ous exposure to fluoride and indicated that bilateral nephrectomy significantly decreased the rate of fluoride absorption by 41.9% during the first 2 hours after fluoride administration and by 41.7% 4 hours thereafter. In the second study, this effect was substantiated using animals exposed to 2 different levels of fluoride. Previous exposure to 125 mg of fluoride decreased the rate of fluoride absorption by 21.9% and when the animals were in addition nephrectomized, a further decrease of 13.7% was noted. After exposure to 250 mg of fluoride, the rate of fluoride absorption was decreased by a similar value of 21.9%. Nephrectomy further decreased the rate of fluoride absorption and the combination of previous exposure and nephrectomy decreased the rate of fluoride absorption by more than 65%. These data suggest that when kidney function becomes impaired the body responds by decreasing the rate of fluoride absorption.

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Received January 21, 1963. P.S.E.B.M., 1963, v113.

Radiochromium-Labeled Lymphocytes in the Rat.* (28368)

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The life cycle of lymphocytes has been investigated by transfusion of blood from patients with chronic lymphocytic leukemia (1), by counting lymphocytes, obtained from the appendix, after injection into rabbits(2), by measuring the volume of myeloid and lymphoid tissue in the rat and counting the number of mitotic figures(3), by studying parabiotic rats(4), by collecting lymph and counting the lymphocytes of dogs(5), cats (6), rats(7) and rabbits(8,9), and by labeling lymphocytes with a fluorescent dye(10).

With the advent of radioisotopes, lymphocytes labeled with phosphate-P^{32} were studied(11,12). More recently McCall and associates(13) and Shorter and Bollman(14) studied lymphocytes labeled with radiochromium (Cr^{51}), Schooley and Berman(15) studied tritiated lymphocytes from the thoracic duct, and Gowans(16,17) studied tritiated-DNA labeled lymphocytes from the thoracic duct.

Despite these studies, clear answers are not yet available to the questions: How long does the lymphocyte live? How long does it remain in the bloodstream? Is there a lymph-to-blood-to-lymph circulation? The blood span is reported as 30 minutes to 12 hours, the life span 3 hours to many days, and whether or not there is recycling of the lymphocytes is disputed. This paper describes part of an extensive consideration (18) of these questions.

Methods. Labeling rat lymphocytes. Intestinal lymph was obtained from Sprague-Dawley rats, weighing 180 to 290 g, by the method of Bollman and associates(19). The

* Abridgment of thesis submitted by Dr. Bunting to the Faculty of the Graduate School of Univ. of Minnesota in partial fulfillment of the requirements for the degree of Master of Science in Medicine.

