tested as described above. The final solutions prepared by the sulfate treatment, were usually about as active as the extracts from which they were prepared; thus, about one-fourth of the activity was recovered. As indicated by Exp. 9, the ratio of heme formed to initial heme was increased by the sulfate treatment. Similar results were obtained using other estimates of heme. Although the concentration of hemoglobin, indicated by the heme content, in the solutions obtained by the sulfate treatment was less than in the extracts, the activity per unit of protein, as indicated earlier for the simpler cell preparation(2), was not sufficient to allow unequivocal assignment of the activity to a catalyst.

Discussion. The present results establish net synthesis of heme from protoporphyrin and ionic iron in the presence of material from duck erythrocytes and indicate, as reported earlier(1-4), that the material from the erythrocytes is necessary for the synthesis. However, it appears that under certain conditions(12-14), notably in the presence of a detergent and globin(14), heme is formed at neutral pH and moderate temperature without addition of cellular material. Under present experimental conditions globin had no effect on heme synthesis in the presence of red-cell extracts (*cf.* Experiment 6) and alone (*cf.* Experiment 10) did not promote synthesis. Nevertheless, it is possible that the material from the avian erythrocytes in some relatively non-specific way simply creates a suitable environment for heme synthesis.

Summary. Soluble preparations of duck erythrocytes were found to effect net synthesis of heme from protoporphyrin and iron.

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Repopulation of Hematopoietic Tissues of X-Irradiated Mice by Cells From Leukemoid Blood. (24815)

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Congdon *et al.*(1) reported that injection of blood showing extreme granulocytosis induced by a tumor prolonged survival of mice that had been given a lethal dose of total-body x-irradiation. Congdon *et al.* suggested that the leukemoid blood might protect against early death by some mechanism other than repopulation. The leukemoid blood might provide a humoral factor which would stimulate recovery of irradiated hematopoietic tissues or large number of leukocytes might help combat infection. Later Smith and Congdon(2) found evidence indicating that injected blood cells probably repopulated at least some hematopoietic tisues. More conclusive evidence that repopulation takes place is reported here.

The evidence was obtained by injecting leukemoid blood from mice of one genetic constitution into x-irradiated mice of another genetic constitution and later testing the irradiated, recipient mice by the Harderian-gland method(3) for presence of donor-type cells.

Materials and methods. The Harderian-gland test is based on the finding that although a nonvascularized graft survives in an homologous host because it does not initiate immunity, the graft is destroyed if host becomes immune(3). Injection of a suspension of homologous cells will immunize a mouse. In these experiments suspensions of cells from irradiated protected mice were injected into test mice of same genetic constitution as the irradiated mice. Each test mouse carried an Harderian-gland graft from a mouse of same genetic constitution as donors of the leukemoid blood. If tissues of irradiated mice contained cells that originated from the leukemoid blood, the grafts disintegrated. To provide a basis for estimating the proportion of cells originating from leukemoid blood, the immunizing capacity of a known number of cells of the same genetic constitution as leukemoid blood was compared with immunizing capacity of an equal number of cells from irradiated protected mice. Irradiated mice were (BALB/c X A) F_1 hybrids. The donors of the leukemoid blood were (BALB/c X C3H) F_1 hybrids. The following substrains were used in obtaining the hybrids: BALB/cAnN, A/LN and C3H/HeN. The tumor arose in a BALB/c mouse but was transferred for these experiments into (BALB/c X C3H)F_1 hybrids so that Harderian-gland grafts would come from a pigmented mouse. Disintegration of the pigment cells in the graft is the best end point for the Harderian-gland test. Squamous-cell carcinoma, A 280, used was kindly provided by Dr. Charles Congdon. He found that in mice supporting subcutaneous growth of this tumor, the leukocyte count was approximately 80 times normal in 4 to 5 months(1). In experiments described here blood was taken 6 months after mice were inoculated subcutaneously with the tumor. A half cc of blood was drawn from the heart of each mouse into a syringe moistened with saline containing heparin. The blood was immediately injected into the tail vein of a mouse that had been exposed, 1 to 6 hours previously, to 800 or 900 r total-body x-irradiation. Physical conditions for x-irradiation were: 200 KV, 15 ma, 126.5 r/minute at focus skin distance of 54 cm with .25 mm Cu and .55 mm Al filters. The irradiated mice were 3½ months of age and kept 3 or 4 to a cage. Three to 12 months after x-irradiation and injection of leukemoid blood, the mice were killed and certain tissues tested for presence of cells originating from leukemoid blood. Suspensions of marrow cells were obtained by pushing the marrow in Hanks' balanced saline through a 23 gauge needle several times. Other tissues were macerated in saline. The pH of saline was approximately 8.0 except in one experiment in which it was approximately 7.5. Dilutions were made so that about 100,000 cells could be injected in 0.5 ml volumes within approximately 30 minutes of time the suspensions were prepared. Further dilutions were made so that 25,000 cells could be injected in 0.5 ml approximately 30 minutes later. For comparative purposes similar preparations were made of cells from normal mice of same genotype as donors of leukemoid blood.

