LEUKEMIA, PSEUROLEUKEMIA AND RELATED CONDITIONS IN THE SLYE STOCK OF MICE

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Enlargement of the lymph glands and spleen has been a frequent finding in this stock of mice. It has seemed possible that a careful examination of this material might throw some light upon the complicated and difficult problem of the origin, nature and classification of leukemias, pseudoleukemias and other diseases of the lymphadenoid system. Accordingly 316 of these mice with enlargement of the lymph glands occurring in the first 15,000 autopsies have been studied for the purposes of (1) establishing criteria for the diagnosis of leukemia and related conditions of the lymph glands of mice; and (2) of comparing the pathology of these conditions in mice with that of the corresponding diseases in man.

For routine work sections of the different organs of the mice have been stained with eosin and hematoxylin. A limited number of mice showing the different types of lesions were selected, and sections of their organs were stained with Unna's, Giemsa's and Levaditi's stains. The indo-phenol oxidase reaction was applied to frozen sections from a considerable number of mice. No examinations were made of the blood before death of the animals. Attempts to examine smears from the heart's blood taken at autopsy proved unsatisfactory, because of the rapid postmortem changes that take place in the blood and tissues of leukemic mice. It has therefore been necessary to establish the diagnosis in each case from a study of the gross and microscopic pathology of each mouse.

In the following discussion the term leukemia is used in its ordinary sense. Pseudoleukemia is employed with the same
meaning as originally given the word by Cohnheim (13), namely, a condition characterized by the same gross and microscopic changes in lymph glands as in true lymphatic leukemia, but lacking the leukemic blood picture. It is thus an entirely different pathologic condition from lymphogranulomatosis or Hodgkin’s disease as differentiated by Reed (61).

There is some advantage in adopting the terminology introduced by Türk (83) and by Helly (31) by which leukemia is designated as leukemic lymphadenomatosis, and pseudoleukemia as aleukemic or subleukemic lymphadenomatosis. But since the names leukemia and pseudoleukemia are firmly established in medical literature by long usage, it does not seem advisable to dispense with them entirely. They will therefore be used in the following discussion, the more descriptive terms being employed only when greater clearness will result from their employment.

LEUKEMIA AND LYMPHOMATOUS CONDITIONS IN LOWER ANIMALS

Leukemia has been reported as occurring spontaneously in many species of animals. The earliest available report of such disease was that of Leisering (40) in 1865, in a pig. Since that time other cases of leukemia in pigs have been reported by Bollinger (8), Fürstenberg (25), Wolff (96), Willach (94), and Gmach (26). At least three of these cases were of the myelogenous type. Leukemia of both forms manifests itself in the pig by the presence of a generalized enlargement of the lymph glands and of the spleen, with leukemic infiltrations of the viscera and marked increase of the nucleated cells of the blood.

In cattle, leukemia also occurs in its two characteristic forms, and cases have been reported by Rössle (64), de Jong (36), Ranozotti (60), Aubertin and Morel (2), and du Toit (82). Zimmermann (99) has recorded the occurrence of pseudoleukemia in a cow, with enlargement of the lymph glands of the mediastinum and shoulder region. The blood and visceral organs were not affected. The record of this case is not sufficiently detailed to enable one to determine whether, in the absence of visceral changes, this may not have been some other type of disease of the lymphadenoid tissues.
Magnusson (45) observed a lymphosarcoma of the stomach of a cow with metastases in the heart, diaphragm, intestine and subperitoneal and subpleural tissues.

Petit and Weil (58a) have reported cases of leukemia in horses. Bollinger (8) mentions "several cases" of this disease in horses but remarks that on account of the peculiar reaction of horses to infection with enlargement of lymph glands and marked increase of leucocytes in the blood, he is of the opinion that the cases reported as leukemia in these animals are only very marked leucocytoses. He states that a leucocytosis in the horse may be so great that the number of leucocytes may equal the erythrocytes of the blood.

Weil and Clerc (92, 93) have recorded ten cases of leukemia in dogs, nine of which were of the myelogenous type. Of Bollinger's (8) two cases in dogs, one was diagnosed "myelogenous and lymphatic leukemia," while the other was considered an early stage of the disease and no diagnosis was stated as to type. Two other cases in dogs have been reported by Milks (49) and by Siedamgrotsky (72). In this animal the spleen is especially affected and is frequently enormously enlarged. In Siedamgrotsky's case the spleen weighed 1175 g. while in one of Bollinger's dogs it was described as "about the size of a child's head." There was generalized enlargement of the lymph glands, and the viscera showed leukemic infiltration.

Sticker (79) described a lymphosarcoma in a dog. The lymphosarcoma-like lesion that sometimes originates in the genital organs of the dog and is apparently transmitted by coitus has been studied by Beebe and Ewing (7) and by Crile and Beebe (14).

Two cases of leukemia in cats have been reported by Siedamgrotsky (72) and by Lellmann (41). Massaglia (43) has recently reported a case of leukemia in a monkey.

No instance of leukemia appears to have been observed in guinea pigs or rabbits. Miguez (48) has described a lymphosarcoma-like tumor in the neck of a guinea pig with areas of infiltration in the liver and kidneys. He succeeded in transmitting the tumor by subcutaneous inoculations through nine
generations of guinea pigs. Wallner (90) has reported the occurrence of lymphosarcoma in a rabbit. Dessy and Aberastury (15) found a similar tumor in the peripancreatic lymph glands of a rabbit with metastases in the heart, ovaries and other organs. Woolley and Wherry (97), and Bulloch and Rohdenberg (11) have described lymphosarcoma in wild rats. McCoy (46) mentions a similar tumor in a ground squirrel. Multiple myeloma has been reported in a gopher (100). A sarcoma of doubtful lymphoid origin has been found in the lymph glands, spleen, liver and lung of the common hedgehog (101). A lymphosarcoma has been described in the liver of a parrot (102) and a "myeloid tumor" in the periosteum and pelvic cavity of an ostrich (103).

Leukemia in fowls has been recently studied by Ellermann (17) and by Schmeisser (66). They have fully reviewed the literature dealing with avian leukemia. From their studies the following facts may be stated with some confidence:

1. Leukemia in fowls is a definitely infectious disease due to a filterable virus.

2. Schmeisser was able to produce only the myelogenous type of leukemia, but Ellermann succeeded in causing both forms of the disease by inoculations with the same virus. In some fowls typical pseudoleukemia was produced in the same manner, differing from leukemia only in the absence of a leukemic blood picture.

3. The organs most prominently affected in chickens are the liver and spleen. Ellermann states that the chicken is devoid of lymph glands. There is usually found in the neck region of leukemic fowls a mass of lymphoid tissue which, according to Kon and Ellermann, is in reality thymus. The spleen is usually several times the normal size, while the liver may be so greatly enlarged that it fills the entire abdominal cavity. There is little difference in the size of the spleen in the two types of the disease, but the liver is much more enlarged in the lymphatic than the myelogenous form.

4. The kidneys show perivascular and intertubular infiltrations with the characteristic cells of the disease. In the lungs
there is a marked intracapillary, but only a slight perivascular infiltration.

5. The disease, in both forms, is definitely invasive in chickens. Schmeisser observed invasion of the capsule of the mass of lymphoid tissue in the neck, with infiltration of the surrounding fat. Ellermann found that in the periportal infiltrations in the liver, the walls of the branches of the portal vein were invaded by the growing cells. In Warthin’s cases the branches of the portal vein were so extensively infiltrated that the lumina of the vessels were, in many instances, obliterated.

LEUKEMIA IN MICE; REVIEW OF LITERATURE

Reports in the literature of leukemia in mice are not numerous. In 1878 Eberth (16) briefly described an instance of myelogenous leukemia in a mouse whose spleen measured 49 x 17 x 7 mm. The spleen showed “general hyperplasia of the pulp.” The liver, kidneys and lungs showed leukemic infiltrations. The heart contained one leukemic nodule. No changes were mentioned in the lymph glands.

Fajersztajn and Kuczynski (19), in 1892, reported three cases of leukemia in mice that had been kept in a cage with some 20 to 30 others. The disease was first found accidentally in a mouse that died. Careful examination of all of the other mice in the cage revealed another case. The third was discovered about four months later. There was no epidemic of any kind among these mice at the time of the appearance of the disease. Examination of the blood of the second and third mice showed a great increase in the number of leucocytes. The spleens and lymph glands of all three mice were greatly enlarged and showed “leukemic hyperplasia.” In the liver, lungs and kidneys, there were leukemic infiltrations.

