Myelogenous Leukemia in the Rat

By William C. Moloney, Andrew D. Dorr, Geraldine Dowd and Antonio E. Boschetti

The fundamental problems involving the etiology, pathogenesis and control of leukemia remain unsolved. In man, research has been greatly restricted by the rarity of the disease and the impossibility of carrying out adequate experiments. Because of biochemical, genetic and cytologic dissimilarities, objections have been raised to the use of data obtained from animal studies. Nevertheless, valuable information has been gathered from research on mice, especially in regard to the leukemogenic effect of radiation and chemical carcinogens, screening of various chemotherapeutic agents, and the virus transmission of leukemia.

While leukemia commonly occurs in some strains of mice and in fowl, it is a rare disease in the dog, rabbit, hamster and guinea pig. In the rat, leukemia was not reported until 1936, but in recent years it has been noted more frequently in this species. Myelogenous leukemia has been produced in the rat by chemical carcinogens, radioactivity and other agents. The chronic and subacute forms are easily identified cytologically, and in the rat the myeloid cells contain readily detectable enzyme activities. Moreover, chronic myelogenous leukemia in the rat has many similarities to the human disorder and the disease has other characteristics of special research interest.

In 1951, Shay et al. reported on the induction of chloroleukemia in the rat by the use of 20-methylcholanthrene (MCA) and the successful transfer of the disease to rat pups. Recently, Zipf and his co-workers produced a transplantable radiation-induced chloroleukemia in Sprague-Dawley rats. Both the MCA and radiation-induced disease have been carried on in subtransfer by a number of investigators. During the past three years in our laboratory the Shay chloroleukemia has undergone 23 transplants and more recently (since June 1960) the Mound Laboratory chloroleukemia has also been studied.

Results of observations on the factors influencing growth of these chloroleukemias and studies on the cytologic and enzymatic characteristics of the leukemic cells will be reported. However, transplants to non-inbred animals pose immunologic problems which invalidate observations on growth and other aspects of these neoplasms. Nevertheless, evidence will be presented that induced myelogenous leukemia in the rat offers a unique opportunity to investigate important aspects of leukemogenesis.

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METHODS AND MATERIALS

The chloroleukemia used in these studies was subtransferred from a Wistar rat furnished by Dr. Shay in February 1958. The donor rat had a large well-localized subcutaneous tumor with no evidence of leukemic cells in the peripheral blood. The tumor was greenish on cut surface and following crude homogenization, saline cell suspensions of varying concentrations were made up. Histologic sections and imprint smears of the cut surface were obtained; cells resuspended in rat serum were "pulled" on cover slips for cytologic and histochemical studies. For biochemical procedures, portions of the tumors were homogenated in the cold and extracted by various methods as noted in the experimental results.

The suspended cells, in the appropriate concentration, were used for intraperitoneal, intracardiac and subcutaneous injection. The numbers of cells, age of donor tumor, age of recipient and route of inoculation are indicated in the experimental results. Following initial experiences the subcutaneous route was used routinely for subtransfers unless otherwise specified. Similar methods were employed in the subtransfer experiments with the Mound Laboratory chloroleukemia obtained from Dr. H. Pott of the Argonne National Laboratory.

EXPERIMENTAL RESULTS

Effect of age of the recipient: During the past three years, 23 subtransfers have been carried out to over 700 non-inbred Wistar rats. In rat pups less than one week old, subtransfers were successful in nearly 90 per cent; with animals over a week old, including full-grown rats, 84 per cent were successful (table 1). However, there was much greater variability among older rats and takes ranged from 50 per cent to 100 per cent within individual litters. Leukemia developed much more commonly in younger rats and was unrelated to the presence or absence of leukemia in the donor animal. Regression of tumors was observed more frequently in older than in younger rats (15 per cent vs. 1.5 per cent). However, most rats in this study were sacrificed when the tumors were well grown for subtransfers or other purposes. In adult recipients not sacrificed for some months following successful chloroma transplant, regression occurred in a much higher percentage of animals. In more recent generations, as the chloroma cells have become more undifferentiated, the per cent of takes and incidence of leukemia increased while the regression rate decreased markedly (table 2).

