MYELOID LEUKEMIA AND NON-MALIGNANT EXTRAMEDULLARY MYELOPOIESIS IN MICE

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Lymphatic leukemia of mice has been extensively studied by many investigators (cf. 1, 2). The anatomical changes are well known and their differentiation from non-leukemic processes offers little or no difficulty. Myeloid leukemia is in most stocks of mice much less frequent than lymphoid leukemia (cf. Emile-Weil and Bousser, 1; Opie, 3). In the Slye stock, however, Simonds found 39 cases of myeloid and 28 of lymphatic leukemia in the first 15,000 autopsies. In our Stock Ak lymphoid leukemia is very common but myeloid leukemia is rare. In Stock Rf, on the other hand, myeloid leukemia occurs frequently but lymphoid leukemia is unusual. In Stock S both types are found, as well as atypical forms. It is not possible from the literature to give exact figures for the incidence of myeloid leukemia because, thus far, it has not been sharply separated from non-malignant extramedullary myelopoiesis.

Simonds (4), who first extensively studied leukemia in mice, states:

"It was necessary to differentiate myelogenous leukemia in mice from an inflammatory leukocytosis. In the latter condition, a focus of acute infection could frequently be found in some organ, such as 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 leukocytes 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 with ring-shaped nuclei being only moderately increased. Nucleated red cells were not encountered in mice with acute infections and leukocytosis.

"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"

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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 process of infection.

"Furthermore, myeloid infiltration of lymph glands and visceral organs differed from localized acute supplicative processes 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."

The difficulty in distinguishing myeloid leukemia from non-malignant extramedullary myelopoiesis is not evident from Simonds' study. He does not describe any cases in detail and speaks only in generalities concerning the involvement of the internal organs.

Hueper (5) diagnosed myeloid leukemia in mice from the following findings:

"The spleen was sometimes enlarged. There was a diffuse replacement of the lymphoid tissue by myeloid cells in the spleen and lymph nodes. Megakaryocytes were often very numerous in these organs. Myeloid infiltrations, usually of diffuse, infiltrative character, were found in the liver, kidney, heart and lung. In two instances the meninges showed very massive myeloid infiltrations. Coexistence of lymphoid foci and myeloid infiltrations in the same organ (liver, lung) was observed several times. The lymphoid foci were then usually located around large veins, while the myeloid cells more or less diffusely infiltrated the interstitial spaces between the liver cells or the interalveolar septa of the lung respectively. Foci consisting of both types of cells were occasionally seen in the liver in the neighborhood of capillaries, suggesting that the lymphoid elements were primary with the myeloid infiltration superimposed. Megakaryocytes were not infrequently seen in the lumina of the hepatic capillaries. The Kupffer cells were always in a state of active proliferation. The liver cells were compressed and atrophic in the areas of massive myeloid infiltration and had completely disappeared in some, leaving a delicate network of interstitial tissue in the meshes of which the leukemic cells were found."

Hueper did not describe in detail any of his cases, and his diagnostic criteria are as general as those of Simonds. He recognizes that "the differential diagnosis was especially difficult in cases with septic complications and carcinosis of the internal organs."

More definite criteria than have hitherto been described are necessary to distinguish between myeloid leukemia and non-malignant extramedullary myelopoiesis. The problem is a difficult one and, as remarked by Emile-Weil and Bousser (1), offers an important obstacle to the study of leukemia in mice.

Leukemia is a progressive, fatal disease due to the unrestricted proliferation of immature blood cells (malignant leukocytes); in non-malignant extramedullary myelopoiesis, on the other hand, the cells are not endowed with the power of unrestricted multiplication. This essential difference between the neoplastic and the hyperplastic states of blood-forming tissues is clearly demonstrable by transmission experiments. Lymphatic leukemia of mice has been transmitted by numerous workers (cf. 1, 2). At present, almost every case of spontaneous lymphatic leukemia occurring among our mice can be transmitted, either to closely related mice or to mice whose resistance has been lowered by x-rays administered preceding inoculation. After subcutaneous inoculation the leukemic cells multiply at the site of injection, with the production of tumors composed of a uniform type of cell similar to that introduced. In 1934 the transmission of myeloid leukemia in mice was re-
ported (6, 7) and subsequently two different strains have been described from this laboratory (8, 9). The transmission of five other strains will be recorded here. Jørgensen (10) reported on the transmission of a myeloblastic leukemia in mice in 1935. In that year a "leukemia coincident with and transmissible by a spindle-celled sarcoma in the mouse" was described by Parsons (11), but a later report indicates that the blood disturbance observed coincident with the sarcoma was non-malignant (12).

It is the purpose of this report to describe the anatomical characteristics of our leukemias the malignant character of which has been definitely established by transmission experiments, and to compare them with the changes that occur in mice with spontaneous and induced non-malignant extramedullary myelopoiesis. We are also recording the results of attempts to produce myeloid leukemia by irradiation and by benzpyrene.

![Myceloid Cells of Mice](image)

**FIG. 1. MYELOID CELLS OF MICE**

**MATERIAL AND METHODS**

The mice used in this study were of three different stocks (A, R and S) that have been inbred since 1928, or first generation hybrids of two of these stocks. The origin of the stocks has been described (13). In attempts to transmit leukemia or non-malignant extramedullary myelopoiesis, inoculations were made intravenously, subcutaneously, or rarely intraperitoneally, with a cellular suspension, usually of the spleen, in Tyrode solution. Inoculated mice that were irradiated received 400 r of x-rays over the entire body from several hours to a few days before inoculation, if not otherwise stated. The factors were: 200 kv., 18 ma., 50 cm. distance, 1.0 mm. Cu + 1.0 mm. Al, 50 r per minute. We are indebted to Dr. J. R. Carty and his associates of the Department of Radiology, Cornell University Medical College, for irradiating our animals. The transmissible strains are designated by the number of the mouse with the spontaneous disease.

Tissues were fixed in a mixture of Zenker's solution (9 parts) and formalin
(1 part) and stained usually with hematoxylin and eosin. "Imprint preparations" were made by gently pressing the organ against a glass slide. These preparations as well as blood smears were stained with Wright and Giemsa solutions.

The terminology used in describing the myeloid cells is indicated in Fig. 1. The myeloblast has a large spherical nucleus and a scant rim of basophilic cytoplasm with no granules. The promyelocyte is similar to the myeloblast but contains a few purple-red cytoplasmic granules. The myelocyte has a spherical or slightly indented nucleus and numerous neutrophilic or purple-red cytoplasmic granules. A conspicuously indented or ring-shaped nucleus is characteristic of the metamyelocyte, while the adult polymorphonuclear leukocyte has an irregular segmented nucleus.

**TRANSMITTED MYELOID LEUKEMIA**

Since 1934, attempts have been made in this laboratory to transmit fourteen cases of myeloid leukemia in mice, diagnosed during the life of the animal. In 1934, four attempts were unsuccessful and two successful. In 1935, two cases could not be transmitted, while two were transmitted. In 1937–38 all of four attempts met with success. These results may be explained in the following way: The earlier attempts to transmit the disease were made while the stocks of mice were being inbred and the mice used for inoculation were genetically impure. More recently, with the stocks highly inbred, transmission of both myeloid and lymphoid leukemia has been almost uniformly successful.

These considerations suggest that transmission experiments since 1937 are suitable for determining the malignant character of the hyperplastic alterations occurring in the blood-forming organs of our highly inbred stocks of mice. This does not apply to stocks of mice not highly inbred, nor to our stock prior to 1937; i.e., failure of transmission does not exclude malignancy.

Of eight cases of spontaneous myeloid leukemia successfully transmitted in this laboratory through one or more passages, four occurred among non-irradiated mice and four among irradiated mice. In three of the latter group the irradiation consisted of a single massive dose of 400 r administered between the ages of six and ten weeks. One mouse received 300 r at the age of one month, and again at two months and three months. Leukemia was recognized in these mice from four and a half to eight months after irradiation. General irradiation with x-rays has been found to increase the incidence of myeloid leukemia of mice approximately eight times (14).

Table I lists significant data concerning the strains of myeloid leukemia that have been transmitted in this laboratory.

**Strain Ar 117**

A complete description of this strain, originating in a non-irradiated mouse, has been presented (7). The morphologic characteristics of the malignant cells have not been altered during more than four years of transmission but the disease can no longer be transmitted to mice of strains other than Ar, even though they are irradiated.
### LEUKEMIA AND MYELOPOIESIS IN MICE

#### TABLE I: Transmissible Strains of Myeloid Leukemia

<table>
<thead>
<tr>
<th>No. of Strain</th>
<th>Irradiation*</th>
<th>Age of Mouse at Death (months)</th>
<th>Malignant Cell Type</th>
<th>Characteristics of the Strain</th>
<th>Transmission Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (r)</td>
<td></td>
<td></td>
<td></td>
<td>Related Mice</td>
</tr>
<tr>
<td>Arl17†</td>
<td>-</td>
<td>-</td>
<td>Myelocyte with large basophilic granules</td>
<td>Multiple myelomata of gray-white color</td>
<td>1934</td>
</tr>
<tr>
<td>Aka51</td>
<td>300 300 300</td>
<td>1 2 3</td>
<td>Myelocyte</td>
<td></td>
<td>1934</td>
</tr>
<tr>
<td>Rfb117</td>
<td>400 2.5</td>
<td>9</td>
<td>Large myelocyte with purple-red granules of moderate size</td>
<td>Conspicuous visceral infiltrations; occasionally of greenish-gray color</td>
<td>1935</td>
</tr>
<tr>
<td>Rfb123</td>
<td>400 1.5</td>
<td>6</td>
<td>Myelocyte with purple-red granules of moderate size</td>
<td>Hemorrhages in lungs</td>
<td>1935</td>
</tr>
<tr>
<td>Slb351</td>
<td>-</td>
<td>12</td>
<td>Myeloblast and promyelocyte</td>
<td>Chloroleukemia</td>
<td>1937</td>
</tr>
<tr>
<td>Arj20†</td>
<td>400</td>
<td>11</td>
<td>Myelocyte</td>
<td></td>
<td>1938</td>
</tr>
<tr>
<td>Akf46†</td>
<td>-</td>
<td>11</td>
<td>Myeloblast</td>
<td></td>
<td>1938</td>
</tr>
<tr>
<td>Akh106†</td>
<td>-</td>
<td>10</td>
<td>Myeloblast and promyelocyte</td>
<td></td>
<td>1938</td>
</tr>
</tbody>
</table>

* The figures in the column “Irradiation” refer to the dose of x-rays received by the mouse with the spontaneous disease and the age at the time of irradiation.

† Cells of these strains are being preserved in the frozen state.

**Strain Slb 351**

This strain of transmissible chloroleukemia has been described by Hall and Knocke (9). The malignant cells have retained their morphologic characteristics and still produce leukemia with the characteristic green infiltration in lymph nodes.

**Strain Akf 46**

This strain of myeloblastic leukemia, studied recently by Dr. C. E. Forkner, originated in a non-irradiated mouse. An ante-mortem smear showed that the leukocytes of the blood were slightly increased in number and that many were immature myeloid cells. There was severe anemia with many erythroblasts in the blood. At autopsy the mouse had a moderately enlarged spleen and lymph nodes; the liver was grossly normal. The spleen, lymph nodes and bone marrow were infiltrated with large mononuclear cells resembling...
myeloblasts, among which were a small number of promyelocytes and occasional metamyelocytes. Approximately 25 per cent of the cells in the lymph nodes were oxydase-positive. The disease was transmitted to related mice through two successive passages. Most of the inoculated animals died after approximately one to two months with alterations in the blood and organs resembling those in the mouse with the spontaneous disease.

