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

Exp. No.	Blood	Lymphocytes, 6-9 μ		Large lymphocytes and prolymphocytes, >10 μ		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 μ 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.

1. Skipper, H. E., Chapman, J. B., Boyd, G. A., Riser, W. H., Jr., and Bell, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 849.

Received November 5, 1951. P.S.E.B.M., 1951, v78.

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

NATHANIEL I. BERLIN AND CAROL LOTZ.

From Donner Laboratory of Medical Physics, University of California, Berkeley.

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

* Supported by the Public Health Service and by the Atomic Energy Commission.

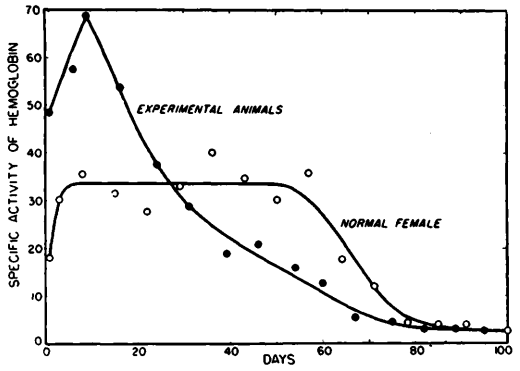

FIG. 1. Average specific activity of hemoglobin of anemic rats and normal rats following administration of glycine-2-C^{14}.

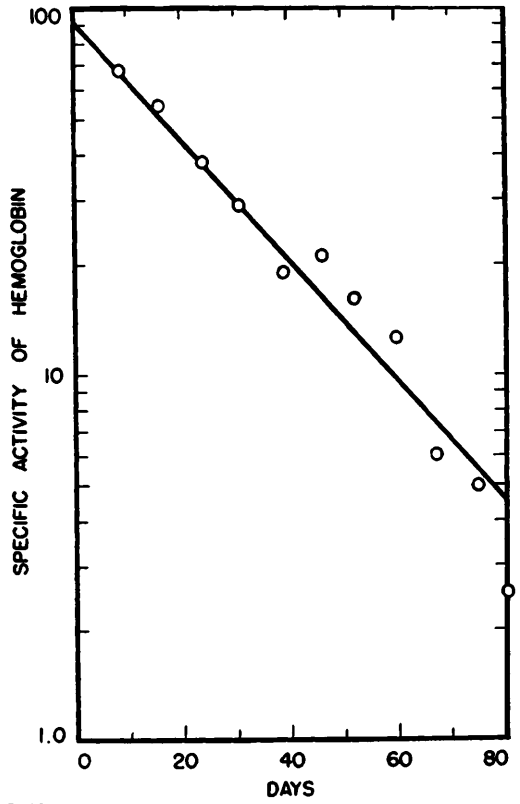

FIG. 2. Rate of fall in specific activity of hemoglobin of experimental animals from Day 10 to Day 70.

anemic rat the C^{14} level reaches a maximum and then, in contrast to the normals, falls rapidly.

Fig. 2 shows that in the anemic animals the rate of fall from day 10 to day 70 can be fitted to a single exponential component having a half-time of 19 days. This would indicate that these cells had a mean life span of 27 days. This value cannot be estimated by the method of Shemin and Rittenberg(3) but merely follows from the fact that the mean life time is 1.44 times the half-time when any process is a simple exponential decay process (4), such as this seems to be. Fig. 1 also shows that there is an approximately 2-fold increase in the amount of C^{14} incorporated in the red blood cells of the anemic animals. The control series of 10 female rats showed almost the same mean red cell life span as the male rats of a genetically similar strain, or 64 days as compared to 68 days(2).

Discussion. Following hemorrhage the red blood count and hemoglobin have returned to normal in 8-15 days (5-9); thus little, if any, of the decrease in the specific activity of the hemoglobin after day 10 can be attributed to dilution resulting from an increased total red cell volume.

The finding of a mean life span of the red blood cell of 27 days in those red cells produced in response to acute hemorrhage in the rat extends the finding of Neuberger and Niven(1) from the rabbit to the rat. These data in the rat and rabbit suggest that the red blood cells produced in response to acute hemorrhage are functionally poor from the standpoint of life span, although adequate in numbers to replace rapidly the red cells lost. For the considerable period of time that these faulty cells are gradually being replaced by red cells having a more normal life span, the bone marrow will be hyperactive. In light of this evidence long-term studies of the transfusion of red cells from one animal to another in which the donor animal has previously undergone repeated bleeding are apt to be misleading.