Table 1.—Results of Subcutaneous Transfers of Rat Chloroma Cells (Shay) to 656 Non-Inbred Wistar Rats

<table>
<thead>
<tr>
<th>Total number of rats</th>
<th>Number of cells</th>
<th>Chloroma</th>
<th>Leukemia</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats under 1 week old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1x10⁵</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>5x10⁵</td>
<td>12</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>1-5x10⁶</td>
<td>16</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>191</td>
<td>&gt;5x10⁶</td>
<td>174 (90%)</td>
<td>119 (62%)</td>
<td>3 (1.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>236</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats over 1 week old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>1-5x10⁶</td>
<td>39</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>373</td>
<td>&gt;5x10⁶</td>
<td>315 (84%)</td>
<td>45 (12%)</td>
<td>56 (15%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>420</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A suspension of five to six million cells was used routinely in transplant experiments.*
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Table 2.—Observations on Results of Recent Subtransfers of Shay Chloroma Cells

<table>
<thead>
<tr>
<th>Generation</th>
<th>Total rats</th>
<th>Tumors</th>
<th>Leukemia</th>
<th>Regression</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>XVII</td>
<td>42</td>
<td>41</td>
<td>16</td>
<td>5</td>
<td>15-20 days</td>
</tr>
<tr>
<td>XVIII</td>
<td>32</td>
<td>32</td>
<td>13</td>
<td>3</td>
<td>11-17 days</td>
</tr>
<tr>
<td>XIX</td>
<td>32</td>
<td>32</td>
<td>23</td>
<td>0</td>
<td>15 days</td>
</tr>
<tr>
<td>XX</td>
<td>42</td>
<td>38</td>
<td>22</td>
<td>1</td>
<td>13-20 days</td>
</tr>
<tr>
<td>XXI</td>
<td>28</td>
<td>28</td>
<td>10</td>
<td>5*</td>
<td>9-17 days</td>
</tr>
<tr>
<td>XXII</td>
<td>24</td>
<td>24</td>
<td>8</td>
<td>0</td>
<td>14 days</td>
</tr>
</tbody>
</table>

*Older recipients used.
|Sacrificed early.

Effect of age of donor tumors: The per cent of successful takes was not significantly altered by the age of the tumor used and this was true whether the tumor was 17 or 175 days old. In the huge tumors found in older rats, necrotic changes were often noted, and this material was avoided when selecting cells for transfer or other purposes.

Time of onset of tumor growth: Following subcutaneous transplant the time of onset ("latent period") of tumor growth was variable in recipient rats of the same age group. Due to the immunologic problems associated with non-inbred rat strains, valid conclusions were not possible in these experiments. In general, however, there was a tendency on repeated subtransfers for a shortening of the latent period. At present, in the 23rd subtransfer, the period is about 15 days and this more aggressive behavior of the chloroleukemia is in keeping with the observations that the leukemic cells have become more undifferentiated (table 2).

Specific Factors Influencing Acceleration or Inhibition of Transplanted Chloromas

Earlier in these studies the effects of antitumor agents, such as 6-mercaptopurine and urethane, were investigated. Unsuccessful attempts were also made to evaluate the effect of serum and splenic extracts obtained from normal, regressed and tumor-bearing animals on growth of transplantable chloromas. At that time the role of immunologic differences between non-inbred Wistar rats was not fully appreciated, but it became obvious that the variable and often high degree of spontaneous remissions invalidated most of these experiments. It is of interest to note that following administration of splenic homogenates, recipient animals developed a condition which simulated "runt disease" described by Billingham.

Immunologic Investigations

Various serologic studies were carried out using suspensions of chloroma cells in serum of normal rats, rats resistant to chloroma transplants, rats with regressed tumors and chloroleukemic rats. No agglutination of chloroma cells could be demonstrated under conditions of varying temperature and time of incubation. Attempts to demonstrate incomplete antibody by use of serum suspended cells and anti-rat globulin (produced in the rabbit) were unsuccessful. Demonstration of heterologous antibodies by injection of whole
chloroma cells and homogenates with Freund’s adjuvant in the rabbit was also unsuccessful. In these studies the use of an anti-rabbit globulin did not demonstrate anti-chloroma cell antibodies.