**Strain Rfb 117**

This strain, derived from an irradiated mouse (Rfb 117), has been partly described in a preliminary report (8).

**The Spontaneous Disease:** When the irradiated mouse, Rfb 117, was seven and a half months old, the spleen was greatly enlarged and a blood smear contained many large myelocytes with medium-sized purple-red granules. One month later the mouse appeared anemic and was dyspneic. It was killed in extremis. An ante-mortem blood count showed 2,270,000 red blood cells and 66,000 white blood cells per c.mm., of which 32 per cent were myelocytes with purple-red granules. These cells were large, varying from 12 to 20 \( \mu \) in diameter, with an average diameter of 14.7 \( \mu \). The cytoplasm was basophilic and contained conspicuous purple-red granules that were smaller than those characteristic of myeloid leukemia Strain Ar 117 (Figs. 4 and 5). The granules did not stain with hematoxylin and eosin. The malignant cells yielded a positive oxydase reaction with benzidine. The nuclei were vesicular, oval or slightly indented, usually slightly eccentric, and in many cases almost completely filled the cells. At autopsy all lymph nodes were slightly enlarged and several contained minute red hemorrhagic spots. There were similar hemorrhagic areas in the lungs. The spleen measured 2.8 by 0.9 by 0.6 cm. and was firm and gray-red. The liver was only slightly enlarged.

Microscopic examination showed the liver sinusoids filled with immature granulocytes, many in mitosis. In the larger veins immature myeloid cells and erythrocytes were present in approximately equal numbers. There was a conspicuous perivascular infiltration by these cells (Fig. 3). Mature cells, however, were seen in many areas. There were no megakaryocytes or erythrogenic foci, although a few nucleated red blood cells were present in the lumina of the large vessels. The pulp of the spleen was densely infiltrated by immature myeloid cells, many in mitosis (Fig. 6). Lymphoid follicles were small. Several megakaryocytes but very few erythrogenic foci were noted. The lymph nodes contained immature myeloid cells, which replaced about 80 per cent of the normal lymphoid tissue. The femoral bone marrow showed a moderately dense infiltration by the same immature myeloid cells found in the other organs (Fig. 2). Part of the marrow was relatively acellular, an alteration repeatedly seen in mice previously irradiated. Many of the small arteries and capillaries of the lung contained multiple thrombi, formed about disintegrating leukocytes. The capillary bed was studded with immature granulocytes.

**Transmission Experiments:** The results of inoculations with a myeloid cell suspension from the spleen of this mouse and of the successive subpassages are summarized in Table II.

Almost every mouse of stock Rf was at one time susceptible to this strain. Inoculations were less often successful in the distantly related Rg stock, still less in the unrelated mice of Stock A, and not at all in unrelated Stock S. Irradiation failed to increase noticeably the susceptibility of the unrelated mice of Stocks A and S to this strain.

Leukemic tissue rapidly frozen to \(-30^\circ\) C. and kept at that temperature for thirty minutes failed to transmit the disease, though transmission was successful with material rapidly exposed to \(-10^\circ\) C. and kept at that temperature for an equal period. These experiments were made before it was discovered that slow freezing does not destroy leukemic cells (15, 16).

In tissue cultures the malignant myelocytes were actively motile, multiplied by mitotic division, and failed to mature into polymorphonuclear leukocytes.
PLATE I. MYELOID LEUKEMIA OF STRAIN RFB 117: THE SPONTANEOUS DISEASE

FIG. 2. Bone marrow: Invasion by malignant myelocytes.  × 500.

FIG. 3. Liver: Moderate diffuse and perivascular infiltration by malignant myelocytes; several in the lumen of the vessel.  × 500.


FIG. 6. Spleen: Replacement of pulp by immature myeloid cells.  × 500.

Note: The tissues for all illustrations were fixed with Zenker-formalin solution. The sections were stained with hematoxylin and eosin; the blood smears with Wright and Giemsa solutions. The magnifications are approximate.
After twenty-four months of transmission, inoculations were successful in only an occasional mouse of Stock Rf, and the last inoculation into eight irradiated mice of this stock was entirely unsuccessful. It is possible that in-breeding resulted in the loss of the genes in the host necessary for transmission, but it is also possible that the transmitted cells underwent modification in the course of successive passages so that ultimately they could not multiply in mice of the family in which the disease originated.

Strain Rfb 123

The Spontaneous Disease: Mouse Rfb 123 was irradiated and at six months of age its spleen was greatly enlarged but the lymph nodes were normal in size. The leukocyte count was 232,000 per c.mm. and a blood smear showed many immature red blood cells and a preponderance of myelocytes. There was moderate anemia, with 5,500,000 erythrocytes per c.mm. The mouse was killed and autopsy revealed a uniformly dark red spleen measuring 3 by 1 cm. The liver and cervical lymph nodes were slightly enlarged. The portal areas and sinusoids of the liver were extensively infiltrated by myeloid cells, many of which were mature (Fig. 11). Mitoses were numerous. No erythrogenic foci were seen. The spleen showed an almost complete replacement of the normal lymphoid structure by early myeloid cells, many in mitosis. In a few areas, however, the structure of the organ was retained but there was an extensive myeloid infiltration in the follicles. Erythroblasts were absent. There were many adult polymorphonuclear leukocytes in the pulp. Both sinuses and cords of the lymph node examined were infiltrated by immature myeloid cells and there was epicapsular infiltration. In the lung there were areas of peri-vascular infiltration by lymphoid cells, myelocytes and metamyelocytes. The large blood vessels were filled with immature myeloid cells. About one half of the marrow of the femur was acellular; sharply demarcated from this region was an area of granulocytic hyperplasia with numerous immature cells. There was extensive myeloid infiltration in the salivary glands.

Transmission Experiments: Injection of a cellular suspension of the spleen was made intravenously or subcutaneously into five non-irradiated mice of the same family. Ten weeks later three of these mice showed signs of myeloid leukemia. At autopsy each of these three mice had a tumor in the abdominal wall at the site of injection. Their spleens were greatly enlarged and their livers slightly so, while the lymph nodes showed little or no increase in size. There were numerous hemorrhagic infarcts in the lungs.

The microscopic picture of the tumor and organs of one of these mice (Rf 265) was typical of all three. The tumor of the abdominal wall was composed of immature myeloid cells with round, reniform, or slightly lobed nuclei and a small amount of slightly basophilic cytoplasm. These were unassociated with metamyelocytes, erythrogenic foci, and mega-
PLATE II. MYELOID LEUKEMIA OF STRAIN RFB 123 (FIGS. 7-10 THE TRANSMITTED DISEASE; FIG. 11 THE SPONTANEOUS DISEASE)

Fig. 7. Lymph node: Replacement by immature myeloid cells; epicapsular infiltration. × 100.
Fig. 8. Tumor of abdominal wall: Malignant myelocytes around a muscle fiber in the abdominal wall. All cells immature. × 1000.
Fig. 9. Spleen: Replacement of pulp by myelocytes. × 250.
Fig. 10. Bone marrow: Invasion by malignant myelocytes. Mitotic figure. × 800.
Fig. 11. Liver: Infiltration by myeloid cells with several metamyelocytes. × 900.
karyocytes (Fig. 8). They replaced the entire corium but the epidermis was intact. Nerve
tissue was surrounded, but only occasional myeloid cells were found between the nerve
fibers. Striated muscle was diffusely infiltrated and breast glands were separated by mye-
loid cells. In the liver there was diffuse and portal infiltration by immature myeloid cells
and in most of the portal vessels these were present in large numbers. The spleen was al-
most entirely replaced by myelocytes (Fig. 9) and only a few follicles persisted. The
immature myeloid cells were homogeneous and resembled those in the subcutaneous tumor.
Mitoses were numerous. There were few metamyelocytes and megakaryocytes. Erythro-
genic foci were absent. One lymph node was almost entirely replaced by similar myeloid
cells, no vestige of lymphoid tissue remaining except in one small subcapsular area. In the
center of this lymph node were numerous small areas of necrosis. There was myeloid in-
filtration of the capsule and of the surrounding areolar tissue (Fig. 7). The greater part of
the marrow of the shaft of the femur was hyperplastic, with neutrophils and eosinophils in
all stages of development, but the epiphysis was densely filled with immature myeloid cells
similar to those found in liver, spleen, and lymph nodes (Fig. 10).

An attempt to transfer the spleen of mouse Rf 265 was made, but with no success. The
mouse had been dead for approximately five hours before inoculations were attempted.

**Strain Arj 20**

The Spontaneous Disease: When irradiated mouse Arj 20 was eleven months old the
number of leukocytes in the blood appeared slightly increased, and there were several
myelocytes. These cells, approximately 10 to 14 \( \mu \) in diameter, had kidney-shaped nuclei
and contained numerous moderately coarse purple and red granules in basophilic cytoplasm.
At autopsy the spleen measured 3 by 0.8 cm. and was brown-red on the cut surface, with
numerous small gray areas. The liver was slightly enlarged and of normal brown-red color.
Cervical lymph nodes were enlarged to 4 mm. in their greatest diameter, but other lymph
nodes were not enlarged.

The liver showed slight portal infiltration by myeloid cells and scattered through the
sinusoids were many groups of from three to twenty immature myeloid cells, most of which
were myelocytes. Mitotic figures were numerous. There was a very small number of
megakaryocytes but erythrogenic foci were absent. Almost all the cells in an imprint prep-
paration of the spleen were oxydase-positive. Microscopic examination showed a loss of
normal splenic architecture due to extensive infiltration by immature myeloid cells with fre-
quent mitoses. While erythrogenic foci were numerous and there were occasional mega-
karyocytes, there were large areas of pale-staining myelocytes among which both were
absent. The general architecture of the two cervical lymph nodes examined was preserved,
but more than two thirds of each node was replaced by plasma cells. There was moderate
hyperplasia of large mononuclear cells but no immature myeloid cells were seen. The bone
marrow was composed almost entirely of myeloid cells with a preponderance of myelocytes
and numerous metamyelocytes. There were several small groups of eosinophils. Erythro-
genic foci and megakaryocytes were few.

Transmission Experiments: Two related mice that had been irradiated one month be-
fore were inoculated and forty days later had advanced myeloid leukemia. At autopsy the
pathological changes were similar in both mice, and resembled those in the spontaneous
disease. The infiltration by immature myeloid cells in the liver was much more conspicu-
ous, however, in the transmitted disease, and the lymph nodes were almost entirely replaced
by myelocytes. The marrow of the middle of the femur was acellular but in the diaphyseal
regions there were moderate fibrosis and infiltration by myeloid cells similar to those in
other organs. These cells extended along the Haversian canals and in several areas the
periosteum was raised by an underlying infiltration (Fig. 16).

**Strain Akh 106**

The Spontaneous Disease: Non-irradiated mouse Akh 106 was killed when ten months
old because its spleen was enlarged. In the blood smear were several myelocytes, although
the number of leukocytes per c.mm. was apparently not increased. The spleen measured
2.5 by 0.7 cm. and was uniformly brown-red. The liver was of normal size and was brown-
LEUKEMIA AND MYELOPOIESIS IN MICE

red. Most of the lymph nodes were slightly enlarged; the cervical and mesenteric nodes were moderately enlarged.

