

strips of cotton were introduced into the marrow cavity with a thin bladed thumb forceps and forced down with a blunt, fairly flexible probe. Both epiphyseal ends were plugged first with soft bone wax which was followed with wax-saturated cotton. Pressure by the harder cotton occasionally produced shock and death of the animals. The entire marrow cavity was packed tightly. This method of packing resulted in the expulsion of remaining bits of marrow and prevented regeneration of marrow in loosely filled places. Many other procedures in filling the marrow cavity were tried such as filling with a paste by means of needle and syringe. The method described, although more time consuming and laborious, was found to conform to the requirements of the experiment.

In 24 animals, the marrow of 1 to 3 bones was replaced with inert material at a single operation at intervals of 2 to 4 weeks. In 6 animals, the marrow of all the long bones on one side of the body, tibia, femur, humerus,

radius and ulna, was replaced at one operation. The marrow of all the long bones of the other side was removed from 5 to 7 days later. These varying time intervals allowed to study the effects of rapid and gradual ablation of bone marrow.

The marrow cavities and the bones were examined at intervals over a period of 3 years. There was an initial thickening of the bone in the first several weeks followed by a slight degree of reduction in thickness. Whenever the packing was not complete, proliferating endosteum and marrow were found formed between bone and packing.

Summary. A method of ablating permanently the marrow of all the long bones in living rabbits is presented. The ablation may be carried out rapidly over a period of a week or gradually over several weeks. Approximately $41 \pm 5\%$ of the total marrow of the animal may be ablated permanently by this method.

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Effects of Permanent Mechanical Ablation of Marrow of Long Bones on Peripheral Blood of Living Rabbits.

BERNHARD STEINBERG AND RUTH A. MARTIN.

From the Toledo Hospital Institute of Medical Research, Toledo, Ohio.

We described in previous articles procedures of removing marrow from long bones of living rabbits and replacing it with an inert material.^{1,2} The total marrow of the body may be reduced by $41 \pm 5\%$ by this method.³ Thirty rabbits were used over a period of 3 years in this experiment. In 24 animals the marrow was removed gradually over a period of 22 days to 5 months. In 6 rabbits the marrow was replaced within one week. The 30 animals were observed

for periods of 10 days to 14 months after ablation of the marrow. Marrow was removed from 2 to 10 of the long bones with a reduction of 11 to 41% of the marrow. The marrow of the radius and/or ulna on one side was left frequently intact. The marrow of these bones is completely inactive in mature animals. Peripheral erythrocyte, platelet and leukocyte counts, hemoglobin determinations and studies of stained blood smears were made at frequent intervals before and after removal of marrow.

After removal of marrow there was an initial rise in the number of peripheral leukocytes in most of the animals. In some of the rabbits there was a drop. The change in the peripheral content of the cells re-

¹ Steinberg, B., and Martin, R. A., *Proc. Soc. Exp. Biol. and Med.*, 1946, **61**, 428.

² Steinberg, B., and Martin, R. A., *Proc. Soc. Exp. Biol. and Med.*, 1946, **63**,

³ Dietz, A., *Proc. Soc. Exp. Biol. and Med.*, 1944, **57**, 60.

TABLE I.

Effect of Rapid Removal of $41 \pm 5\%$ of Marrow from Long Bones on Cell Content of Peripheral Blood.

Time examined	Hemoglobin, in g	Erythrocytes, millions	Leucocytes, thousands	Platelets, thousands	Differential study of stained blood smears		
					Granulo-cytes	Lympho-cytes	Mono-cytes
Before removal of marrow	11.5 to 12.5 in 1 wk	5.3 to 6.0	8,200 to 9,200	330,000 to 350,000	34	62	4
One day after removal	9	4.8	7,100	210,000	34	64	2
In 3 days	8.7	4.4	19,600	280,000	31	68	1
" 5 "	8	4.5	20,800	300,000	22	76	2
" 7 "	10	5.0	12,200	270,000	22	76	2
" 20 "	10.5	5.0	9,800	320,000	41	56	3

TABLE II.

Infrequent Effects of Gradual Removal of Marrow from Long Bones on Cellular Content of Peripheral Blood.

Time examined	Hemoglobin, g	Erythrocytes, millions	Leukocytes, thousands
Before removal of marrow	12.8	5.2	12,250
After removal of 1 marrow, 7 da. later	13.9	6.0	15,200
3 marrows, 14 da. later	12.5	5.2	5,550
4 marrows, 14 to 19 da. later	12.0	5.1	16,900 to 25,850
7 marrows,* 5 da. later	13.0	6.3	49,800
Before removal of marrow	9.5	4.5	13,000
9 da. after removal of 2 marrows†	7.3	2.8	46 polys.
			54 lymphs
			135,300
			52 segmented polys.
			11 stab polys.
			13 juveniles
			14 myelocytes
			10 lymphs

* Animal was ill. It was killed for study of tissues. Platelets were slightly reduced for a week after removal of 3 marrows, but returned to normal. The differential cell content was not significantly altered.

