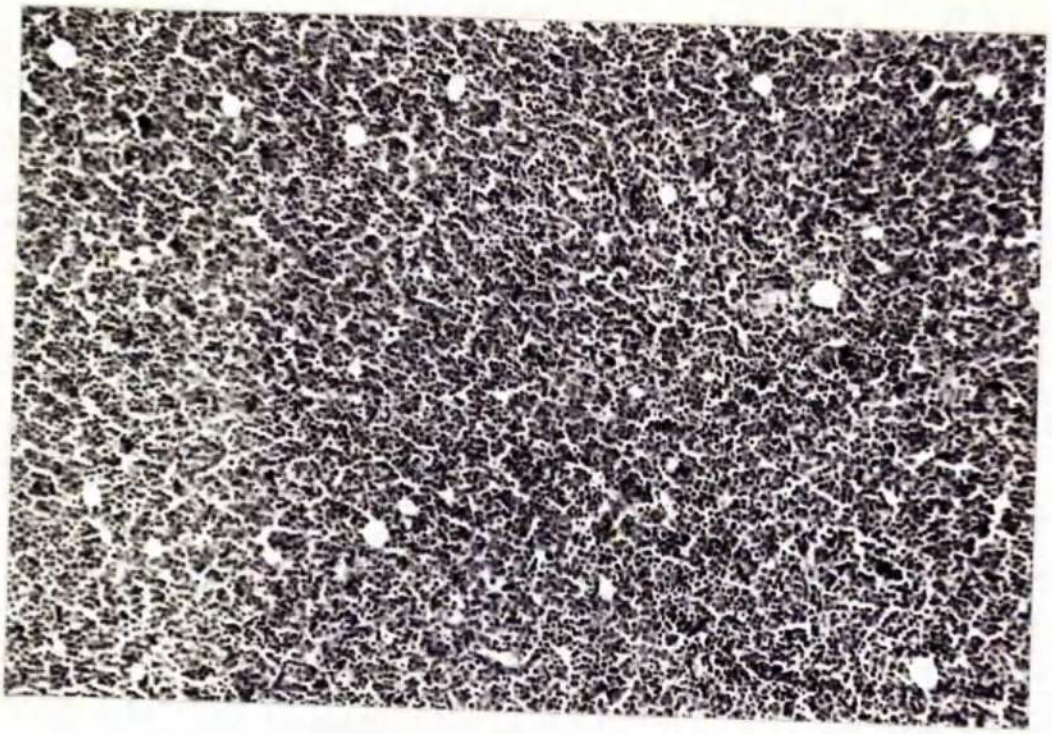

Figure 75. Cat 22868/22. The bone marrow cell sheet. H. & E. x 150.