Microscopic examination of the liver showed slight to moderate portal infiltration by myeloid cells and scattered small foci of these cells, most of which were metamyelocytes. Many small groups of cells were composed entirely of myelocytes; in other areas polymorphonuclear leukocytes were conspicuous. Mitotic figures abounded. Megakaryocytes and erythrogenic foci were absent. The large blood vessels did not contain an increased number of leukocytes. In the spleen there was extensive hematopoiesis. Here also most of the myeloid cells were metamyelocytes and myelocytes, but polymorphonuclear leukocytes were numerous and in a few areas predominant. There were many mitotic figures. Large numbers of erythrogenic foci were scattered throughout the spleen and megakaryocytes were very numerous. The lymph nodes were extensively invaded by myeloid cells in all stages of maturity. Metamyelocytes were predominant and mitotic figures were numerous. There was no transcapsular invasion. No erythrogenic foci or megakaryocytes were found. The bone marrow showed extensive hyperplasia of the myeloid cells; most of them were metamyelocytes. There were a few megakaryocytes but erythrogenic foci were inconspicuous.

Transmission Experiments: In mice with the transmitted disease there was massive infiltration in the liver, spleen, lymph nodes, bone marrow, and other organs by myeloblasts and promyelocytes. Most cells in imprint preparations of lymph nodes were oxidase-positive. Compared to the mouse with the spontaneous disease, the mice of the first transfer had fewer metamyelocytes, megakaryocytes, and erythrogenic foci among the malignant immature myeloid cells in the spleen. In the mice of the second transfer metamyelocytes, megakaryocytes, and erythrogenic foci were inconspicuous in the spleen, and the organ was composed almost exclusively of immature myeloid cells.

While metamyelocytes were numerous in the liver of the mouse with the spontaneous disease, they were inconspicuous in the livers of mice with the transmitted disease. Similar changes were noted in the lymph nodes.

Strain Aka 51

The Spontaneous Disease: When mouse Aka 51 was eleven months old, the spleen was found to be very large and a blood smear showed a leukocytosis with many mature and young granulocytes and occasional large basophilic cells with round nuclei, presumably myeloblasts. The white cell count was estimated at approximately 70,000 per c.mm. At autopsy the spleen was moderately large and gray-brown. The lymph nodes were of approximately normal size. The liver was gray-red and about twice the usual size.

There was extensive diffuse and perivascular infiltration of the liver with immature myeloid cells, which packed the sinusoids and compressed the hepatic cords (Fig. 12). Mitoses were abundant among these cells, which also filled the large blood vessels. There were a diffuse fatty degeneration and focal necrosis of liver cells. The structure of the spleen was destroyed, the organ appearing as a dense homogeneous collection of myeloid cells, which also invaded the capsule (Fig. 13). There were few remaining lymphocytes. Areas of erythropoiesis were present in small numbers only, and megakaryocytes were rare. The lymph nodes were occupied by masses of myeloid cells and a few small foci of lymphocytes (Fig. 15). The marrow of most of the femur was relatively acellular, but in a few areas near the epiphysis there were masses of immature myeloid cells (Fig. 14).

Transmission Experiments: Three related mice were inoculated with this strain, after irradiation. Two weeks after injection one of these showed generalized weakness and paralysis of the hind legs. (Paralysis occurs in mice with several strains of leukemia and is often the first sign of the transmitted disease.) In this mouse the blood picture was essentially normal except for an occasional immature myeloid cell. The animal was killed and at autopsy the spleen measured 2.6 by 0.8 cm.; the lymph nodes and liver were normal.

Microscopic examination of the liver and lymph nodes showed no myeloid infiltration but in the spleen there were many areas of immature myeloid cells with occasional mitotic figures. Many megakaryocytes, large mononuclear cells, and erythroblasts were scattered
PLATE III: SPONTANEOUS MYELOID LEUKEMIA OF MOUSE No. AKA 51

All sections show advanced invasion by immature myeloid cells. Fig. 12. Liver \( \times 800 \). Fig. 13. Spleen \( \times 900 \). Fig. 14. Bone marrow \( \times 1200 \). Fig. 15. Lymph node \( \times 250 \).
throughout the organ. In the bone marrow of a *lumbar vertebra* there was a dense collection of immature myeloid cells with many mitoses infiltrating the dura mater, subdural and epidural spaces, and paravertebral muscle tissue, but the spinal cord was not invaded (Fig. 26). There were many eosinophils scattered among the immature myeloid cells.

Myeloid leukemia occurred in only one of the animals inoculated with Strain Aka 51. We believe, however, that the disease in this mouse was transmitted and not spontaneous, for the following reasons: The incidence of spontaneous myeloid leukemia among these mice at the time of inoculation was very low; hence the development of spontaneous leukemia was unlikely. Young animals were used for inoculation and spontaneous leukemia has not been observed in mice below the age of five months. The injected animal developed signs of the disease after the usual incubation period. The malignant cells closely resembled those of the spontaneous disease.

**Comment**

The characteristics of eight strains of undoubted myeloid leukemia that were transmitted in this laboratory have been presented. The spontaneous disease that developed in irradiated mice was similar to that in non-irradiated animals. Among the latter there was a chloroleukemia, a basophile myelocytic, a myeloblastic, and a myeloblastic and promyelocytic leukemia. Among the irradiated animals there were four neutrophile myelocytic leukemias.

In each case the characteristics of the malignant cells remained unaltered through the various transfers. Gray-white myelomata about the bones were formed when a few cells of Strain Ar 117 were injected intravenously. Cells of Strain Slb 351 produced green discoloration of the infiltrated lymph nodes. Visceral tumor masses were produced by cells of Strain Rfb 117.

In the organs of several of the mice with spontaneous leukemia (notably Strain Akh 106) the malignant immature myeloid cells were associated with numerous mature myeloid cells, with or without megakaryocytes and cells of the erythrogenic series. In the course of successive transfers the myeloid cells that invaded the tissues of the host were almost exclusively immature, and mature myeloid cells, megakaryocytes and erythrogenic foci were inconspicuous or absent.

In none of the animals in which inoculations were successful was there any evidence of infection, and the death of the mice could be attributed to the transmitted leukemia. The uniform fatality of the transmitted disease, when once evident, its similarity to the spontaneous disease, and the absence of infection firmly established the diagnosis of leukemia.

**Myeloid Leukemia: Unsuccessful Attempts at Transmission**

Unsuccessful attempts were made to transmit six cases of spontaneous myeloid leukemia. In each instance the disease was clinically and pathologically evident. Data relevant to these mice and the attempted transmissions are given in summary in Table III. All of the mice were irradiated at ages varying from one to five months with single doses of 200 to 450 r units. Myeloid leukemia was diagnosed five to sixteen and a half months thereafter. Injections of cellular suspensions of spleens and lymph nodes were made into non-irradiated mice of the same family and irradiated mice of closely related families; but in no case did leukemia occur.

A brief description of each of these mice follows:
### Table III: Unsuccessful Attempts to Transmit Myeloid Leukemia

<table>
<thead>
<tr>
<th>Number of Mouse</th>
<th>Irradiation</th>
<th>Age of Mouse at Death (months)</th>
<th>Age of Mouse at Death (months)</th>
<th>Leukocyte Count at Death (per c.mm.)</th>
<th>Predominant Cell Type</th>
<th>Attempts at Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Af372</td>
<td>450</td>
<td>1.5</td>
<td>18</td>
<td>268,000</td>
<td>Myeloblasts and myelocytes</td>
<td>1934</td>
</tr>
<tr>
<td>Ala36</td>
<td>400</td>
<td>3.0</td>
<td>12</td>
<td>246,000</td>
<td>Large myelocytes with purple-red granules</td>
<td>1934</td>
</tr>
<tr>
<td>Slb24</td>
<td>200</td>
<td>1.0</td>
<td>11</td>
<td>Slightly elevated</td>
<td>Myeloblasts and myelocytes</td>
<td>1934</td>
</tr>
<tr>
<td>Sic21</td>
<td>200</td>
<td>1.0</td>
<td>14</td>
<td>120,000</td>
<td>Myeloblasts and myelocytes</td>
<td>1934</td>
</tr>
<tr>
<td>Afb291</td>
<td>400</td>
<td>5.0</td>
<td>10</td>
<td>87,300</td>
<td>Myelocytes</td>
<td>1935</td>
</tr>
<tr>
<td>Rfb113</td>
<td>400</td>
<td>2.5</td>
<td>9</td>
<td>605,000</td>
<td>All transitional forms from immature to mature granulocytes</td>
<td>1935</td>
</tr>
</tbody>
</table>

**Mouse Af 372:** At autopsy the spleen was found to be moderately enlarged (2.7 by 0.8 cm.); it was red, mottled with several gray areas. The lymph nodes were small and yellow-gray. There was conspicuous perivascular and intrasinusoidal infiltration in the liver by immature myeloid cells, with many mitotic figures. Megakaryocytes were rare and erythrogenic foci infrequent. Immature myeloid cells diffusely invaded the spleen and its capsule. Most of the marrow in the diaphysis of the femur was relatively acellular, but in the region of the metaphysis there was myeloid hyperplasia with many immature myeloid cells.

**Mouse Ala 36:** When ten months old, this mouse had greatly enlarged superficial lymph nodes. The left inguinal node was excised and found to contain numerous large myelocytes with purplish-red granules. A blood smear taken at the same time gave no evidence of leukemia. At twelve months the mouse developed progressive weakness and its blood was leukemic (Table III). At autopsy the lymph nodes were greatly enlarged and yellow-red. The spleen was moderately enlarged (2.7 by 0.7 cm.) and pale yellow-brown. The liver was spotted with yellow and green areas. Microscopic examination of the liver showed extensive portal and diffuse infiltration with immature myeloid cells. The larger blood vessels were filled with similar cells. Erythrogenesis was inconspicuous and there were no megakaryocytes. The architecture of the spleen was almost entirely destroyed by infiltrating myeloid cells that compressed the follicles. Megakaryocytes and erythrogenic foci were few. The medulla of the lymph nodes was infiltrated with early myeloid cells and there was compression of the cortical tissue and invasion of the capsule. The marrow of the mid-diaphysis of the femur was necrotic; elsewhere in the marrow there was myeloid hyperplasia with numerous myelocytes.

**Mouse Slb 24:** At autopsy the lymph nodes were yellow-green in color. The spleen measured 2.8 by 1 cm., and was uniformly green-gray. The liver was slightly enlarged and contained a green-gray nodule. There was massive invasion of the liver by immature myeloid cells. The appearance was almost identical with that of the liver illustrated in Fig. 2. The spleen was almost entirely filled with immature granulocytes. In the lung many of the smaller vessels contained masses of immature myeloid cells.

**Mouse Sic 21:** At autopsy the spleen was uniformly pale brown and measured 2.6 by 0.7 cm. The liver was yellow-brown and one and one-half times the normal size. Most of the superficial lymph nodes were slightly enlarged. Microscopic examination showed massive infiltration of the liver by immature myeloid cells. Megakaryocytes and erythrogenic foci were absent. The large vessels of the liver contained relatively few myeloid cells. The spleen and lymph nodes were filled with cells similar to those in the liver. They invaded the capsule of the lymph node and the surrounding areolar tissue. The bone marrow was
leukemia and myelopoiesis in mice

acellular, but from one part of the endosteum there was a growth of fibroblast-like cells with several mitotic figures. Numerous myeloblasts and myelocytes invaded areolar tissue about the periosteum.