Results. Of 16 mice inoculated with tumor A 280, 2 showed no tumor growth at 6 months and leucocyte count was nearly normal. The 2 x-irradiated mice given blood from these animals died within 2 weeks, indicating no protection. Leukocyte counts for the 14 remaining animals ranged from 19,000 to 483,000/mm^3. Five irradiated mice received less than 50 million leukocytes, 4 received between 50 and 100 million and 5 received over 100 million. Six of these 14 mice were used from 3 to 12 months after treatment to determine origin of cells of certain of their tissues. Of the 8 other mice, 2 had died by 2 months after treatment, 5 more by 8 months and 1 more by 10 months.

A mouse tested at 88 days and one at 150 days gave positive tests for presence of homologous cells in marrow, lymph nodes, thymus, spleen and blood. A known number of marrow or lymph node cells from 4 other mice

TABLE I. Results of Harderian-Gland Test to Compare Antigenicity of Known Number of Cells from Irradiated Mice, (BALB/c × A)F_1, Protected by Leukemoid Blood from Mice of Another Genotype, (BALB/c × C3H)F_1, with Antigenicity of the Same Number of Cells from Mice with Same Genotype as Leukemoid Blood Donors.

Cells inj.		No. of test mice in which graft disintegrated/Total No. of test mice		
No. (×1000)	Tissue	Cells from irradiated mice protected with leukemoid blood	Cells from mice with same genotype as leukemoid blood donors	No. of days after irradiation
100	Lymph node	5/5		180
25		3/5		
100	*Idem*	3/6	3/6	218
25		2/6	2/5	
100	Bone marrow	2/3	3/3	271
100	Lymph node	4/4	4/4	370
25		2/4	2/4	

were tested at 180 to 370 days. Twenty-five thousand or 100,000 cells from the irradiated mice or from mice of the genotype of donors of leukemoid blood were injected into test mice. Preliminary tests with mice of genotypes used here as well as other experiments, some of which have been reported previously (4) with mice of other genotypes, showed that fewer than 25,000 homologous cells usually gave mostly negative tests. Further, with these numbers of cells a dilution of ¼ usually gave a detectable change in proportion of positive to negative tests. The results (Table I) indicate that at least ¼ and possibly almost all cells from marrow or lymph nodes of irradiated and protected mice were derived from the leukemoid blood.

Appearance of lymph nodes of the irradiated, protected mice at time the tests were made, varied greatly. The nodes were nearly normal in size and appearance in the 2 heaviest and most normal appearing mice, the mice killed at 150 and 218 days. The lymph nodes were smallest in the mouse that was least healthy, the mouse killed at 271 days. Almost no cells were freed when nodes from this mouse were macerated and virtually no lymphocytes were present in the node studied histologically. Tests had to be made on marrow cells from this mouse. In the rest of the mice, lymph nodes were smaller than normal and had yellowish opaque masses in them.