In 1909, Tyzzer (88) described a case of lymphatic leukemia in a mouse. The mesenteric glands appear to have been the only ones enlarged. There was lymphoid infiltration in the periportal connective tissue and to a less extent in the hepatic sinusoids. Lymphocytes, larger than the ordinary type, were found in greater numbers than normal in the vessels of all of the organs of the body.
In three other mice Tyzzer (87) encountered conditions which he placed in a group of "unclassified tumors." No enlarged glands were mentioned in the description of these cases. In all three mice the spleen was greatly enlarged. This organ and the liver were infiltrated with cells having "large and hollow nuclei" and relatively large amount of cytoplasm coloring pink or purple with eosin and methylene blue. These cells were intimately mixed with others definitely recognized as myelocytes, both in the blood vessels and in the perivascular infiltrations. The blood in the vessels of the different organs contained considerable numbers of these cells, which Tyzzer appears to consider, in some cases, to be ingrowths of tumor tissue directly into the lumen of the vessel. These three cases seem to be closely related to those in this series placed in the group of myelogenous leukemias. The large cells in the blood and perivascular infiltrations gave the indophenol oxidase reaction in our cases.

Haaland (29), in 1911, reported a study of 353 primary tumors of various kinds in mice. Of these, 3 were leukemia, all of which were in female mice, two being mother and daughter, respectively. In all three of these mice, the cervical, axillary, inguinal, mesenteric and retroperitoneal glands were greatly enlarged, and in one the thymus was also involved. The spleen was greatly increased in size in all. "Histologically the picture was that of lymphatic leukemia," in one mouse. In the other two the microscopic findings were essentially the same except that the cells of the lymph glands and visceral infiltrations were described as of the "large mononuclear type."

Levaditi (42) (1914) observed the lesions of leukemia in two mice. The first was a male that had to be sacrificed on account of an infection. The autopsy revealed a "watery condition of the blood." The spleen measured 30 x 7 mm. There was a "slight hyperplasia of the mesenteric glands." The second case was in a female mouse obtained from a dealer whose stock had shown numerous cases of spontaneous cancer. The spleen of this mouse was not so large as that of the first mouse. In the mediastinum there was a tumor-like mass the size of a hazelnut. Both mice showed an enormous leucocytosis, especially an in-
crease in the large and small lymphocytes. The spleen and lymph glands had lost their normal histologic structure. The liver, kidneys and lungs showed "enormous lymphomas, chiefly perivascular." The tumor-like mass in the mediastinum showed the same structure as the enlarged lymph glands.

The details in the reports of the above 13 cases of leukemia in mice are not sufficient to enable one to recognize with certainty the type of disease present, but it appears probable that seven of them were myelogenous leukemia. It is at least evident from these recorded cases that mice suffer from both myelogenous and lymphatic forms of leukemia. It is not possible, from the data available in the literature, to form a conjecture as to the relative
frequency of each type or as to the morbidity of the disease in any given stock of mice.

**FIG. 2. LEUKEMIC INFILTRATION OF THE LIVER, MYELOGENOUS TYPE, SHOWING MYELOCYTES AND YOUNG POLYMORPHONUCLEAR LEUCOCYTES WITH RING-SHAPED NUCLEI.**

Magnified 1200 diameters.

**LEUKEMIA IN THE SLYE STOCK OF MICE**

Among the 316 mice with lymphadenoid enlargements occurring in the first 15,000 autopsies on the Slye stock, which form the basis of this study, 67 showed pathologic processes which have been diagnosed leukemia. Of these, 28 were of the lymphatic, and 39 of the myelogenous type. Three other mice have been diagnosed myelogenous leukemia in this study, but were given different diagnoses at the Sprague Institute, namely, 2, pseudoleukemia, and 1, lymphoid hyperplasia.
The fundamental basis for the diagnosis of leukemia in this investigation has been the presence of an enormous increase in the nucleated cells of the blood in the larger vessels of all of the organs of the mouse, associated with a more or less generalized enlargement of the lymph glands and of the spleen. Ordway (52), however, has recently stated that "because leukemias have been seen without abnormal blood pictures, ... cellular hyperplasia is a more exact criterion of leukemia than the blood picture." It has seemed advisable, however, for the purpose of this study to retain in this list only those mice which showed the characteristic blood picture.

The larger vessels of the liver, lungs and kidneys were found
to be the most satisfactory locations in which to study the cells of the blood. Even in the most typical cases of leukemia in these mice there were frequently observed distinct variations in the number of nucleated cells in different blood vessels, but in all organs their numbers exceeded the normal.

**LYMPHATIC LEUKEMIA**

In normal mouse blood lymphocytes constitute fifty per cent or more of the nucleated cells. Lymphatic leukemia has been diagnosed, therefore, only in those mice in which lymphocytes so greatly outnumbered all other leucocytes that only an occasional nucleated cell of any other type was seen in the blood. In most of these mice the lymphocytes of the blood were slightly larger than normal lymphocytes, and in a few cases they were distinctly of the large lymphocyte type. There was also a distinct uniformity in the size of the nucleated cells in the blood vessels in these lymphocytic leukemias. Nucleated red cells were seen only rarely.

Those mice with lymphatic leukemia showed an enlargement of at least one and usually of several groups of lymph glands. The superficial groups were somewhat more commonly involved than the internal ones. The size reached by these glands varied, but was in nearly all instances, relatively great, sometimes even enormous. The spleen was always enlarged, and ranged from 3 to 30 times the normal size.

Upon microscopic examination of the lymph glands, they were usually found to have completely lost their normal histologic architecture as a result of the marked proliferation of the lymphoid cells. These cells sometimes were only slightly larger than ordinary lymphocytes, but as a rule they belonged to the large lymphocyte variety. The lymph glands, especially if quite large, were transformed into a homogeneous mass of closely packed lymphoid cells. Occasionally, the follicles and cords could be made out, but these were larger than normal, frequently confluent, their germinal centers were not distinguishable, and the lymph sinuses were reduced to narrow slits in which lymphocytes were numerous but less densely crowded than within the
follicles and cords. Mitotic figures were usually numerous. The reticulum was not noticeably affected. In some mice large phagocytic cells containing fragments of nuclei were seen scattered among the lymphoid cells. Very little blood was visible in these glands.

A very characteristic and constant feature has been the invasion of the capsules of the lymph glands by lymphoid cells. These could be seen lying in rows between the layers of the capsule, sometimes stretched out and elongated in the tissue spaces. From the capsule the cells streamed off into the surrounding tissues, frequently to considerable distances. In the loose retroperitoneal and mesenteric tissues, the enlarged glands
were usually plumply ovoid in shape, and the extensions beyond the capsule appeared to progress about equally in all directions. The enlarged subcutaneous glands were ovoid in shape, with the long axis parallel to the skin. The infiltration of the tissues about these glands was most marked at the ends. This may be the result of mechanical pressure by the overlying skin. The infiltrations from two or more neighboring glands were frequently observed to coalesce, thus fusing them into a lobulated mass. The extraglandular growth of lymphoid cells frequently involved the salivary glands in the neck region; the fat and skeletal muscles in the axillae and groins; fat and pancreas if the mesenteric or retroperitoneal glands were affected; and the bronchi and vena cava if the leukemic glands were within the mediastinum. This invasive property was not limited to one gland or to one group of glands, but all of the enlarged glands in any given animal showed this characteristic in more or less marked degree. Invasiveness was less evident in those lymph glands in which some semblance of the normal histologic architecture was retained, but even here it was often not wholly lacking.

Microscopic examination of the spleen usually showed loss of normal histologic structure, but occasionally dim outlines of it could be distinguished. The splenic pulp was so extensively infiltrated with lymphoid cells that it could not be differentiated from the splenic corpuscles. Mitotic figures were relatively numerous. Megakaryocyte-like cells, normal to the mouse spleen, were present; sometimes they appeared in increased numbers.

The lungs, liver and kidneys, one or more of them depending upon the case, showed perivascular and intracapillary infiltration with lymphoid cells. In the liver in most of the cases, large masses of lymphoid cells surrounded the branches of the portal vein invading its walls and frequently narrowing its lumen by the formation of considerable masses of cells immediately beneath the endothelium. These periportal infiltrations were of sufficient extent to infringe on the liver lobules, sometimes presenting a fairly even line of contact with the hepatic cells,
sometimes sending finger-like processes into the adjacent sinusoids. Mitoses were numerous among the cells composing these periportal masses. The cells lay in a delicate reticulum not unlike that of lymph glands. Within the lobules, the sinusoids were crowded with lymphoid cells, frequently arranged in rows, sometimes collected in small groups within a dilated sinusoid.

The perivascular infiltration of the lungs, when present, was most marked in those mice in which there was a growth of lymphoid tissue in the mediastinum. In many mice the pulmonary vessels and bronchi were surrounded by thick branching cylinders of infiltration continuous with the mass at the hilus of the lung. The capillaries in the alveolar walls were crowded with lymphoid cells causing a reduction in the size of the alveolar spaces.

In most of these leukemic mice there was a large mass of lymphoid tissue in the hilus of the kidneys. From this, thick cylinders of infiltration followed the renal vessels into the kidneys invading the walls of the veins and frequently a sheet of lymphoid cells spread into the capsule for varying distances. In some mice the entire kidney appeared to be encased in a shell of closely packed lymphoid cells. In the cortex of the kidney the invading lymphoid cells occupied the tissue spaces and capillaries between the tubules, sometimes forming an almost continuous latticework of connecting cords, sometimes occurring in more localized irregular masses of closely packed cells.