Mouse Afb 291: At autopsy the spleen measured 3 by 1 cm. and the liver was slightly enlarged. The lymph nodes were slightly enlarged and several were green-gray. Microscopic examination of the liver showed an extensive perivascular and intrasinusoidal infiltration by immature myeloid cells, many with mitotic figures. There were few metamyelocytes. The lumina of the larger vessels were almost filled with similar early myeloid cells. Erythrogenic cells were rare and no megakaryocytes were noted. The substance of the spleen was almost totally replaced by myelocytes, which also invaded the capsule. Cells of the erythrogenic series were few; megakaryocytes were rare. The lymph nodes showed a variable picture; some were completely invaded by immature myeloid cells, others showed but a slight invasion. There were large numbers of myeloid cells in their capillaries. The bone marrow was the seat of granulocytic hyperplasia with many immature myeloid cells and a great number of mitotic figures.

Mouse Rfb 113: At autopsy the spleen measured 3.5 by 1.2 cm. and was mottled gray and red. Lymph nodes were slightly enlarged. The liver, twice its normal size, contained several small gray nodules. There were two hemorrhagic areas in the lung. Microscopic examination showed that the liver was infiltrated by myeloid cells with many adult forms. There was advanced central fatty degeneration. The spleen and lymph nodes were dense masses of similar myeloid cells with numerous mitotic figures. Erythrogenic foci and megakaryocytes were absent. Extensive myeloid hyperplasia with small areas of necrosis was observed in the bone marrow. There was slight subperiosteal infiltration by immature myeloid cells, a few in mitosis.

Comment

The pathological changes, both gross and microscopic, in the six mice in which transmission failed show no essential difference from those in the mice in which transfers were successful. These changes, characteristic of myeloid leukemia and present in both groups, consist of infiltrations, usually extensive, by immature myeloid cells in the liver, spleen, lymph nodes, bone marrow and other organs. The only conspicuous individual variations are the state of maturity of the malignant cells and the extent of the infiltration in the different organs. In most cases the predominant cells are myelocytes, with which myeloblasts and metamyelocytes are commonly found in varying numbers. There is usually massive diffuse infiltration of the liver with distention of the sinusoids and frequent necrosis of compressed hepatic cords. Immature myeloid cells densely packed together usually surround portal veins and form subendothelial masses. Mitotic figures are frequent. Megakaryocytes are few or absent. Occasionally there are small foci of erythroblasts. In the blood in the larger vessels there is usually a greatly increased number of leukocytes with a preponderance of immature myeloid cells. The spleen is conspicuously infiltrated with myeloid cells which almost completely replace the pulp and cause infiltration, compression, or complete obliteration of the malpighian corpuscles. Mitotic figures are numerous. There are usually only small scattered foci of erythroblasts, and megakaryocytes are few. Masses of immature myeloid cells with no other hematopoietic cells are characteristic of the leukemic infiltration. The lymph nodes are extensively infiltrated, although for the most part they escape complete obliteration of normal structure. The myeloid cells often invade the capsule. Massive collections of early granulocytes frequently block the lumen of the capillaries of the lung and form the
characteristic "leukemic thrombi." Perivascular collections of myeloid cells are more rare. The bone marrow usually contains numerous immature myeloid cells which replace the mature elements in many areas. Myeloid cell infiltration may be found in the kidney, adrenal, hypophysis, ovary, broad ligament, muscle, meninges, and elsewhere.

The six cases of myeloid leukemia in which attempts at transmission failed illustrate the difficulties of conducting transmission experiments without highly inbred stocks of mice. In some of our early experiments as many as 68 mice were inoculated and, although massive doses of x-ray were given to many of them, transmission of leukemia failed.

**Myeloid Leukemia: Transmission Not Attempted**

Many cases of spontaneous myeloid leukemia were studied, transmission of which was not attempted. In this section a few such cases of special interest will be described briefly.

*Mouse Rfb 402: Myeloid Leukemia with Numerous Mature Myeloid Cells:* This mouse, irradiated two months after birth, died at the age of twelve months of intestinal obstruction. Its spleen was greatly enlarged and the lymph nodes but slightly so. Microscopic examination of the liver showed an extensive diffuse infiltration of the sinusoids and perivascular areas by myeloid cells, among which metamyelocytes and myelocytes were predominant and polymorphonuclear leukocytes numerous (Fig. 19). Only occasional mitoses were observed. There were few erythroblasts. A few megakaryocytes were encountered in hepatic sinusoids but there was none in foci of perivascular infiltration. The number of leukocytes in the blood vessels was greatly increased; most of the cells were myelocytes and metamyelocytes. The spleen was extensively invaded by cells similar to those in the liver but here megakaryocytes were very numerous. Erythroblasts, present in moderate numbers, were not as conspicuous as in typical cases of non-malignant extramedullary myelopoiesis. The lymphoid follicles were either compressed or disrupted by the myeloid cells. The lymph nodes were almost completely replaced by similar myeloid cells with many metamyelocytes. Only occasionally were mitoses seen. The capsule was invaded in several areas. There was a marked hyperplasia of granulocytes of the femoral marrow with conspicuous maturation, but immature myeloid cells had infiltrated the periosteum and the surrounding areolar tissue.

A major distinction between non-malignant extramedullary myelopoiesis and myeloid leukemia is the maturation defect present in most cases of leukemia. When maturation of most of the myeloid elements occurs, diagnostic difficulties may arise, albeit other criteria are considered. In this case the diagnosis of myeloid leukemia was suggested by the extent of the infiltration, the conspicuous leukocytosis, comparative paucity of erythroblasts and megakaryocytes, invasion of the capsule of a lymph node and destruction of the normal architecture of spleen and lymph nodes by myeloid cells.

*Mouse Aka 15: Mesenteric Myeloid Tumor:* This mouse was irradiated with 400 r at the age of two months and died fourteen months later. At autopsy the spleen measured 3 by 0.7 cm. and was pale gray-brown on the external surface. At one end was a hematoma measuring 0.7 cm. in diameter. The lymph nodes were not enlarged. Both ovaries were moderately enlarged, each measuring approximately 3 or 4 mm. in diameter. Several firm gray-white nodules varying from 5 to 8 mm. in diameter were attached to the small intestine and mesentery.

Microscopic examination of the liver, spleen, lymph nodes, and bone marrow showed extensive diffuse infiltration by early myeloid cells similar to those illustrated in Figs. 12–15. There was a small tumor in the left ovary composed of lutein cells. Several foci of im-
PLATE IV: SPONTANEOUS AND TRANSMITTED MYELOID LEUKEMIA

FIG. 16. Transmitted Myeloid Leukemia (Strain Arj 20): Bone marrow, showing dense infiltration by immature myeloid cells in the metaphysis with extension through an Haversian canal and subperiosteal invasion. There is fibrosis in the marrow of the diaphysis.

FIG. 17. Spontaneous Myeloid Leukemia of Mouse Aka 15: Tumor-like infiltration of myeloid cells in the wall of the small intestine. ×40.

FIG. 18. Spontaneous Myeloid Leukemia of Mouse Rm 121: An acellular area in the bone marrow, with slight fibrosis and masses of myelocytes. ×40.

FIG. 19. Spontaneous Myeloid Leukemia of Mouse Rfb 402: Diffuse invasion of liver by myeloid cells. ×100.
mature granulocytes were scattered among the lutein cells. The mesenteric tumor was composed of myelocytes with numerous mitotic figures. There was extensive infiltration of the surrounding areolar and muscular tissue and the wall of the intestine was invaded to the submucosa in several areas (Fig. 17).

In this case of myeloid leukemia there was no enlargement of lymph nodes but the myeloid tumor in the mesentery may have originated in an infiltrated lymph node. Myeloid tumors are uncommon in mice with spontaneous leukemia but can be readily produced experimentally in the viscera or subcutaneous tissue with certain transmissible strains (Fig. 8).

Mouse Rm 121: Necrosis of Femoral Bone Marrow: This mouse, irradiated with 400 \( r \) units at one month of age, died at thirteen months. It had myeloid leukemia with characteristic invasion of the viscera by immature myeloid cells. A large part of the middle third of the femoral marrow was almost devoid of cells (Fig. 18). In and at the periphery of this area were moderate numbers of fibroblast-like cells, and surrounding it were vast numbers of myelocytes, among which erythroblasts and megakaryocytes were very scarce.

In many irradiated mice that develop spontaneous myeloid leukemia there is, usually in the middle third of the femoral marrow, a necrotic or relatively acellular area bordered by fibroblasts and immature myeloid cells. It is probable that this change is produced by x-rays. If so, the genesis of leukemia in these cases may be analogous to the genesis of carcinoma following x-ray burns.

Myeloid Leukemia among Mice That Had Been Injected with Benzpyrene: In experiments in this laboratory, reported elsewhere (16, 17), benzpyrene was injected into the spleens of 96 mice in attempts to produce neoplasms of cells of the hematopoietic system. Nine mice developed monocytic leukemia and four myeloid leukemia. Among an equal number of controls monocytic leukemia was not observed and only one questionable case of myeloid leukemia occurred. Myeloid leukemia that developed in the mice injected with benzpyrene differed in no essential way from that occurring spontaneously or following general irradiation. It has been found that the time interval between general irradiation and the peak of incidence of myeloid leukemia is approximately eight months (14). The interval between the injection of benzpyrene and the appearance of myeloid leukemia varied from four to thirteen months.

While it appears that monocytic leukemia may be produced by the intrasplenic injection of benzpyrene (17), the available data, although suggestive, are insufficient for the conclusion that myeloid leukemia can be produced by that hydrocarbon. The leukemia-like condition described by Parsons (11, 12) in mice that had been injected with carcinogenic chemicals is, we believe, non-malignant extramedullary myelopoiesis.

Non-Malignant Extramedullary Myelopoiesis

It is generally believed that in adult mammals the formation of myeloid elements under physiological conditions is confined to the bone marrow (18). In older mice, however, myelopoiesis in the spleen is very common, but whether this is physiological or pathological is not known. In extramedullary blood-forming foci all the elements of the normal marrow are usually present; the
granulopoietic elements most often predominate over the erythropoietic and megakaryocytic.

In experimental animals, as for example the rabbit and mouse, the heterotopic development of myeloid tissue has been produced by repeated bleeding, by subcutaneous or intravenous injections of carmine or various blood poisons, as sapotoxin, pyrogallol, phenylhydrazene, pyrodin, benzol (cf. 19), and by live or dead bacteria, e.g., Proteus X 10 (20) and B. coli (19, 21). Opie (22), studying in guinea-pigs the relation of cells with eosinophile granulation to bacterial infection, noted the presence of myeloid elements in the spleen a short time following intraperitoneal inoculations of bacterial suspensions. He says: "the occurrence of this phenomenon within from two to four hours after inoculation demonstrates that these elements are derived from the bone marrow and are not formed in the spleen."

Jaffé (23) noted extramedullary myelopoiesis and erythropoiesis in the liver and spleen of mice following successful inoculations with transplantable tumors, and Hueper (5) found an increased incidence of extramedullary myelopoiesis in mice with spontaneous breast tumors. Lewis (24, 25) observed that mice bearing transmitted tumors (originally produced by 1:2:5:6-dibenzanthracene) developed pronounced myeloid hyperplasia and an enlarged spleen. These changes were interpreted as "a severe neutrophilia rather than a leukemia." In the transmissible "myeloid leukosis" originally described by Krebs, Rask-Nielsen and Wagner (26), and more recently studied by H. C. and R. Rask-Nielsen (27, 28), "the lymphocytosis normally existing in mice is changed to a prolonged relative and absolute neutrophilia." There was hyperplasia of the bone marrow, and the pulp of the spleen "in some instances showed pronounced myeloid hyperplasia; in other instances there was accumulation of myeloblasts; transitional stages between these changes were also observed."

