TABLE II.
Concentration of Histamine in Plasma of Rabbit Blood After *in vitro* Additions of Sheep Erythrocytes, Saline Solution Washings of the Erythrocytes, and Physiologic Salt.

	μg histamine/cc plasma of blood to which had been added:		
Rabbit	0.5 cc of physiologic salt sol.	0.5 cc of final washing sol.	0.5 cc of 50% suspension of washed sheep erythrocytes
12	.25	.20	1.25
	.09	.21	
13	.13	.33*	1.02
	.11	.36*	1.13

* Blood shaken to effect adequate mixing.

histamine was produced by the reaction and much more probable that its distribution was altered through a liberation from the cells of the blood. If this was the case, the plasma histamine values following the *in vitro* hemolytic reactions were so high that the histamine could not have been derived from the red cells alone but must have come in the main from the elements of the buffy coat. This participation of the white cells and platelets in the hemolytic reaction was further indicated by the reduction in the size of the buffy coat layer in those samples in which hemolytic reactions occurred.

The mechanism by which the hemolytic reaction releases histamine from the formed elements of the blood is not known. A release of histamine into the plasma of drawn blood takes place after such simple trauma as contact of the blood with glass or with fragments of debris or by clotting or shaking of the blood. The release of histamine in the course of the hemolytic reaction is most likely the result of some form of chemical or physicochemical trauma.

The results of this study indicate that release of histamine from blood cells into plasma may contribute to the symptoms observed during hemolytic transfusion reactions.

Summary. Rabbits were sensitized by the repeated intravenous injection of washed sheep erythrocytes. After an appropriate period, addition of the washed sheep red cells to the rabbit blood *in vitro* produced prompt hemolysis. This *in vitro* hemolytic reaction in the rabbit blood was always accompanied by an increase in the histamine content of the plasma.

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The Influence of the Spleen on Hematopoietic Recovery After Irradiation Injury.* (17711)

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It has been reported by Jacobson and associates(1,2) that if the spleens of mice are mobilized surgically and protected with lead during whole body exposure to a dose of 600

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1. Jacobson, L. O., Marks, E. K., Gaston, E., Robson, M., and Zirkle, R. E., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 740.

2. Jacobson, L. O., Marks, E. K., Robson, M., Gaston, E., and Zirkle, R. E., *J. Lab. Clin. Med.*, 1949, v34, 1538.

TABLE I.
Preparation and Treatment of Animals.

Group	No. of mice	Preparation of animals	Treatment
I	25	None	None
II	35	Anesthetic (nembutal) Surgical mobilization of spleen	,,
III	105	Same	1025 r whole body X radiation including spleen
IV	100	,,	1025 r whole body X radiation, spleen lead protected

r of X radiation, compensatory ectopic blood formation appears in the protected spleens within 24 to 48 hours. Concurrently, a transient leucopenia and thrombocytopenia unassociated with anemia appear in the peripheral blood. An anemia and a severe and more persistent leucopenia and thrombocytopenia invariably occur if the spleen is not lead protected during whole body X radiation with dosages of 600 r or above. Even with a dose of 1025 r whole body X radiation which is 300 roentgens above LD_{100} for intact mice, ectopic blood formation appears rapidly in the lead protected spleen and no anemia and only a modest and transient leucopenia and thrombocytopenia develop. By the third to fourth day after irradiation, the bone marrow of mice exposed to dosages of 600 r and above is largely depleted of free nucleated cells with the exception of a few scattered erythroblasts, plasma cells, and unidentified round cells. Typically the marrow at this stage consists of reticular cells, dilated blood sinuses, hemorrhage, fat, and a homogeneous material which stains slightly blue with hematoxylin-eosin-azure. The $LD_{50}/28$ days for mice which have had lead protection of the surgically mobilized spleen during X irradiation is about 1025 r; the LD_{50} for mice without lead protection of the spleen is about 550 r. Thus lead protection of the spleen increases the LD_{50} by a factor of nearly two. This report relates observations on the regeneration of the hematopoietic tissue of mice exposed to 1025 r with or without lead protection of the spleen. A complete report, including the hematologic data and more extensive illustrations and descriptions of the histopathologic findings, will be published elsewhere.