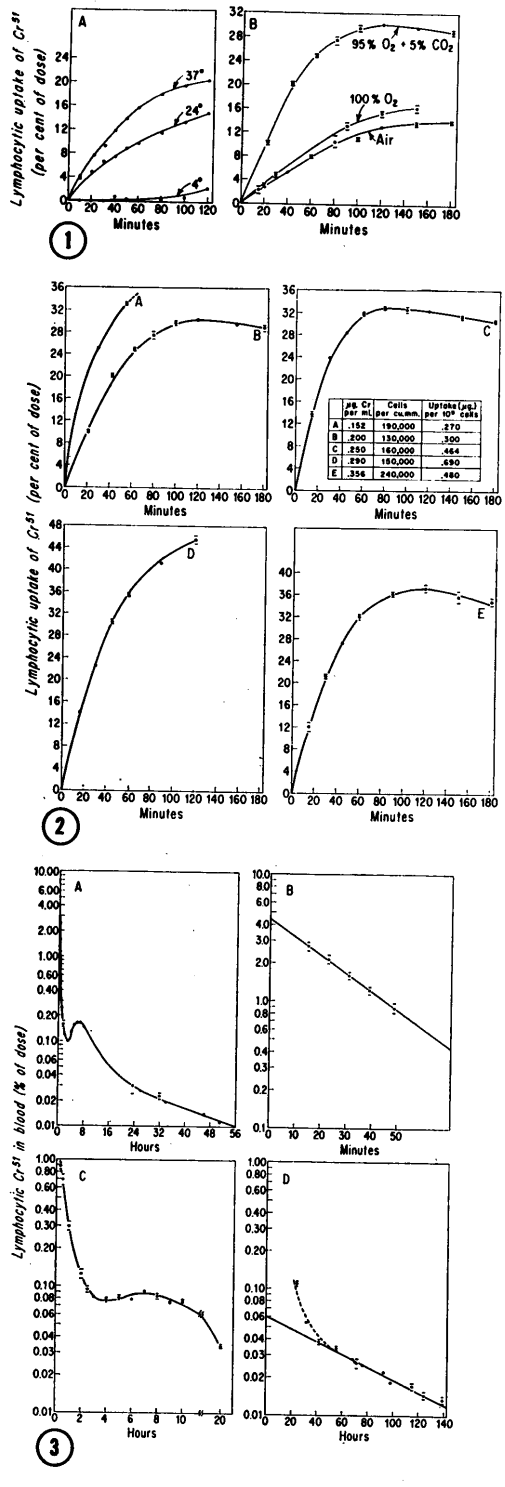

FIG. 1. Effects of temperature and atmosphere on uptake of Cr^{51} by rat lymphocytes. A. To measure effect of temperature, 110,000 cells per cubic millimeter were suspended in 0.9% saline with 0.1 μg Cr per milliliter. Atmosphere was air. B. To measure effect of atmosphere, 130,000 to 160,000 cells per cubic millimeter were suspended in 10% lymph (see text) with 0.2 to 0.25 μg Cr per milliliter. Temperature was 37°C.

FIG. 2. Effect of various concentrations of chromium and lymphocytes on uptake of chromium. Incubations were at 37°C in an atmosphere of 95% O_2 and 5% CO_2. Cells were suspended in 10% lymph (see text).

FIG. 3. Disappearance of labeled lymphocytes from blood. Values given are per cent of dose contained in 1 ml of rat blood. A. Entire curve. B. First part of curve on extended time axis. C. Second part of curve. D. Third part of curve. A, B, C, and D were plotted from data selected from 4 different experiments.

technic was similar to that used by Gesner and Gowans(20) in unanesthetized mice except that higher concentrations of lymphocytes are obtained from the intestinal lymph, as in our studies, than from the thoracic duct, as in Gesner and Gowan's. The lymph was collected for about 12 hours in siliconized tubes chilled to 4°C. The cells appeared to be lymphocytes exclusively, and more than 95% were of the "small" variety.

Maximal labeling of lymphocytes was achieved by centrifuging the lymph in the cold, resuspending the cellular button in defibrinated lymph diluted 10-fold with .0033 M phosphate buffer, pH 7.2, in 0.9% solution of NaCl, incubating with a solution of $Na_2Cr^{51}O_4$ for 1 hour at 37°C (Fig. 1A) in an atmosphere of 95% oxygen and 5% carbon dioxide (Fig. 1B). The optimal concentrations were found to be about 200,000 lymphocytes per cubic millimeter and 0.2 μg of chromium per milliliter of suspension (Fig. 2).

In the absence of lymph, isolated lymphocytes quickly changed microscopically and tended to clump. Suspending the lymphocytes in undiluted lymph prevented these changes (primarily loss of motility) but reduced the cellular uptake of Cr^{51}. The optimal concentration of lymph to prevent clumping and other changes and yet permit maximal labeling was about 10%. When the atmosphere above the cell-Cr^{51} incubation mixture was air or oxygen, the pH of the medium increased steadily; this could be prevented by using the oxygen-carbon dioxide mixture. Concentrations of lymphocytes

in excess of 200,000 per cubic millimeter led to clumping. Adding glucose to the incubation mixture did not seem to improve viability or labeling.

To evaluate the state of the Cr^{51} within the lymphocytes, labeled lymphocytes were lysed with distilled water and a suspension of erythrocytes was added. Less than 5% of the Cr^{51} was found associated with the erythrocytes after 15 minutes at 37°C. When an amount of chromate-Cr^{51} equal to that in the lysed lymphocytes was added to another suspension of erythrocytes, about 80% of the Cr^{51} was incorporated into the erythrocytes within 15 minutes. In a comparable experiment with lymphocytes, less than 2% of the Cr^{51} from lysed lymphocytes was taken up, but 22% of free chromate-Cr^{51} was incorporated in the suspension of lymphocytes.

