Transfer of Myelogenous Leukemia Induced by Gastric Instillation of Methylcholanthrene in Wistar Rats

By HARRY SHAY, M.D. MARGOT GRUENSTEIN, CHARLES HARRIS, M.D.* AND LILLIAN GLAZER

Six cases of lymphatic and 2 of myelogenous leukemia developed in a group of random bred Wistar rats in which methylcholanthrene was instilled into the stomach.1 Attempts were made to transfer each type of leukemia to 30 to 40 day old rats of our colony with only indifferent success in lymphatic leukemia and complete failure to achieve any transfers in myelogenous leukemia.

Following the report of Gross,2 we used animals for transfer that were under 7 days of age and have been uniformly successful in transmitting the lymphatic leukemia to animals of our random bred Wistar colony.3 About a year ago another of our rats receiving methylcholanthrene by stomach catheter developed myelogenous leukemia. This has been transferred successfully to very young rats.

Material and Results

Three tissues, namely, spleen, liver and blood were used for transfer. The solid tissues were first passed through a special masher, which forced the tissue through a screen with perforations 1 mm. in diameter. The material so prepared was mixed thoroughly with an equal volume of normal saline and 0.2 cc. of this cell suspension was injected intraperitoneally for transfer.4 Transfer has also been attempted in a group of animals using the subcutaneous route. Best results for transfer of the myelogenous leukemia have thus far been obtained with whole blood. The donor animal is anesthetized with ether, the abdomen is opened and blood is drawn from the abdominal aorta with a syringe and 20 gauge needle which have been rinsed with heparin. Two-tenths cc. of the blood is immediately injected intraperitoneally for transfer. The peripheral white blood count of the donor animals at time of transfer has varied between 20,000 and 300,000.

Diagnosis of a successful transfer is first made by peripheral blood smear (fig. 1) and confirmed by bone marrow smear (fig. 2), and tissue sections (figs. 3 to 8). Although none of the animals with induced myelogenous leukemia showed green discoloration of the infiltrated tissues, all of the transfers developed as chloroleukemias. As a result, we are now able to make accurate diagnoses of successful transfers from the peripheral blood smear and the striking green color of the leukemic infiltration. The criteria for diagnosis of our original myelogenous leukemias have already been detailed.1 After correlating many autopsies with blood smears, and with the transferability of the same

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Fig. 1—Peripheral blood smear, myelogenous leukemia. 1000X

Fig. 2—Bone marrow smear. 1000X
Figure 3.—Liver, dark areas showing leukemic infiltration in portal areas. 5X

Figure 4.—High power view from area of infiltration in liver. 1000X
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Fig. 5. — Spleen. 1000X

Fig. 6. — Lymph node showing good examples of the large, plump-leucocyte cells. 1000X
Fig. 7.—Kidney cortex shows small areas of infiltration with leukemic cells. The dark area is perirenal fat densely infiltrated with leukemic cells. The adrenal is not infiltrated. 55X.

Fig. 8.—Section through portion of sternum also showing infiltration of adjacent muscle. 55X.
sample of blood, we feel that the presence of 1 per cent abnormal myelocytes in the peripheral blood smear in our transfers can be used as evidence for a successful "take" in our animals. By abnormal myelocytes we refer to those myelocytes which are not rounded but rather polygonal, often with processes resembling pseudopodia, which sometimes appear to separate from the cell-body. These cells are seen in cover slip preparations as well as in smears (fig. 1).

Pathologic findings at autopsy are signalized by the striking green color of all organs and tissues that are heavily infiltrated with leukemic cells. These tissues usually include the intra-abdominal fat, lymph nodes, thymus, bone marrow, portal areas of the liver, galea, scalp, and the dura covering the brain, and at the base of the skull, ribs and spinal column. Microscopically the cellular infiltrations are composed of small anaplastic cells differing from each other only slightly in size and staining characteristics. Ringform polys are scattered throughout but the majority of cells appear to be myelocytes. Also commonly found are large phagocytes, their cytoplasm filled with red staining material resembling red cell debris with the nucleus often pushed to one side (fig. 6).

Although the spleen invariably shows marked leukemic infiltration, it does not have any of the green color. Successful transfers by the intraperitoneal route result usually in leukemia alone and only occasionally does a local tumor develop as well. To date of 54 animals injected subcutaneously with 0.2 cc. of whole blood, 24 developed chloromata locally in one to nine weeks and 19 developed leukemia as well. Eight animals developed chloromata without leukemia and 3 developed leukemia but no local tumors.