Methods. Four groups of CF-1 female mice†, 10 to 12 weeks of age and weighing approximately 25 g, were prepared as indicated in Table I. Mice in Group I were untreated controls. The mice in Groups II, III, and IV were anesthetized with nembutal (0.072 mg/g), an incision made in the left upper quadrant of the abdomen, and the spleens mobilized through the incision with the main pedicle intact. Group III and Group IV mice were irradiated with 1025 r whole-body X radiation while the spleens were without the abdominal cavity. During irradiation the spleens of Group III were wrapped in gauze and kept moist with physiological saline; whereas, the spleens of Group IV were placed in one-quarter inch thick lead boxes with openings for the splenic pedicle only. Thus the whole body including the spleen of Group III mice was irradiated whereas Group IV mice received whole body irradiation but the spleens were lead shielded during irradiation. The irradiation required about 15 minutes. After irradiation the spleens of Groups II, III, and IV were returned to the abdominal cavity and the incision sutured. The X-rays were generated in a 250 Kv machine operating at 15 ma. A 0.25 mm copper filter was used. The half-value layer of the filtered beam was 1.0 mm. The mice were weighed daily for the first 9 days and every 4 days thereafter. Mice from each

† CF-1 (Carworth Farms albino with brown, non-agouti background) Homozygous for the recessive genes aa bb cc.

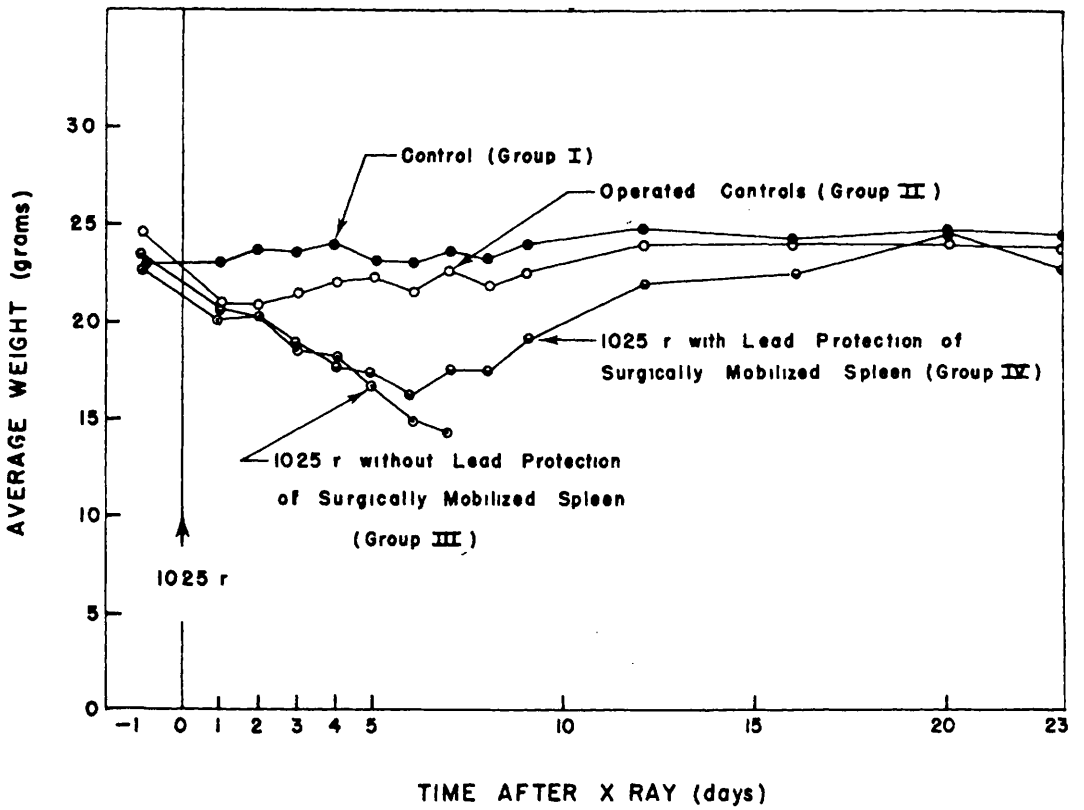

FIG. 1.
The mean weight of control and irradiated mice.

group were killed at daily intervals and tissues fixed in Zenker-formol embedded in nitrocellulose, sectioned at 5 to 8 mu and stained with hematoxylin-eosin-azure.

