Observations on Leukemia in Wistar and Wistar Furth Rats

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

This investigation is concerned with studies on leukemia occurring in a series of noninbred Wistar and inbred Wistar Furth (W/Fu) rats treated with whole body x-ray and oral administration of 3 methylcholanthrene (3MCA). The animals were followed by serial leukocyte counts and examination of peripheral blood smears. At the time of sacrifice coverslip smears were obtained from the peripheral blood, bone marrow, spleen, liver, lymph nodes and other tissues for cytologic and histochemical studies. Observations were also made on chromosome preparations obtained from the bone marrow of leukemic rats. All rats were followed until sacrificed or natural death and autopsies were carried out on all leukemic rats and on 87 per cent of the entire series of animals. Results of these studies are presented and discussed in this report.

METHODS AND MATERIALS

A total of 673 male and female rats were used in these experiments: 341 noninbred Wistar and 332 inbred W/Fu rats. The animals were maintained 2 in a cage and fed a Purina Laboratory chow diet. At the age of 3 to 4 months 161 Wistar and 145 W/Fu rats were exposed to a single total body dose of x-ray. The factors were: 250 Kvp., 15 ma., thorax 1 filter (0.25 m; 0.25 Cu; 1 Al) 50 cm. TSO, HVL 2.00 mm. Cu, TD 430 rads. Rate 30-32 rads per minute. One hundred and twenty Wistar and a similar number of W/Fu rats were fed 10 mg. of 3MCA in olive oil by stomach tube for a total dose of 180 mg. given over a 3-week period. Sixty Wistar and 67 W/Fu rats were first irradiated, allowed to recover and 3 months later given 180 mg. of 3MCA. All animals were examined and weighed at least once a week. Prior to treatment white blood cell counts and differentials were obtained from coverslip smears of tail blood, and these studies were repeated 4 to 7 days following x-ray and after the last dose of 3MCA. Subsequently leukocyte counts (using a Coulter Counter) and differentials were carried out at 4-week intervals. If a rat developed leukocytosis or abnormal cells were noted in the smear, or if the animals became sick, white blood cell counts and examination of the stained blood smears were done as frequently as indicated. Wright stained smears were

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Fig. 1.—Histogram showing number and age at death of W/Fu rats and rats with leukemia.

carefully examined for presence of abnormal cells and, as an additional aid to identification, histochemical methods for detection of leukocyte alkaline phosphatase (LAP), verdo peroxidase (VPO) and esterase were employed.\(^1\)

Rats were sacrificed by ether anaesthesia before they reached a terminal stage in order to obtain adequate whole blood specimens and fresh tissues for histologic, cytologic and histochemical preparations. Grossly the size and weight of organs and presence of greenish-colored tissues and tumors were especially noted. If green-colored tumors were discovered, immediate exposure to Wood light was carried out for detection of fluorescence characteristic of chloromas. Tissues were generally fixed in 10 per cent unbuffered formalin solution and stained with hematoxylin and eosin. Imprint and paintbrush smears were made on coverslips from the bone marrow, spleen, liver, enlarged lymph nodes and tumors if present. The smears were air dried and stained with Wright and Giemsa stains; histochemical studies were also carried out on these preparations.

Chromosome preparations were obtained directly from the bone marrow or spleen employing modifications of earlier methods described by Nowell and Hungerford.\(^2\) Also, the system advocated by Hungerford and Nowell\(^3\) was used for classification of the rat chromosomes (figs. 3 and 4).