To summarize, lymphatic leukemia in these mice is characterized by (a) an enormous relative and absolute increase of the lymphocytes of the blood; (b) a vigorous proliferation of the lymphoid cells of the body causing enlargement of the spleen and of widely separated groups of lymph glands; (c) loss, usually complete, of the normal histologic architecture of the lymph glands and spleen with marked invasion of the capsules of the former and infiltration of the surrounding tissues; and (d) leukemic infiltrations, invasive in character, in one or more of the viscera, especially the liver, lungs and kidneys.
MYELOGENOUS LEUKEMIA

In myelogenous leukemia in these mice, there was an enormous increase in the nucleated cells of the blood, 90 per cent or more being of myeloid origin, that is, polymorphonuclears especially the young forms with ring-shaped nuclei (73), and myelocytes. As in this disease in man, so also in myelogenous leukemia in these mice, there was observed marked variations in the size of the nucleated cells of the blood, thus presenting a sharp contrast to the uniformity in size of the cells that characterize lymphatic leukemia. Nucleated red cells were moderately abundant.

In myelogenous leukemia, furthermore, multiple groups of lymph glands were enlarged. The spleen was increased in size, in all cases, varying from 10 to 24 times the normal. Microscopically, the enlarged lymph glands showed loss of normal histologic structure. The lymphoid cells in many glands were largely replaced by myelocytes and young polymorphonuclears with ring-shaped nuclei. Similar cells invaded the capsules of the glands and infiltrated the adjacent tissues. Within the lymph glands mature polymorphonuclears were present in moderate numbers, but it was only the immature forms, myelocytes and cells with ring-shaped nuclei, that took a noteworthy part in the process of infiltration. Occasionally in this group a slightly enlarged lymph gland was found in which the increase in size was due to lymphoid hyperplasia, without invasion of the capsule. But even in these glands, careful search usually revealed one or more small groups of myeloid cells.

The enlargement of the spleen was seen on microscopic examination to be due chiefly to a marked infiltration of its pulp with myeloid cells. Megakaryocyte-like cells were present, but in no greater numbers than in lymphatic leukemia.

The lungs, liver or kidneys, one or more, showed invasive perivascular infiltrations, similar to those seen in lymphatic leukemia except that they were composed of myeloid instead of lymphoid cells. The capillaries of these organs were also crowded with myeloid cells. In those mice with perivascular and intracapsular infiltrations of the kidneys, there was usually a mass of myeloid cells in the hilus.
In brief, then, myelogenous leukemia in these mice is distinguished by (a) a very great relative and absolute increase of myeloid cells,—myelocytes and young polymorphonuclears (73)—in the blood; (b) widespread enlargement of the lymph glands and of the spleen due to infiltration with myeloid cells which obliterate the normal microscopic markings of the glands and infiltrate the surrounding tissues; (c) and leukemic infiltrations of the lungs, liver, or kidneys, one or more, with myeloid cells.

It was necessary to differentiate myelogenous leukemia in mice from an inflammatory leucocytosis. In the latter condition, a focus of acute infection could frequently be found in some organ, such as a pneumonia, an abscess or a pyelitis. In such infections the blood was frequently remarkably rich in nucleated cells and these might almost equal the number seen in myelogenous leukemia. The vessels in the immediate vicinity of the focus of infection were usually richer in polymorphonuclear leucocytes than vessels elsewhere in the body. In acute infections, also myelocytes, if present at all, were in relatively small numbers, and never equal the percentages seen in myelogenous leukemia. Moreover, in infections the polymorphonuclears of the blood were of the more mature types, the younger forms (73) with ring-shaped nuclei being only moderately increased. Nucleated red cells were not encountered in mice with acute infections and leucocytosis.

In myelogenous leukemia, the myelocytes and young polymorphonuclears with ring-shaped nuclei, invaded the capsules of the lymph glands and the walls of veins in the liver and such other organs as showed leukemic infiltrations. In acute infections infiltrations of visceral organs were either absent or were restricted to such organs as were directly concerned in the inflammatory process, while invasion of the capsules of the glands, if present at all, was restricted to those glands in the immediate vicinity of the focus of infection.

Furthermore, myeloid infiltration of lymph glands and visceral organs differed from localized acute suppurative process in these locations in the absence of (a) an exudate containing fibrin; (b)
liquefaction necrosis; and (c) edema and hyperemia of the tissues immediately surrounding the focus of infection. Myeloid infiltration of the kidney, for example, was not accompanied by pus cells within the renal tubules or pelvis; while in both ascending and descending infections of this organ, free pus could usually be found in one or both of these locations. In pneumonia and abscesses of the lungs, pus cells were found in the alveoli and bronchioles, while in myelogenous leukemia, the myeloid cells were restricted to the perivascular tissue spaces and to the capillaries in the alveolar walls and were not found free in the air spaces of the lungs. Furthermore, the remarkably widespread character of the infiltrations as well as the distinctive types of cells which constituted them, in myelogenous leukemia helped to differentiate this disease from most cases of pyogenic infection.

In addition to myelogenous leukemia, three mice of this series had one or more abscesses in some part of the body. These distinctly localized suppurative processes differed from the more generalized myeloid infiltrations in the same animal in the manner outlined above.

Amyloid degeneration of the spleen and, to a less extent, of the kidneys and liver, was a not uncommon finding in these 316 mice. Of the 58 cases of leukemia here reported, 6 were found to show amyloid degeneration, and of these mice, 3 had myelogenous and 3 lymphatic leukemia.

The size of the spleens of these mice was estimated and the figures are, therefore, only approximate. But as all of the estimates were made by the same person (Maud Slye) they have comparative value. In lymphatic leukemia the spleens varied in size from 3 to 20 times the normal, with an average of twelve times the normal. In myelogenous leukemia, they ranged from 10 to 24 times the normal, with an average of 15 times normal. It is evident, therefore, that there was a more marked increase in the size of the spleen in lymphatic leukemia in mice than in the corresponding disease in man. The largest spleens encountered in man have been found in patients with myelogenous leukemia. This has not been true of mice, for 2 spleens of this series were estimated to be 50 times enlarged, and neither of these mice had either form of leukemia.
On the other hand, there was a widespread and marked enlargement of the lymph glands of mice in myelogenous leukemia. The average number of different sets of lymph glands enlarged in myelogenous leukemia was slightly less than in lymphatic leukemia, but the difference was so slight as to be practically negligible. In this respect, therefore, leukemia in mice differed from the corresponding types of the disease in man, for in the latter, the myeloid infiltration is limited largely although not exclusively to the spleen. Possibly the relatively slight response of the lymph glands in man to myeloid infiltration is the cause of the tremendous increase in the size of the spleen. In man the spleen is only moderately enlarged in lymphatic leukemia, while in the mouse the spleen reaches dimensions almost equal to those observed in myelogenous leukemia in this animal. Of some significance in this connection may be the fact that in the mouse the lymphocyte, derived from lymphoid tissues, is the chief nucleated cell of the blood, while in man, polymorphonuclear leucocytes, derived from myeloid tissues, predominates in the blood.

In 4 of these leukemic mice (3 with myelogenous and 1 with lymphatic leukemia), there was infiltration of the walls of the stomach or intestine. Nine of these mice (2, or 8 per cent, with myelogenous, and 7, or 21 per cent, with lymphatic, leukemia) showed leukemic infiltrations of the skin. In many instances the invading cells lay immediately beneath the epithelium and surrounded hair follicles and skin glands. In practically every focus of infiltration of the skin, there was an underlying subcutaneous gland from which the infiltration appeared to proceed, by growth of lymphoid or myeloid cells through its capsule.

Of the 67 leukemic mice, 11 had associated tumors, of which 7 were carcinomas of the mammary gland, 3 were adenomas of the lung, and 1 a sarcoma of the mouth.

**LEUCOSARCOMA**

Five of the mice of this series showed lesions which correspond in all essential respects to Sternberg's leucosarcoma (74). The site of the primary growth was in the mesenteric glands in two
cases, in the region of the thymus in one, and undetermined in the other two. The local growths showed the property of invasiveness to an extreme degree, breaking through the capsule and spreading widely in the surrounding tissues. The cells were of the lymphocyte type, and mitoses were numerous. In all of these mice the liver was markedly infiltrated with cells similar to those in the primary growth. The infiltration of the kidneys was only moderate as was also that of the lungs except in the case in which the primary growth occupied the site of the thymus. The cells in the areas of infiltration in the liver, lungs and kidneys showed the same powers of penetration and invasion as those in the primary growth. The nucleated cells of the blood of all of the organs were notably increased but not to such an extreme degree as in true leukemia. The condition in these mice, therefore, resembles lymphosarcoma except in the blood picture and the areas of infiltration in distant organs. They resemble leukemia except in the definitely localized involvement of the lymph glands, and the somewhat more extensive invasion of the tissues surrounding the involved glands.