It is still disputed whether the extramedullary hematopoietic foci are formed by cells that emigrate from the bone marrow or by cells that develop locally from preexisting elements. Extramedullary myelopoiesis is found most frequently in the spleen and liver. In mice distribution of the extramedullary hematopoietic tissue in the organs is similar to that in myeloid leukemia, both spontaneous and transmitted, the sites usually involved being the pulp of the spleen, the perivascular areas in the liver, and the medullary tissue of the lymph nodes. The lymphoid follicles may be reduced in size, but their centers rarely contain myeloid cells. These observations do not support the view that the myeloid and erythrogenic foci arise from cells of the germinal centers (cf. 29).

In order to ascertain whether an infective agent is responsible for the development of non-malignant heterotopic myeloid foci and to determine the possible presence of malignant cells, normal mice were inoculated with cell suspensions of the spleens of mice with non-malignant extramedullary myelopoiesis. Table IV summarizes the results of several such attempts. None of the inoculated mice developed a progressive fatal disease with the characteristics of leukemia. The five mice inoculated with a splenic suspension from mouse Az46 died within a week, of an unidentified acute infection. A
TABLE IV: Attempts to Transmit Non-Malignant Extramedullary Myelopoiesis

<table>
<thead>
<tr>
<th>No. of Mouse</th>
<th>Age at Death (months)</th>
<th>Associated Lesions</th>
<th>Attempted Transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Year</td>
</tr>
<tr>
<td>Az46</td>
<td>K13</td>
<td>Focal necrosis in liver and spleen</td>
<td>1932</td>
</tr>
<tr>
<td>Rfb124*</td>
<td>K11</td>
<td>Subcutaneous abscess</td>
<td>1935</td>
</tr>
<tr>
<td>Rfb220</td>
<td>D12</td>
<td>Ulcer on neck</td>
<td>1936</td>
</tr>
<tr>
<td>Afb314</td>
<td>K13</td>
<td>Cyst of cervical lymph node and adenomata of lung</td>
<td>1936</td>
</tr>
<tr>
<td>Rf473†</td>
<td>K11</td>
<td></td>
<td>1937</td>
</tr>
<tr>
<td>Rfb515</td>
<td>K13</td>
<td></td>
<td>1937</td>
</tr>
<tr>
<td>Rf615</td>
<td>K15</td>
<td>Tumor of lung</td>
<td>1937</td>
</tr>
<tr>
<td>M264</td>
<td>K</td>
<td></td>
<td>1938</td>
</tr>
<tr>
<td>M281</td>
<td>K</td>
<td></td>
<td>1938</td>
</tr>
<tr>
<td>M282</td>
<td>K</td>
<td></td>
<td>1938</td>
</tr>
<tr>
<td>M283</td>
<td>K</td>
<td></td>
<td>1938</td>
</tr>
</tbody>
</table>

* Irradiated with 400 r at six weeks of age. † Injected with benzpyrene at two months of age.

few mice in the other experiments died of diverse causes several months after injection and the others, apparently normal, were killed.

The following description illustrates the gross and microscopic characteristics of non-malignant extramedullary myelopoiesis.

Mouse Rfb 124: This mouse had a slight leukocytosis with many immature myeloid cells. At autopsy the spleen measured 3 by 1 cm. and was uniformly gray-red. Lymph nodes were slightly enlarged; the liver was normal.

Microscopic examination of the liver showed small foci of metamyelocytes and a few myelocytes diffusely scattered throughout the sinusoids and many grouped around portal areas. Mitotic figures were infrequent. Eosinophils were occasionally seen. There were but a few erythroblasts and megakaryocytes. The blood in the larger blood vessels contained a normal number of leukocytes. There was extensive infiltration of the spleen, especially in the subcapsular areas, with granulocytes in all stages of development. The capsule was not invaded. Metamyelocytes were preponderant, but myelocytes, megakaryocytes, and eosinophils were numerous. Dispersed among the granulocytes were great numbers of cells of the erythrogenic series (Fig. 20). The cortical area of the lymph nodes was well preserved but the medulla was densely filled with metamyelocytes and polymorphonuclear leukocytes and a few myelocytes. Occasional mitoses were seen. Monocytes and plasma cells were abundant. The capsule was not invaded. The bone marrow was moderately hyperplastic; mature myeloid cells were predominant, with many eosinophils and megakaryocytes.

Mouse Rfb 220: The ante-mortem leukocyte count of this mouse was 32,000 per c.m.m. and the differential count was: adult polymorphonuclear leukocytes, 24 per cent; immature polymorphonuclear leukocytes, 37 per cent; metamyelocytes, 7 per cent; lymphocytes, 28 per cent; monocytes, 3 per cent; eosinophils, 1 per cent. The spleen measured 3 × 1 × 0.4 cm. and was gray-red. Between the spleen and the diaphragm was a thick-walled abscess. The liver was brown, slightly gray, and of normal size, and the lymph nodes were not enlarged.
The pulp of the spleen contained many myeloid cells, mostly metamyelocytes, among which were scattered numerous megakaryocytes and erythroblasts. There were many histiocytes laden with golden brown pigment. In the portal area of the liver were large numbers of lymphoid cells, with occasional myeloid cells and pigment-laden histiocytes. The Kupffer cells were moderately increased in number and several of them contained similar pigment. In several areas numerous adult polymorphonuclear leukocytes occurred among the metamyelocytes. The lumina of the large vessels contained many polymorphonuclear leukocytes. The bone marrow was diffusely filled with myeloid cells; almost all were metamyelocytes. Eosinophilic myelocytes and histiocytes with golden brown pigment were also abundant in the marrow.

Mouse Afb 314: A firm nodule 1 cm. in diameter was present just beneath the left ear when this mouse was eleven months old. At autopsy there was an ulcer on the neck at the site of the nodule, which had disappeared. The spleen, dark brown-red in color, measured 3 × 0.9 cm. The liver was normal; the lymph nodes were slightly enlarged.

The liver contained several small groups of metamyelocytes. The spleen was the site of far-advanced non-malignant extramedullary myelopoiesis. The vast majority of cells were metamyelocytes and polymorphonuclear leukocytes and there were many eosinophils. Among these cells erythrogenic foci were numerous, and as many as seven megakaryocytes were seen in one high-power field. The lymphoid follicles were reduced in size. In one of two lymph nodes examined the medullary cords were distended by masses of metamyelocytes, polymorphonuclear leukocytes, and many plasma cells. In the other lymph node lymphoid hyperplasia was conspicuous, with large numbers of plasma cells. There was conspicuous myeloid hyperplasia in the femoral bone marrow with preponderance of metamyelocytes. In several areas polymorphonuclear leukocytes were present almost to the exclusion of other cells. Erythrogenic cells were inconspicuous, but megakaryocytes were present in moderate numbers.

Mouse Rf 473: This mouse received an intrasplenic injection of benzpyrene (2.5 mg. in 0.05 c.c. of lard) when about two months of age. An ante-mortem blood count (at eleven months) showed 2,300,000 erythrocytes and 44,000 leukocytes per c.mm. In the smear erythroblastic cells in all stages of development were seen and there were numerous monocytes, many with conspicuous basophilic cytoplasm. The spleen measured 3 × 1.2 × 0.5 cm., it was intensely red, and the trabeculae were conspicuous. The liver was brown and of normal size. The lymph nodes were not enlarged except for one cervical node which contained a small cyst.

The liver was free from myeloid infiltration. The pulp of the spleen was extensively infiltrated by myeloid cells in all stages of maturity, erythrogenic cells approximately half as numerous, and scattered megakaryocytes. The bone marrow was similar to that of Mouse Rfb 124. There were several adenomata in the lung.

Mouse Rfb 515: At autopsy the spleen was uniformly gray-red and measured 3 × 1 cm. The liver did not appear unusual, and most of the lymph nodes were slightly enlarged.

The liver contained numerous clumps of from four to fifteen metamyelocytes in the portal areas. The lymphoid follicles of the spleen were hyperplastic. There were large numbers of immature myeloid cells with numerous mitotic figures and many eosinophils and megakaryocytes in the pulp. Erythrogenic foci were not conspicuous. Near the periphery were several small areas of necrosis. One moderately enlarged lymph node showed lymphoid hyperplasia with vast numbers of plasma cells, and numerous myeloid cells in all stages of maturity in many areas (Figs. 21, 22). Polymorphonuclear leukocytes and eosinophils were conspicuous, but erythrogenic cells were not seen. Mitotic figures were frequent. In the bone marrow there was conspicuous myeloid hyperplasia and almost all the cells were adult polymorphonuclear leukocytes and neutrophilic or eosinophilic metamyelocytes. Megakaryocytes were present in moderate numbers, but erythrogenic foci were not conspicuous.

Mouse Rfb 615: An ante-mortem blood smear was entirely normal. Post-mortem examination showed non-malignant myelogenesis to be extensive in the spleen, moderate in the liver, and slight in the lymph nodes, with the usual myeloid hyperplasia of the bone marrow.

Mouse Az 46: At autopsy the spleen measured 4 × 1.3 cm. The superficial lymph
nodes were gray and slightly enlarged, with several minute hemorrhages in the cervical nodes. The liver was brown with lighter gray areas and numerous yellow spots of pinpoint size. There was one yellow necrotic area measuring 2 mm. in diameter. The lung contained minute hemorrhages.

On microscopic examination the liver contained several small areas of necrosis. The liver cords in most areas were thin and the cytoplasm of the liver cells was vacuolated. Infiltrating the perivascular areas and scattered throughout the sinusoids were many myelocytes and metamyelocytes, and a few polymorphonuclear leukocytes. Erythrogenic foci were conspicuous. Occasional megakaryocytes were seen. The architecture of the spleen was in general retained. There was moderate congestion of the pulp and numerous myeloid cells were present in all stages of maturity, among which cells of the erythrogenic series and large mononuclear cells occurred in moderate numbers. Megakaryocytes were observed in small numbers. There was one small area of necrosis in the pulp. The lymph nodes examined appeared normal except for numerous plasma cells and an occasional myeloid cell. In the bone marrow there was conspicuous myeloid hyperplasia with very numerous metamyelocytes. Erythrogenic foci and megakaryocytes were also conspicuous. There was no myeloid infiltration in the lungs or kidneys.

Focal necrosis is usually not found in non-malignant extramedullary myelopoiesis and the result of this transmission experiment indicates that this animal had an acute infectious disease. All injected mice died within one week after inoculation. Their spleens were very slightly enlarged and dark red, containing small yellow spots of necrosis.

Comment

The cases described illustrate the alterations that occur in mice with non-malignant extramedullary myelopoiesis.

The liver rarely shows as conspicuous involvement as in myeloid leukemia. There may, however, be moderate portal, or less commonly, diffuse infiltration by myeloid cells in all stages of maturity, though metamyelocytes usually predominate. Erythrogenic foci may occur, but less frequently than in the spleen. Megakaryocytes are commonly present. There may be occasional small collections of lymphocytes or, less often, of plasma cells in the portal areas.

The cells characteristic of non-malignant extramedullary myelopoiesis may replace the greater part of the spleen and cause considerable enlargement of that organ, but the changes can usually be distinguished from those of myeloid leukemia. The composite cellular picture is that of normal bone marrow. Erythrogenic cells are scattered among the myeloid cells and megakaryocytes are numerous throughout the organ. Neutrophils are preponderant among the granulocytes but eosinophils are often conspicuous. Mitotic figures may be numerous among the myeloid cells but there is evidence of maturation. Plasma cells are frequently present in the pulp.