**EXPERIMENTAL RESULTS**

Rats were followed from the time of treatment until natural death or sacrifice. In 583 autopsies there were 28 leukemias among 403 benign and malignant tumors in 302 animals. The largest number of tumors were of mammary gland origin but there were many subcutaneous, epithelial, renal and pancreatic neoplasms as well as a variety of other tumors (table 1). Since a number of animals died within a few months following x-ray or 3MCA, mainly from pulmonary infections, the incidence of leukemia might have been higher had these animals survived long enough to develop the disease. Deaths of animals with leukemias and the nonleukemic deaths are plotted in 4-month units in figure 1 and 2.
OBSERVATIONS ON LEUKEMIA

The largest number of leukemias in the noninbred Wistars occurred following radiation alone (table 1). With the exception of two lymphatic cases, all the leukemias in the Wistar rats were of the granulocytic type (table 2). Even in the acute form of the disease the leukemic myeloid cells could be readily identified by the nature of the cytoplasmic granules, the character of the chromatin and usually the presence of a positive peroxidase reaction. In the subacute and chronic cases the granulocytes were better differentiated and the cells were positive not only for VPO but also for LAP and esterase. Another feature of leukemia in the Wistar rats was the occurrence of 5 chloromas among 9 granulocytic cases. These leukemic rats demonstrated marked infiltration of the bone marrow, spleen, liver and other tissues. This was especially noted in paintbrush and imprint smears stained with Wright and Giemsa stains; histologic studies confirmed these findings in autopsied animals. One rat (No. 470) presented the unusual picture of subacute granulocytic leukemia of the basophilic type. In both Wistar and W/Fu rats the combined x-ray and 3MCA treatment proved to be toxic resulting in many early deaths; a number of these animals died before the time leukemia or tumors would have developed.

In contrast to the Wistar strain, leukemia in the W/Fu rats was more difficult to classify and presented a number of dissimilar features. Only two leukemias appeared following radiation; one an acute lymphatic with a greatly elevated leukocyte count and typical leukemic infiltration of the spleen, liver, bone marrow and lymph nodes. In the other animal the leukemia was similar to those cases occurring in 3MCA-treated W/Fu rats. In both breeds of rats the duration of the disease was relatively short lasting a few days to several weeks. The course was rapidly progressive in the acute lymphatic and most blast and mononuclear cell leukemias while the disease was more prolonged in the animals with subacute and chronic granu-
Table 1.—Tabulation of Tumors and Leukemias in a Series of Wistar and W/Fu Rats

<table>
<thead>
<tr>
<th>AGENT</th>
<th>BREED</th>
<th>TOTAL NO. RATS</th>
<th>RATS AUTOPSIED</th>
<th>RATS WITH TUMORS</th>
<th>LEUKEMIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
</tr>
<tr>
<td>X-RAY</td>
<td>W</td>
<td>161</td>
<td>140</td>
<td>87.0</td>
<td>75</td>
</tr>
<tr>
<td>3MCA</td>
<td>W</td>
<td>120</td>
<td>104</td>
<td>86.7</td>
<td>75</td>
</tr>
<tr>
<td>X-RAY</td>
<td>W/Fu</td>
<td>67</td>
<td>45</td>
<td>67.2</td>
<td>25</td>
</tr>
<tr>
<td>TOTALS</td>
<td></td>
<td>341</td>
<td>303</td>
<td>88.9</td>
<td>190</td>
</tr>
<tr>
<td>X-RAY</td>
<td>W/Fu</td>
<td>145</td>
<td>134</td>
<td>90.2</td>
<td>54</td>
</tr>
<tr>
<td>3MCA</td>
<td>W/Fu</td>
<td>120</td>
<td>101</td>
<td>84.2</td>
<td>33</td>
</tr>
<tr>
<td>TOTALS</td>
<td></td>
<td>332</td>
<td>280</td>
<td>84.3</td>
<td>112</td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td></td>
<td>673</td>
<td>583</td>
<td>86.0</td>
<td>302</td>
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</table>