The presence of rat chloroma antibodies was further investigated by the use of fluorescent anti-rabbit globulin technics. No antibody could be demonstrated on the chloroma cells under the U.V. microscope by this technic.

Cytologic, Histochemical and Biochemical Studies

Cytology: The characteristics of chloroma and leukemic myeloid cells in the peripheral blood have been the subject of special interest. The tumor cell preparations were obtained by imprints, smears and by the “paint brush” technic from the cut surface of the tumor. However, the cell preparations found to be the most suitable for cytologic and histochemical studies were made from homogenates of the tumor; the cells suspensions were resuspended in rat serum and “pulled” in the manner of blood smears on cover slips. Peripheral blood smears were prepared by the cover slip technic. The imprint and paint brush preparations were too thick and contained background material which interfered with staining and histochemical procedures. Cells from the tumor washed in saline and resuspended in serum retained excellent cytoarchitectural detail, but enzyme activity was diminished in some cells.

The Shay chloroma rats, obtained in February 1958, were in good general condition with large subcutaneous tumors. On Wright-Giemsa staining, the chloroma cells consisted of an immature population of promyelocytes with a small proportion of neutrophilic myelocytes and myeloblasts. The cells were large, round with a deep blue, opaque cytoplasm. Numerous and comparatively fine, reddish-purple granules were scattered diffusely throughout the cytoplasm and over the nucleus in some cells. The nucleus was large with somewhat spongy, loosely arranged chromatin which stained a deep purple; the nuclei contained one or more large nucleoli. A striking feature was the absence of eosinophilic and basophilic granulocytes so commonly encountered in human subacute and chronic myelogenous leukemia. Moreover, in the numerous studies carried out, no Auer rods were found in these leukemic cells nor were nuclear anomalies of the Pelger-Hüet type observed. When leukemia developed, small numbers of promyelocytes first appeared in the peripheral blood. These cells contained a rhomboid-shaped nucleus with relatively light-staining chromatin. The cytoplasm was not as deep blue as the chloroma cell and granules tended to be fine and less numerous. While some rats developed typically high white cell counts, in most instances only a slight to moderate leukocytosis was noted. In later generations the leukemic myeloid cells in the peripheral blood resembled the chloroma cells more closely.

With each subtransfer the chloroma and peripheral blood cells were studied serially. During this period there was a gradual change in cell characteristics, mainly due to loss of granules with an increasing myeloblast population. In many blast cells the nucleus seemed to become more condensed with a reduction of the nuclear-cytoplasmic ratio. The chromatin, instead of developing a fine spongy pattern, appeared to knot, giving rise to a nucleus which looked somewhat like that of a megaloblast. In recent generations the population has
Fig. 1.—Cover slip preparation of chloroma cells suspended in rat serum and stained with Wright-Giemsa (reduced from x 450).

consisted of nearly all early promyelocytes and myeloblasts (fig. 1). Another characteristic feature, noted both in the tumor and peripheral blood cells, has been the presence of pseudopodia. Frequently round cell-like bodies measuring about 3 to 5 μ in diameter were noted in the smears. These bodies contained opaque blue cytoplasm and often had dark purple and sometimes bright red granules. In many of these cells there were unusual looking nuclear fragments of a dark-staining homogenous chromatin. Often the nuclear chromatin appeared to be undergoing further karyorrhexis with several nuclear fragments remaining in the globule. These cell-like bodies were identified as myeloid elements, probably “pinched off” from the myeloblasts, and this was confirmed by the fact that histochemically, they gave positive stains for peroxidase, alkaline phosphatase and esterase activity.

Histochemical Studies

Alkaline Phosphatase (A.P.): The cytoplasm of granulocytic leukocytes in the rat contain a large amount of A.P. activity. Unlike human granulocytes, this enzyme is also present in immature myelocytic cells found in rat bone marrow. In 1957 Hollingsworth, working with the Shay chloroma, noted that in the chloroleukemic cells, marked A.P. activity was present. This finding has been repeatedly confirmed in our laboratory by histochemical and biochemical methods. In the rat, leukemic myelocytes and promyelocytes demonstrate strong A.P. activity, while in man even the segmented polymorphonuclear leukocytes in chronic myelogenous leukemia are practically devoid of this enzyme.

