

Uniform Transfer to Random Bred Rats of Lymphatic Leukemia Induced by Gastric Instillation of Methylcholanthrene. (18331)

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We have recently reported(1) the development of lymphatic leukemia in various members of our random bred colony of Wistar rats following the gastric instillation of methylcholanthrene. Initial efforts to transmit the leukemia to a group of rats, 32 to 44 days of age, met with only indifferent success. Recently Gross(2) reported the transmission of lymphatic leukemia from Ak mice to C₃H subline by using animals 1 to 7 days old. By employing rats in this age group, we not only obtained a uniform transfer to animals of our own colony, but to rats obtained from the current Wistar Institute colony and to random bred rats of the Sherman and Long-Evans strains. All of the transfers described in this report were accomplished by the intraperitoneal injection of a suspension of leukemic spleen cells.

Procedure. The spleen from a leukemic animal was put through a special masher* and then mixed thoroughly with an approximately equal volume of normal saline solution. Injections of 0.2 cc of this cell suspension per animal were used. Table I contains the total number of transfers made and thus far represents transmission of the disease through 14 generations. It also lists the groups of animals older than 7 days to which transfer of the leukemia was attempted. Similar to the results of Gross in mice, we found

a sharp decline in the number of successful transfers when animals past the age of 15 days were used.

Results. Among 18 rats from 32 to 44 days of age injected intraperitoneally with suspensions of spleen cells from animals which had developed leukemia after the gastric instillation of methylcholanthrene, only 3 developed leukemia. These were diagnosed 19, 29, and 30 days after injection and at the time of sacrifice the white counts ranged from 47,000 to 340,000 with 31% to 95% blast forms. Histologically, the tissues were widely infiltrated with leukemic cells. Of the 18 animals in this group those under 35 days of age are included in the 30-day-old group in Table I, while those older than 35 days are included in the 40-day-old group. In contrast, 100% success was achieved with the 161 rats from 3 to 7 days of age injected in a similar manner. These animals all showed peripheral blood evidence of lymphatic leukemia within 8 to 18 days after inoculation and if not sacrificed died usually within 25 days of injection.

Uniform transfer of the disease was also achieved in 2 litters totaling 9 rats born to females obtained from the current random bred Wistar colony of the Wistar Institute from which the original animals for the development of our colony were obtained more than 12 years ago, as well as in 13 rats of the Sherman strain and 18 of the Long-Evans strain born to mothers obtained from random bred colonies of these strains (Rockland Farms). The ages of these rats at the time of transfer were 3 to 7 days and all developed leukemia as readily as did the young rats of our own colony.

Comment. The uniform transfer of the lymphatic leukemia, developed through the gastric instillation of methylcholanthrene, to very young animals of 3 different strains of rats and only a limited transfer to 40-day-old animals, suggests the presence of some

TABLE I. Lymphatic Leukemia.

Age of animals at transfer, days	No. of animals	No. developing lymphatic leukemia
1-7	161	161
15	27	24
30	33	18
40	27	7

1. Shay, H., Gruenstein, M., Marx, H. A., and Glazer, L., *Cancer Research*, in press.

2. Gross, L., *PROC. SOC. EXP. BIOL. AND MED.*, 1950, v73, 246.

* We are indebted for the detailed design of this instrument to Ernest Sturm of the Rockefeller Institute.

mechanism that resists invasion by foreign cells, which appears to be absent at birth and for a short time thereafter, but which then develops rapidly. The possibility of such a mechanism appears in the studies of Duran-Reynals(3) with birds, those of Gross with mice and in our own with rats.

Summary. Lymphatic leukemia induced in Wistar rats by the gastric instillation of

3. Duran-Reynals, F., and Bunting, H., *Cancer Research*, 1942, v2, 343; Duran-Reynals, F., *Cancer Research*, 1943, v3, 569.

methylcholanthrene, was transferred to 40-day-old animals of our random bred Wistar colony with only indifferent success. When transfers were made to animals not older than 7 days, these were successful in 100% of the animals. Transfer of the leukemia was also made uniformly to equally young animals of the Sherman and Long-Evans strains. The intraperitoneal injection of leukemic spleen cells was used in these experiments.

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Effect of Crystalline Vitamin B₁₂ on Experimental Anemia in Mice.* (18332)

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Crystalline vitamin B₁₂ has been shown to produce favorable hematological response and general clinical improvement in cases of Addisonian pernicious anemia. It has been reported(1) that vit. B₁₂, when effective, is active in smaller doses than folic acid or thymine but the interrelations between these antianemic substances have not been clearly established. The potency of vit. B₁₂ preparations has been determined on the basis of the clinical response of pernicious anemia patients(2,3) and assays have been made based on the growth of microorganisms (*Lactobacillus leichmannii* and *Lactobacillus lactis* Dorner)(4-8).

The experiments reported here were per-

formed to determine the effect of vit. B₁₂ on the regeneration of red blood cells and hemoglobin in mice made anemic by the intraperitoneal injection of phenylhydrazine. Phenylhydrazine has been employed as a mild hemolytic agent in *Polycythemia vera*(9). According to Long(10) the mature circulating red cells of laboratory animals and humans are hemolyzed but the erythroblastic elements of the bone marrow are not injured by this drug. In other studies on the hemopoietic activity of proteins and amino acids phenylhydrazine was used to induce severe hemolytic anemia in rats and rabbits(11,12). Damodaran and Vijayaraghavan(13) observed that liver extract (Cohn's fraction G) caused a

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10. Long, P. H., *J. Clin. Invest.*, 1926, v2, 315, 329.