Table 2.—Classification of Leukemias in Wistar and W/Fu Rats

<table>
<thead>
<tr>
<th>AGENT</th>
<th>BREED</th>
<th>BLAST CELL</th>
<th>ALL</th>
<th>AML</th>
<th>AGL</th>
<th>SAGL</th>
<th>CGL</th>
<th>TOTAL</th>
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<tr>
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<td>WISTAR</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>3MCA</td>
<td>WISTAR</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>X-RAY</td>
<td>W/Fu</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
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<tr>
<td>TOTAL</td>
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<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>X-RAY</td>
<td>W/Fu</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<td>2</td>
</tr>
<tr>
<td>3MCA</td>
<td>W/Fu</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>TOTAL</td>
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<td>5</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17</td>
<td>28</td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td></td>
<td>5</td>
<td>3</td>
<td>11</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>28</td>
</tr>
</tbody>
</table>

ALL = ACUTE LYMPHATIC
AML = ACUTE MONONUCLEAR CELL
AGL = ACUTE GRANULOCYTIC
SAGL = SUBACUTE GRANULOCYTIC
CGL = CHRONIC GRANULOCYTIC

Lycytic leukemia. However, since animals were sacrificed before clinical deterioration was too far advanced, estimates on duration of the disease are somewhat invalidated in this study.

The largest number of leukemias appeared in the 3MCA-treated W/Fu rats; these were all of the acute variety but the leukocyte counts were only moderately elevated ranging from 11,800 to 67,500 per cu. mm. Clinically,
the rats often appeared listless and bleeding from the nose and around the eyes was a common finding. Associated with the leukemia, hemolytic anemia was frequently present in the terminal stage of the disease and was characterized by marked polychromasia, normoblastemia, and a striking erythroid hyperplasia in the bone marrow. The gross findings at autopsy revealed moderate splenomegaly, little or no hepatomegaly and enlargement of the
peripancreatic and mesenteric lymph nodes. Thymic enlargement and infiltration in the kidneys was noted in some animals. However, no chloromas were found and in only a few leukemic W/Fu rats were there associated benign or malignant tumors. A high incidence of pulmonary infections was a common finding and a frequent cause of death.

Considerable difficulty was experienced in classifying the leukemic cells noted in the W/Fu animals. In the 3MCA-treated rats 4 were designated acute blast cell and 9 acute mononuclear cell leukemia (table 2). Both groups presented many features in common and differed only in the presence of absence of granules or esterase activity in the cytoplasm of the leukemic cell. The peripheral blood contained from 30 per cent to 70 per cent large mononuclear cells exhibiting a bean-shaped, indented or somewhat segmented nucleus. The purplish chromatin varied from a smooth homogeneous lymphoid type to a more spongy blast-cell-like nucleus and one or two large indistinct nucleoli were often present. In many cells the clear, light blue cytoplasm contained scattered reddish colored granules. In some cells the granules were very large and bead-like; in others they were finer and resembled those found in promyelocytes (figs. 6 and 7). Histochemical stains showed in 9 of 13 cases one plus to two plus VPO or slight to moderate esterase activity in the cytoplasm. This type of reaction resembles the histochemical staining of human monocytes in contrast to the coarse and strongly positive staining found in rat and human granulocytes. Leukemic infiltration was noted in the spleen, liver, and lymph nodes whereas the bone marrow usually contained surprisingly few of these cells. Postmortem histologic studies confirmed the lack of marrow replacement and the marked infiltration in the spleen. Characteristically, the liver was slightly to moderately involved with a variable periportal and sinusoidal infiltrate.

*Leukemoid reactions:* Following x-ray and 3MCA administration, infections were commonly encountered and over 50 per cent of the autopsied
Fig. 6.—Acute mononuclear cell leukemia in the W/Fu rat. Peripheral blood smear showing population of cells with cytoplasmic granules. Note presence of nucleated red blood cells.