In vivo *studies with labeled lymphocytes.* Lymphocytes were labeled with Cr^{51} as described. At the end of the 1-hour incubation for labeling, the cells were sedimented by centrifugation at 800 rpm (500 × *g*) for 7 minutes at 4°C. The cells were suspended in 15 ml of the phosphate buffer-saline-lymph solution, centrifuged, and resuspended in order to wash off unbound Cr^{51}. The final suspension, 1 to 2 ml, was kept in siliconized glassware until used for injection. A 50-μl aliquot was set aside for motility studies and to serve as a standard for the *in vivo* test. The specific activity of the Cr^{51} varied from 195 to 702 μc/μg, averaging 403. The dose to the rats averaged approximately 25 μc.

Each rat was anesthetized with ether, and 1 to 2 ml of the suspension of labeled lymphocytes was injected into a leg vein. The animal was then placed in a restraining cage with free access to food and water. Samples of blood were obtained by nicking the tail across the vein. After discarding the first drop of blood, 25 μl was collected in a pipette and this sample was rinsed out with saline containing heparin into a test tube for assay of radioactivity. At the same time, 2 glass microhematocrit tubes were partially filled with blood and sealed. After centrifugation for 30 minutes at 3800 rpm (2400 × *g*), measurements for calculation of the hematocrit value were made, the tubes were snapped at the cell-plasma junction, and 10 or 20 μl from each segment was transferred to a counting tube, the pipettes again being rinsed out with saline.

The Cr^{51} was counted in a thallium-activated sodium iodide well-type counter. Radioactivity was expressed simply as counts per second per volume, or as per cent of dose per 10 ml of blood or plasma by comparison of the average radioactivity of duplicate samples with that in the standard.

Results. When lymphocytes labeled with Cr^{51} were given intravenously to intact rats, the radioactivity disappeared from the blood very rapidly (within the first hour), rebounded with a maximum at about 6 to 8 hours, then declined again, but much more slowly than initially (Fig. 3A). More than 50% of labeled lymphocytes left the circulation within 30 minutes of their intravenous administration, and about 90% had disappeared within 2 hours.

In 13 experiments, the half-time of the "first" disappearance rate averaged 22.4 minutes, a rate constant of 3.2% per minute when plotted semilogarithmically (Fig. 3B).

The second phase was evaluated in 9 experiments. The secondary increase in radioactivity in the blood began at 3.7 hours, on average, and reached a maximum at 6.7 hours. Maximal radioactivity averaged 25% above the preceding minimum (Fig. 3C).

The third phase was a straight line on a semilogarithmic plot. In 5 experiments, the rate of disappearance averaged 1.13% per hour. Extrapolation of this line back to time = 0 gave an intercept which averaged 0.06% of the injected dose per milliliter of blood or about 1% of the dose in the entire blood volume of a 200- to 250-g rat (Fig. 3D).

When a suspension of labeled lymphocytes was heated to 56°C for 4 minutes and then given intravenously to rats, almost all of the radioactivity disappeared from the cellular elements of the blood before the first sample could be drawn, and there was no rebound (Fig. 4).

Lymph was collected by cannulation of an intestinal lymphatic trunk after intravenous

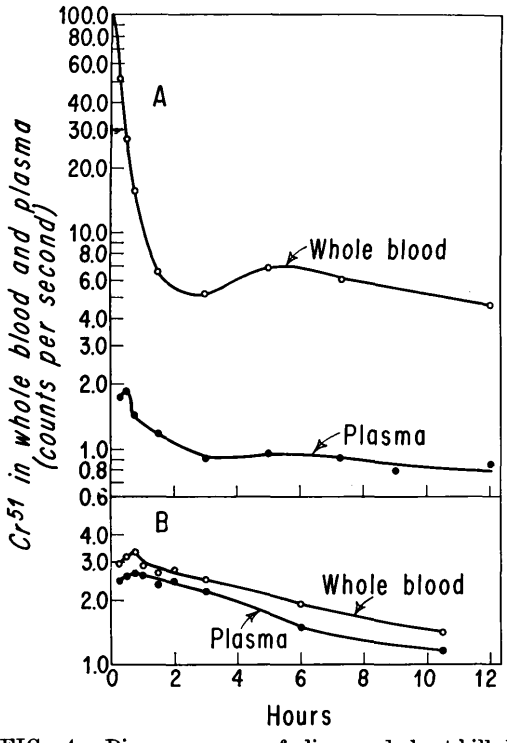

FIG. 4. Disappearance of live and heat-killed lymphocytes (labeled with Cr^{51}) given intravenously to rats. Values are counts per second in 25 μl of rat blood. A. Live lymphocytes. B. Heat-killed lymphocytes.