In the first rats used in this study, the chloroma cells were mainly promyelocytes. These chloroleukemic cells, except for the most immature myeloblasts,
Table 3.—Observations on Cytology and Histochemistry of Recent Shay Chloroma Transplants

<table>
<thead>
<tr>
<th>Generation</th>
<th>Cytology</th>
<th>Histochemistry</th>
<th>Red fluorescence</th>
<th>Green color</th>
</tr>
</thead>
<tbody>
<tr>
<td>XVII</td>
<td>promyelocytes and myeloblasts</td>
<td>4+ weak</td>
<td>weak</td>
<td>marked</td>
</tr>
<tr>
<td>XVIII</td>
<td>promyelocytes and myeloblasts</td>
<td>4+ 4+ weak</td>
<td>marked</td>
<td>present</td>
</tr>
<tr>
<td>XIX</td>
<td>increased myeloblasts</td>
<td>4+ 4+ rare cell</td>
<td>marked</td>
<td>present</td>
</tr>
<tr>
<td>XX A*</td>
<td>promyelocytes and myeloblasts</td>
<td>4+ 4+ rare cell</td>
<td>marked</td>
<td>present</td>
</tr>
<tr>
<td>XX C</td>
<td>myeloblasts</td>
<td>1+ 1+ rare cell</td>
<td>1+ marked</td>
<td>present</td>
</tr>
<tr>
<td>XX</td>
<td>promyelocytes and myeloblasts</td>
<td>4+ 4+ rare cell</td>
<td>marked</td>
<td>present</td>
</tr>
<tr>
<td>XXII</td>
<td>myeloblasts and promyelocytes</td>
<td>4+ 4+ rare cell</td>
<td>1+ marked</td>
<td>present</td>
</tr>
</tbody>
</table>


*Litter A rats were one month old at time of transplant; Litter C rats one week old.

showed strong cytoplasmic A.P. activity. In subtransfers this enzyme activity has persisted up to the present generation, and there has been little variation from litter to litter within each generation; biochemical determinations of A.P. activity on homogenated chloroma cells have substantiated these findings (table 3).

Peroxidase: Verdoperoxidase or myeloperoxidase (MPO), commonly found in the cytoplasm of human and animal granulocytes, was strongly present in most chloroleukemic cells. In these studies various histochemical methods were employed, but the Graham-Knoll technic,12 which permits the use of Giemsa counterstain, was found to give the most satisfactory results. Peroxidase activity was demonstrated by golden yellow, granular deposits in the cytoplasm; nearly all granulocytes, including most promyelocytes and some myeloblasts, were positive to this reaction. Peroxidase activity markedly decreased in dried smears, and after seven days the reaction was practically absent. In thick smears and imprints, peroxidase activity was especially intense. Chloroma cells resuspended in rat serum were strongly peroxidase positive; however, cells repeatedly washed in saline were often found to have diminished enzyme activity. In uncounterstained granulocytes, peroxidase activity was more obviously demonstrable than in stained smears.

Serial observations on chloroma cells and leukemic granulocytes for 14 subtransfers showed consistent and strong peroxidase activity. In subsequent generations peroxidase activity varied from litter to litter and, in general, the reaction was less strongly positive. In some litters of 18th and 19th generations, the peroxidase reaction was greatly diminished. However, in the 22nd generation, very strong peroxidase activity was present.

Esterase: Gomori in 1953 observed that granulocytic leukocytes contained a "specific" cytoplasmic enzyme capable of hydrolyzing a naphthol AS choloro-
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acetate substrate. Utilizing a recent modification for the synthesis of napthol ASD chloroacetate and with the use of the azo dye, Fast Garnett GBC, a highly chromogenic, substantive and well-localized staining was obtained at the site of esterase activity in myeloid cells. Gomori pointed out that in the blood this esterase activity occurred only in myeloid cells and was present in cells as immature as the promyelocyte, whether normal or leukemic. An extensive experience with this histochemical technic has confirmed Gomori’s observations in human leukocytes, and studies on rat chloroleukemic cells have shown that myeloid cells of all degrees of immaturity, except primitive myeloblasts, demonstrate strong esterase activity. Serial observations have been carried out over the past three years and in the most recent subtransfers, esterase activity has been greatly diminished. Beginning with generation 18, a few promyelocytes demonstrated red granular cytoplasmic staining, but the great majority of the cells were esterase negative.