Fig. 7.—Leukemic mononuclear cells in peripheral blood smears showing cytoplasmic granules and characteristic nuclei.
Table 3.—Chromosome Studies on Direct Marrow Preparations from Leukemic Rats

<table>
<thead>
<tr>
<th>RAT NO</th>
<th>AGENT USED</th>
<th>TYPE OF LEUKEMIA</th>
<th>BREED</th>
<th>TOTAL CELL COUNT</th>
<th>CHROMOSOME COUNTS</th>
<th>MORPHOLOGIC ABNORMALITIES</th>
<th>BONE MARROW INFILTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>566</td>
<td>X-RAY</td>
<td>AGL</td>
<td>W</td>
<td>93</td>
<td>2</td>
<td>67</td>
<td>NONE</td>
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<tr>
<td>452</td>
<td>X-RAY</td>
<td>AGL</td>
<td>W</td>
<td>58</td>
<td>1</td>
<td>57</td>
<td>NONE</td>
</tr>
<tr>
<td>466</td>
<td>X-RAY</td>
<td>AGL</td>
<td>W</td>
<td>18</td>
<td>61</td>
<td>131</td>
<td>NONE</td>
</tr>
<tr>
<td>459</td>
<td>X-RAY</td>
<td>AGL</td>
<td>W</td>
<td>50</td>
<td>1</td>
<td>61</td>
<td>NONE</td>
</tr>
<tr>
<td>451</td>
<td>X-RAY</td>
<td>SAGL</td>
<td>W</td>
<td>59</td>
<td>43</td>
<td>44-46</td>
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</tr>
<tr>
<td>470</td>
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<td>SAGL</td>
<td>W</td>
<td>29</td>
<td>28</td>
<td>28</td>
<td>NONE</td>
</tr>
<tr>
<td>859</td>
<td>3MCA AC BLAST</td>
<td>W/Fu</td>
<td>W</td>
<td>13</td>
<td>1</td>
<td>12</td>
<td>NONE</td>
</tr>
<tr>
<td>826</td>
<td>3MCA AML</td>
<td>W/Fu</td>
<td>W</td>
<td>50</td>
<td>12</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
<td>860</td>
<td>3MCA AML</td>
<td>W/Fu</td>
<td>W</td>
<td>12</td>
<td>2</td>
<td>16</td>
<td>NONE</td>
</tr>
<tr>
<td>609</td>
<td>X-RAY</td>
<td>3MCA AML</td>
<td>W/Fu</td>
<td>26</td>
<td>1</td>
<td>24</td>
<td>YES</td>
</tr>
<tr>
<td>826</td>
<td>3MCA AML</td>
<td>W/Fu</td>
<td>W</td>
<td>50</td>
<td>12</td>
<td>3</td>
<td>35</td>
</tr>
<tr>
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<td>3MCA AML</td>
<td>W/Fu</td>
<td>W</td>
<td>19</td>
<td>2</td>
<td>17</td>
<td>NONE</td>
</tr>
</tbody>
</table>

*Large abnormal chromosome present in 3 of 5 karyotypes.

Rats were found to have benign or malignant tumors. Among the autopsied animals there were 23 leukemoid reactions with leukocyte counts ranging from 30,000 to 100,000 per cu. mm. Severe pulmonary infections and necrotic tumors were the major causes of leukemoid reactions which were of the granulocytic type in 18 of 23 animals. In 5 rats the cells were lymphoid or mononuclear in character and many contained coarse to moderately fine granules. At postmortem, 4 of these rats had various kinds of tumors but no evidence of leukemia. One animal with a leukocyte count of 37,000 per cu. mm. and a large per cent of granule-containing mononuclear cells was considered to be leukemic on the basis of hematological studies. However, at autopsy the rat had bronchopneumonia and splenitis but no evidence of leukemic infiltration was found.

Chromosome studies: While adequate metaphase preparations may be obtained by 4-day cultures of peripheral blood leukocytes from normal rats, in leukemic animals this method was almost uniformly unsuccessful. Satisfactory metaphase plates were obtained from direct bone marrow preparations in 6 Wistar rats with granulocytic leukemia. While aneuploidy was present in 1 case, no morphologic abnormalities of the chromosomes were observed in these animals (table 3). In 6 leukemic W/Fu rats satisfactory metaphase plates were obtained and significant aneuploidy was noted in 2 animals whereas in 1 rat (609) a large abnormal "marker" chromosome was found in 14 per cent of the metaphases counted (fig. 5). It should be pointed out that while the bone marrow was almost completely replaced with leukemic granulocytes in the Wistar rats, the W/Fu rats with few exceptions showed only a small number of leukemic cells infiltrating the bone marrow. Hemolytic anemia with erythroid hyperplasia in the marrow was a common feature of leukemia in the W/Fu rat, and most of the metaphase plates were
probably from red-cell precursors; this undoubtedly accounted for the low incidence of chromosome abnormalities noted in the W/Fu rats in this series.