In the lymph nodes myeloid alterations are found less often in mice with non-malignant extramedullary myelopoiesis than in mice with myeloid leukemia. The architecture of these nodes is preserved. Most of the myeloid cells are metamyelocytes and polymorphonuclear leukocytes, although myelocytes are often numerous. Megakaryocytes are occasionally seen, but erythrogenic cells are usually absent.

Small foci of myelopoiesis have occasionally been observed in the kidneys, but organs other than those of blood formation have not been studied systematically by us.

The genesis of non-malignant extramedullary myelopoiesis is still disputed.
Plate V: Spontaneous Non-Malignant Extramedullary Myelopoiesis (Figs. 20–22) and Non-Malignant Extramedullary Myelopoiesis Produced by B. coli (Figs. 23–25)

Fig. 20. Mouse Rfb 124: Spleen pulp containing all forms of myeloid cells, small erythrogenic foci, and a megakaryocyte. × 800.

Fig. 21. Mouse Rfb 515: Lymph node, showing preservation of normal architecture. × 30.

Fig. 22. Higher magnification of the cords of cells shown in Fig. 21. Numerous myeloid cells. × 500.

Fig. 23. Spleen: Myeloid cells in all stages of development, erythrogenic foci, and a megakaryocyte are seen. × 550.

Figs. 24, 25. Liver: Collections of myeloid cells. × 500.
The etiologic agent in many cases is undoubtedly infectious; in others it is unknown. The process is first evident and is most massive in the spleen. A moderate degree of hematopoiesis is present in that organ in many mice with no demonstrable pathological changes. Next in frequency of involvement is the liver, followed by the lymph nodes.

Frequently when only one of these organs (usually the spleen) is studied, it is impossible to decide whether the myeloid alterations are leukemic or non-leukemic in character but when several organs are examined the differential diagnosis can usually be made.

**NON-MALIGNANT EXTRAMEDULLARY MYELOPOIESIS PRODUCED BY INJECTIONS OF B. COLI**

In order to compare the histologic characteristics of spontaneous non-malignant extramedullary myelogenesis with that produced experimentally, several experiments were conducted in which mice were given repeated injections of a suspension of living *B. coli* in an attempt to produce that condition. Opie (22) noted the occurrence of myeloid elements in the spleens of guinea-pigs following intraperitoneal inoculation of bacterial suspensions.

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**Table V: Production of Non-Malignant Extramedullary Hematopoiesis by the Injection of B. Coli**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Life After Injections Begun, in Days*</th>
<th>Extent of Extramedullary Hematopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. I Injected</td>
<td>Intraven. Intraper.</td>
<td>K5</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Controls 5 and 6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Exp. II Injected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
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</tr>
<tr>
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<td>8</td>
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<tr>
<td>Controls 6 and 7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Exp. III Injected</td>
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<td></td>
</tr>
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<td>1</td>
<td>0</td>
<td>7</td>
</tr>
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<td>2</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Controls 5 and 6</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*K = killed. D = died.*
The mice used were between two and three months old, an age at which extramedullary myelogenesis is usually absent or very slight, and were members of family Ak, in which spontaneous extramedullary hematopoiesis is relatively rare. A twenty-four-hour agar slant culture of B. coli of murine origin was suspended in 10 c.c. of normal saline solution. The mice received daily intravenous and/or intraperitoneal injections of this suspension for five to ten days and were killed five to eleven days after the first injection.

Table V shows the extent of extramedullary hematopoiesis in the spleen and liver.

The changes occurring in the injected mice were similar and varied only in degree. In the spleen there were numerous metamyelocytes, adult polymorphonuclear leukocytes, and eosinophils, with many erythroblasts and normoblasts (Fig. 23). There was conspicuous lymphoid hyperplasia. Some of the follicles were hyalinized. The liver contained small foci, occasionally perivascular, of myeloid cells, mainly metamyelocytes, and cells of the erythropoietic series (Figs. 24, 25). There were several mitotic figures. In the mice injected intravenously there were a few areas of focal necrosis with cell debris and amorphous eosinophilic material. No hematopoietic foci were seen in the lymph nodes. The bone marrow showed moderate granulocytic hyperplasia with preponderance of polymorphonuclear leukocytes.

In the spleen of the control mice there were very few myeloid cells, mainly metamyelocytes, a few megakaryocytes, and occasional erythroblasts. The liver and lymph nodes were entirely free from myeloid infiltration.

The difference between spontaneous and induced extramedullary myelopoiesis is merely in the degree of infiltration by relatively mature myeloid and erythrocytic cells and megakaryocytes. The extramedullary myelopoiesis produced experimentally was of only moderate degree compared to that observed in many spontaneous cases.

Extramedullary Myelopoiesis of Questionable Malignancy

In a group of cases the differential diagnosis between myeloid leukemia and extensive non-malignant extramedullary myelopoiesis could not be made with certainty. The difficulty was especially great in those cases where one or more organs showed evidence of one condition and the rest of the other. Diagnostic difficulties are illustrated by the cases listed in Table VI.

Mouse Rxa 2: Probable Myeloid Leukemia: The liver contained numerous focal clumps of immature myeloid cells both in the sinusoids and perivascularly. In a few areas, however, there were extensive perivascular infiltrations by many polymorphonuclear leukocytes and a few immature myeloid cells. Mitoses were infrequent. Erythroblasts were scattered singly among the myeloid cells. Occasional megakaryocytes were seen. The large blood vessels contained normal numbers of leukocytes. In the spleen, lymphoid tissue persisted but most follicles were invaded and the pulp was diffusely infiltrated by myeloid cells in different stages of development. There was very conspicuous infiltration in the subcapsular areas, where immature myeloid cells were found to the almost complete exclusion of the lymphoid tissue. Erythrogenic foci were prominent among the myeloid elements. There was a slight invasion of the capsule by myeloid cells. The structure of the lymph nodes was preserved but they were extensively infiltrated by myeloid cells. Mature cells predominated, although there were abundant myelocytes and mitotic figures. In one area there was an invasion of the capsule and of the surrounding areolar tissue by myeloid cells. The femoral marrow was hyperplastic and contained granulocytes in all stages of development, erythrogenic foci, and megakaryocytes.

In this case there was a predominantly perivascular infiltration in the liver, in which foci of immature cells were as numerous as mature ones. Changes in the spleen were compatible with an extensive non-malignant hema-


**Table VI: Extramedullary Myelopoiesis of Questionable Malignancy**

<table>
<thead>
<tr>
<th>No. of Mouse</th>
<th>Probable Diagnosis</th>
<th>Age at Death (months)</th>
<th>Autopsy Findings</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver Enlargement</td>
<td>Spleen Size (mm.)</td>
</tr>
<tr>
<td>Rxa2</td>
<td>Myeloid leukemia</td>
<td>20</td>
<td>None</td>
<td>28x9</td>
</tr>
<tr>
<td>Rfb401</td>
<td>Non-malignant extramedullary myelopoiesis</td>
<td>11</td>
<td>None</td>
<td>23x9</td>
</tr>
<tr>
<td>Rfb390</td>
<td>Non-malignant extramedullary myelopoiesis</td>
<td>11</td>
<td>None</td>
<td>20x7</td>
</tr>
<tr>
<td>Afb278</td>
<td>Non-malignant extramedullary myelopoiesis</td>
<td>9</td>
<td>None</td>
<td>25x6</td>
</tr>
<tr>
<td>Aka482</td>
<td>Lymphomatosis and non-malignant extramedullary myelopoiesis</td>
<td>9</td>
<td>Slight</td>
<td>30x10</td>
</tr>
</tbody>
</table>

Topoipoiesis, although there were areas where there was little maturation of myeloid cells. The bone marrow was apparently non-leukemic, but the very conspicuous involvement of lymph nodes with the invasion of surrounding fat suggested a malignant process.

**Mouse Rfb 401: Probable Non-Malignant Extramedullary Myelopoiesis:** The sinusoids and portal areas in the liver contained moderate numbers of granulocytes in all stages of maturation. In the portal areas the cells were lying in a loose stroma and were not densely packed as in a typical malignant infiltration. Most of the cells were metamyelocytes and adult polymorphonuclear leukocytes. Many erythroblastic cells with a densely basophilic, pyknotic nucleus and eosinophilic cytoplasm were found singly among the myeloid cells. The blood in the larger vessels did not contain an increased number of leukocytes. Pigment-laden macrophages lined the sinusoidal walls and were found occasionally in the periportal areas. The general architecture of the spleen was preserved. The follicles, pulp, and subcapsular areas were diffusely invaded by metamyelocytes and young polymorphonuclear leukocytes and occasional myelocytes. The cells were not densely packed as in the usual leukemic proliferation. Mitotic figures were infrequent. Many scattered erythroblasts were present, as well as numerous megakaryocytes. The capsule was not invaded. In the sinuses of the lymph nodes were scattered myelocytes and metamyelocytes. The bone marrow was hyperplastic with delayed maturation of myeloid elements. Most cells were either myeloblasts or myelocytes. Metamyelocytes and adult forms were infrequent. There were occasional megakaryocytes.

In summary, invasion by myeloid cells was slight in the lymph nodes, moderate in the liver, and very conspicuous in the spleen. Most of the cells were more adult than in a typical case of leukemia. The lack of maturation of the myeloid cells in the bone marrow is unusual in a non-leukemic hyperplasia. The presence of an abscess in the neck of this animal probably ac-
counts for at least part of the hematopoietic changes. We are inclined to regard this case as non-malignant extramedullary myelopoiesis because of the predominance of the more mature types of myeloid cells with almost complete absence of mitoses, the intimate association of the myeloid cells with numerous megakaryocytes and erythrogenic foci, the very slight involvement of the lymph nodes, the preservation of normal splenic architecture, and the comparatively slight degree of infiltration in the liver.

Mouse Rfb 390: Probable Non-Malignant Extramedullary Myelopoiesis: The liver showed a moderately extensive, diffuse and perivascular infiltration by myeloid cells. Most of the large blood vessels were partially surrounded by the cellular proliferation. Maturation was conspicuous, but in a few areas, particularly in the sinusoids, there were small clumps of immature cells. Erythroblasts were frequent but not numerous among the granulocytes. Occasional mitoses were seen. The blood in the larger hepatic vessels showed an extensive leukocytosis with very many mature granulocytes and an occasional myelocyte. There was much golden-yellow pigment in the liver; most of this was contained in polymorphonuclear leukocytes and the rest in Kupffer cells. An occasional megakaryocyte was seen. The structure of the spleen was preserved. The pulp was diffusely and extensively infiltrated with myeloid cells, mostly metamyelocytes and polymorphonuclear leukocytes. In many areas, however, there were clumps of immature cells and many mitotic figures. Erythroblasts were interspersed among the granulocytic cells, being, however, less abundant in the foci with delayed maturation. Megakaryocytes were common and there were many macrophages laden with golden-yellow pigment. The capsule was not invaded. One lymph node was fibrotic and contained many plasma cells and numerous large mononuclear cells. In one area near the hilus there were many adult polymorphonuclear leukocytes. A few small foci of metamyelocytes, polymorphonuclear leukocytes, and occasional myelocytes were scattered throughout this node. Several capillaries showed an extensive leukocytosis with mature cells predominating. There was no capsular invasion. Other lymph nodes, although with less fibrosis, were similar. There was granulocytic hyperplasia in the femoral bone marrow but maturation was conspicuous, with numerous metamyelocytes and polymorphonuclear leukocytes.

