The University of British Columbia

FACULTY OF GRADUATE STUDIES

PROGRAMME OF THE

FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

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M.D., Severance Medical College, 1955
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MONDAY, MAY 3, 1965, AT 10:00 A.M.
IN PHYSIOLOGY SEMINAR ROOM
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Abstract

Factors affecting bone blood flow were studied in rabbits and dogs. A method for estimating bone blood flow was developed using the initial bone clearance of radioactive Strontium (Sr\textsuperscript{85}) from blood. The method is based on Fick Principle and is similar to that used in estimating renal plasma flow from clearance of PAH or diodrast.

The validity of the method depends on the efficiency of Sr\textsuperscript{85} removal from blood by bone as indicated by the Extraction Ratio (ER). This represents the proportion of the Sr\textsuperscript{85} which has been removed from the blood flowing through bone. It was determined in 10 dogs by injecting Sr\textsuperscript{85} and plasma dye, T=1824, into the nutrient artery of tibia. During the next 5 minutes, 87.3 ± 2.9% of the plasma dye and 20.21 ± 1.68% of the Sr\textsuperscript{85} were recovered from the corresponding femoral vein. The Extraction Ratio calculated from the data was 0.764 ± 0.066 (SE) and is comparable to the ER of 0.90 for extraction of PAH by kidney. The high ER appears to justify the use of initial (0-5 min) bone clearance of Sr\textsuperscript{85} as a measure of effective bone blood flow. Divided by the Extraction Ratio, the clearance gives an indirect measure of total bone blood flow.

Using the above technique, the mean effective bone blood flow for 270 bones from 80 rabbits was found to be 9.60 ± 0.19 (SE) ml/min/100 g fresh weight, and for 46 bones from 10 dogs, the average value was 10.15 ± 0.61 (SE) ml/min/100 g fresh weight. Total skeletal blood flow was estimated to be 7.1 ± 0.25 (SE) % of the resting cardiac output in the rabbits and 7.3 ± 0.95 (SE) % of the resting cardiac output in the dogs.

The nutrient artery of femur was found to supply 70% of the blood flow to the shaft and 1/3 of the blood flow to the ends. Blood flow to the ends of bone was significantly higher than that to the shaft.
Various factors affecting bone blood flow were studied. Section of the sciatic nerve increased blood flow to the bones of the leg and foot, presumably due to interruption of vasomotor fibers. In contrast, infusion of epinephrine (2-4 μg/kg/min) reduced blood flow to tibia and humerus by 78-81% and sharply reduced calcium exchange between blood and the labile calcium storage pool in bone. Immobilization of the leg in a plaster cast for 2 weeks resulted in some decrease in blood flow in tibia and calcaneus but more prolonged immobilization (2 months) caused disuse osteoporosis in these bones and a relative increase in blood flow. The surgical problem of fractures of the neck of femur was studied, and it was found that such fractures reduced blood flow to the femoral head by 52-83%. This interference with blood flow may account for high incidence of aseptic necrosis of the femoral head associated with such fractures.

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QUANTITATIVE STUDIES OF FACTORS AFFECTING BONE BLOOD FLOW
BASED ON BONE CLEARANCE OF RADIOSTRONTIUM (Sr$^{85}$)

by

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
in the Department
of
PHYSIOLOGY

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

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ACKNOWLEDGEMENTS

The author would like to express his deep appreciation to Dr. D. Harold Copp for his sponsorship of the author's graduate study and research work, and for his encouragement and guidance; to Dr. Edgar C. Black, Chairman of the Committee for the author's graduate study, for his tireless encouragement, direction and advice; and to Dr. Hugh McLennan and Dr. Carl F. Cramer for their counsel and valuable suggestions.

The author is grateful to Dr. Frank P. Patterson for his co-sponsorship of the author's graduate study, and to Dr. Allan M. McKelvie of Washington, D.C. who introduced the author to Dr. Patterson in January, 1961.

The author owes many thanks to Mr. Kurt Henze who prepared the graphs and photographs, to Miss Mary Hashimoto who assisted in preparation of the manuscript, and to other staff members of the Departments of Physiology, Surgery and Pediatrics for their help and co-operation.