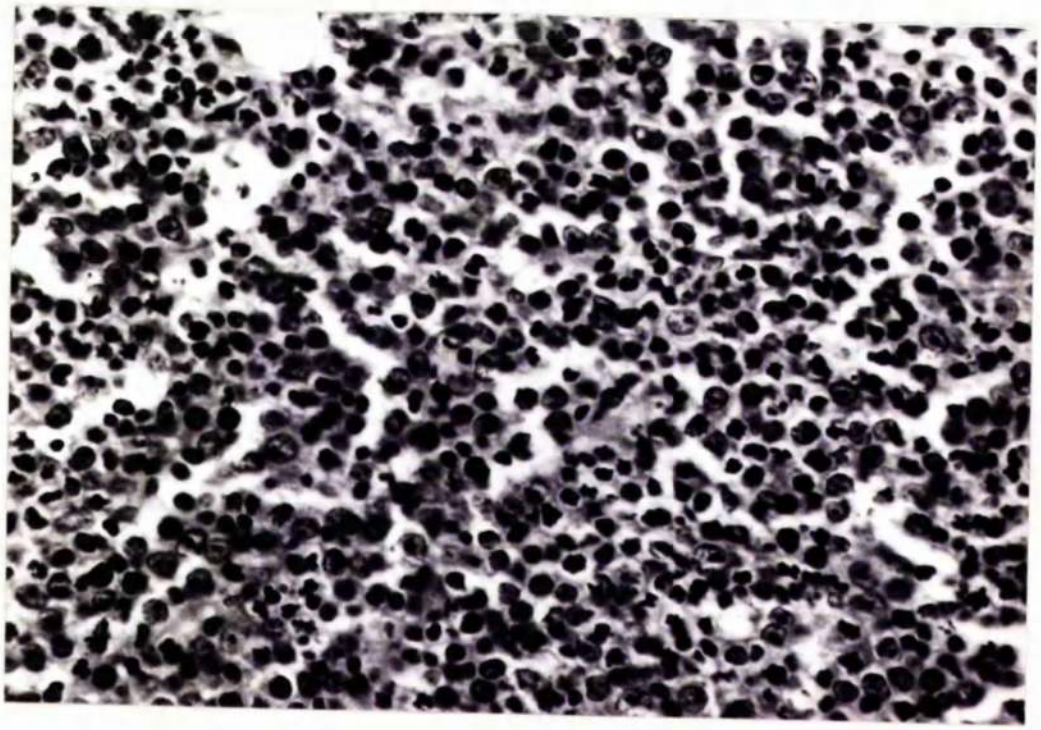

Figure 76. Cat 22868/22. The bone marrow to show the large undifferentiated lymphoid cells which are present in high numbers. H. & E. x 150.

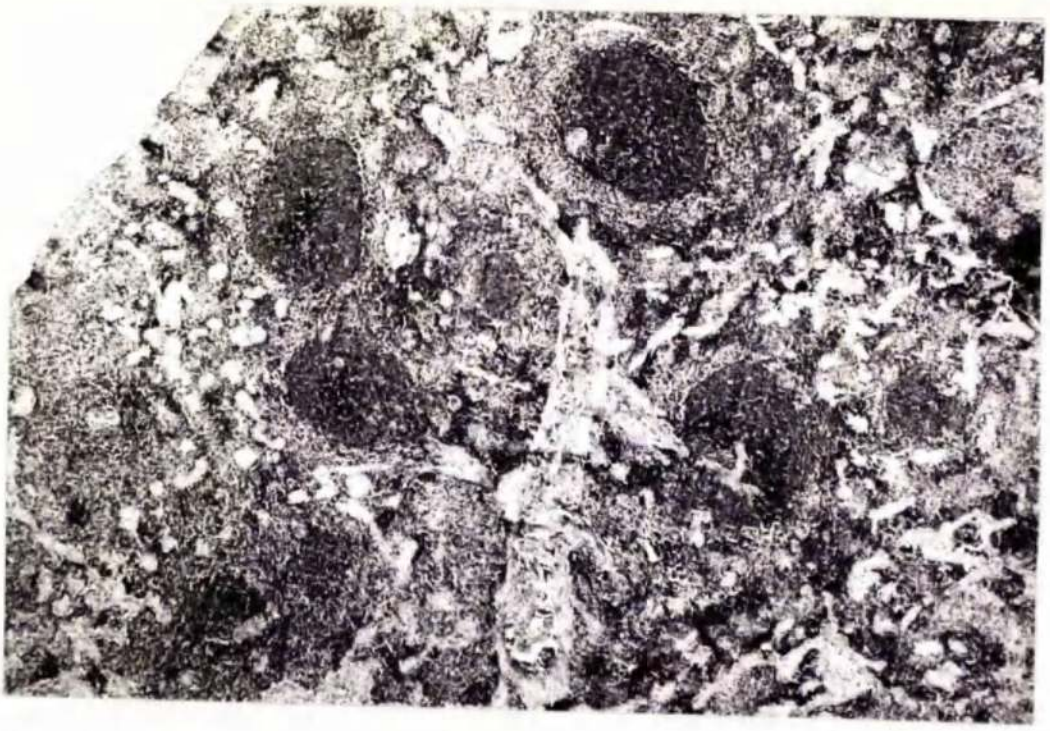

Figure 77. Cat 22868/25. Tumour follicles in the spleen. H. & E. x 50.