Türk (83), Naegeli (51), and others refuse to recognize leuкоsarcoma as an entity. These five cases in these mice, however, seem to occupy a definite position between the leukemias and lymphosarcomas, partaking of certain characteristics of each, yet showing such distinct differences that they can not be placed in either of these groups. The name applied by Sternberg to such lesions appears, therefore, to be distinctly useful.

PSEUDOEOUKEMIA IN MICE

No case of pseudoleukemia in mice appears to have been reported in the literature under that name. Haaland (28) has described a pathologic condition in mice under the name of malignant lymphoma which apparently corresponds to the lesion here classified as pseudoleukemia. Haaland's first paper concerns five mice which are not described in detail. These mice show a marked enlargement of all of the lymph glands of the body, together with an increase in the size of the spleen and frequently of the liver. The normal histologic architecture of
the lymph glands and spleen was more or less completely obliterated by the great increase of the lymphoid cells in these organs. The lungs showed marked invasion of the perivascular tissues. There were large masses of infiltration with lymphoid cells about the intrahepatic branches of the portal vein. These cells were also numerous in the hepatic sinuses. At the hilus of the kidneys there was a mass of lymphoid cells from which cylindrical masses of perivascular infiltration penetrated the organ, often extending to and involving the intertubular connective tissue of the cortex. The blood did not show a leukemic picture.

In a later paper, Haaland described with varying amount of detail seven other mice, some of which unquestionably belong to this group. They had in common a generalized enlargement of the lymph glands of the body with infiltration of the visceral organs, but lacked a leukemic blood picture. Haaland found nematode worms in the tissues of two of his mice and believed that they were concerned with the causation of the changes observed in the lymphadenoid apparatus.

Jobling described a mouse which showed enlargement of all of the external lymph glands which were freely movable and were composed of cells of the large lymphocyte type. It is not stated whether the capsules of the glands were invaded. The liver, lungs, and kidneys showed perivascular and intracapillary infiltration with cells similar to those in the lymph glands.

In Murray’s series of cases, Mouse No. 2 probably belonged to this group, although the details given are not sufficient to classify it definitely. In this animal all of the lymph glands of the body were enlarged; the liver and spleen were “affected;” and the “chest was full of growth.”

Of the 316 mice which have formed the basis of this study, 111 showed lesions which have been diagnosed pseudoleukemia with more or less certainty. In 102 of these mice my own diagnoses have agreed with those of the Sprague Institute; in the remaining 9 there was a difference of opinion as to the nature of the pathological condition found.

Pseudoleukemia in these mice stands between the leukemias
and the lymphosarcomas, and these 111 cases can be separated into three groups: (1) Typical pseudoleukemias, 74 cases, including 5 in which there was a difference of opinion; (2) Subleukemic lymphadenomatous, or pseudoleukemias which appear to be developing into leukemias, 23 cases, including 4 that were questionable; (3) Pseudoleukemias with some of the characteristics of lymphosarcoma, 14 cases, with 4 in which there was disagreement as to the diagnosis.

1. Typical Pseudoleukemia.—This condition resembles leukemia in its gross and microscopic pathology but is not accompanied by the characteristic leukemic blood picture. It will therefore be necessary only to state briefly the distinguishing features upon which the diagnosis of typical pseudoleukemia has been made in these mice.
A. The lymph glands of one or more groups were enlarged. In some cases five different groups of glands showed enlargements. The thymus was also frequently increased in size. The normal histologic architecture of the enlarged lymph glands was either completely lost, the gland being transformed into a homogeneous mass of closely packed cells; or there was a marked increase in the size of the follicles and cords, which became confluent, thus narrowing the lymph sinuses. The component cells were usually of larger size with paler nuclei and more abundant cytoplasm than normal lymphocytes. Mitotic figures were numerous throughout the affected glands. There was always an invasion of the capsule, the cells lying in rows between its layers, and often infiltrating adjacent structures to a moderate degree. In the more marked cases the infiltration extended beyond the capsules and involved the surrounding tissues—connective tissues, fat, skeletal muscle, salivary gland, pancreas, etc. The invasive character of the changes in the lymph glands was little if at all, less marked than in true leukemia.

B. The spleen was enlarged in this condition as in leukemia. The size varied from 4 to 20 times normal, with an average of 12.5 times the normal. The normal histologic architecture of the spleen was lost or was greatly obscured by the dense infiltration of its pulp with lymphoid cells. Megakaryocyte-like cells were present in about the same proportion as in lymphatic leukemia. Amyloid degeneration of the spleen was observed in a few cases.

C. Marked perivascular infiltration occurred in one or more of the viscera, especially the lungs, liver or kidneys, frequently with a moderate increase in the number of nucleated cells in the capillaries of these organs. The walls of the veins were invaded as in leukemia and the infiltrating cells lay immediately beneath the endothelium. The extent of the perivascular infiltration of these organs depended somewhat upon whether their regional lymph glands were enlarged. This applies to the lungs and kidneys more than to the liver, for the latter organ was found infiltrated in every case of this group whether the mesenteric lymph glands were enlarged or not. In the case of the lungs and
kidneys the involvement of the regional lymph glands caused quantitative rather than qualitative differences in the degree of infiltration of these organs. In nearly all of these mice, the sections of the kidney showed a mass of lymphoid tissue in the hilus, from which invading lymphoid cells extended along the renal vessels into the kidney or into the capsule for varying distances as in leukemia. Two mice of this group showed a lymphoid infiltration of the skin similar to that seen in leukemia. In one mouse there was a small focus of infiltration in the wall of the intestine.

D. The blood in the larger vessels of the different organs showed no noticeable increase in the number of leucocytes.

2. Subleukemic Lymphadenomatosis.—In this subgroup the gross and microscopic pathology of the lymph glands, spleen and viscera was similar in all essential respects to that of the leukemias and typical pseudoleukemias. The blood in the larger vessels of different organs contained a distinctly greater number of leucocytes than normal. The increase affected the lymphocytes chiefly, perhaps solely, but was not great enough to justify a frank diagnosis of lymphatic leukemia. This group may represent either the transformation of pseudoleukemia into true leukemia, or, possibly, some of these may be cases of leukemia in which there has occurred a remission accompanied by a marked fall in the leucocyte count.

3. Pseudoleukemia with Some of the Characteristics of Lymphosarcoma.—In this group there was a widespread enlargement of lymph glands, with invasion of the capsules by lymphoid cells and all of the other characteristics of pseudoleukemia. But in each case the growth in at least one group of glands showed an extreme degree of invasiveness. The proliferating lymphocytes grew widely into adjacent structures—skeletal muscle, salivary gland, etc. In at least 7 cases the extremely invasive lesion was in the region of the thymus; in 4 instances it involved the retroperitoneal, and once the cervical, lymph glands. The enlargement of the lymph glands was too widespread,—i.e., involved too many different groups—to warrant a diagnosis of lymphosarcoma; and the infiltrating character of the lesions in
the glands other than the one group in which the growth was markedly invasive, was not sufficient to justify a diagnosis of generalized lymphosarcomatosis. Whether these cases are examples of the coexistence of two pathologic processes—lymphosarcoma and pseudoleukemia—or whether they represent a pure pseudoleukemia of a peculiarly malignant type, it is not possible, from the data available, to determine. These cases do, however, suggest a similarity in the fundamental nature of pseudoleukemia and lymphosarcoma, just as the preceding group points to a relationship between leukemia and pseudoleukemia.

**LYMPHOSARCOMA**

Fifty-one of the mice studied in this series showed lesions diagnosed as lymphosarcoma. In 16 of these there was a difference of opinion as to the diagnosis. This constitutes a rather well defined group that is neither so limited as Kundrat’s (38) conception of the disease in man, for it includes cases of generalized lymphosarcomatosis as described by Hirschfeld (33) and by Kaufmann (37); nor so broad as that of Webster (91), who groups under this title “all cases of lymphoid tumor or general lymphoid hyperplasia not accompanied by leukemia.” Webster would, therefore, probably include among the lymphosarcomas the cases classified here as pseudoleukemias.

Pathologic processes in mice that correspond to the lymphosarcomas as observed in this stock have been described in the literature as either lymphosarcomas, or as malignant lymphomas, or as malignant lymphoid hyperplasias. In Murray’s (50) series, Mouse No. 4 showed a lymphosarcoma originating in the region of the thymus and involving the heart and lungs extensively. Even the chest wall had been invaded by the growth. Mouse No. 3 showed very similar lesions. In Haaland’s (28, 29), collection of cases at least three appear to have been lymphosarcomas. One of these, with the primary tumor in the region of the thymus, showed in addition a carcinoma of the mammary gland. He mentions six other mice without giving details that showed lesions of the lymphadenoid apparatus, some of which were described as “decidedly sarcomatous.” Of these 6 mice,
one showed involvement of the kidneys; in three the mesenteric and retroperitoneal glands were the site of the growth; while in the other two the primary tumor occupied the site of the thymus. In still another mouse, the axillary, mediastinal, mesenteric and retroperitoneal glands were greatly enlarged and the "pelvis was filled with growth." Haaland was of the opinion, however, that "these conditions can not be distinguished with certainty from hyperplasias and infectious processes."

