Biochemical Investigations on a Transplantable Rat Leukemia*

I. Transplantability of Strains 302 and J

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SUMMARY

Two strains of lymphatic leukemia, J and 302, have been maintained through hundreds of successive intraperitoneal injections of spleen tissue from leukemic donors. The two strains appeared to be similar pathologically, but were chemically dissimilar. The J leukemia had white cell counts of 500,000–1,000,000 cu. mm. with enlarged spleen and no jaundice. The 302 strain had markedly enlarged liver, slightly enlarged spleen, and easily observable jaundice terminally. The ease of maintenance of the two strains of leukemia provides an opportunity for biochemical, metabolic, nutritional, and chemotherapeutic studies.

Major efforts to define the etiology of leukemia have been accomplished in the mouse, since the disease occurs spontaneously in this animal and because tissue fragments from a previously affected animal can be transmitted to mice of a genetically pure strain or into first-generation hybrids. The rat has also been used in studying leukoneogenesis but less frequently than the mouse. During the past 6 years it has been possible to develop and maintain, in noninbred rats, clinical signs and hematologic findings similar to those of human leukemia. The pathologic changes have been uniformly observed after several hundred transplants in two strains of rat leukemia, and the first paper in this series describes the characteristics of these rat leukemias. Our major interest has been to study the metabolic changes in leukemic rats, and other reports in this series will describe some of our studies on amino acid and carbohydrate metabolism.

MATERIALS AND METHODS

Strain 302.—The first donor of this strain was obtained from Shay1 who originally observed the leukemia after intragastric administration of methylcholanthrene (3). After anesthetizing the donor rat with ether, a portion of the excised spleen was weighed as rapidly as possible and placed in a hand tissue mincer which forced the tissue through a fine sieve by means of a plunger on a screw. Liver, blood, and kidney could also be used, but these gave less consistent results. An amount of physiological saline equal to twice the weight of the tissue was added to the homogenate, and approximately 0.1–0.2 ml. of the suspension was injected intraperitoneally through a No. 20 needle into recipient rats of known age, obtained from the Holtzman Company, Madison, Wis. Another piece of the spleen or other tissue was placed in 10 per cent formalin for subsequent histologic examination. All rats received a commercially pelleted feed that was nutritionally adequate for growth and reproduction.

Strain J.—This leukemia resulted from feeding 2-acetylamino-phenanthrene to rats and was first observed by Drs. Elizabeth and James Miller several months after the drug was discontinued. A preliminary description of the initial experiments has been published by Miller et al. (2), and the pathological findings have been presented by Hartmann et al. (Case No. 2, Table 2) (3). Our experiences with this strain and the changes observed after many transplantations will be described here. Blood and spleen from the donor rats were injected intraperitoneally into 2- or 7-
day-old rats in a manner similar to that described for 302 leukemia. The age of the recipient was increased from 2 to 14 days when it became necessary to use older rats in an attempt to prolong survival. Before an injection with spleen was made, a white cell count was usually made from tail blood. Successive transplants were always performed with spleens from rats with a white cell count of no less than 100,000 cu. mm. After the strain was stabilized it was not necessary to do blood counts prior to injections.

To obtain an evaluation of the cellular elements in liver and spleen, fresh imprints of tissues from both leukemic strains were made immediately after the animal was sacrificed. A portion of the cut tissue was blotted to absorb free blood, and the surface was gently applied several times to different areas of the slide in the usual fashion. After fixing, the slides were stained with Kingsley's solution.