Biochemical Studies

Porphyrrins: While biochemical methods for the extraction and identification of porphyrins were not employed in these studies, the tumors on fresh-cut surface retained a striking green color and, when exposed to U.V. light, continued to demonstrate the bright pink fluorescence indicating the presence of various porphyrins and other substances.

Peroxidase: Biochemical assay of homogenated chloromas, using the guaiacol procedure, showed intense myeloperoxidase activity. In recent generations of tumors, histochemical methods indicated that the peroxidase activity was greatly diminished while biochemical assays continued to demonstrate a high degree of myeloperoxidase activity.

Alkaline phosphatase: Quantitative assays for alkaline phosphatase content of homogenated chloroma tissue amply confirmed the extraordinary intense activity of this enzyme demonstrated by histochemical technic. To date, serial studies on all generations of chloroma transplants have continued to show undiminished alkaline phosphatase activity. Utilizing this exceptionally rich source of alkaline phosphatase and employing butanol fractionation and continuous flow electrophoresis for partial purification, studies have been carried out on various characteristics of this enzyme. The results of these experiments will be the subject of another report.

Investigations on the Zipf-Miller Chloroleukemia

In addition to the Shay chloroma, similar studies were undertaken on a radiation-induced chloroleukemia occurring in non-inbred Sprague-Dawley rats obtained in June 1960. The rats bore large subcutaneous tumors and represented about the 37th generation of subtransplants carried on at first by the Mound Laboratory and later at Argonne Laboratory. The initial tumor-bearing rats and six subtransfer generations to Sprague-Dawley pups have been studied cytologically and histochemically. The tumors on cut surface were beige rather than green, and did not show fluorescence when exposed to U.V. light except for rare, minute focal areas in some cases. The cells were mainly of a peculiar myeloblast type and looked very much like those of the
more immature Shay chloroma cells. Histochemical studies revealed a complete lack of alkaline phosphatase and peroxidase activity. However, esterase was strongly present in a large percentage of the cells, appearing as bright red granules within the cytoplasm. Quantitative assays for myeloperoxidase and alkaline phosphatase confirmed the complete lack of these enzymes in the myeloblastic tumors.

Zipf and his co-workers have described growth and other characteristics of the radiation-induced chloroleukemia emphasizing the similarities between this tumor and the Shay chloroma. From their report, until 1958, or 4 years after induction, this chloroma still possessed the green color, strong peroxidase activity and red fluorescence under U.V. light. However, our recent observations make it apparent that this line of the tumor has undergone cytologic, enzymatic and biochemical changes during the past two years. On the other hand the Shay chloroma, maintained since 1950 by subtransfers, has become less differentiated but to date has retained the green color and red fluorescence as well as peroxidase and alkaline phosphatase activity.

**Comments and Discussion**

The results obtained in these studies are in general agreement with those of other investigators. It is apparent that in non-inbred rats the major factors influencing growth of subcutaneous chloroma transplants are (1) age of recipient, (2) number of donor cells, (3) degree of immaturity of donor cells, (4) route of administration. Although humoral antibodies were not detected in our studies, it seems obvious that immunologic mechanisms were involved in the rejection of transplants, regression of chloromas, localization of tumors and failure of chloromas to progress to leukemias. With repeated subtransfers, the cells became less differentiated and the disease more aggressive. Whether these neoplastic cells lost their "immune recognition signals" or achieved complete autonomy by release from maturation or other regulating influences remains to be determined.

Shay and his co-workers have stated that chloroleukemia in the rat "is almost indistinguishable from chronic myelogenous leukemia in man." In Shay's original animal with leukemia, following administration of 20 MCA, the published hematologic data supported this diagnosis. However, the cytologic picture in the subtransferred disease was typical of acute and subacute myelogenous leukemia; this has been repeatedly confirmed in our studies. Various investigators, on the assumption that the disease was closely similar to human chronic myelogenous leukemia, have attempted to use this preparation for chemotherapeutic trials. These experiments provide no support for the reported similarity between **transplanted chloroleukemia** in the rat and **chronic myelogenous leukemia** in man.