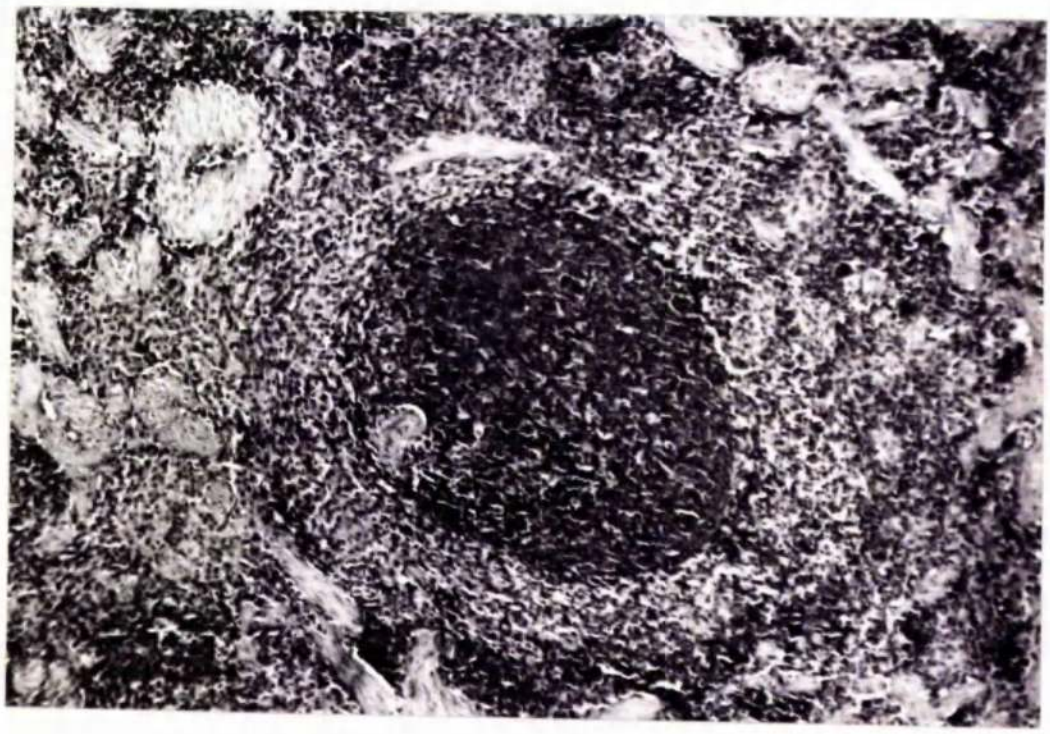

Figure 78. Cat 22868/25. A higher magnification of a follicle in the spleen. H. & E. x 150.