### RESULTS

**Strain 302.**—The sequence of signs in 302 leukemia was: palpably enlarged spleen, enlarged liver, anemia followed by the rapid appearance of jaundice and staining of the genital area by highly concentrated bile substances in the urine, and death. After more than fifteen successive intraperitoneal injections the signs of the leukemia were constant, and the appearance of the jaundice served as a simple objective criterion of an animal with 302 leukemia. It was not unusual for these rats to die within 5–7 hours after jaundice was first detected, usually by the 7th or 8th day after the transplantation of the spleen homogenate. The age of the donor animal was relatively unimportant if signs of the leukemia had progressed to near maximum. Most animals used as donors were 28–35 days old, or approximately 7–10 days after the injection with spleen. The age of the recipient was more important for continued and reliable perpetuation of the strain. The spleen injection was made intraperitoneally into 7-, 10-, or 12-day-old rats in the early experiments with 80–100 per cent success. At any specific time, however, recipient rats could be no more than 3 days older than the previous recipients, or decreased incidence of leukemia resulted. After more than 100 transplant generations the percentage of animals with leukemia nearly always approximated 100 (Table 1); however, there were occasional animals in some litters that failed to show signs and symptoms of the disease, and these failures could not be ascribed to transplant procedures. Now that this strain has been used for nearly 4 years, the age of the recipient has been established at 21 days, and the average survival time after transplant is 7–8 days.

The type of tissue used for the transplant was important, and from evaluation of pathological sections it appeared that the amount of infiltration of leukemic cells into the respective tissue would determine how good this tissue would be for donor material. Spleen was better than liver, and blood too variable to rely on for satisfactory transplants. Successful leukemia was always obtained with spleen that weighed at least 1 per cent of the body weight. Large spleens were a reflection of the extent of the leukemic process, since this tissue enlarged because of infiltration with leukemic cells. In some attempts to prolong survival of donor tissue some spleens were stored in a deep freeze at $-20^\circ C$ before being homogenized and transplanted. The tissues were frozen within 20 minutes. Storage for as little as 4 hours after excision resulted in inactivity. Attempts to perpetuate the leukemia from spleen preserved in glycerol in the usual manner were not altogether satisfactory.

When blood or spleen tissue from 302 leukemia was inadvertently or purposely injected into the subcutaneous spaces, a solid tumor developed at the site of the injection. The tumor originated in the subcutaneous tissue as a circumscribed, discrete mass of pinkish hue, and was very firm when cut. It was possible to inject homogenates of this tumor intraperitoneally and recover typical 302 leukemia in rats. The capacity to convert this solid tumor to the leukemic phase has been demonstrated by Perlman.

Gross pathological findings at autopsy showed pallor and jaundice of all tissue, enlarged spleen, and mildly enlarged thymus. The most outstanding...
ing feature was the pale, markedly enlarged liver, the weight of which ranged between 10 and 12 per cent of the body weight. (Normal is approximately 3–5 per cent.)

The microscopic examination of the bone marrow revealed complete infiltrations with immature cells, mainly the lymphatic series. However, many cells could not unequivocally be so identified, since many granules were seen in the cytoplasm. The nuclei were more typical of the lymphatic series, however. The liver of leukemic rats was heavily infiltrated with immature cells, mainly around the central vein, and often the finger-like projections enveloped entire lobules. The spleen showed loss of Graffian follicles and loss of characteristic architecture, typical of most leukemias. The lungs were normal and were never infiltrated with leukemic cells. The kidneys showed invasion by abnormal cells, but this tissue was not usually as severely involved as the liver. Imprints of the spleen were used to identify abnormal cells. Typical photomicrographs are shown in Figures 1–6.

The peripheral white blood cell count was not grossly elevated in early experience with 302 leukemia, but in later and in most recent transplants white blood counts of 100,000 cu. mm. or more on the 6th day after injections were found. Occasionally immature white cells could be seen in the blood smear, together with nucleated red cells. The jaundice increased rapidly once the animal became anemic, and by chemical analysis of the blood was revealed to be mainly direct-reacting bilirubin. Several total bilirubin determinations revealed values between 5 and 8 mg/100 ml blood. Fragility tests on the red blood cells were not conclusive.

At the present time it is not possible to explain why a mature rat cannot be used as the recipient to perpetuate leukemia with early transplants. Our experience has shown that increasingly older rats need to be used as recipients in successive transplants; otherwise, death would occur too rapidly. This need to increase the age of susceptible recipients probably is due to an adaptation to the heterozygous host. It is possible that, with continued transplants, more compatible cells for the recipient are selected.