Tyzzer (88) mentioned without giving details, 10 mice in which he diagnosed lymphosarcomas. Of these the primary tumor was in the inguinal glands in two mice; in four cases it was in the region of the thymus and had invaded the lungs in three, and the heart in two; and in one mouse the growth originated in the mesenteric glands. In the remaining three mice the primary site of the tumor could not be determined because the growth was so disseminated that it was not possible to decide the question of origin. But even in these three animals the lesion was not a generalized hyperplasia for the growth was limited to some portion of the lymphadenoid system. In an earlier paper, Tyzzer (87, 88), describes in greater detail two cases of lymphosarcoma in mice, one of which originated in the inguinal glands, the other in the mediastinum. In one of these, certain portions of the tumor contained many phagocytic cells. The spleen contained great numbers of cells similar to those seen in the tumor. In the other mouse there was invasion of the pericardium and lungs.

Of these 24 cases collected from the literature, the primary tumor was intrathoracic in 10; intra-abdominal in 5; in the inguinal region in 3; generalized in 4; and source not stated in 2.

The characteristic lymphosarcoma was found to begin in one lymph gland, or at least in one group of glands. The tumor was composed of round cells closely packed together, larger than ordinary lymphocytes, and showing mitoses in great abundance. The stroma of the tumor was the reticulum of the gland. Large phagocytic cells containing fragments of nuclei of ingested cells were numerous in some of the affected glands in some of these mice. Similar cells were observed by Tyzzer (88) in lympho-
sarcoma in mice and by Petrow (59) in the same disease in man. All vestiges of the normal histologic structure of the enlarged lymph glands were obliterated. As a rule, blood vessels were not numerous in these growths, although in a few instances fairly wide spaces filled with blood and lined with a single layer of flat cells could be seen in the tumor growths.

The proliferating lymphoid cells of the tumor invaded the capsules of the glands, and infiltrated the surrounding tissues widely. There was no definite evidence of blood borne metastases in distant organs with the possible exception of the spleen. In a few mice this organ was enlarged and showed rather sharply differentiated masses of proliferating cells similar to those in the primary tumor.

Among the fifty-one lymphosarcomas of this series the primary tumor was intrathoracic in 32 mice; intra-abdominal in 8; subcutaneous in 9; and too generalized to determine the original site in 2 mice.

In the 32 cases of intrathoracic growths in this series, the lungs were invaded in every case, and the heart in six. In most of these cases in which it was possible to determine the matter, the tracheal wall was infiltrated with the growth; in a smaller number of mice the walls of the esophagus and vena cava showed invasion; and in a few instances there was some infiltration of the wall of the aorta. The extension into the lungs was distinctive, and occurred in the form of very thick cylinders of infiltrating cells growing out from the primary tumor and following the branchings of the bronchi and pulmonary vessels.

In these 32 cases of primary mediastinal (and thymic) lymphosarcomas, the liver was found infiltrated 11 times and the kidneys 12 times. In reaching the liver, the growth in some instances appears to have followed the lymphatics through the diaphragm and along the vena cava, for circles of infiltration could be seen about the branches of the hepatic veins.

In some of these mice with primary tumors in the thoracic cavity as well as those with the primary site in the retroperitoneal glands, the involvement of the kidneys was truly enormous. Fabian (18), Symmers (80) and others have emphasized the tendency of lymphosarcomas to involve paired organs.
Generalized lymphosarcomatosis, as in two of these mice, was differentiated with some difficulty from pseudoleukemia. In the latter condition, however, one or more of the internal organs—liver, lungs, and kidneys—were always infiltrated to a greater or less extent with lymphoid cells, and these infiltrations bore no definite relations to the location of the affected glands. In lymphosarcoma, even of the generalized type, this relation could usually be made out. The liver was found infiltrated in every case of pseudoleukemia, but in only 11 cases of lymphosarcoma.

As stated above, there was a difference of opinion as to the diagnosis in 16 mice of this group. The following diagnoses were made in these 16 mice: Pseudoleukemia, 5; lymphoma, lymphadenoma or diffuse lymphoma, 9; diffuse lymphogranulomatosis, 1; and diffuse chronic lymphadenitis, 1. This difference of opinion in approximately one-third of the cases indicates that the difficulties inherent in the diagnosis of lymphosarcoma in mice are no less than in the recognition of the corresponding lesion in man.

LYMPHOMA

Ten of these mice showed a localized, non-invasive enlargement of one or more lymph glands, usually of a single group. These have been diagnosed lymphomas. In 9 of these, the thymus was involved, and in the tenth the spleen was 50 times the normal size.

LYMPHOID HYPERPLASIAS IN MICE

After eliminating those mice which showed lesions of the lymphadenoid apparatus—leukemia, pseudoleukemia, and lymphosarcoma—whose outstanding feature was their invasiveness, there is left a heterogeneous residue that showed lesions of more varied and less distinctive forms. These have been included under the title of lymphoid hyperplasias.

1. Lymphoid cells are being constantly regenerated in the lymph glands and spleen to replace those which escape into the bloodstream and are lost to the tissues which produce them. Bunting and Huston (12) state that more lymphocytes leave the
blood stream in twenty-four hours than are present in the blood at any one time. These lost lymphocytes are neither destroyed in the blood stream nor returned to the tissues whence they came, but escape into the mucous membranes, especially that of the gastro-intestinal tract. Rous (65) showed that the lymphocyte content of the blood is remarkably constant (in dogs). For the maintenance of the lymphocytes of the blood at their normal level, replacement must be uninterrupted. The regenerative powers of lymphoid cells are therefore great.

2. An irritant of proper quality and concentration intensifies this regenerative activity of the lymphoid tissues. Paltauf (54) has shown that the effect of a stimulus may persist and the augmented proliferation of lymphoid cells may continue for long periods after the irritant has ceased to act.

3. The total effect of any stimulus upon the lymphadenoid tissues of the body depends upon (a) the nature of the irritant, especially upon the exact degree of virulence of the infecting micro-organisms, if these furnish the cause of the irritation; (b) on the route by which the irritant reaches the lymph gland, whether by the lymphatics when the result may remain localized or by the blood stream when it will be generalized; and (c) on the species of the animal, some of the lower animals showing a much more marked lymphoid reaction than man.

4. As pointed out by Arnold (1), Ribbert (62) and others, there are present in the perivascular tissues of many organs, especially of the liver, kidneys and lungs, small accumulations of lymphoid cells that are potential lymph glands. A stimulus or irritant circulating in the blood and inducing proliferation of typical lymph glands will also stimulate these cells to reproduction. Hence, it is possible in purely hyperplastic processes affecting the lymphadenoid tissues of the body, to have accumulations of lymphoid cells in the viscera that resemble the infiltrations of these organs in leukemia and pseudoleukemia. Schridde (69) has expressed the opinion that the perivascular tissues should be classed with the blood forming organs because they contain cells capable of giving rise to blood cells. As will be shown later, small localized collections of myeloid cells in the
perivascular tissues of internal organs, especially in the liver, are fairly characteristic of one group of lymph gland hyperplasias in mice.

All of these considerations increase the difficulty of separating the enlargements of lymph glands of these mice into well defined and characteristic groups. A considerable number do not fit perfectly into any one group. It is possible, however, to establish reasonably definite criteria whereby the hyperplasias may be differentiated from the more invasive types. The presence or absence of definite evidence of infection serves as a basis for further subdivision of the hyperplasias into two subgroups. The infectious subgroup may be further subdivided into those with acute and those with chronic infections.

1. Acute Infections.—These include abscesses in various organs,—subcutaneous tissues, lymph glands, liver, spleen,—pneumonia, pyelitis, etc. The lymph glands regional to the focus of infection are enlarged, hyperemic, frequently edematous and contain many polymorphonuclear leucocytes. In only a few mice, however, has the enlargement been limited to the regional glands. The increase in size of the remote glands was moderate, the normal histologic architecture was sometimes distinct, the lymphoid cells were of about normal size, mitoses were not numerous, and the capsule was not invaded. In most of these enlarged glands careful search revealed one or more small localized accumulations of myeloid cells readily recognized as such by the presence of young polymorphonuclear leucocytes with ring-shaped nuclei.

The spleen was found enlarged as in acute infections in other animals, and showed small areas of myeloid infiltration. The largest spleens were seen in cases of pneumonia. Not infrequently the liver showed isolated narrow rings of perivascular infiltration which, on close examination, was found to contain myeloid cells, along with lymphoid cells. Small groups of myeloid cells were frequently seen in the sinusoids within the hepatic lobules. In two mice there were areas of focal necrosis in the liver.

Only rarely did the lungs and kidneys show infiltrations unless
they were the seat of infection. Nucleated cells of the blood were increased in number, sometimes enormously, so that the first impression was of a myelogenous leukemia. Myelocytes were either absent or present in small numbers only. Young polymorphonuclears with ring-shaped nuclei were relatively less numerous than in myelogenous leukemia.