**Discussion**

Spontaneous leukemia rarely occurs in the rat. However, in 1936 Wilens and Sproul described 11 cases of leukemia in a colony of 365 old inbred Osborne Mendel rats and more recently Furth reported 6 cases of leukemia occurring in 66 inbred old W/Fu rats. In 1962, Schreiner and Will described 3 cases of chloroleukemia in a colony of adult Wistar rats, and these authors carried out successful transplants of the disease.

Leukemia is not readily induced in the rat, and there are only a few reports of this disease following exposure to radiation. Hlavayova noted that 2 cases of myeloid leukemia developed in 20 Wistar rats treated with 800 r. total body x-ray and later given 3MCA. Since it is well known that methylcholanthrene is leukemogenic in rats, leukemia in Hlavayova's animals cannot unequivocally be attributed to x-ray. In 1959 Zipf et al. found among several hundred Sprague-Dawley rats treated with radioactive actinium 227 2 animals with chloroleukemia. Since spontaneous leukemia is extremely rare in Sprague-Dawley rats, these 2 cases were considered to be radiation-induced.

Hunstein et al. have extensively investigated the effects of fractionated whole body radiation of Wistar rats. They describe a variety of hyperplastic and neoplastic changes in the hematopoietic and reticuloendothelial tissues. In these experiments, leukemia occurred in 2.27 per cent of the animals and the authors discuss the difficulties, not only in classifying leukemias histologically, but also in differentiating between benign and malignant changes. In view of the low incidence of spontaneous leukemia among Wistar rats it is noteworthy that in our studies 8 leukemias occurred in 140 autopsied rats following a single exposure to 450 r. total body exposure.

Various chemical agents have been reported to produce leukemia in the rat. Shay in 1950 induced 6 lymphatic and 2 myeloid leukemias in a large series of Wistar rats fed 20 MCA by gastric tube. Recently, Nowell, et al. reported the results of chromosome studies in 9 myeloid and three lymphatic leukemias induced by 20 MCA in Wistar rats. In our studies, 2 granulocytic leukemias were found in 104 3MCA-treated Wistar rats (table 1). Studies by Hartmann et al. on the leukemogenic effect of 2-acetylaminophenanthrene (2AAP) are of particular interest. These authors noted that among 117 2 AAP-treated albino rats, 56 developed extensive blast-cell infiltration of the spleen, liver, bone marrow and other organs. In only 3 animals was the peripheral blood picture diagnostic of leukemia but green chloromatous tissue was found at autopsy in 5 animals. Hartmann et al. considered it impossible, except in the peripheral blood, to distinguish lymphoblasts from myeloblasts in these animals.

Leukemias in the W/Fu rats were found to be markedly different in cell-type and other features from those occurring in the Wistar strain. The
average age at death of the leukemic 3MCA-treated W/Fu rats was 19.8 months. In contrast leukemia occurred earlier in the Wistar strain with age at death averaging 12.4 months. Only two leukemias, one acute lymphatic and the other acute blast-cell, developed in irradiated W/Fu rats in this series. Among these animals approximately a 10 per cent incidence of spontaneous leukemia should have been anticipated; possibly the x-ray exposure prevented the occurrence of the disease in these W/Fu rats.