Strain J.—Signs of the developing disease were protruberant abdomen, enlarged spleen, labored respirations, anemia, and elevated white cell counts of 50,000 to 500,000 cu. mm. In the early experiments it was observed that the rats survived more than 14 days after weaning (Table 2). Later, in spite of increasing the age of the recipient, the life span was 11–14 days. After several years' experience the survival time stabilized so that 21-day-old recipients were all dead between 8 and 10 days after injection.

It was clear that the age of the donor was of little consequence if the animal was typically leukemic. The care and technic of the injections as well as the age of the recipient were more important. The tissue used for transplanting this strain presumably made little difference, since spleen, blood, or thymus was equally effective.

The gross pathological changes usually showed an enlarged thymus, enlarged pale liver, and a spleen that was grossly enlarged up to 2.5–4 per cent of the body weight. The lungs were usually hemorrhagic, and the kidneys were pale. Microscopic evaluation of these tissues showed that the bone marrow was hyperplastic, and the individual elements were immature. The impres-

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<th>Time</th>
<th>Age of recipient (days)</th>
<th>Incidence of leukemia (per cent)</th>
<th>Av. survival after injection (days)</th>
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<tr>
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<td>10</td>
<td>100</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>50</td>
<td>39</td>
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<td>14</td>
<td>100</td>
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</tr>
<tr>
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<td>21</td>
<td>100</td>
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<td>Aug., 1958</td>
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sion of predominating lymphocytes supports the thesis that the J strain is a lymphocytic leukemia (1). The spleen may be only mildly involved in typical J leukemia and in some sections follicles were still easily seen. When heavy infiltration occurred in the bone marrow, the spleen and other organs were also infiltrated as described by Hartmann et al. (1). Interestingly, despite the greatly elevated white cell counts of 500,000–750,000 cu. mm. the leukemic infiltration in some tissues was no greater than in animals whose count was 50,000. The length of survival of an animal with leukemia may have influenced the degree of infiltration, but our data are not extensive enough to make this correlation. In some animals studied, the ovaries and bronchi showed infiltrations, and in others the thymus was usually involved. Figures 1–6 shows typical imprints of rat liver and spleen from normal, J, and 302 leukemia. The immature cells are predominant in both the 302 and J liver and spleen.
DISCUSSION

The ability to perpetuate rat leukemias provides a further tool for nutritional, biochemical, and chemotherapeutic studies. The two strains described here demonstrate the contrast in the leukemias induced by two diverse carcinogens. Strain 302, originally induced with methylcholanthrene, can be readily transplanted to give the enlarged liver, mildly affected white cell count, enlarged spleen and jaundice in either young or older rats. The J strain carried through more than 100 transplants from its first occurrence following administration of 2-acetylaminophenanthrene in the diet showed grossly enlarged spleen and unusually high white cell count, and no jaundice. The dissimilarity in the clinical appearance of the two strains was not found in the pathological sections. The bone marrow in both instances had immature cells not completely defined but reminiscent of the lymphatic series. Whether the two drugs used to induce leukemia were able to express different manifestations of the disease chemically but similar influences on the bone marrow remains to be determined by future work. The details of the transplantation were strictly adhered to in order not to lose the strain. In spite of the great care, an occasional litter would not show leukemia in all animals.

ACKNOWLEDGMENTS

Excellent technical assistance was provided by Donna Paske, Maryland Erhart, and Marcella Krakowski.

REFERENCES


FIG. 1.—Normal rat liver imprint—Kingsley stain, X900. Note many dark stained normoblasts among white cell series.

FIG. 2.—302 leukemia liver imprint—Kingsley stain, X900. Many immature cells from lymphocyte series. Some normoblast cell series seen.

FIG. 3.—J leukemia liver imprint—Kingsley stain, X900. A variety of young and immature lymphocytes.

FIG. 4.—Normal rat spleen imprint—Kingsley stain, X900. Note many dark stained normoblasts among white cell series.

FIG. 5.—302 leukemia spleen imprint—Kingsley stain, X900. More uniform distribution of immature white cells.

FIG. 6.—J leukemia spleen imprint—Kingsley stain, X900. Monotonous closely packed immature lymphocytes. No red cell series seen.
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