In some mice no focus of infection could be found. But the enormous polymorphonuclear leucocytosis indicated that an acute infection was present, either an undiscovered local inflammatory process or a generalized infection of the nature of a septicemia. In these cases the spleens were increased in size and multiple lymph glands were enlarged. In the spleen, liver and lymph glands, and occasionally in the kidneys, localized collections of myeloid cells could be found.

2. Chronic Infections.—These consisted of (a) chronic abscesses, encapsulated, containing granular material with fragments of nuclear substance and a few intact pus cells; (b) chronic mastitis, with small cysts, increase of connective tissue and infiltration of the mammary gland with small lymphocytes; and (c) chronic metritis, sections of the uterus showing thickened walls infiltrated with lymphocytes. In these chronic infections the regional lymph glands were enlarged, and there was a much more widespread and more marked enlargement of lymph glands in distant parts of the body. The increase in size of the glands was due chiefly to hyperplasia of their lymphoid elements. Invasion of the capsule by these cells was lacking or slight, and the surrounding tissues were not infiltrated.

In many of the glands there was a marked proliferation of the endothelial lining of the sinuses, so that these spaces were often almost occluded by the large endothelial cells lying free within them. In these mice occasionally also a lymph gland was found that was partly replaced by connective tissue, indicating, perhaps, a healed former acute suppurative process that partially destroyed the gland.

The spleen was rather constantly enlarged in this subgroup. The increase in size was due to infiltration of the pulp with lymphoid cells, with, occasionally, a small focus of myeloid cells.
In many cases there was an increase of connective tissue in the spleen and in some a noticeable amount of hemosiderin.

The liver, lungs and kidneys frequently showed a slight or moderate degree of infiltration. This was limited to a narrow ring of cells about the blood vessels and was more common in the periportal tissues of the liver than in other organs. This perivascular infiltration did not reach the proportions seen in leukemia and pseudoleukemia and did not infringe on the parenchyma of the liver; it was usually unaccompanied by intrasinusoidal accumulations of cells except in some mice with a marked leukocytosis or with a marked hyperplasia of the spleen. In the latter cases the "leucostasis" in the liver was probably agonal, as claimed by Ellermann (17).

The nucleated cells of the blood were usually moderately increased. Either lymphocytes or polymorphonuclear leukocytes predominated.

3. Cases Not Associated with Demonstrable Infection.—This subgroup was by far the most populous single subdivision. The lymphoid hyperplasia in these cases may be due to an unrecognized or latent infection corresponding to the latent tuberculosis of lymph glands described by Bartels; or to a residual regenerative impulse in the lymphoid tissues of the body after the original stimulus has ceased to be active; or to some toxic substance produced somewhere in the body, with a more or less specific stimulative effect on the lymphadenoid tissues of the body.

In this group the lymph glands reached considerable size but not usually the enormous dimensions seen in leukemia, pseudoleukemia and lymphosarcoma. A greater disparity existed in the size of the glands of different groups than in leukemia and pseudoleukemia. In some mice the superficial glands were more markedly enlarged; in others, the internal. The increase in the size of the lymph glands was due to proliferation of lymphoid cells. In general, the individual cells were more nearly normal in size and appearance than in leukemia, pseudoleukemia and lymphosarcoma, although this was not a pathognomonic difference. Mitoses were less numerous than in the three invasive
Invasion of the capsule of the gland occurred, but not with such regularity as in leukemia and pseudoleukemia, and, as a rule, not all of the hyperplastic glands in a given mouse showed it in the same degree. Infiltration of the tissues about the glands was very rare, and if it occurred at all it was limited in distribution to one gland or to one set of glands.

The size of the spleen was variable in this group, but it was usually enlarged. Its pulp was infiltrated with lymphocytes. There was nothing about the type of enlargement of the spleen in this group that was characteristic.

Periportal infiltrations with lymphoid cells were frequently present in the liver. These cells did not show evidence of extremely rapid proliferation. On the whole, these infiltrations were less massive than those in leukemia and pseudoleukemia; they appeared either as rather narrow circles of cells about the portal vessels, or, at most, infringed only slightly in the adjacent lobules; and the walls of the portal veins were only moderately, if at all, invaded. The huge piling up of lymphoid cells immediately beneath the endothelium of the portal vein with narrowing of its lumen, such as was seen in many cases of leukemia and pseudoleukemia, was not encountered. The hepatic sinusoids were not excessively rich in lymphoid cells. They were present here in the greatest numbers in those mice which showed a leucocytosis.

Perivascular and peribronchial infiltration in the lungs was not uncommon in this group, but it differed from the corresponding infiltrations of the lungs in leukemia, pseudoleukemia and lymphosarcoma in that it did not form, as a rule, an unbroken cylindrical mass completely surrounding the vessel or bronchus, and continuous with a mass of similar cells in the mediastinum. On the contrary, these infiltrations occurred more commonly in the form of rather small isolated masses lying in the perivascular and peribronchial connective tissue.

Occasionally a mass of lymphoid cells was present in the hilus of the kidneys. A few scattered areas of perivascular infiltration were occasionally seen in the substance of the kidneys, but the massive intertubular infiltrations of the cortex so common in
leukemia, pseudoleukemia and lymphosarcoma, when the latter involved the kidney, were lacking.

The leucocytes of the blood were usually moderately increased, most frequently as a result of lymphocytosis.

It is evident from the above that many cases of lymphoid hyperplasia have characteristics whereby they may be differentiated from other lesions of the lymphadenoid apparatus. These may be summarized as follows:

1. In lymphoid hyperplasia, the lymph glands do not reach the massive size seen in leukemia and pseudoleukemia, and there is greater variation in the size of the different glands of the same mouse.

2. Invasion of the capsule and infiltration of the surrounding tissues, if present at all in lymphoid hyperplasia, are relatively slight and usually limited in their distribution to one gland, or a single group of glands, while in leukemia and pseudoleukemia, this is a marked feature of the disease and is evident in all of the affected glands.

3. In lymphoid hyperplasia, the lymphocytes composing the glands are more nearly normal in size and appearance than in leukemia, pseudoleukemia and lymphosarcoma, and mitoses are less numerous.

4. Periportal infiltrations in the liver, when present in lymphoid hyperplasia, are less extensive, infringe less noticeably on the liver lobules, and invade the walls of the portal vein less markedly, than in the corresponding lesions in leukemia and pseudoleukemia.

5. Leukemic infiltration of the hepatic sinusoids is absent in lymphoid hyperplasia, but intra-sinusoidal accumulations of lymphocytes of moderate degree may occur in mice with a leucocytosis, and in some others as, apparently, an agonal leucostasis.

6. Perivascular infiltrations of the lungs and kidneys are rare and very moderate in grade in lymphoid hyperplasia.

7. It is not uncommon in leukemia and pseudoleukemia to find the lungs, liver and kidneys of the same mouse all markedly infiltrated with lymphocytes; in lymphoid hyperplasia it is rare to find all three of these organs infiltrated at the same time.
LYMPHOCYTE MATURATION IN THE MAMMALIAN RETICULO-ENDOTHELIAL SYSTEM

Ziegler (98) states that "Hodgkin's disease" is common in the lower animals. Jobling (35) has reported the only case of this disease in a mouse that corresponds in any way with the conception of it expressed above, namely, a lymphogranulomatous process, essentially different from pseudoleukemia. In Jobling's case only a section of one of the inguinal lymph glands was available for examination. The "tumor" was composed for the most part of lymphoid cells. Other cells with large pale vesicular nuclei were also present. Many eosinophilic cells were scattered throughout the gland. There were large areas composed of a reticulum coarser than the usual reticulum and inclosing large endothelioid cells, of which some were multinuclear. In these areas the lymphocytes were relatively few in number and the other types of cells relatively numerous.

In this series of mice, four showed lesions that had a close resemblance to lymphogranulomatosis as seen in man. In one mouse the retroperitoneal glands alone were affected; in the second, the cervical and inguinal glands; in the third, the inguinal and mesenteric; and in the fourth, the mesenteric glands alone were involved. Invasion of the capsule was not present in any of these glands. Their normal histologic structure was obscured or entirely obliterated by the presence of irregular accumulations of large cells, some of which had elongated vesicular nuclei and resembled fibroblasts, while others had large round or indented nuclei or multiple nuclei that were oval in shape. The lymphocytes were large replaced by these cells. Cells with cytoplasm that stained diffusely with eosin were scattered through the sections of the lymph glands, but no cells with definite eosinophilic granules were observed (73).

The spleen was enlarged in all four of these mice, being 16 times the normal size in one mouse, and 4 times the normal in the other three. The normal histologic structure of the spleen was indistinct, and in it were patches of large epithelioid and multinuclear cells (not the normal megakaryocyte-like cells of the mouse spleen) scattered throughout the sections. The spleen of one mouse was definitely fibrosed. In three of these mice, the
liver showed some periportal infiltration with lymphocytes and large cells similar to those seen in the lymph glands. In the first mouse there was a mass at the hilus of the kidney similar in its microscopic appearance to the retroperitoneal glands. In this mouse there was a very moderate perivascular infiltration of the kidney similar to that described in the liver. There was no amyloid degeneration in the organs of any of these mice. Two of the four had acute infections, one a pyelitis, the other a pneumonia.