A more fundamental and important difficulty, emphasized by Kim et al., arises from the immunologic differences which greatly limit the investigational usefulness of chloroleukemia transplanted to non-inbred rats. If the disease could be induced in an inbred strain of rats and subtransferred to litter mates, an opportunity would be provided for the study of growth, enzyme activities and other features of chloroleukemia uncomplicated by immunologic consider-
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exceptions. Nevertheless, transfer of cells which have already undergone neoplastic metamorphosis will not furnish a situation which is analogous to spontaneously occurring or induced leukemia in man or animals.

In spite of the above criticisms, experience in this study and a review of the literature offer convincing evidence that myelogenous leukemia in the rat may be an important preparation for research in the field of leukemia. Since 1936 there have been only 15 reports on the spontaneous or induced occurrence of myelogenous leukemia in the rat, and the low incidence of the disease in common laboratory strains has been well documented. However, the disease may be overlooked since chronic myelogenous leukemia is found only in older rats and frequently experimental animals are sacrificed at an early age. Moreover, unless abnormalities in the peripheral blood, enlarged spleen and liver, or green-colored organs or tumors are noted at autopsy, the disease may go undetected. It is also apparent that myelogenous leukemia occurs more often in some strains than in others; to date it has not been reported as a spontaneously occurring disease in Wistar or Sprague-Dawley rats. On the other hand, Wilens and Sproul found a remarkable incidence of myelogenous leukemia in a colony of old Osborne-Mendel rats. Among 365 autopsied animals they identified 11 cases of myelogenous leukemia and 36 cases of myeloid metaplasia. These animals were used in nutritional experiments, but no relationship between the type of diet and the blood dyscrasia was noted. Since the rats were subject to a number of pyogenic infections the authors were of the opinion that this may have played a role in the development of the myeloproliferative disorder. Other instances of spontaneously occurring myelogenous leukemia were reported by Rask-Nielsen, Oberling et al. and Arai (table 4).

The first induced myelogenous leukemia was reported in 1937 by Gennero and Grazio. They observed one case among 15 rats whose skin was painted with benzpyrene in benzene. Myelogenous leukemia was also produced by Ito in one rat among 20 fed o-aminoozotoluene followed by methylene blue. In 1951 Shay et al. reported the induction of myelogenous leukemia in non-inbred Wistar rats fed 20MCA. Subsequently, in a series of papers, Shay and his associates published observations on the successful subtransfer of this chloroleukemia to rat pups along with studies on the growth and other characteristics of these leukemic cells. Hlovayova administered 800 r total body radiation to 20 Wistar rats and then fed the animals MCA. In this experiment two rats developed myelogenous leukemia. More recently Hartmann and his co-workers reported that 48 per cent of 116 rats fed 2-acetylaminophenanthrene developed leukemia. One animal had a blood picture of chronic myelogenous leukemia and several others at autopsy were found to have green-colored infiltration of the thymus or lymph nodes. Ikeda fed 74 adult rats 2-acetylaminophenanthrene and leukemia developed in 16 animals. Of these, two were acute and two aleukemic leukemias. Twelve were chronic myelogenous leukemias with typical peripheral blood findings. Leukemia was more common in females than males following 2 AAP, and this was also noted by Hartmann et al.

In 1959, Kim working in J. Furth's laboratory, induced chronic myelogenous
Table 4.—The Reported Occurrence of Spontaneous and Induced Myelogenous Leukemia in the Rat