Results. As indicated in Fig. 1, the mean weight of the 2 groups of irradiated mice followed the same pattern of decline. However, all the mice without lead protection of the spleen had died by the ninth day; whereas, the mice which had lead protection of the spleen reached a minimum weight by the sixth day and thereafter rose to approximate the controls by the twentieth day after irradiation. In the mice prepared for histologic study, no regeneration of bone marrow, thymus, or lymph nodes was as yet apparent on the tenth day and only focal areas of regeneration were apparent in the bone marrow on the eleventh day in mice which received 1025 r without lead protection of the spleen; death of all animals in the group precluded study of hematopoietic regeneration in this group beyond this interval. In the mice which received this dose but which had lead protection of the surgically mobilized spleen during irradiation, regeneration of the bone marrow was already apparent at 4 and 6 days and normal in cellularity at 8 days. (Fig. 2) Reconstitution of the lymph nodes in terms of cellularity was also within normal limits by 8 days. The thymi appeared to resume normal cellularity more slowly. In mice exposed to 600 r X radiation with or without lead protection of the spleen, the difference in the hematopoietic recovery rate of the two groups is less striking but nevertheless clear-cut. The bone marrow of rats which have been exposed to dosages of 600 r or 800 r with or without lead protection of the spleen has been examined and demonstrates the same phenomenon(3). Recovery of the irradiated

3. Jacobson, L. O., Simmons, E. L., Marks, E. K., Robson, M. J., and Gaston, E. O., 1949, unpublished data.

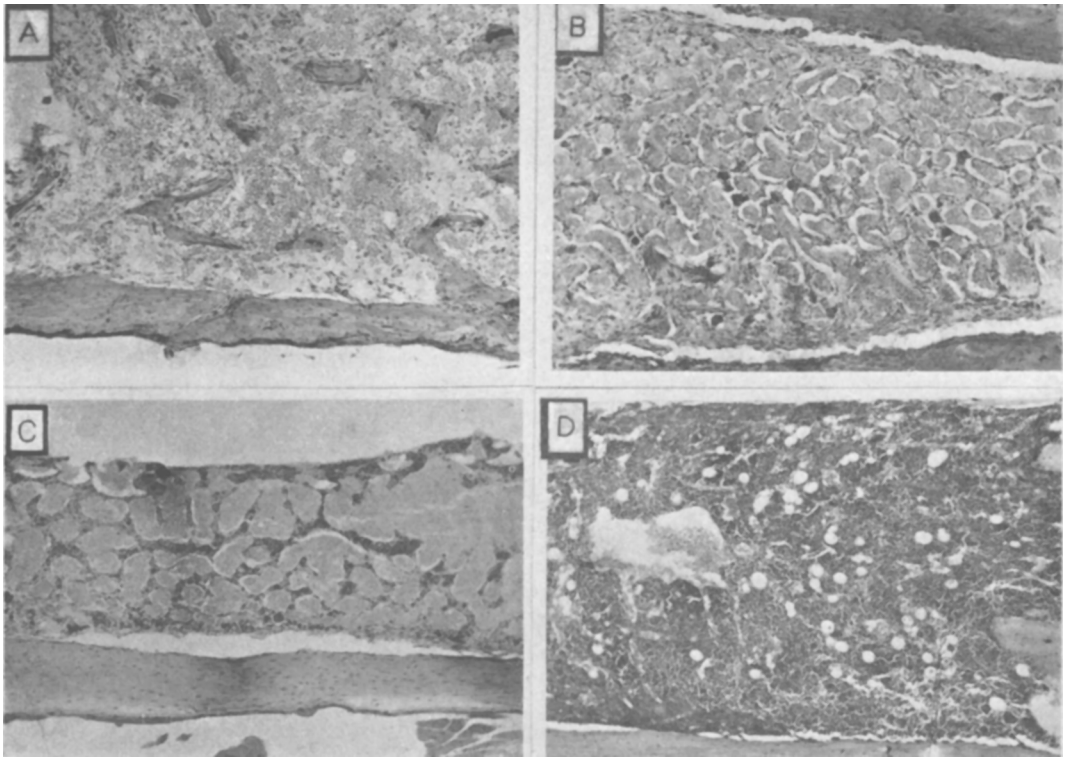

FIG. 2.