"MESENTERIC DISEASE"

Five mice of this series showed a peculiar lesion which has been conveniently designated as "Mesenteric Disease." It was characterized by a marked enlargement of the mesenteric lymph glands with little or no changes in the glands of other organs. These mesenteric glands showed a characteristic picture in the form of wide blood-filled spaces which disrupted the normal histology. Endothelial linings could be seen in some of these spaces but not in all. Between these spaces there were lymphocytes in a delicate reticulum, usually less thickly crowded than in normal lymph glands. In other words, the residual lymphoid tissue was atrophic. The capsules of the glands appeared to be intact, but the capsule was not present in all sections. In one of the five mice the appearance of the mesenteric gland differed somewhat from the others in that the blood-filled spaces were limited to one sector of the gland, while the remainder was composed of large round cells resembling large lymphocytes.

The spleen of these mice varied from 4 to 10 times the normal size. In one instance there was amyloid degeneration of the spleen. The histologic structure of this organ was not markedly changed in two mice; in two others there was a considerable degree of lymphoid infiltration of the pulp that rendered the normal architecture indistinct; while in the fifth mouse no sections of the spleen were available for examination. In one of these mice the retroperitoneal glands showed a condition similar to that in the mesenteric glands.

The liver and kidneys were not markedly changed, except that in each there was a very slight degree of perivascular infiltration.
The nucleated cells of the blood were noticeably increased in only one mouse, and in this animal the increase was due to a moderate excess of lymphocytes. The nature of this condition is obscure. Whether it is the result of an infection is open to question. It has some resemblance to a hemangioma such as has been seen in the spleens of two or three of these mice. In a few other mice a hemangiomatous condition was observed in the liver. The mesenteric glands of these five mice did not resemble the hemolymph nodes found in the retroperitoneal tissues of sheep and other animals. Borst (10) mentions hemangioma of lymph glands, but the condition to which he refers appears to be congenital and to be associated with a widespread similar change in other organs. Shennan (71) has reported a case of metastasizing hemangioma of the spleen with secondary tumors in the thymus and mediastinal lymph glands, in which the structure resembled somewhat the lesions described in these mice. Hutchinson has described a special form of hemangioma which he believes to be of infectious origin. The condition present in the mesenteric glands of these mice has some resemblance to the hemangioma-like areas sometimes seen in multiple myelomas. Wells (93a) has suggested that these latter are due to a sort of hemorrhagic necrosis and replacement by vascular tissue. Although the conditions surrounding the affected lymph glands are quite different from the environment of a myeloma, it is not impossible that “mesenteric disease” in these mice may have an origin similar to the vascular transformation of these tumors of bone.

ENLARGEMENTS OF THE SPLEEN

In two of these mice the spleen was estimated to be 50 times the normal size. In one of these the lymph glands were not enlarged; in the other, there was a moderate increase in the size of the axillary, inguinal and retroperitoneal glands. The liver, lungs, and kidneys showed little or no change. In the spleen of one mouse the normal histologic architecture was completely obliterated by the dense infiltration of the pulp with lymphoid cells. In the second mouse, which showed enlargements of the lymph glands, there were large epithelioid cells in great numbers mingled with lymphoid cells in the splenic pulp. The lymph
glands were crowded with similar cells. The spleen of this mouse has some resemblance, microscopically, to the type of enlargement of this organ designated as Gaucher's splenomegaly in man.

In three other mice the spleens were enlarged to from 10 to 24 times the normal size. The splenic pulp was densely crowded with lymphoid cells which completely obliterated the normal histologic picture. There was little or no enlargement of the lymph glands of the body in two of these mice; in the third the mesenteric and retroperitoneal glands were moderately enlarged. The lungs, and kidneys, showed no noteworthy changes. The most marked and striking alteration was in the liver. In each mouse the hepatic sinusoids were so packed with small round cells that the entire capillary system of the liver appeared to be completely occluded. There was a moderate degree of periportal infiltration with some invasion of the walls of the branches of the portal vein. Mitoses were not numerous, but this may be due to the fact that postmortem changes were advanced.

It does not seem possible at present correctly to interpret this condition. Since the cells are in the capillaries and not in the larger vessels of the liver, and not in the larger vessels of other organs; and since these cells resemble those present in the spleen, it appears probable that the latter organ is the source whence they came.

With the exception of the cases of lymphosarcomas, there has been a definite tendency throughout these 316 mice for the lymph glands and spleen to be enlarged together. From this it would appear that in the mouse, the lymph glands and spleen are affected by the same stimuli and react to them in much the same manner. It has hardly seemed likely, therefore, that any such stimulus would have so definite a selective action on the spleen that the lymph glands would be almost entirely uninvolved when the spleen was so markedly changed. For these reasons an infectious origin of this process is doubtful. On the other hand, the condition can not readily be classed as a sarcoma of the spleen with metastases in the liver, because, (a) the spleens of these mice were uniformly involved, while sarcoma of this organ usually gives it a nodular appearance; and (b) because the
invading cells of the liver do not have the arrangement nor the distribution of metastases in this organ from sarcoma of the spleen. An interpretation of this condition has, therefore, not been attempted.

DISCUSSION

This has been an anatomical and histologic study and has, therefore, furnished little opportunity for the investigation of the problem of the etiology of the various pathological conditions described above. The following observations, however, are related to the question of the cause of these diseases. Sections of numerous mice stained with Giemsa’s and Levaditi’s stains showed no microorganisms. Nematode worms were found in the tissues of four mice. Their presence had no recognizable relation to the type or location of the lesion observed in the infected mouse. Haaland (28, 29) and Borrel (9) found similar parasites in the tissues of some of their mice with lymphadenopathies. They believed that these worms were etiologic factors in the disease. But since only four mice of this large series showed nematode worms in their tissues, it was not thought that they were related to the changes observed in the lymph glands.

Heredity has apparently been a factor worthy of consideration in some cases of leukemia in mice. One of Haaland’s (28) leukemic mice was the daughter of another mouse that died of the same disease. Bashford and Murray (6) report the development of a malignant lymphoma in a mouse belonging to their cancer strains. Levaditi (42) states that his leukemic mice were obtained from a dealer whose stock had shown a large percentage of carcinoma. One of Haaland’s (29) mice that had pseudoleukemia had, in addition, an adenoma of the lung. The association of tumors with the different types of lesions of the lymph glands described in this group of mice is shown in the following Table:

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total Number Cases</th>
<th>Number with Carcinoma</th>
<th>Number with Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td>67</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Pseudo leukemia</td>
<td>111</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Lymphosarcoma</td>
<td>51</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>229</td>
<td>11</td>
<td>6</td>
</tr>
</tbody>
</table>
From this Table it is seen that of 67 cases of leukemia, 10, or slightly more than 14 per cent had associated tumors; and of 111 mice with pseudoleukemia, 6, or slightly more than 5 per cent had associated tumors. The relation of heredity to leukemia and pseudoleukemia will be the subject of another paper by Maud Slye.

Furthermore, it is of interest, in view of Murphy's work on the relation of lymphocytes to immunity to cancer, to note that in many of these mice of this stock that showed the most marked lymphoid proliferation and the most abundant lymphocytes, carcinoma developed and grew apparently unhindered.

The theories which have been advanced to account for the nature of leukemia and related conditions fall into three groups:

1. Banti (4, 5), Benda, Ribbert (63), and others believe that leukemia is a definitely neoplastic process, because of the atypical character of the cells which show numerous mitoses, actively invade the capsules of the lymph glands and surrounding tissues and the walls of veins, and produce metastases in the form of leukemic infiltrations in distant organs.

2. Ferrata (22), Grawitz (27), Naegeli (51), Fabian (18), and others consider leukemia a purely hyperplastic condition of the lymph glands and bone marrow, and not in any sense a neoplasm.

3. Pappenheim (56, 57) takes a middle ground and is of the opinion that the leukemias and pseudoleukemias represent a mixture of lesions, some hyperplastic, others neoplastic. v. Hansemann (30) thinks that leukemia may represent a secondary manifestation of some long infectious disease. The microorganisms may have been discharged from the body, but the stimulus which they gave to the lymphadenoid tissues may remain, and the proliferation of these cells continue indefinitely. Pappenheim (57) believes that some "infectious noxa" is necessary to initiate the hyperplastic process.

From this anatomic and histologic study it is evident that leukemia, pseudoleukemia and lymphosarcoma in mice have certain features in common, especially the marked invasiveness of the growths affecting the lymph glands and infiltrating the viscera, by which they can be differentiated from lymphoid
hyperplasias. In their type forms they present characteristics by which they can be distinguished from each other.

