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Erythrocyte Repopulation in X-Irradiated Recipients of Nucleated, Peripheral Blood Cells of Normal Mice. (25966)

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Unitarian and polyphyletic theories of blood cell formation have been discussed for many years(1). Many hematologists agree that the fixed stem cell may be pluripotent, but the capacity of more mature forms, and particularly of circulating cells, to dedifferentiate toward less-mature blast forms is not universally accepted. Studies with tissue culture technics have indicated that numerous morphological types can be derived from apparently pure blood cell cultures(2). Much information about blood cell differentiation may also be obtained by use of an *in vivo* culture system in mice, in which normal hematopoiesis has been destroyed by X radiation. In such mice, Merwin(3) showed that leukocytes of leukemoid blood produce immunologically competent cells, and Smith *et al.*(4) reported that leukemoid leukocytes differentiated into erythroblasts. Since leukemoid blood contains many immature cells, preliminary observations that circulating leukocytes from normal mice may produce erythroblasts in irradiated recipients are herein reported because of their important implications about the dynamics of normal hematopoiesis.

Materials and methods. A program was initiated to produce coisogenic strains of mice that differ at the hemoglobin locus. The scheme was essentially similar to that of Snell (5). The "single" type hemoglobin of strain C57BL mice was genetically introduced in strain BALB/c, RF, AKR, and 101 mice, which possess "diffuse" type hemoglobins, and the "diffuse" hemoglobin of 101 mice was introduced into C57BL mice. Technics of starch gel electrophoresis(6) and hemoglobin solubility(7) were used to identify mice that carried the introduced, genetically controlled, type of hemoglobin. At present, the coisogenic status of these strains varies from 5 to 8 generations of backcrossing.

Sixteen-week-old BC_5 coisogenic 101 mice, which were progeny of the BC_4 backcross to normal 101 mice and were heterozygous for diffuse and single hemoglobins, were given 600 r of X rays and used as recipients. X ray conditions were 250 kvp, 31.5 ma, hvl 0.43 mm of Cu; the dose rate in air was approximately 80 r/min, tissue to source distance was 93.7 cm. Donor mice were anesthetized with Nembutal, ventral skin was removed, and the sternum was excised to expose the heart. Cardiac blood was withdrawn by means of a hypodermic needle and syringe that contained 2% sodium citrate as anticoagulant. Twelve to 15 ml of blood was pooled from 10 normal 101 donors, 3-4 months old. White blood cells were separated in 15-ml centrifuge tubes by centrifugation at 600 X *g* for 15 minutes. The buffy coat and many underlying red cells were removed with a pipette and placed in Wintrobe hematocrit tubes to be centrifuged a second time at 600 X *g* for 20 minutes. Ap-

* Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.

TABLE I. Percentage of Donor-Type Erythrocytes.

Exp.	Animal	Days after treatment 15	29	46	49	63
I	1	0		0		
	2	30		60*		
II	3		†		40	25
	4		0		0	0
	5		60		100	100

* Mouse died on day 69.

† Analysis indicated that the graft was partially successful, but the mouse was very anemic and quantity of donor-type erythrocytes cannot be determined accurately under such conditions.

proximately 100 million nucleated, buffy coat cells were injected intravenously *via* the tail vein into the 600 r X-irradiated recipients. Microscopic examination of stained preparations showed that the buffy coat contained many platelets and all forms of normal circulating white blood cells. Aggregation of white blood cells was prevented by use of 2% sodium citrate. The technic of HbCO solubility(7) was used to determine the percentage of donor-type erythrocytes in irradiated recipients 30-60 days after treatment.

Results. Five X-irradiated, coisogenic BC_5 101 mice, heterozygous for diffuse and single hemoglobins, have been injected with ~ 100 million buffy coat cells removed from normal 101 mice. The percentage of donor-type red cells derived from the injected white cell mixture is indicated in Table I for each recipient.

Discussion. Although none of the circulating cells of normal blood are classified as erythroblasts, the data suggest that cells that separate with the white blood cell fraction can differentiate into erythropoietic cells. Similarly, no leukemoid blood cells are classified as erythroblasts, but leukemoid leukocytes do incorporate tritium-labeled thymidine *in vitro* and do differentiate into erythroblasts *in vivo* (4).