Photomicrographs of femoral bone marrow of mice at 4 and 8 days after 1025 r whole body X radiation. HEA × 70. (a) Whole body X radiation without lead protection of the spleen (4-day interval). (b) Whole body radiation without lead protection of the spleen (8-day interval). (c) Whole body X radiation with lead protection of the spleen (4-day interval). (d) Whole body X radiation with lead protection of the spleen (8-day interval). Note that the marrow in A and B is devoid of free hematopoietic cells and consists of diffuse hemorrhage and dilated venous sinusoids; whereas, in C active hematopoiesis exists about the dilated venous sinuses and a normal cellularity exists in D.

hematopoietic tissues occurred sooner in rabbits which had lead protection of either the spleen or the appendix during whole body exposure to 800 r X radiation than in rabbits irradiated without lead protection of the spleen or appendix(4). Ectopic blood formation in the lead protected spleen of rats and mice is greatly intensified after irradiation; whereas, in the lead protected spleen of the irradiated rabbit, ectopic blood formation is minimal and absent in the lead protected appendix of the irradiated rabbits.

Discussion. Several possible explanations for these findings may be mentioned. After exposure to whole body X radiation, the irradiated spleen may, by some humoral mechanism, suppress regeneration of hematopoietic tissue in the marrow and lymphatic organs. Perhaps irradiation injury is so great in the group without lead protection of the spleen that failure of hematopoietic recovery is simply a "toxic" phenomenon. Against this latter suggestion is the fact that the weight loss of both irradiated groups closely paralleled each other during the period in which marrow and lymphatic tissue of the spleen-protected group had already begun to regenerate; whereas, no regeneration was apparent in the other. The essentially normal spleen of the lead protected group which quickly assumes a major role in blood formation may reduce radiation toxicity sufficiently

4. Jacobson, L. O., Marks, E. K., Robson, M. J., and Bethard, W. F., 1949, unpublished data.

to allow for normal hematopoietic regeneration. Since lead protection of either the spleen or the appendix of the rabbit during irradiation allows a more rapid regeneration of the irradiated hematopoietic tissue than in rabbits without such protection, it is obvious that neither is it the spleen specifically nor the ability of the lead protected tissue to produce ectopic blood formation that accounts for the observed phenomenon. These findings strongly suggest that lymphatic tissue exerts a humoral control over hematopoiesis. It might be postulated that the bone marrow which is not normally lymphopoietic, and the thymus would produce a comparable effect were it possible to test this postulate by exclusive protection of these tissues during irradiation. Such experiments are probably necessary before one can assume that the entire hematopoietic system functions as a unit in the maintenance of a steady state. Colonization or seeding from the lead protected lymphatic tissue may actually provide cells from which the irradiated marrow and lymphatic tissue become repopulated. If this latter suggestion were true, then it would follow that the lymphocytes coming from a protected lymphatic organ such as the appendix and going to the bone marrow were capable of giving rise to all types of hematopoietic cells.

If the irradiated lymphatic tissue is acting to temporarily suppress hematopoietic regeneration and recovery under the conditions of these experiments, then the process may be related to the "indirect" therapeutic effect observed in myelogenous leukemia when the spleen alone is irradiated.

It should perhaps be pointed out that lead protection of lymphatic tissue of irradiated animals may possibly reduce "radiation toxicity" in a non-specific manner and that a more rapid regeneration of tissue in the body other than hematopoietic tissue such as skin, gonads, and intestinal mucosa will be apparent on examination.

Summary and conclusions. Young adult female mice were exposed to 1025 r whole-body X radiation. The spleens of these mice were mobilized surgically through an abdominal incision and lead protected during irradiation in one group and mobilized but not lead protected during irradiation in another group.

The bone marrow and lymphatic tissues were destroyed and no regeneration was as yet apparent on the tenth day in the animals irradiated without lead protection of the spleen. In the animals with lead protection of the spleen during irradiation, these tissues were never depleted of free hematopoietic cells and the bone marrow and the lymph nodes were normal in cellularity by the eighth day after irradiation.

Several possible interpretations of the role of the spleen under the conditions of these experiments are discussed.

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Effects of Vitamin B_{12} and Liver Residue on Growth of Hyperthyroid Male Rats.* (17712)

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Available data indicate that requirements for a number of nutrients are markedly increased in the hyperthyroid animal(1). This is particularly true for some of the B vitamins. An increased requirement for thiamine(2,3), pyridoxine(4), pantothenic acid

* Communication No. 249 from the Department of Biochemistry and Nutrition, University of Southern California.

1. Drill, V. A., *Physiol. Rev.*, 1943, v23, 355.

2. Drill, V. A., PROC. SOC. EXP. BIOL. AND MED., 1938, v39, 313.

3. Drill, V. A., and Sherwood, C. R., *Am. J. Physiol.*, 1938, v124, 683.

4. Drill, V. A., and Overman, R., *Am. J. Physiol.*, 1942, v135, 474.