But this ready differentiation applies only to typical cases of each disease. In this material, borderline or transition forms were numerous. Cases of lymphosarcoma occurred in which the glandular involvement was as extensive and the infiltration of the viscera almost as marked as in pseudoleukemia. The chief points of differentiation in such cases have been the extent of involvement of lymph glands and the relation of the infiltrations of the viscera to the involved lymph glands. If the invasive growth was limited to one group of glands, or if a regional extension along afferent or efferent lymphatics could be established for each organ showing infiltration, the case should be classed as a lymphosarcoma. On the other hand, if the involvement of lymph glands was widespread or if an organ was found infiltrated while its related lymph glands were free from growth, pseudoleukemia was considered the more correct diagnosis.

While the number of nucleated cells in the blood has been made the basis of differentiation between pseudoleukemia and leukemia, cases were frequently met with in which it was difficult to decide whether the number of these cells in the blood was sufficient to warrant the diagnosis of leukemia. There were thus many cases in this series that stood between these two conditions in their characteristic forms. Such lesions would be more accurately described by Türk’s term, subleukemic lymphadenomatosis, or subleukemic pseudoleukemia.

A few cases were encountered among these mice that partook of the characteristics of lymphosarcoma and leukemia. These corresponded closely to Sternberg’s leucosarcoma and have been described under that title.

The distinguishing features by which leukemia, pseudoleukemia and lymphosarcoma are differentiated from each other are thus seen to be quantitative rather than qualitative. This strongly suggests that these three pathologic conditions are fundamentally of the same nature. That is to say, they are all either neoplastic or hyperplastic processes. The histologic changes observed in the examples of these diseases in these mice
correspond more closely to the usual conception of a neoplasm than to that of a simple hyperplasia, for the following reasons:

1. In all three of these conditions, the actively proliferating cells were atypical. The lymphoid cells were larger and had paler nuclei than ordinary lymphocytes. In myelogenous leukemia the invading cells in the lymph glands and visceral infiltrations were definitely of a less mature type than those commonly found outside the bone marrow, namely, myelocytes and young polymorphonuclears with ring-shaped nuclei.

2. The energy of the proliferative process in the lesions was shown by the great numbers of mitoses seen in the lymph glands and in the areas of infiltration in the viscera.

3. The quality of invasiveness was the most marked and most striking feature of these conditions. In the leukemias the cells even invaded the blood stream in enormous numbers. In leukemic and sarcomatous lymph glands the invasion appeared to be due in part to amoeboid movement on the part of the invading cells,—they could often be seen stretched out between the layers of the capsule—and in part to the mechanical pushing along of the cells by the force of the growing mass.

However, by the injection into fowls of a filterable virus, Ellermann (17) and Schmeisser (66) have produced leukemia, and with another virus Rous (65) has produced sarcoma. Although definitely infectious in origin, these processes have the same quality of invasiveness possessed by malignant tumors in other animals. Their component cells were distinctly atypical, they were markedly invasive, they destroyed and replaced the cells of the invaded organ, and, in leukemias at least, produced infiltrations and metastases in distant organs. There is no record of leukemia having been successfully transmitted in mice, although Tyzzer (86), Haaland (29) and others have made repeated attempts. In the present state of our knowledge it is therefore impossible to harmonize the transmissible, infectious, invasive tumor-like lesions in leukemia in fowls with the non-transmissible, apparently non-infectious, invasive, histologically similar tumor-like leukemic conditions in mice.

Two opposing views have been held as to the origin of the areas
of infiltration of the viscera in leukemia and pseudoleukemia: (a) that they result from the proliferation of cells brought to the locality by the blood; and (b) that they arise autochthonously from cells normally present in the tissues at the site of the infiltrations. Awrarow and Timofejewsky (3) made "tissue cultures" of leukemic blood and observed multiplication of the nucleated cells. They therefore believe that the infiltrations arise from cells deposited from the blood. Arnold (1) demonstrated the presence of small accumulations of lymphoid cells in the perivascular tissues of the lungs, liver and kidneys. Schridde (69) believes that the perivascular tissues should be classed with the blood-forming organs, because there are cells in these locations which possess the power of forming blood cells. The definite relation in these mice of the infiltrations to blood vessels and their presence in cases that do not show either a leukemic blood picture or evidence of marked proliferative power on the part of the cells of the enlarged lymph glands, seems to lend support to the view that the perivascular infiltrations in these mice originated from cells already present in the tissues that became infiltrated. This autochthonous origin of the infiltrating cells speaks against the neoplastic nature of leukemia, as pointed out by Ferrata (21).

Leukemia in mice differs from the similar disease in man chiefly in three respects: In the first place, the invasion of the capsules of the lymph glands and surrounding tissues is much more marked in mice. In the second place, in myelogenous leukemia in the mouse, two characteristic cells appear in the blood and in the areas of infiltration, namely, true myelocytes and the readily recognized young polymorphonuclears with ring-shaped nuclei. Third, the spleen is more constantly involved in lymphatic leukemia, and the lymph glands in myelogenous leukemia, in these mice than in the corresponding diseases in man.

SUMMARY

1. Among the 316 mice in the first 15,000 necropsies of the Slye stock, studied because they showed enlargements of the lymph glands and spleen, there were found 67 leukemias, of
which 28 were of the lymphatic, and 39 of the myelogenous, type; 111 pseudoleukemias; and 51 lymphosarcomas.

2. Typical instances of these conditions can be recognized without great difficulty. Leukemia and pseudoleukemia possess in common marked invasive power of the cells of the lymphoid tissues, the cells growing through the walls of veins and the capsules of lymph glands and infiltrating the surrounding tissues. In both there is a widespread involvement of the lymph glands of the body with infiltration of one or more of the viscera without relation to the affected glands. They differ from each other in the number of nucleated cells in the blood. Lymphosarcoma is distinguished by its greater invasiveness and by being localized in one group of glands from which it may invade related organs. But generalized lymphosarcomatosis does occur in which the manner of spread is not readily apparent.

3. Border-line or transition cases are numerous. A generalized lymphosarcoma may closely resemble a pseudoleukemia; a subleukemic pseudoleukemia is not easily differentiated from a true leukemia; a leucosarcoma partakes of the characteristics of both leukemia and lymphosarcoma; in some cases of pseudoleukemia the lesion in one lymph gland or one group of glands may be so extremely invasive as to resemble a lymphosarcoma. An attempt has been made to establish criteria whereby these difficult cases may be correctly classified.

4. Lymphoid hyperplasia may be either local or generalized. This condition differs from the above-mentioned diseases chiefly in the absence of the quality of invasiveness.

5. Anatomically and histologically, leukemia, pseudoleukemia and lymphosarcoma in these mice have distinctive features which indicate that they are probably fundamentally of the same nature and probably belong among the true neoplasms.

REFERENCES
(1) Arnold: Virchows Arch., 1880, lxxx, 315.
(2) Aubertin and Morel: Arch. mal. de Coeur, 1913, vi, 201.
(3) Abramow and Timofejevsky: Virchows Arch., 1914, cccvi, 184.
(8) Bollinger: Virchows Arch., 1870, lix, 341.
(13) Cohnheim: Virchows Arch., 1865, xxxii, 452.
(14) Chile and Beebe: J. M. Res., 1907, xviii, 452.
(17) Ellermann: Die übertragbare Hühnerleukose (Leukämie, Pseudoleukämie, Anämie), Berlin, 1918.
(20) Fawcett and Boycott: J. Path. and Bacteriol., 1910, xiv, 404.
(22) Ferrata: La Emopatie, Milan, 1918.
(23) Fox and Farley: Am. J. M. Sc., 1922, clxiii, 313.
(31) Helly: Ergeb. der allge. Path., 1914.
(32) Herskheimer and Reinke: Ergeb. der allge. Path., 1913, xvi, 1.
(34) Hutchinson: Arch. Surg., 1900, xi, 221.
(36) de Jong: Virchows Arch., 1903, cxxixii, 511.
(42) Levaditi: Compt. rend. Soc. de biol., 1914, lxvii, 258.
(43) Massaglia: Lancet, 1923, i, 1056.
(55) Pappenheim: Folia Hematol., 1911–12, Ref. xii, 257.
(55a) Petit and Weil, cited by Weil and Clerc.
(59) Petrow: Centralbl. f. allge. Path., 1914, xix, 676.
(61) Reed: Johns Hopkins Hosp. Rep., 1902, x, 133.
(65) Rous: J. Exper. M., 1908, x, 238.
(69) Schridde: Centralbl. f. allge. Path., 1908, xix, 865.
(82) Türk: Arch. f. wissenschl. u. prakt. Tierheil., 1917, xliii, 145.
(84) Türk: Wien. med. Presse, 1903, xliv, 1360.
(85) Tyzzer: 4th Rep. of the Caroline Brewer Croft Cancer Commission, 1907, p. 27.
(86) Tyzzer, J. M. Res., 1907, xvii, 199.
(92) Weil and Clerc: Arch. med. exper., 1904, xvi, 462.