† Animal died on tenth day. Platelets not significantly affected.

mained for 4 to 7 days and was reflected by a rise or drop either in granulocytes alone or in lymphocytes and granulocytes. The erythrocytes and the hemoglobin either remained unchanged or were slightly decreased in some animals. The leukocytes, erythrocytes and the hemoglobin content eventually returned to normal, but in a few instances remained slightly decreased. The platelets were at first reduced in number, but in most animals returned to a normal level (Table I).

When more than 6 marrows were ablated, some of the animals developed a permanent slight reduction in leukocytes, erythrocytes, platelets and hemoglobin. In 7 out of 30 rabbits there occurred significant changes in the peripheral cell content. The leuko-

cytes were increased appreciably, reaching from 20,000 to 135,300 mm^3 of blood. The rise was contributed by granulocytes. In 2 animals there were immature granulocytes, which constituted from 17 to 27% of the total number of cells. The hematologic picture was that of leukemia. The animals either became very ill and were killed or they died. The erythrocytes and hemoglobin did not reveal changes in those rabbits with an increase in mature granulocytes. However, immaturity of granulocytes appeared to be associated with a decrease in hemoglobin and erythrocytes and with presence of normoblasts and poikilocytes (Table II).

Summary. Marrow was removed from long bones of rabbits and replaced by inert

material. This procedure resulted in a permanent suppression of marrow. From 11 to 41% of the total marrow was obliterated by this method. The effects on cell and hemoglobin content of peripheral blood were investigated. In most of the animals there were no significant permanent changes. Extirpation of the marrow was followed frequently by an increase in leukocytes and a decrease in hemoglobin, erythrocytes and platelets. When more than 6 marrows were

ablated, some of the animals developed a slight reduction in all the elements investigated. An infrequent animal showed significant changes. Leukocytes were increased in number and the peripheral blood was suggestive of leukemia. Appearance of immature granulocytes and a high leukocyte count was associated with a reduction of hemoglobin and erythrocytes and morphologic abnormalities of red blood cells.

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Tissue Changes in Rabbits with Ablation of Marrow of Long Bones.

BERNHARD STEINBERG.

From the Toledo Hospital Institute of Medical Research, Toledo, Ohio.

In previous articles a method of replacing marrow of long bones with inert material was described.^{1,2} The procedure reduces the marrow content of the body of the rabbit by approximately $41 \pm 5\%$.³ In this paper tissue changes incident to the substitution of marrow by inert material are described. Thirty rabbits were used in this experiment over a period of 3 years. Some of the animals died and others were killed in from 10 days to 14 months after removal of the marrow. In the 30 rabbits, 11 to 41% of the marrow was ablated in from 4 days to 5 months. Gross and histologic observations were made of all the thoracic and abdominal viscera, the remaining marrow and occasionally of the pituitary gland.

In 6 animals $41 \pm 5\%$ of the total marrow was replaced by inert material over a period of 2 to 5 months and the animals were allowed to survive for total periods of 4 to 14 months. Of the 6 animals, 4 showed infiltration by mature and immature granulocytic leukocytes of liver, kidneys,

spleen and in 2 instances of the heart muscle and lungs. One animal showed a slight degree of infiltration of liver and kidneys and in the sixth there were no infiltrative changes (Fig. 1).

In 14 animals, $37 \pm 5\%$ of the marrow was ablated. The animals were allowed to survive from 2 to 14 months. Nine of the rabbits developed cell infiltration of liver, kidneys, spleen and in some there was involvement of the adrenals. Four rabbits showed a slight infiltration of liver and spleen only. One animal did not have any infiltrative changes.

In 7 rabbits 29 to 37% of the marrow was replaced by inert material. The total survival period of each animal did not exceed 2 months. Very slight cell infiltration was found in one animal. There were no infiltrative changes in the others.

In 3 animals from 11.5 to 23% of the bone marrow was removed and the total survival period varied from 1 to 4 months. There was no cell infiltration in any of the organs (Table I).

The unextirpated marrow from ribs, ulna and radius was correlated with the infiltrative changes in the other organs. The radius and ulna in an adult rabbit are acellular and were used as a criterion for the demand

¹ Steinberg, B., and Martin, R. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1946, **61**, 428.

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