Recipient mice in these experiments are not completely coisogenic; nevertheless, the infusion of white blood cells was performed in a parent-to-F_1 manner, *i.e.*, parental white blood cells to partially coisogenic recipients, so that the tendency for rejection of the graft at midlethal doses of X rays was minimized (8). Analyses made 15-30 days after infusion of white blood cells may have been somewhat affected by presence of donor-type erythrocytes in the white cell fraction injected; however, analyses at 60 days should not have been affected by contamination of donor red cells since the life span of mouse red blood cells is reported to be approximately 60 days(9). Furthermore, increasing quantities of donor-type red cells in succeeding tests indicate that grafted cells must have been actively proliferating. The strain 101 mice used in this particular experiment were supplied by a commercial breeder, and it has recently been discovered that his 101 stock was not isogenic (C. C. Congdon, personal communication); perhaps the failure of transplantation in mice Nos. 1 and 4, and graft regression between days 49 and 63 in mouse No. 3 (Table I), resulted from graft rejection. The proportion of donor-type rbc in the latter is greater than what might have occurred without at least transitory production of new cells by grafted progenitors.

Data presented support the unitarian theory of blood cell formation in that they suggest that circulating cells are capable of differentiating into erythroblasts. Perhaps one of the morphological types we classify as fairly mature (*i.e.*, the monocyte or large lymphocyte) is really a free stem cell capable of differentiation into many cell types. Bond *et al.* (10) showed that a small number of such cells in peripheral blood of normal human beings incorporate tritium-labeled thymidine and consequently presumably can divide. From studies of blood cultures, Maximow(11) and Downey(12) concluded that a distinction between the lymphocyte and hemocytoblast is perhaps artificial. Furthermore, Pierce(13) and Downey and Stasney(14) showed that staining characteristics of a cell do not clearly indicate its maturity since mature cells may have more basophilic cytoplasm than less-mature forms. Whether our results can be ascribed to the lymphocytic, granulocytic, or erythrocytic series, or perhaps all of these, is being investigated by using tissues primarily composed of such elements and peritoneal exudates.

The adoptive transfer of transplantation immunity conferred by use of whole blood

(15) and the deleterious effect of isologous whole blood for homologous bone marrow transplantation in mice(16) should be reinterpreted in the light of these findings. Transplantation immunity can be conferred with as few as 50,000 cells of a Peyer's patch(17) and 80,000 isologous bone marrow cells are able to protect against hematopoietic failure induced by X radiation(8). Hence, since the white blood cell count is ~ 8 million per ml of whole blood, the proportion of the circulating cells that are able to divide may be as high as 1%.

Summary. Strains of mice are being produced that are isogenic with inbred stocks except for the hemoglobin locus. With mice of such strains, experiments were carried out to ascertain whether peripheral blood of normal mice contain cells capable of repopulating the marrow of irradiated recipients. In 3 of 5 recipients injected intravenously with ~ 100 million nucleated peripheral blood cells after 600 r, increasing numbers of graft-derived erythrocytes were noted within 60 days after treatment, indicating the presence in peripheral blood of cells that are able to give rise to mature erythrocytes.

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A Study of the So-Called "Cat Effect" of Cruickshank and Whitfield. (25967)

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Some years ago Cruickshank and Whitfield (1) claimed on rather scant evidence from time-concentration curves on cats that "gobbling" of T-1824 by the reticulo-endothelial system leads to an overestimate of plasma volume upon first injection of this dye. Furthermore, they suggested that "a previous 'saturating' dose of dye should be injected before a determination is made in order to abolish the 'absorption curve' ". Judging from the questions directed to our laboratory from time to time, even as recently as the present year, we have the impression that Cruickshank and Whitfield's report, often referred to as the "cat effect," is still considered unchallenged. To be sure, the experiments of Cruickshank and Whitfield have not, to the best of our knowledge, been exactly repeated but since the question has been raised with us again, we decided to report the results of experiments made by one of us in 1946-47 (E.N.) which were designed to test the "cat effect." Our reasoning was that if the claims regarding this phenomenon were true, then the plasma volume obtained by the second of 2 successive injections of T-1824 should be

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