Discussion. Smith and Congdon(2) found that the general picture of recovery after irradiation and injection of leukemoid blood was the same as after injection of marrow. They believed that the cells of leukemoid blood repopulated at least the granulopoietic elements. The present results indicate that lymphocytes also are derived from leukemoid blood. Several studies have indicated that the lymphocytic cells in mice irradiated and given marrow are derived from the introduced marrow. One of these studies was made in this laboratory using the Harderian-gland test (4). Tests have been made on lymph node cells of 3 more mice at 358 to 450 days after irradiation and marrow injection. The antigenicity of 25,000 and 100,000 cells from protected mice was about the same as that for an equal number of cells from mice of the same genetic constitution as marrow donors. Thus the previously reported observations have been confirmed and extended.

Although in the present experiments, mice were protected from early deaths after irradiation by injection of homologous blood, Congdon *et al.*(1) and Smith and Congdon(2) were able to get prolonged survival with isologous blood only. The graft-against-host reaction, which seems to be important in length of survival of irradiated mice after injection with homologous hematopoietic tissues(5,6), may be less severe in the hybrid combination used here, because some of the components at the H2 locus are common to C3H and A strains (7). Further the genes that are different in the 2 hybrids are present in only ½ of the

dosage that would be found if 2 different strains were used. The possible role of dosage effect in increasing the protection afforded irradiated mice by injection of homologous cells is discussed by Uphoff(8).

Summary and conclusions. Survival of lethally x-irradiated (BALB/c X A)F_1 hybrid mice was greatly prolonged by injection of leukemoid blood from (BALB/c X C3H) F_1 hybrid mice. Quantitative estimates indicate that many, if not all cells in lymph nodes or marrow of irradiated protected mice were derived from leukemoid blood.

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Molecular Weight and Plasma Substituting Effectiveness of 3 Plasma Expanders. (24816)

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On the assumption that only compounds of high molecular weight were capable of substituting for blood plasma, dextrans of high molecular weight were initially employed in treatment of shock. These dextrans gave rise to a high incidence of side effects which were attributed to high molecular weight of the compound(1). Wilkinson and Storey(2) essayed a dextran considered to be "highly fractionated" (65% of molecules weighing between 130,000 and 250,000). They found in normal volunteers that it still produced a high incidence of urticaria and vasomotor instability. Since then the molecular weight of clinically used plasma expanders has been greatly diminished without significant change in their therapeutic effectiveness. Most of those now in use have an average molecular weight between 30,000 and 40,000(3). The study of a new compound of much lower molecular weight was considered interesting since it might produce fewer side effects than reported for the higher polymeric expanders. In the present investigation 3 plasma expanders were compared as to their capacity to restore normal blood pressure and plasma volume as well as to prevent death from an experimentally produced hemorrhage.

Materials and methods. The low polymeric plasma expander studied was a partially methoxylated polymer of galacturonic acid whose molecular structure resembles that of dextran (Fig. 1). Its average molecular weight was only 6,200. This substance was used in a stabilized and buffered 1% solution in saline (Graplasmoid, L. I. F. E.). The relatively higher polymers employed for comparison were: 6% dextran in saline (Intradex, Glaxo) with average molecular weight of 40,000, and

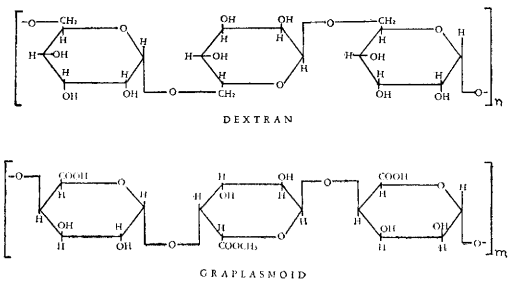

FIG. 1. Chemical structure of a moiety of dextran and Graplasmoid.