In this mouse the myeloid cells in the liver showed conspicuous maturation in most of the periportal areas, but in many instances of intersinusoidal infiltration almost all the cells were myelocytes. Erythrogenesis was present, but only to a slight degree. In the spleen there was myelogenesis with occasional areas of delayed maturation. Conspicuous maturation and erythrogenesis, numerous megakaryocytes, and good preservation of structure supported the diagnosis of non-malignant myelopoiesis. The bone marrow was hyperplastic but not leukemic. These hematopoietic changes were probably due to streptococcic abscesses in the perineum and cervical lymph nodes.

Mouse Afb 278: Probable Non-Malignant Extramedullary Myelopoiesis: This mouse was injected intravenously with 0.2 c.c. of dibenzanthracene at the age of two months. Three months later, 0.4 c.c. of the same drug was injected intravenously and this dose was repeated six times during the course of the next twelve weeks.

Microscopic examination of the liver showed a very slight portal infiltration by myeloid cells. Throughout the sinusoids there were many small foci of metamyelocytes and polymorphonuclear leukocytes with an occasional myelocyte. There were occasional mitoses. A few megakaryocytes were present in the sinusoids; erythroblasts were scant. The blood in the larger vessels was normal. There was moderate fibrosis of the spleen, especially around the follicles, which were small. Vast numbers of metamyelocytes and numerous adult polymorphonuclear leukocytes were scattered throughout the pulp, with occasional small foci of myelocytes. There were many mitotic figures. Large mononuclear cells and megakaryo-
cytes were numerous, but there were only a few scattered erythroblasts. The femoral bone marrow was moderately hyperplastic, with conspicuous maturation. Mitotic figures were frequent.

In this case there were slight non-malignant extramedullary myelopoiesis in the liver and moderate hyperplasia of granulocytes in the bone marrow. The spleen showed extensive myeloid cell proliferation with many mitotic figures and areas containing solely immature myeloid cells. There was very slight evidence of erythrogenesis. These findings cast doubt on a diagnosis of non-malignant myelopoiesis. This diagnosis is supported, however, by the predominance of mature granulocytes and the presence of numerous eosinophilic polymorphonuclear leukocytes and megakaryocytes in the spleen. Furthermore, extensive myeloid involvement was limited to the spleen. Nevertheless, leukemia cannot be excluded with certainty.

**Mouse Aka 482: Lymphomatosis and Extramedullary Myelopoiesis, Probably Non-Malignant:** The morphologic characteristics of the spontaneous and transmitted disease of Strain Aka 482 resemble those of lymphoid and myeloid leukemia and non-malignant extramedullary myelopoiesis.

**The Spontaneous Disease:** The strain originated in a non-irradiated mouse which, at the age of nine months had a slightly enlarged spleen and superficial lymph nodes. The leukocytes numbered 150,000 per c.mm. In a blood smear most of the cells were metamyelocytes and polymorphonuclear leukocytes; myelocytes were few. The mouse was killed and at autopsy the superficial lymph nodes were found to be moderately enlarged and yellow-gray. The mesenteric lymph nodes were greatly enlarged and of a green-gray color. The spleen measured 3 X 1 cm. and was uniformly brown-red externally. The thymus was large and gray. The liver showed but a slight increase in size.

Microscopic examination of one lymph node showed an almost complete transformation into a myeloid organ. Most of the cells were polymorphonuclear leukocytes and metamyelocytes; a smaller number were myelocytes. Most myelocytes were neutrophilic; a few were eosinophilic. The foci of immature cells were surrounded by mature ones. Mitotic figures were very numerous. Megakaryocytes and erythroblasts were not seen. Another lymph node was densely packed with a homogeneous collection of cells resembling large lymphocytes with large and round or slightly indented nuclei and a small amount of cytoplasm. The possibility that these cells were myeloblasts cannot be excluded. There was a very small number of metamyelocytes among them. Megakaryocytes and erythrogenic foci were absent. In the liver there was extensive periportal infiltration by myeloid cells similar to those seen in the lymph node (Figs. 27 and 29). In many fields the myeloid cells were breaking through the vessel walls. The spleen contained vast numbers of myeloid cells in the pulp; most of them were metamyelocytes, but among these in several areas adult polymorphonuclear leukocytes abounded. In different areas round or oval cells with large round or slightly irregular nuclei were present to the almost complete exclusion of other cells, and here numerous mitotic figures were noted (Fig. 28). Cells of the erythrogenic series were seen in moderate and megakaryocytes in small numbers, usually in areas of the pulp where the more mature myeloid cells occurred. The pancreas was invaded by myeloid cells similar to those in the lymph node, and they surrounded the islands of Langerhans in several areas. In the bone marrow there was a very extensive hyperplasia of granulocytes. Maturation was conspicuous in many areas.

**Transmission Experiments:** One of eleven non-irradiated, closely related mice that were inoculated with a suspension of cells of the spleen and lymph node died with leukemia one month after injection.

In the liver of this mouse (A 9509) there was an extensive portal infiltration of myeloid cells with numerous mitotic figures. Mature and immature granulocytes occurred in approximately equal numbers but in a few areas there was a preponderance of polymorphonuclear leukocytes. Among the myeloid cells were numerous large cells with round or slightly indented, pale-staining nuclei, usually containing an eccentric nucleolus. It was
Fig. 26. Transmitted Myeloid Leukemia of Strain Aka 51. Spinal canal, showing subdural and epidural infiltration by myeloid cells which caused compression of cord. The leptomeninges are at the upper part of the picture. × 100.

Figs. 27–29. Spontaneous Leukemia of Mouse Aka 482. Fig. 27. High-power view of liver, showing numerous mature myeloid cells. × 800. Fig. 28. Replacement of spleen pulp by round cells. Several mitotic figures. × 650. Fig. 29. Liver: Conspicuous perivascular infiltration by myeloid cells. × 100.
uncertain whether these cells were lymphoblasts or myeloblasts. Erythroblasts and megakaryocytes were rare, but eosinophils were present in moderate numbers. There was an extensive subendothelial myeloid infiltration in the larger veins, which narrowed the lumen considerably. The blood in the larger vessels contained many large round cells and immature myeloid cells. The spleen was largely replaced by myeloid cells and only a few small atrophic follicles remained. Most of the myeloid cells were mature granulocytes, but there were many young myeloid cells with numerous mitotic figures about the trabeculae and in and beneath the capsule. There were large numbers of megakaryocytes and erythroblasts among the myeloid cells. Among the definite myeloid cells were numerous large round cells similar to those in the liver. The lymph nodes showed complete destruction of the normal architecture by an extensive infiltration of large round cells, among which were many immature myeloid cells. The capillaries and large vessels of the lung were densely packed with these round cells and young myeloid cells. Immature myeloid cells filled the bone marrow. Most cells were myelocytes, but large round cells were also numerous.

Briefly, the mouse with the transmitted disease showed, histologically, extensive myelogenesis in the liver, spleen, lymph node and pancreas, with the characteristics of both non-malignant and malignant myelopoiesis. In several areas in the spleen and throughout one lymph node there were cells indistinguishable from malignant lymphocytes. The contrast between these two types of infiltration is shown in Figs. 27 and 28.

These alterations in the organs may be interpreted as follows. It is possible that there was a mixed myeloid and lymphoid leukemia, inasmuch as one lymph node was infiltrated by cells indistinguishable from those in lymphomatosis, while the spleen, liver and bone marrow were diffusely invaded by vast numbers of myeloid cells. It is also possible that the myeloid alterations were those of non-malignant extramedullary myelopoiesis coincident with lymphomatosis. This interpretation is strengthened by the preponderance of mature myeloid cells. It may be that the large cells with round nuclei were myeloblasts and the leukemia was entirely myeloid.

We believe that mouse Aka 482 probably had lymphomatosis with non-malignant extramedullary myelopoiesis secondary to that condition or to an unidentified infection. In many cases of spontaneous lymphomatosis in mice we have observed leukocytosis estimated at from 15,000 to 50,000 cells with a preponderance of metamyelocytes and polymorphonuclear leukocytes. This case of transmitted lymphomatosis was associated with granulocytic leukocytosis and non-malignant myelogenesis in the spleen, liver, and pancreas. These changes may be analogous to the increase of myeloid cells in the blood and tissues that has been observed in association with transmissible sarcomata of mice by Parsons (11, 12) and Lewis (24, 25) and with transmissible leukemia by Rask-Nielsen (27, 28).

**Myeloid Leukemia and Non-Malignant Extramedullary Myelopoiesis in Relation to X-rays and Breast Tumors**

The coincidence of cancer with leukemia is rare in man and in our own experience it is uncommon also in mice. In our stock Ak, for example, the incidence of leukemia is now approximately 70 per cent but other neoplasms are almost unknown. The reverse is true for our stocks Af and Ar: most adult females of these stocks develop breast tumors, but leukemia is rare.
LEUKEMIA AND MYELOPOIESIS IN MICE