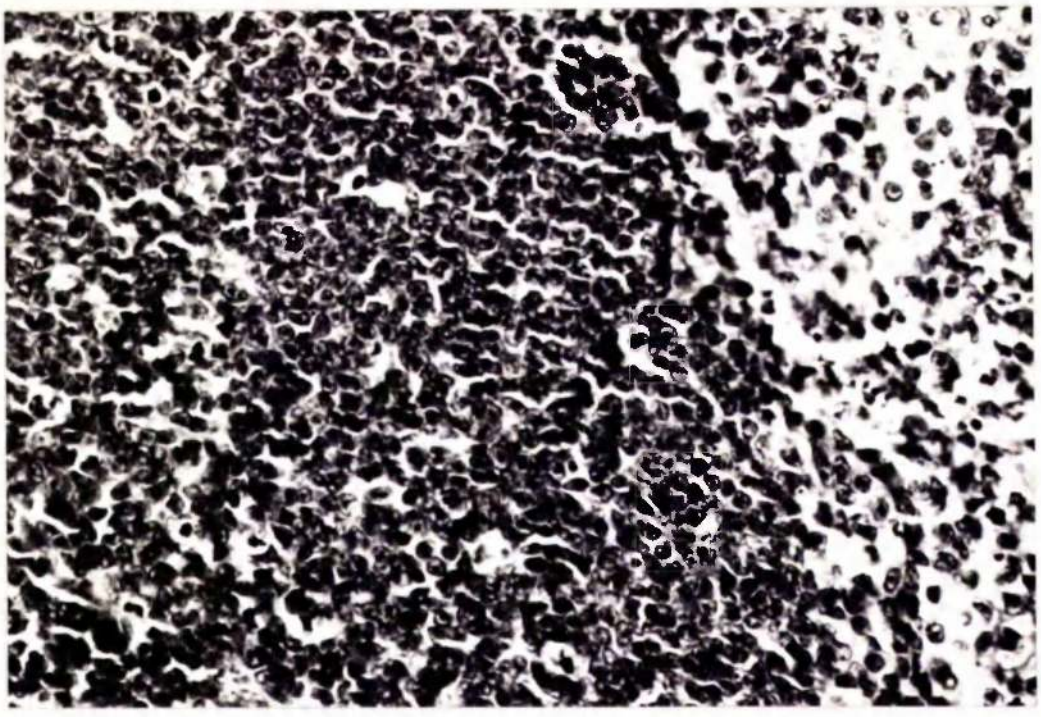

Figure 79. Cat 22868/25. The relatively uniform and immature lymphoid cells in the splenic follicles. H. & E. x 500.

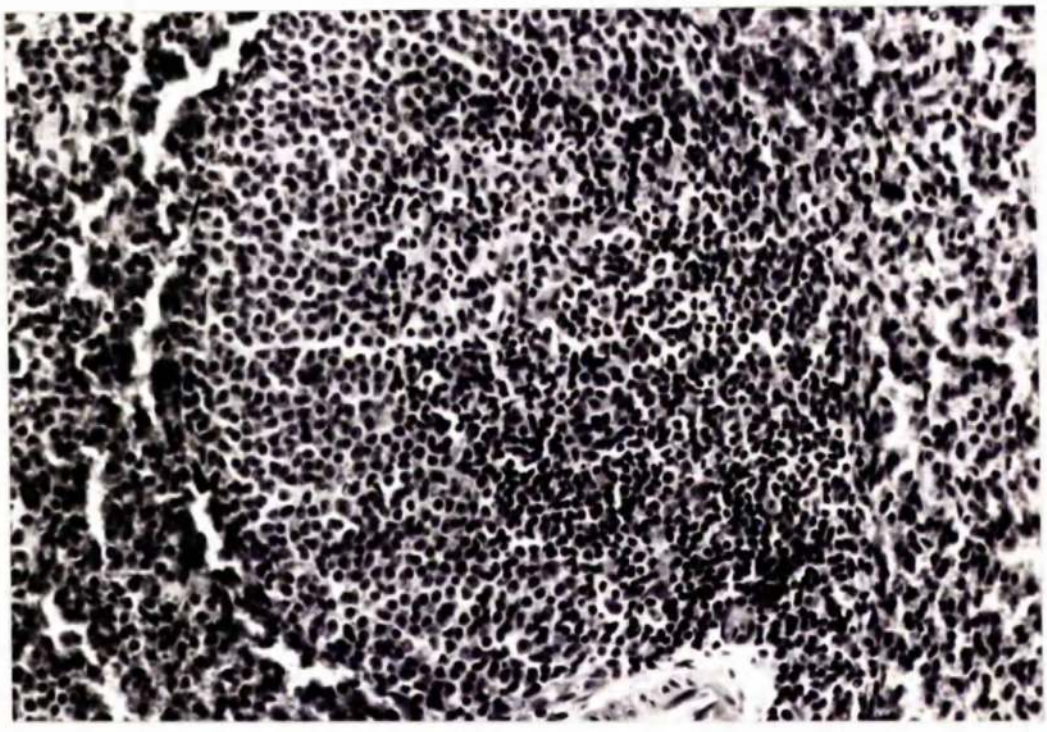

Figure 80. Cat 23908/45. A Malpighian corpuscle in the spleen to show the peripheral hyperplasia and formation of lymphoblasts which are already entering the red pulp. H. & E. x 300.

ACKNOWLEDGEMENTS.

I welcome this opportunity to place on record my indebtedness to Professor W.I.M. McIntyre, who helped me to enter academic life after more than a decade in veterinary practice and who was instrumental in my obtaining a research fellowship from the Carnegie Trust for the Universities of Scotland. To the Carnegie Trust, I also wish to record my appreciation.

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Finally, I am pleased to record my great indebtedness to Mary, my wife, for her encouragement and support.

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