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## Further Studies on Formate Incorporation by Leukemic Blood Cells.* (19219)

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It has been observed that at one hour after injection of $C^{14}$-formate into mice with advanced Ak-4 leukemia, the greater portion of the leukemic prolymphocytes give positive autoradiograms on nuclear track emulsion while very few normal-appearing lymphocytes and practically none of the polymorphonuclears or erythrocytes are positive(1). Two preliminary explanations of these results were considered: (a) that the $C^{14}$-formate was incorporated during the anabolic phases of cell division and that the leukemic cells were dividing very rapidly as compared to normal cells, or (b) that the formed leukemic cells were metabolizing formate much more rapidly than were normal blood elements. The present study was designed to obtain information which might shed light on this question. In these experiments, leukemic blood has been exposed to $C^{14}$-formate away from sites of hematopoiesis for subsequent autoradiographic examination.

*Experimental.* Blood was aspirated from the heart of Akm mice with advanced transplanted Ak-4 leukemia (a rather acute lymphoid strain). This blood was heparinized and placed in a small cellophane bag which was then introduced through an incision into the abdominal cavity of leukemic mice (Ak-4). These mice (with leukemic blood-containing cellophane bags) were injected IP. with 100 microcuries of $C^{14}$-formate. After one hour, the mice were sacrificed and blood smear autoradiograms were prepared on cardiac and cellophane-bag blood after each had been washed twice with inactive sera. The details of the procedure employed in making the autoradiograms have been presented(1). A number of blood smears on NTB plates (10 $\mu$ emulsion thickness) were made on each sample. The plates were developed for 2 or 20 minutes after exposure periods in the dark from 1 to 10 weeks. All of the NTB plates were examined under the microscope and a hundred or more cells on each were classified as grossly positive or negative. The results of these experiments are summarized in Table I.

*Discussion.* The white blood counts on the leukemic host animals in Exp. No. 1 and 2 were 7,500 and 34,800, respectively, somewhat

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TABLE I. Summary of Autoradiogram Results on Cardiac and Cellophane-Bag Blood.

| Exp. No. | Blood | Lymphocytes, 6-9 $\mu$ | | Large lymphocytes and prolymphocytes, >10 $\mu$ | | Segmented cells | |
|---|---|---|---|---|---|---|---|
| | | Fraction positive | % positive | Fraction positive | % positive | Fraction positive | % positive |
| 1 | Cardiac | 16/428 | 3.7 | 98/192 | 51 | 0/198 | 0 |
| | Cellophane bag | 5/261 | 1.9 | 62/162 | 38 | 0/200 | 0 |
| 2 | Cardiac | 12/240 | 2.9 | 67/120 | 56 | 0/214 | 0 |
| | Cellophane bag | 3/120 | 2.5 | 62/134 | 46 | 0/255 | 0 |

In Exp. 1, the donor supplying the bag blood and the host animal were in the fifth leukemic day. Mice used in Exp. 2 were in the sixth leukemic day. Cells were counted in many randomly picked fields. All cells in each field were classified as positive or negative. Two observers counted each slide with rather good reproducibility.

lower than animals used in earlier experiments (1). The values of 51% and 56% positive large lymphocytes plus prolymphocytes (cells larger than 10 $\mu$ in diameter) in the "host" mice are lower than the 90% positive prolymphocytes previously reported on cardiac blood in leukemic mice.

The per cent positive large lymphocytes and prolymphocytes in the cellophane bag blood was only slightly less than observed in cardiac blood from the "host" mice. This would seem to suggest that the profound difference between formate utilization in normal lymphocytes and leukemic prolymphocytes previously reported (at one hour after injection of $C^{14}$-formate) is a result of the more rapid uptake of the radioactive compound by formed leukemic cells. It seems unlikely that blood cells immobilized in a cellophane bag in the abdominal cavity of a mouse are proliferating as rapidly as peripheral blood cell precursors; however, this possibility cannot be excluded by the present results.

*Summary.* Experiments have been carried out which demonstrate that leukemic cells immobilized in a cellophane bag implanted in the abdominal cavity of a host mouse incorporate $C^{14}$-formate much more rapidly than do normal lymphocytes.

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## Life Span of the Red Blood Cell of the Rat Following Acute Hemorrhage.* (19220)

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Neuberger and Niven(1) showed with $N^{15}$ labeled glycine in rabbits made anemic by bleeding that the life span of the red blood cell was considerably shortened. These findings are of sufficient interest to investigate for confirmation in the rat.

*Methods.* A series of 10 adult female rats of a highly inbred Curtis-Dunning strain was given 20 microcuries (1.64 mg) of glycine-2-$C^{14}$ intraperitoneally 6 hours after an acute hemorrhage of 5 cc by cardiac puncture. For control, 10 female rats of the same species were given the same amount of glycine-2-$C^{14}$. The rats were handled, and the blood samples obtained and analyzed in the manner described previously(2).

*Results.* Fig. 1 shows the average specific activity of the hemoglobin of the rats as a function of time after administration of the $C^{14}$ labeled glycine. This shows that in the

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