Although the largest number of leukemias occurred in the 3MCA-treated W/Fu rats, the occurrence of 13 leukemias among 101 autopsied animals probably does not represent a significant increase due to the leukemogenic affect of 3MCA. As noted above, Furth found 6 cases of leukemia in 66 untreated W/Fu rats and in our control series of W/Fu rats presently under observation, we have encountered 11 leukemias among 100 untreated rats; 95 of these animals are dead and 5 are still under observation. All 13 leukemias in the 3MCA-treated W/Fu rats were similar in most respects and were classified as blast-cell or mononuclear-cell type; the blast-cell type contained no granules and lacked esterase activity in the cytoplasm. The striking red granules found in most of these leukemic cells was a distinctive and unique feature which proved of value in studies on bone marrow smears and organ imprints. Under Wright stain the granules clearly distinguished the presence of leukemic-cell infiltration, and this was of special assistance in detection of invasion in organs such as the spleen, lymph nodes and thymus gland.

One of the main objectives of this study was the investigation of the early phase of leukemia in an attempt to discover whether the disease starts in a single locus and subsequently disseminates. The so-called latent period (from the time of treatment with the leukemogenic agent to development of obvious leukemia) is very prolonged in the rat. During the later part of the latent period the distinctive granule-containing mononuclear cells begin to appear in the peripheral blood smears. While large lymphocytes containing coarse cytoplasmic granules are noted in some nonleukemic rats, these occur in small numbers and are not found infiltrating organs at autopsy. In some rats the mononuclear cells were observed to increase with a rising leukocyte count but later the abnormal cells disappeared and no evidence of leukemia was noted at autopsy. Five animals with significant leukocytosis and a large per cent of mononuclear cells were sacrificed and 4 were found to have tumors or infections. In 1 animal postmortem examination provided no adequate explanation for the mononuclear leukocytosis. These observations suggest that leukemic mononuclear cells may escape from localized colonies, perhaps arising and proliferating in the spleen or lymph nodes. The leukemic cell line may wax and wane before finally predominating at the expense of normal cells, and it is possible that occasional leukemic stem lines fail to proliferate successfully, and the leukemia may spontaneously disappear. Observations are presently being carried out on x-ray treated Wistar, 3MCA-treated W/Fu and control W/Fu rats in an effort to shed further light on this aspect of the problem.
Considerable interest has been aroused in the role of chromosome abnormalities in leukemia. In rat leukemia no consistent morphologic or numerical abnormalities have been reported. Nowell et al.\(^2\) noted that 8 of 9 rats with chloroleukemia and 3 with ALL showed only minor numerical and no morphologic changes in the chromosomes. This has been our experience in the Wistar rats with myeloid leukemia.\(^4\) However, in subsequent studies on the W/Fu leukemias several rats with significant aneuploid and one animal (809) with a large “marker” chromosome have been encountered. In more recent investigations, employing the methods described by Nowell and Hungerford\(^2\) and utilizing the spleen rather than the bone marrow, much better metaphase preparations have been obtained. During observations on the karyotypes of 16 additional primary rat leukemias, 4 showed “marker” chromosomes and other morphological abnormalities.

As predicted by Furth\(^6\) some years ago, we have found that the inbred W/Fu rat provides unusual opportunities for studies on leukemogenesis. In our laboratory further investigations are in progress on the leukemogenic effect of whole body x-ray and orally administered 3MCA on Wistar and W/Fu rats. Moreover, a series of untreated W/Fu rats are being followed throughout their life span to establish the incidence of spontaneous leukemia and to carry out further observations on the hematology and pathology of leukemia in these inbred animals. In view of recent developments, special efforts are also being directed to the investigation of the viral and cytogenetic aspects of leukemogenesis in the rat.

**SUMMARY**

1. Six-hundred and seventy-three Wistar and W/Fu rats were treated at the age of 4 months with whole body x-ray, orally administered 3MCA or a combination of these agents. All animals were followed until death and 87 per cent were autopsied.

2. Among 303 autopsied animals there were 403 benign and malignant tumors, including 28 leukemias.

3. Differences were noted in the incidence and types of leukemia occurring in both breeds of rats. In Wistars 9 of 11 leukemias were myelogenous and 8 of these followed x-ray exposure.

