Bone Marrow Blood Flow After Marrow Removal or Nutrient Vessel Ligation[1] (35161)

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This study is concerned with changes in blood flow during the reconstitution of rabbit bone marrow in a medullary cavity depleted by dextran perfusion. Since the procedure of dextran perfusion disrupts the nutrient vessels, the effects of nutrient vessel ligation *per se* were also studied. Blood flow was estimated by the medullary uptake of radioactively labeled microspheres of various sizes in the experimental relative to the contralateral femur marrow.

Materials and Methods. New Zealand rabbits, age 10 weeks and weighing 2 to 2.5 kg, were used as the experimental animal. In one group of rabbits, bone marrow was removed from a segment of the right femur diaphysis by an aspiration and dextran perfusion technique which has been described elsewhere (1). In a second group, the nutrient vessels of the right femur were isolated just proximal to the nutrient foramen and then ligated and cut. The procedure was the same as that described by Huggins and Wiege (2).

Blood flow to the femoral marrow was measured in 5 normal rabbits, in 25 rabbits at various stages of regeneration after localized depletion, and in 19 rabbits at various times after ligation of the nutrient vessels. The method used for determining blood flow has been described by Rudolph and Heyman (3) and adapted to the rabbit by Neutze *et al.* (4). In the present study a mixture of microspheres of 15 $\pm$ 5, 25 $\pm$ 5, and 50 $\pm$ 10 μ labeled with either ^{125}iodine, ^{85}strontium, or ^{141}cerium[2] were injected simultaneously through a catheter whose tip was in the left ventricle of the heart of the anesthetized rabbit. The spheres are thus distributed to each tissue in direct proportion to its blood flow. The microspheres are trapped in tissue arterioles and do not recirculate. About 3×10^4 microspheres of each of the 3 sizes were injected. Each size microsphere was labeled with a single and different nuclide. Within 5 min after injection the rabbits were sacrificed, the kidneys and medullary tissue from both femurs were removed and weighed and the radioactivity was measured in a gamma scintillation counter. A pulse height analyzer divided the scintillation counter output into 100 channels of 10 keV each. Since the nuclides used have a known gamma emission with a characteristic peak and isotope overlap within this range, the amount of each isotope present in a given sample can be determined (3). Each sample was counted for 100 min. In the control and contralateral marrow, the minimum total count for each nuclide was 7×10^3.

Results and Discussion. Microsphere uptake (cpm/g of tissue) in the right relative to the left femoral marrow and the right compared to the left kidney in the 5 control rabbits is shown in Table I. Since there was no significant difference between the right and left femur marrow of normal rabbits, the uptake of radioactive microspheres in the experimental (right) femur was related to that in the contralateral (left) femur. The relative distribution of radioactive microspheres in the kidneys served as an additional control. A ratio of 1 would indicate that there was no preferential streaming of the spheres as they descended the aorta (4). Microsphere

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[2] Product of Minnesota Mining and Manufacturing Company, Nuclear Products Division, St. Paul, Minnesota.

TABLE I. Uptake of Labeled Microspheres in Right Relative to Left Femoral Marrow or Kidney in Five Normal Rabbits.[a]

Microsphere (μ)	Marrow	Kidney
15	1.06 ± 0.10[b]	1.06 ± 0.04
25	1.11 ± 0.09	1.03 ± 0.05
50	0.93 ± 0.09	1.08 ± 0.06

[a] Measurements represent counts per minute per gram of one tissue or organ relative to the other.
[b] Standard error.

uptake is dependent upon the size of the vessels delivering blood to the tissues. Because of rapid changes in size and/or caliber of the vasculature during marrow regeneration, we felt that the best estimate of total blood flow would be provided by the average uptake of all 3 sizes of microspheres considered as a single population.

Since the nutrient vessels are disrupted during removal of the marrow from the femur shaft, the first part of this study was concerned with the normal contribution of nutrient vessels to marrow blood flow. The data are summarized in Table II. Twenty-four hr after ligation, there was no radioactivity above background. It is possible that some blood flow might occur at this time through a collateral circulation but that the diameter of the vessels was not large enough to allow entry of the microspheres. Branemark (5) has noted that the diameter of vessels in the Haversian canals and of capillaries in epiphyseal marrow is 6–8 μ. Thus, blood flow through these vessels would not allow passage of the smallest microspheres used. Blood flow was restored rapidly after the first day and approached normality 4 days after nutrient vessel ligation. It is of interest that the flow as measured by the 50-μ spheres alone was also normal at this time. Thus, this study indicates that the nutrient artery is the main source of blood flow for the diaphyseal marrow, which has been generally accepted since the early work of Drinker *et al.* (6) in 1922. The present results also reveal that there is an effective collateral circulation in the absence of nutrient artery flow. At the time of sacrifice, the nutrient foramen was inspected to confirm that the nutrient vessels had been severed. Although Huggins and Wiege (2) noted beginning capillary infiltration from the epiphyseal marrow 4 days after nutrient vessel ligation, our findings suggest that reestablishment of normal flow must have been primarily through vessels in the periosteum and Haversian canals.

The second phase of this study was concerned with changes in blood flow after marrow removal by dextran perfusion. As shown in Table III, blood flow was negligible at 1 day. Flow was gradually reestablished during the first week and reached a level of about twice normal during the fourth week. Since the uptake of labeled microspheres is expressed per unit weight of medullary tissue and regeneration occurs sooner in some areas than in others (7, 8), it follows that the blood flow to active sites in the evacuated femur may be even greater than normal before the fourth week. It is of interest that some 5 to 8 weeks are required for return to normal blood flow which corresponds to the time necessary for the regenerated marrow to be indistinguishable from normal marrow (1, 7, 9). It is understandable that more time would be required for reestablishment of normal flow in an evacuated medullary cavity than in a marrow which has simply been deprived of its nutrient vessels. This follows from the requirement for blood vessel restoration.

Summary. Studies were made of the changes in blood flow in rabbits after localized depletion of femoral marrow or nutrient ves-

TABLE II. Influence of Nutrient Vessel Ligation on Microsphere Uptake in Femoral Marrow.

After ligation (days)	No. of rabbits	Femoral marrow (cpm/g of ligated vs contralateral)	Kidney (cpm/g of right vs left)
1	2	0	1.12
2	4	0.32	1.02
4	4	0.86	1.00
8	2	0.93	1.07
12	2	1.14	1.13
21	2	1.19	0.98
70	3	1.01	1.04

TABLE III. Microsphere Uptake During Regeneration of a Depleted Medullary Cavity.

After marrow removal (days)	No. of rabbits	Femoral marrow (epm/g of ligated vs contralateral)	Kidney (epm/g of right vs left)
1	2	0.01	1.05
2	3	0.24	1.10
4	4	0.19	1.10
8	3	0.82	0.98
16	3	0.95	1.20
24	4	2.47	1.03
35	2	1.25	1.00
55	4	1.01	0.97

sel ligation. Our findings confirm that the nutrient artery is the principal source of flow to medullary tissue in the diaphysis of long bones. They indicate further that (i) there is a highly effective collateral circulation to the medullary cavity, (ii) marrow regeneration is associated with an increased blood flow, and (iii) normal blood flow is restored at a time when the regenerated marrow after a localized depletion is indistinguishable from normal marrow.

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