injection of labeled lymphocytes into 3 rats. The first labeled lymphocytes were detected within 1 to 2 hours, and maximal radioactivity developed between the 24th and 36th hours; the total lymphocytic Cr^{51} in the lymph in these 3 rats, followed for 88 to 118 hours, was 3.6, 5.9, and 7.4% of the original dose, respectively. In a similar group of rats, labeled lymphocytes were injected subcutaneously. Only a trace (0.1%) of the injected Cr^{51} appeared in the intestinal lymph, and it did not appear for 4 to 12 hours; the radioactivity was within the thoroughly washed lymphocytes.

Comment. A satisfactory, reproducible method for labeling rat lymphocytes with radiochromium was developed. Viability of the cells, based on their ameboid motility when examined under the phase microscope, could be maintained only if the cells were continuously bathed in a solution containing lymph. However, the uptake of Cr^{51} was hampered by too high a concentration of lymph. At 4°C, the lymphocytes seemed to maintain viability for more than 24 hours but were incapable of acquiring the Cr^{51} label at that temperature. Viability and ability to take up Cr^{51} were pH-dependent, but exposure of the suspension to a mixture of 95% oxygen and 5% carbon dioxide served to hold the pH near neutrality.

The concentration of chromium was kept at 0.2 μg per 200,000,000 lymphocytes per milliliter for two reasons: Gibson and Scheitlin(21) found this concentration to be noninjurious to erythrocytic enzymes; and it did not produce visible alterations in the lymphocytes. When the concentration was more than doubled, the lymphocytes tended to show decreased motility. With still greater concentrations, there was a loss of the brilliant, light green appearance of the cells under the phase microscope. Actual death of the lymphocytes was preceded by loss of their spherical shape, then swelling and fragmentation. It is possible, of course, that even at a concentration as low as 1 μg of chromium per 10^9 lymphocytes there were invisible alterations which might have affected the viability of the cells once they were returned to the circulation. This was not tested *in vivo* because further reduction in the amount of radioactivity would have limited the accuracy of the counting. However, within the range of chromium actually used, 0.05 to 0.13 μg, there was no correlation between dose and rate of initial or final disappearance of injected lymphocytes.

If the slope of the final part of the curve of radioactivity in blood plotted against time were taken to be a true representation of rate of survival of lymphocytes, one could estimate their life span to be about a week. This figure is too small if the lymphocytes were injured during the labeling, or if elution of the Cr^{51} from the lymphocytes occurred before their death. The value is too great, however, if the originally sequestered lymphocytes continued to return to the circulation after the first few hours.

One might interpret the initial rapid loss of injected lymphocytes from the circulation as indicating inadequate resuspension of the lymphocytes after centrifugation. The clumps

then presumably would be trapped during their circulation through the capillary beds. Microscopic examination of the suspensions of cells led us to doubt this explanation.

Another possibility is that the cell-labeling technic was so drastic that almost no lymphocytes survived more than a few minutes in the circulation. This also seems unlikely since up to 7.4% of the labeled cells appeared, in a motile state, in the intestinal lymph within 4 days. Furthermore, the disappearance-curve of heat-killed labeled lymphocytes differed significantly from that of viable cells. The evidence is strongly against relabeling of lymphocytes with the Cr^{51} from dead cells, so that live cells appear to be sequestered following their intravenous injection and to emerge tardily in the intestinal lymph. If the results of these experiments approximate the physiologic state, about 99% of the lymphocytes are extravascular and a bilateral exchange between blood and sequestered lymphocytes is occurring continuously.

Summary. A technic is described for labeling rat lymphocytes, collected from intestinal lymph, with radiochromium without impairing their motility. After intravenous administration, these labeled cells disappeared rapidly from the circulating blood, reappeared to a limited extent within 6 hours, then continued to disappear at a very slow rate (about 1.1% per hour). When the rat's intestinal lymphatic trunk was cannulated, 4 to 7% of the labeled lymphocytes appeared in the lymph within 4 days, with maximal lymphatic radioactivity occurring between the 24th and 36th hours.

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Received February 18, 1963. P.S.E.B.M., 1963, v113.