4. Approximately a 10 per cent incidence of spontaneously occurring mononuclear cell leukemia was found in W/Fu rats; exposure to x-ray apparently decreased the occurrence of leukemia in these rodents. Following oral administration of 3MCA, 13 per cent of W/Fu rats developed acute mononuclear cell leukemia.

5. Results of hematologic, cytologic and histochemical studies on rat leukemia are discussed in this article, and the possible significance of the appearance and disappearance of the distinctive mononuclear cells in the peripheral blood is pointed out.

6. Pathologic findings indicate that the majority of deaths, other than those due to leukemia, were due to pulmonary infections and a great variety of tumors. In leukemic animals enlargement of the spleen was almost univer-
sally present and adenopathy, especially involving mediastinal and retroperitoneal glands, was frequently noted. Among 9 myelogenous leukemias in Wistar rats green chloromatous tissues were found in 5 animals.

7. Cytogenetic studies were carried out on direct preparations from the bone marrow in most of the leukemic rats in this series. Aneuploidy was noted in several animals and one W/Fu rat demonstrated a morphologically abnormal “marker” chromosome. In more recent studies improved technics and use of the spleen as a source of metaphase preparations have afforded better opportunities to obtain adequate chromosome preparations in leukemic rats. Further investigations are in progress and special studies are being directed to the viral and cytogenetic aspects of leukemia in the rat.

**Summario in Interlingua**

1. Sex centos septanta-tres rattos Wistar e Wistar/Furth esseva tractate al etate de 4 menses con roentgeno-irradiation del corpore total, con administratones oral de 3-methylcholanthreno, o un combination de iste duo agentes. Omne le animales esseva tenite sub observation usque a lor morte, e 87 pro cento de illos esseva necropsiata.

2. In le 303 necropsias, 403 benigne e maligne tumores esseva constatate, incluse 28 casos de leucemia.

3. Esseva notate differentias in le incidentia e le typos de leucemia inter le duo lineas de rattos. In rattos Wistar 9 de 11 casos de leucemia esseva myelogene, e 8 de istos sequeva le exposition a roentgeno-irradiation.

4. Un incidentia de approximativemente 10 pro cento de spontaneemente occurrente leucemia a cellulas mononuclear esseva censtatate in le rattos Wistar/Furth. Le exposition a roentgeno-irradiation pareva reducer le occurrence de leucemia in iste rodentes. Post le administration oral de 3-methylcholanthreno, 13 pro cento del rattos Wistar/Furth disveloppava acute leucemia a cellulas mononuclear.

5. Le resultatos de studios hematologic, cytologic, e histochimic in leucemia de rattos es discutite in le presente communication e le signification possibile del apparition e disparition del distinctive cellulas mononuclear in le sanguine peripheric es signalate.

6. Constatationes pathologic indica que le majoritate del mortes, non considerante illos causate per leucemia, esseva attribuibile a infectiones pulmonar e un grande varietate de tumores. In animales leucemic, allargamento del splen esseva notate quasi universalmente e adenopathia—afficienete particularmente glandulas mediastinal e retroperitoneae—esseva frequente. In un grupo de 9 rattos Wistar con leucemia myelogene, tissu chloromatose verde esseva inconstrate 5 vices.

7. Studios cytogenetic esseva effectuate in preparatos directe de medulla ossee in le majoritate del rattos in le presente serie. Aneuploidismo esseva notate in plure animales, e un ratto Wistar/Furth monstrava un morphologicamente anormal chromosoma—“marcator.” In plus recente studios, meliorate technicas e le uso de splen como fonte de preparatos metaphasic ha providite plus favorable ocasiones pro obtener adequate preparatos de chromosomes.
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in rattos leucemic. Investigaciones additional es in progresso, e studios special es orientate verso le aspectos cytogenetic e virusal de leucemia in le ratto.

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REFERENCES


Observations on Leukemia in Wistar and Wistar Furth Rats

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