TABLE VII: Non-Malignant Extramedullary Myelopoiesis in Mice with Breast Tumors

<table>
<thead>
<tr>
<th>No. of Mouse</th>
<th>Dose of X-rays (30 r weekly)</th>
<th>Period of Observation</th>
<th>Size of</th>
<th>Enlarge-ment of</th>
<th>Accessory Findings</th>
<th>Degree of Myelopoiesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumor</td>
<td>Spleen</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(mm.)</td>
<td>(mm.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9552</td>
<td></td>
<td>9 days</td>
<td>6 X 6</td>
<td>30 X 7</td>
<td>Slight</td>
<td>Ulcer in groin</td>
</tr>
<tr>
<td>A9553</td>
<td></td>
<td>25 days</td>
<td>20 X 10 X 10</td>
<td>23 X 5</td>
<td>Tumor ulcated; ulcates in groin</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>A9555</td>
<td></td>
<td>25 days</td>
<td>17 X 10</td>
<td></td>
<td>-</td>
<td>Pneumonia with abscesses</td>
</tr>
<tr>
<td>A9557</td>
<td></td>
<td>21 days</td>
<td>6 X 6</td>
<td>25 X 7</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>A9559</td>
<td></td>
<td>36 days</td>
<td>8 X 5</td>
<td>Not enl.</td>
<td>Moderate</td>
<td>Tumor infected</td>
</tr>
<tr>
<td>A9560</td>
<td></td>
<td>32 days</td>
<td>5 X 5</td>
<td></td>
<td></td>
<td>Slight</td>
</tr>
<tr>
<td>A9561</td>
<td></td>
<td>54 days</td>
<td>40 X 30 X 30</td>
<td>Not enl.</td>
<td>None</td>
<td>Lung tumor and sarcosporidiosis</td>
</tr>
<tr>
<td>A9599</td>
<td></td>
<td>33 days</td>
<td>10 X 10 X 10</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9600</td>
<td></td>
<td>5 days</td>
<td>20 X 15 X 10</td>
<td>26 X 7</td>
<td>Slight</td>
<td>Hemorrhages in lung</td>
</tr>
<tr>
<td>Arb172</td>
<td></td>
<td>25 mos.</td>
<td>8 X 8</td>
<td>20 X 6</td>
<td>-</td>
<td>Moderate</td>
</tr>
<tr>
<td>Arb164</td>
<td></td>
<td>18 mos.</td>
<td>10 X 10</td>
<td>20 X 5</td>
<td>-</td>
<td>Slight</td>
</tr>
<tr>
<td>Arb201</td>
<td></td>
<td>16 mos.</td>
<td>10 X 10</td>
<td>20 X 6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arb197</td>
<td></td>
<td>8 mos.</td>
<td>30 X 25 X 20</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rsc2</td>
<td></td>
<td>26 mos.</td>
<td>10 X 8 X 2</td>
<td>25 X 6</td>
<td>Slight</td>
<td>Bladder calculi; ovarian tumor</td>
</tr>
<tr>
<td>Slb160</td>
<td></td>
<td>10 mos.</td>
<td>12 X 12 X 4</td>
<td>28 X 7</td>
<td>Slight</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>Irradiated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A9564</td>
<td>150 r</td>
<td>22 days</td>
<td>20 X 15 X 1</td>
<td>18 X 5</td>
<td>Slight</td>
<td>Sarcosporidiosis</td>
</tr>
<tr>
<td>A9565</td>
<td>150 r</td>
<td>20 days</td>
<td>10 X 10 X 10</td>
<td>20 X 5</td>
<td>-</td>
<td>Three breast tumors</td>
</tr>
<tr>
<td>A9569</td>
<td>200 r</td>
<td>27 days</td>
<td>10 X 10 X 10</td>
<td>20 X 4</td>
<td>None</td>
<td>Tapeworm cyst in liver</td>
</tr>
<tr>
<td>A9572</td>
<td>350 r</td>
<td>47 days</td>
<td>14 X 10</td>
<td>28 X 5</td>
<td>Slight</td>
<td>Tapeworm cyst in liver and abscesses of neck</td>
</tr>
<tr>
<td>A9573</td>
<td>300 r</td>
<td>39 days</td>
<td>5 X 5</td>
<td>18 X 4</td>
<td>None</td>
<td>Severe diarrhea</td>
</tr>
<tr>
<td>A9576</td>
<td>150 r</td>
<td>18 days</td>
<td>30 X 10 X 15</td>
<td>25 X 5</td>
<td>Moderate</td>
<td>Tapeworm cyst in liver</td>
</tr>
<tr>
<td>Afb165</td>
<td>150 r</td>
<td>18 mos.</td>
<td>7 X 5</td>
<td>25 X 7</td>
<td>None</td>
<td>-</td>
</tr>
<tr>
<td>Afb187</td>
<td>350 r</td>
<td>17 mos.</td>
<td>10 X 10 X 3</td>
<td>25 X 5</td>
<td>Slight</td>
<td>Volvulus</td>
</tr>
<tr>
<td>Afb208</td>
<td>200 r</td>
<td>16 mos.</td>
<td>20 X 20</td>
<td>Not enl.</td>
<td>None</td>
<td>Necrosis in tumor</td>
</tr>
<tr>
<td>Afb237</td>
<td>300 r</td>
<td>12 mos.</td>
<td>28 X 15 X 1</td>
<td>28 X 7</td>
<td>-</td>
<td>Necrosis in tumor</td>
</tr>
<tr>
<td>Afb241</td>
<td>200 r</td>
<td>13 mos.</td>
<td>1 X 1 X 1</td>
<td>20 X 5</td>
<td>Slight</td>
<td>Necrosis of cervical nodes</td>
</tr>
<tr>
<td>Arc68</td>
<td>450 r</td>
<td>22 mos.</td>
<td>1 X 1 X 1</td>
<td>20 X 5</td>
<td>Slight</td>
<td>Lung tumor</td>
</tr>
</tbody>
</table>

In stock S both leukemia and cancer are moderately frequent, but seldom occur together in the same mouse.

Emile-Weil and Bousser (1) state that the coexistence of cancer and leukemia is frequently noted in mice. Hueper found that leukemia occurred in 19 per cent of the non-irradiated mice bearing breast tumors in a stock from which our Ak, Ar and Af mice originated (5). He reported that when these mice with spontaneous breast tumors were exposed to small doses of x-rays (30 or 80 r once a week over a period up to six weeks) the incidence of myeloid leukemia increased. Very large doses (250 to 2300 r at intervals of from three to seven days) had the reverse effect. Therapeutic irradiation of man is not known to produce leukemia, but x-rays play a role in the occurrence of leukemia among radiologists (30). An increase in the incidence of myeloid leukemia in mice that had received a single massive dose of x-rays over the entire body has been described in previous communications (8,14). In irradiated mice leukemia occurred after a very long period of incubation, approximately eight months.

It seemed advisable to repeat the study on the effect of general irradiation on the blood-forming organs of mice with spontaneous mammary carcinoma. Our experiments, performed four years ago, failed to confirm Hueper’s results and are now presented because we have found no reports that confirm or cast doubt upon the conclusions drawn from the work cited (5).

Tumor bearing mice of the same stock (“A”) from which Hueper obtained his animals were used, as well as mice from our own stocks. Irradiation consisted of 50 r units administered at weekly intervals until the death of the
There were 45 mice with spontaneous breast tumors in the irradiated group; 25 similar tumor-bearing mice were controls. In none of these mice, either in the control or in the irradiated group, did leukemia of any type occur, but non-malignant extramedullary myelopoiesis was common in both groups. Table VII indicates the degree of myelopoiesis in spleen and liver of twelve irradiated and fifteen control mice, in most of which these organs were enlarged. Microscopic examination was made in all cases.

Mice A 9552 to A 9600, inclusive, were of the stock from which Hueper obtained his animals. The degree of non-malignant extramedullary myelogenesis is indicated by the following symbols: 0 = none; + = slight; ++ = moderate; +++ = extensive; ++++ = very extensive. All mice died or were killed in extremis.

None of the tumor-bearing mice irradiated with 50 r at weekly intervals developed leukemia. They lived for from one to nine weeks (average about three weeks) after irradiation had been commenced. This period is less than that usually required for production of leukemia by x-rays (14).

Non-malignant extramedullary myelopoiesis was found to occur in the spleen and liver of these tumor-bearing mice, as reported by Hueper, but irradiation produced no increase in the extent of extramedullary myelopoiesis within the period of this experiment. On the contrary, in the livers of the irradiated mice of this series, myeloid foci generally were smaller and less numerous than in the livers of the non-irradiated controls.

DISCUSSION

Attention has been called in this communication to the frequent occurrence of extramedullary blood formation in older mice. This is often associated with infection and with neoplasms that may show extensive necrosis but many times is present without evident cause. The mouse has little reserve marrow, and when the demand for myeloid cells is great the spleen and liver participate in myelogenesis. Whether this myelogenesis takes its origin in these organs (myeloid metaplasia) or originates in primitive cells that are carried to the liver and spleen by the blood stream (colonization) has been discussed for decades. The study of our material does not aid in solving this problem. While we have not observed the transformation of fixed cells of the liver and spleen into blood cells, we cannot deny this possibility.

The causes of seemingly spontaneous non-malignant extramedullary myelogenesis require further investigation. It is noteworthy that it is frequent in one of our stocks of mice (Rf) but relatively rare in two others (A and S). Knowledge of non-malignant extramedullary myelopoiesis is essential in the study of neoplastic changes of blood-forming organs, the two disturbances being often confused. Numerous earlier articles describing the experimental production of leukemia by various agents may have dealt with non-malignant extramedullary myelogenesis (cf. 2). The leukemia-like conditions described recently in association with neoplasms produced by cancerogenic chemicals appear to be non-malignant extramedullary myelopoiesis (11, 12, 24).

In characteristic cases myeloid leukemia can be readily distinguished from non-malignant extramedullary myelopoiesis. A review of many cases of mye-
Leukemia and myelopoiesis in mice

Loid leukemia and of non-malignant extramedullary myelopoiesis suggests the following criteria as aids in distinguishing between malignant and non-malignant myelopoiesis.

**Myeloid Leukemia**
- Most myeloid cells are immature
- Erythrogenic foci are absent among myeloid cells
- Megakaryocytes are few and present only in the organs (spleen, liver, and lymph nodes) where they are found in non-leukemic conditions
- Myeloid cells often invade muscle and other non-hematopoietic tissues
- Blood usually contains immature myeloid cells
- Liver is usually enlarged and gray-brown
- Most of the lymph nodes are usually enlarged
- Hemorrhages are frequent in viscera (lungs, lymph nodes, etc.)
- Transmissible to other mice
- Not shown to be produced by bacteria

**Non-Malignant Extramedullary Myelopoiesis**
- All stages in the development of myeloid cells are present
- Erythrogenic foci are usually present
- Megakaryocytes are usually numerous
- Cells are non-invasive
- Blood is either normal or there is leukocytosis with numerous mature forms
- Liver is usually not enlarged and is brown-red
- Most of the lymph nodes are usually of normal size
- Hemorrhagic manifestations are absent
- Not yet shown to be transmissible
- Can be produced by bacteria

There are cases, however, in which even after gross and microscopic study of the organs no definite conclusions can be drawn as to the character of the myeloid disturbance. The unrestricted multiplication of cells in a new host indicates a neoplastic disease and numerous studies support the view that leukemia is a neoplastic disturbance (1, 6, 7, 8, 9). Transmission experiments may be of value in distinguishing between leukemic and non-leukemic myeloid changes, provided closely inbred related mice are used. Failure to transmit a neoplastic disease under such conditions is not likely, although it may occur.

One reason already noted for the occasional difficulty in distinguishing myeloid leukemia from non-malignant extramedullary myelopoiesis is the frequent concurrence of these conditions, usually in the spleen or less frequently in the spleen and liver. If a cellular suspension of the spleen or liver of a mouse with leukemia be injected subcutaneously into a related mouse the resulting tumor is composed of the malignant myelocytes typical of the strain. The tumor is free from erythrogenic cells, megakaryocytes and adult polymorphonuclear leukocytes. This observation indicates that the erythrogenic foci, megakaryocytes and adult leukocytes that may be present in the liver and spleen of leukemic animals, are not an integral part of the process but result from a secondary disturbance such as occurs in mice with tumors (23, 24).

**Summary and Conclusions**

Eight transmissible strains of myeloid leukemia that have been studied possessed characteristics which, with rare exceptions, remained unaltered in the course of successive subpassages.

Transmission experiments indicated the neoplastic nature of these disturb-
ances. The malignant cells in five cases were myelocytes, in three cases myeloblasts maturing into promyelocytes or myelocytes. Although morphologically the individual cells of these leukemias with one exception (Strain Ar 117) resembled normal cells, they differed from the latter as well as from one another in their behavior in inoculation experiments. These differences included ability to produce tumors, color of the leukemic infiltration, localization in various tissues, and transmissibility to different stocks of mice. The myelocytes characteristic of one strain underwent mitotic division in tissue cultures, and, like malignant cells, failed to mature.

In six additional cases of myeloid leukemia attempted transmissions to mice that were not highly inbred were unsuccessful.

The infiltrations in non-malignant extramedullary myelopoiesis in mice may be as extensive as those in myeloid leukemia. Non-malignant extramedullary myelopoiesis is frequent in apparently healthy old mice of one of the stocks studied. It often accompanies suppurative inflammations, particularly those of long standing, and spontaneous and transmitted neoplasms. The differentiation of non-malignant extramedullary myelopoiesis from leukemia is occasionally difficult. Features of the non-malignant disturbance include conspicuous maturation of myeloid cells, association with erythrogenesis, presence of megakaryocytes, absence of epicapsular and tumor-like infiltrations, and failure of transmission to other mice.

Parenteral administration of suspensions of B. coli is a simple procedure to stimulate extramedullary hematopoiesis in mice.

Exposure of mice with spontaneous breast tumors to small doses of x-rays did not produce myeloid leukemia, and failed to increase the extent of non-malignant extramedullary myelopoiesis.

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References