The results of transfer with blood, liver and spleen in our random bred Wistar rats, as well as the random bred Long Evans and Sherman rats are shown in table 1. We have also back transferred the leukemia from positive Long Evans rats to our Wistar strain. In table 2 are listed the periods in weeks when successful transfer was first diagnosed by peripheral blood smear. While the percentage of transfers of the myelogenous leukemia has not reached the uniformity obtained with the lymphatic leukemia, it is nevertheless very high.

**Table 1.—Myelogenous Leukemia Transfers**

*All Animals Less than 7 Days Old at Transfer*

(Transfer intraperitoneally)

<table>
<thead>
<tr>
<th></th>
<th>Number of Animals Injected</th>
<th>Number of &quot;Takes&quot;</th>
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<tbody>
<tr>
<td><strong>Wistar strain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random-bred rats (own colony)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 cc. blood</td>
<td>130</td>
<td>103</td>
</tr>
<tr>
<td>0.2 cc. liver</td>
<td>51</td>
<td>28</td>
</tr>
<tr>
<td>0.2 cc. spleen emulsion</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td><strong>Long Evans strain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 cc. blood</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>0.2 cc. liver</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sherman strain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 cc. blood</td>
<td>18</td>
<td>13</td>
</tr>
</tbody>
</table>
Table 3 shows the number of successful transfers in our Wistar rats for each litter compared to the number of animals injected as well as the over-all percentage of successful transfers. The $T$ values indicate the generation of transfer, $T_1$ representing the first transfer from the originally induced leukemia. Some of the groups are small in number because of occasional deaths of injected animals from causes other than leukemia, but more often this is due to maternal cannibalism. Several months ago, investigators of the Research Division of Lederle Laboratories Division, American Cyanamid Company transferred the myelogenous leukemia from one of our animals to their Sherman random bred colony. In more than nearly two thousand animals they obtained successful

<table>
<thead>
<tr>
<th>Number of weeks after injection when animals became positive</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
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<th>15</th>
<th>16</th>
<th>18</th>
<th>19</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>1</td>
<td>9</td>
<td>7</td>
<td>6</td>
<td>25</td>
<td>17</td>
<td>10</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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To date 2-18-52.

Table 3.—Myelogenous Leukemia Transfers

All Animals Less than 7 Days Old at Transfer; Random Bred Wistar Strain

(0.2 cc. blood intraperitoneally)

<table>
<thead>
<tr>
<th></th>
<th>Number of Animals Injected</th>
<th>Number of &quot;Takes&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>T2</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>T3</td>
<td>32</td>
<td>24</td>
</tr>
<tr>
<td>T4</td>
<td>34</td>
<td>28</td>
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<tr>
<td>T5</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>T6</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>T7</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>103 = 79%</td>
</tr>
</tbody>
</table>

transfers in 76 per cent. Their untreated transfers have survived 4 to 25 days after the development of the leukemia. Of 36 transfers of our own group that have been allowed to die of the disease, 1 survived 57 days and 3 for 46 days after diagnosis of the leukemia was made, while the others fell within the 4 to 25 day life span.

The myelogenous leukemia is chronic in type. The total white cell count as a rule does not exceed 300,000 with the abnormal cells consisting largely of myelocytes. These have made up 10 to 30 per cent of the white blood cells, and only a relatively small number of myeloblasts (5 per cent) are present. We have been impressed with the fact that the readiness with which a transfer is accomplished is dependent upon the percentage of myelocytes in the donor blood rather than upon the total white count.
Discussion

The infrequency with which myelogenous leukemia has occurred spontaneously or has been transferred successfully in the rat, and the obvious usefulness of such a leukemia if readily transferable, have prompted this report. To the best of our knowledge, the 2 cases of myelogenous leukemia already reported and the third that developed since, are the first cases of myelogenous leukemia produced with carcinogenic hydrocarbons in rats. These animals not only showed the peripheral blood picture of a myelogenous leukemia but characteristic bone marrow and tissue infiltrations that have already been described for 2 of them. The third animal that developed myelogenous leukemia served as the donor from which the transfers were derived.

Successful transmission of typical leukemia in mammals was first accomplished by Snijders in 1926, when he transferred spontaneous lymphogenous leukemia in guinea pigs. In rats, leukemia was not reported until 1936, when Wilens and Sproul found 11 instances of myelogenous and 1 of lymphatic leukemia among 365 animals of an inbred strain of Osborne-Mendel albino rats. Green coloration of the leukemic infiltrations was observed in 4 of the 11 with myelogenous leukemia. Attempts to transmit the leukemia from 1 of the 4 failed.