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference</th>
<th>Strain</th>
<th>No. rats</th>
<th>No. leukemia</th>
<th>Method of induction</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilens, S. L., and Sproul, E. E.</td>
<td>Am. J. Path. 12: 249-253, 1936</td>
<td>Osborne-Mendel</td>
<td>365</td>
<td>11</td>
<td>spontaneous</td>
<td>autopsied old rats; also 56 cases of myeloid metaplasia</td>
</tr>
<tr>
<td>Rask-Nielsen, R.</td>
<td>Acta path. et microbiol. scandinav. 15: 285, 1938, also 36 cases of myeloid metaplasia</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Arai, M.</td>
<td>Gann 34: 137-143, 1940</td>
<td>not stated</td>
<td>500</td>
<td>1</td>
<td>spontaneous</td>
<td>age not stated</td>
</tr>
<tr>
<td>Gennero, A. de, and Grasic, A. di</td>
<td>Hematologia 14: 107, 1937</td>
<td>not stated</td>
<td>15</td>
<td>1</td>
<td>benzpyrene in benzene to skin</td>
<td>first report</td>
</tr>
<tr>
<td>Ito, S.</td>
<td>Gann 34: 133-137, 1940</td>
<td>not stated</td>
<td>20</td>
<td>1</td>
<td>6-aminoacotholene with methylene blue p.o.</td>
<td>series of articles on chloroleukemia transplants</td>
</tr>
<tr>
<td>Shay, H., et al.</td>
<td>Cancer Res. 11: 29-34, 1951</td>
<td>Wistar</td>
<td>999</td>
<td>3</td>
<td>2B-methylcholanthrene p.o.</td>
<td>series of articles on chloroleukemia transplants</td>
</tr>
<tr>
<td>Hartmann, H. A., et al.</td>
<td>Cancer Res. 19: 210-216, 1959</td>
<td>Holtsman (S-D)</td>
<td>160</td>
<td>1</td>
<td>2-acetylaminophenanthrene p.o.</td>
<td>also green infiltration of thymus and lymph nodes in five rats at autopsy</td>
</tr>
<tr>
<td>Ikeda, Y.</td>
<td>Osaka M. J. 11: 267-268, 1959</td>
<td>?</td>
<td>74</td>
<td>16</td>
<td>2-acetylaminophenanthrene</td>
<td>twelve with typical CML peripheral blood</td>
</tr>
<tr>
<td>Kim, U., and Furth, J.</td>
<td>Fischer (inbred)</td>
<td>Fischer</td>
<td>19</td>
<td>1</td>
<td>thyroidectomy and 3MCA</td>
<td>peripheral blood typical of CML</td>
</tr>
<tr>
<td>Kim, U., and Furth, J.</td>
<td>to be published</td>
<td>Wistar-Furth (inbred)</td>
<td>7</td>
<td>2</td>
<td>3MCA</td>
<td>peripheral blood typical of CML</td>
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leukemia in one of 10 inbred female Fischer rats following thyroidectomy and administration of 3MCA.31 Smears of the peripheral blood and imprints taken of the liver and spleen at autopsy were kindly provided to us by Dr. Kim. The peripheral blood smear showed a greatly elevated leukocyte count with the cells consisting of segmented neutrophils, neutrophilic metamyelocytes and myelocytes; there were relatively few promyelocytes and blast cells (fig. 2). The organ imprints showed a more immature myeloid population, but prac-
tically all cells, except the most primitive, gave strongly positive histochemical stains for alkaline phosphatase and esterase activity. The peroxidase reaction was faint to negative, but the preparations were too old for accurate testing. Kim, using fresh smears, found the cells to be strongly peroxidase positive. Unfortunately, this leukemia was not transplanted. Subsequently Kim was able to induce two additional cases of myeloid leukemia in inbred male Wistar-Furth rats treated with 3MCA without thyroidectomy. Stained smears from one of these animals showed a picture identical to that of the leukemic Fischer rat.

In 1958 Hlavayova and Sveg produced myelogenous leukemia in three of 10 Wistar rats following splenectomy and inoculation of a cell-free filtrate from a rat carcinoma. One leukemic rat also demonstrated a Pelger-Huet nuclear anomaly. (This is the first report of Pelger-Huet anomaly in the rat). This rat was sacrificed and cell suspensions from various organs were used for subtransplants to eight infant rats. Chloroleukemia with Pelger-Huet anomaly developed in two recipients. The relationship between the Pelger-Huet anomaly and myelogenous leukemia is obscure; nevertheless an acquired form of this anomaly has been described by several authors in human patients with acute and chronic myelogenous leukemia as well as in cases of non-leukemic myeloproliferative disorders. In man and in rabbits no connection between the familial heterozygous Pelger-Huet anomaly and myelogenous leukemia has been noted. However, in view of the well-established genetic characteristics it is reasonable to assume that some somatic mutagenic mechanism may be involved in the acquired form of this anomaly. The recent discovery of a “minute” autosome in patients with chronic myelogenous
leukemia lends further emphasis to the genetic aspects of myeloproliferative disorders.\textsuperscript{35,36}