Conclusion. The red blood cells formed as a response to acute hemorrhage in the rat have a considerably shortened life span.

1. Neuberger, A., and Niven, J. F. S., *J. Physiol.*, 1951, v112, 292.

2. Berlin, N. I., Meyer, L. M., and Lazarus, M., *Am. J. Physiol.*, 1951, v165, 565.

3. Shemin, D., and Rittenberg, D., *J. Biol. Chem.*, 1946, v166, 627.

4. Siri, W., *Isotopic Tracers and Nuclear Radiations*, 1949, 267.
5. Boycott, A. E., *J. Path. Bact.*, 1911, v16, 269.
6. Geiling, E. M. K., and Green, H. H., PROC. SOC. EXP. BIOL. AND MED., 1921, v18, 191.
7. Jencks, Z., *Am. J. Physiol.*, 1922, v59, 240.
8. Cartland, G. F., and Koch, F. C., *Am. J. Physiol.*, 1928, v87, 249.
9. Harne, O. G., Lutz, J. F., and Davis, C. L., *Bull. School Med. Univ. Maryland*, 1938, v23, 143.

Received November 7, 1951. P.S.E.B.M., 1951, v78.

Effect of Ultracentrifugal Fractions of Small Intestinal Tissue upon Transplanted Lymphosarcoma.*† (19221)

LESLIE R. BENNETT, FRANK E. CONNON, AND MELVIN D. SCHOENBERG.
(Introduced by J. F. Mead.)
From the School of Medicine, University of California, Los Angeles.

The literature contains numerous reports of attempts to isolate from various normal tissues substances which are inhibitory to the growth of malignant tumors. The majority of these reports indicate highly variable results. Since it is generally known that malignant tumors rarely originate in the small intestine, the present work has been concerned primarily with efforts to derive inhibitory material from this organ. Previous work with this tissue has involved chemical extraction of material from the whole organ(1). In the present studies, ultracentrifugal fractionation has been employed. Similar methods have been used successfully in the separation of hemolytic and antihemolytic substances from liver(2).

Results of preliminary investigations based on centrifugal fractionation are reported in this communication.

Materials and methods. Small intestinal tissue of normal mice or rats was frozen on dry ice, then thawed and ground with sand. A saline suspension of this homogenate was fractionated in the following manner. A fraction corresponding to the mitochondrial-nuclear fraction of normal tissue(3) was separated by centrifugation for 10 to 20 minutes at 4000 x g (Fraction 4). The supernate from this was further centrifuged for 45 to 60 minutes at 90000 x g to yield a microsomal fraction (Fraction 90). The fractions were prepared for use by resuspending in saline. Fractions 4 and 90 and the supernatant from the latter were tested for inhibitory action on lymphosarcoma (Gardner). Lymphosarcoma cells in saline suspension were incubated *in vitro* at 5° C for 10 to 20 minutes with an equal volume of the tissue fraction suspensions described above. Two-tenths ml of these tissue fraction-tumor cell incubates was inoculated subcutaneously in CBA mice (Strong). Controls received saline-tumor cell inoculations.

Results. Results are summarized in Tables I and II. The technic employed yielded 100% takes of the tumor in 124 control mice. Tumors developed to palpable size in 4 to 7

TABLE I. Results of Pre-Inoculation Treatment of Lymphosarcoma Cell Suspensions with Mouse Tissue Fractions. Subcutaneous Injection of Tumor Cells and Tissue Fractions in CBA Mice.

Tissue fraction		No. cases	Survivals	Deaths
Intestine:	Fraction 90	49	47	2
	" 4	13	9	4
	Supernatant*	51	16	35
Liver:	Fraction 90	8	0	8
	" 4	8	0	8
	Supernatant	8	0	8
Spleen:	Fraction 90	6	0	6
	Supernatant	6	0	6
Muscle:	Fraction 90	6	0	6
	Supernatant	6	0	6
Controls:	Tumor + saline	65	0	65

* Supernatant fraction in all cases was the fluid portion remaining after precipitation of Fraction 90.

* This paper is based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.

† Portions of this study were carried out in the Department of Radiology, School of Medicine, and were partially supported by a grant from the University of California cancer research funds.