In 1938 Hall and Knocke transferred chloroleukemia in mice and the following year Oberling, Guérin and Guérin first accomplished successful transfer in the rat. These investigators reported 6 cases of lymphogenous and 3 of myelogenous leukemia from autopsies on nearly 6,000 rats, whose exact strain was not stated. They inoculated subcutaneously, at 5 months of age, 12 rats from the same colony with fragments of liver and spleen and 8 with blood. All remained negative except one rat which died 11 months later with a leukemia and a subcutaneous myelocytoma at the site of transplant. This rat had been transplanted with fragments of liver and spleen. Ten days before death, the peripheral blood of this animal showed a leukocytosis with large numbers of young myeloid cells, characterized by large nuclei, nucleoli and a basophilic cytoplasm filled with fine granules. On autopsy, at the site of transplant two greenish tumors were found as well as a green colored zone along the spinal column, the sternum and over the cranial vault.

For the second passage, these investigators used 30 rats, 3 months of age from the same colony. Twenty were implanted subcutaneously with liver and spleen and 10 with fragments of the subcutaneous chloromas. Among the 10 rats, 3 developed small subcutaneous tumors as large as a small nut. From one of these, a third transplant was attempted. From this material Roussy, Guérin and Guérin were able to complete 6 transfers in all. In some 350 rats which they inoculated, 34 tumors developed until the deaths of the animals and 5 tumors regressed after having attained the volume of a small nut. The overall percentage of takes was low, approximately 10 per cent. In only 5 of the transfers was a true leukemia found.

The development of the additional case of myelogenous leukemia brings to a total of 9 (6 lymphatic and 3 myelogenous) the number of instances of leukemia that developed in our rats treated with methylcholanthrene by stomach catheter. To date 609 rats have received methylcholanthrene in this manner in our labora-
tory. Of these, 187 died or were sacrificed before the age of 6 months. Our ear-
liest case of leukemia developed in an animal 7 months of age. We have, there-
fore, chosen to consider the 9 cases of leukemia as probably representing the
development of leukemia in the remaining group of 422 animals. No instance
of spontaneous myelogenous leukemia has been reported in the Wistar rat.

Ratcliffe in a study of 468 rats selected for autopsy from the colony at the
Wistar Institute when suspected of having developed a tumor of any type,
found 1 lymphoblastoma of the mediastinum and 1 lymphosarcoma of the
mesenteric lymph nodes but makes no mention of any type of leukemia. In a
more recent study from the same institute in which 630 rats were autopsied
for a study of the aging process and in which blood counts were done, 2 in-
stances of lymphoid leukemia were reported. These represent the first 2 in-
stances of any type of spontaneous leukemia that has been reported in the Wistar
rat. Murphy and Sturm in 1941, observed the development of lymphatic
leukemia in one Wistar rat following the accidental injection of dibenzanthracene
into a lymph node of the animal.

If we compare the incidence of leukemia in our rats treated with methy-
cholanthrene with that previously reported in the rat, then the occurrence of
leukemia in our animals represents a 15 fold increase over that observed by
Oberling and his associates and at least a 7 fold increase over that reported by
Kesten in animals of the Wistar Institute colonies. It is, therefore, reasonable
to assume that the gastric instillation of methylcholanthrene probably stood
in a causal relationship to the development of the leukemias in our Wistar
rats, especially since 3 of them were myelogenous leukemia.

SUMMARY AND CONCLUSIONS

A third case of myelogenous leukemia that followed the gastric instillation
of methylcholanthrene in Wistar rats is herein recorded. These 3 cases represent
the first instances of myelogenous leukemia produced in rats by carcinogens
that answer all the criteria for a true myelogenous leukemia; namely, positive
peripheral blood picture, bone marrow, tissue infiltration and transferability.

A high percentage of transfer of the myelogenous leukemia was obtained with
peripheral blood given intraperitoneally to very young animals of our random
bred colony. Successful transfer was also accomplished to very young rats of
the Long Evans and Sherman strains. While none of the three animals in which
myelogenous leukemia was induced showed any green coloration, all transfers
developed as chloroleukemia. Animals with leukemia will be made available to
investigators.

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1 Personal communication.
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