The leukemogenic effect of ionizing radiation has been well established. Following radiation exposure, leukemia in man has been of the acute variety or chronic granulocytic. It is of interest that Zipf and his co-workers discovered two cases of myelogenous leukemia among several hundred Sprague-Dawley rats given actinium 227. The evidence that this leukemia was radiation-induced is circumstantial and based on only one experiment. Nevertheless, as noted previously, spontaneous myelogenous leukemia has not been reported in the Sprague-Dawley strain and two cases in "several hundred" rats represents a relatively high incidence of a rare disease. At any rate, if myelogenous leukemia can be consistently produced by ionizing radiation in the rat, it would provide an important approach to the study of leukemogenesis.

Recent developments in cytogenetics and improved methods of in vitro cell culture have provided new information on the characteristics of human chromosomes. A number of chromosomal abnormalities have been described in acute leukemia and in CML. Nowell and Hungerford have noted a consistent autosomal defect which has been confirmed by other investigators.\textsuperscript{35} Baikie and his co-workers ascribe the presence of this "minute" chromosome to a deletion of a portion of one of the autosome 21 pair by somatic mutation.\textsuperscript{36} Because of the associations of mongolism with leukemia and trisomy of autosome 21, these authors advance the interesting hypothesis that this chromosome may be the site of a hematogenic locus. In man, investigation of the cytogenetic aspects of leukemia is handicapped by the rarity of the disease and the lack of a practical experimental approach. However, studies have already been undertaken and chromosome changes noted in myeloid cells of spondylitic patients following x-ray therapy.\textsuperscript{37} In this regard a remarkable (and as yet unconfirmed) observation has been reported by Iwakashi and Setsuda.\textsuperscript{37} These Japanese investigators fed 4-aminopteroyl glutamic acid to adult rats and noted preleukemic changes in the blood of 3rd-generation offspring with three cases of myelogenous leukemia appearing in the fourth and one case in the fifth generation. The implications of this extraordinary report, if substantiated, are obvious. While chromosome changes noted in human CML have not been reported in the rat to date, similar defects may be demonstrable. Myelogenous leukemia, induced in the rat, represents a unique opportunity to evaluate the role of mutagenic agents in leukemogenesis.

In this laboratory the reported high incidence of myelogenous leukemia and myeloid metaplasia in old Osborne-Mendel rats is under investigation. Studies are also being carried out on the leukemogenic effect of ionizing radiation, MCA and other agents in non-inbred and inbred rats. In addition to cytologic, histochemical and histologic procedures, these animals will be followed by cytogenetic studies on the myeloid cells of the peripheral blood and bone marrow.

**Summary and Conclusions**

Observations on growth and other characteristics of subcutaneously transplanted chloroleukemia in non-inbred rats have been described in this report.
MYELOGENOUS LEUKEMIA IN THE RAT

This chloroleukemia cytologically is an acute or subacute, not chronic, myelogenous leukemia. Because of serious immunologic differences, the experimental usefulness of subtransferred chloroleukemia is greatly limited. However, myelogenous leukemia in the rat, induced by chemical agents or ionizing radiation, may offer a valuable approach to the study of leukemogenic mechanisms.

SUMMARIO IN INTERLINGUA

Es reportate observationes in re le crescentia e altere characteristicas de subcutaneemente transplantate chloroleucemia in rattos ab parentes non consanguinee. In terminos cytologic iste chloroleucemia es un subacute (non chronic) leucemia myelogene. A causa de serie differentias immunologic, le utilitate experimental de subtransferite chloroleucemia es grandemente restringite. Tamen, leucemia myelogene in le ratto, inducite per agents chimic o per radiation ionisante, offere possibilemente un preciose methodologia pro le studio de mechanismos leucemogenic.

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Myelogenous Leukemia in the Rat

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