The author would particularly like to express appreciation to the Medical Research Council of Canada which for the past three years has provided support through a Medical Research Fellowship. He would also like to acknowledge
assistance from the Defence Research Board of Canada and the Trauma Research Unit of the Department of Surgery at the University of British Columbia.
ABSTRACT

Factors affecting bone blood flow were studied in rabbits and dogs. A method for estimating bone blood flow was developed using the initial bone clearance of radioactive strontium (Sr$^{85}$) from blood. The method is based on Fick Principle and is similar to that used in estimating renal plasma flow from clearance of PAH or diodrast.

The validity of the method depends on the efficiency of Sr$^{85}$ removal from blood by bone as indicated by the Extraction Ratio (ER). This represents the proportion of the Sr$^{85}$ which has been removed from the blood flowing through bone. It was determined in 10 dogs by injecting Sr$^{85}$ and plasma dye, T-1824, into the nutrient artery of tibia. During the next 5 minutes, 87.3 ± 2.9% of the plasma dye and 20.21 ± 1.68% of the Sr$^{85}$ were recovered from the corresponding femoral vein. The Extraction Ratio calculated from the data was 0.764 ± 0.066 (SE) and is comparable to the ER of 0.90 for extraction of PAH by kidney. The high ER appears to justify the use of initial (0-5 min) bone clearance of Sr$^{85}$ as a measure of effective bone blood flow. Divided by the Extraction Ratio, the clearance gives an indirect measure of total bone blood flow.

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Various factors affecting bone blood flow were studied. Section of the sciatic nerve increased blood flow to the bones of the leg and foot, presumably due to interruption of vasomotor fibers. In contrast, infusion of epinephrine (2-4 micro-gram/kg/min) reduced blood flow to tibia and humerus by 74-81% and sharply reduced calcium exchange between blood and the labile calcium storage pool in bone. Immobilization of the leg in a plaster cast for 2 weeks resulted in some decrease in blood flow in tibia and calcaneus but more prolonged immobilization (2 months) caused disuse osteoporosis in these bones and a relative increase in blood flow. The surgical problem of fractures of the neck of femur was studied, and it was found that such fractures reduced blood flow to the femoral head by 52-83%. This interference with blood flow may account for high incidence of
aseptic necrosis of the femoral head associated with such fractures.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II REVIEW OF THE LITERATURE</td>
<td>4</td>
</tr>
<tr>
<td>1. Vascular anatomy of bone</td>
<td>4</td>
</tr>
<tr>
<td>2. Nerve supply of bone</td>
<td>7</td>
</tr>
<tr>
<td>3. Physiology of bone blood circulation</td>
<td>8</td>
</tr>
<tr>
<td>A) Rate of bone blood flow</td>
<td>8</td>
</tr>
<tr>
<td>B) Mechanisms of regulation of bone blood flow</td>
<td>12</td>
</tr>
<tr>
<td>C) Factors affecting bone blood flow</td>
<td>14</td>
</tr>
<tr>
<td>D) Functional aspects of bone blood flow</td>
<td>15</td>
</tr>
<tr>
<td>III THE METHOD OF MEASUREMENT OF BONE BLOOD FLOW</td>
<td>18</td>
</tr>
<tr>
<td>DEVELOPED AND USED IN THIS STUDY</td>
<td>18</td>
</tr>
<tr>
<td>1. Techniques and procedures</td>
<td>18</td>
</tr>
<tr>
<td>2. Principle</td>
<td>20</td>
</tr>
<tr>
<td>3. Validity of the method</td>
<td>25</td>
</tr>
<tr>
<td>4. Possible sources of error</td>
<td>27</td>
</tr>
<tr>
<td>5. Advantages and limitations</td>
<td>30</td>
</tr>
<tr>
<td>IV EXPERIMENTAL STUDIES</td>
<td>31</td>
</tr>
<tr>
<td>Purpose, specific method, results and discussion, summary and conclusion in each study</td>
<td></td>
</tr>
<tr>
<td>1. Dependence of bone uptake of Sr$^{85}$ on bone blood flow</td>
<td>32</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>IV</td>
<td>EXPERIMENTAL STUDIES (cont'd)</td>
</tr>
<tr>
<td>2. Sr$^{85}$ extraction ratio for bone</td>
<td>35</td>
</tr>
<tr>
<td>3. Normal blood flow to various regions in femur</td>
<td>44</td>
</tr>
<tr>
<td>4. Normal rates of blood flow through various bones and estimates of total skeletal blood flow</td>
<td>48</td>
</tr>
<tr>
<td>5. Relative contribution of the three arterial systems in long bone</td>
<td>51</td>
</tr>
<tr>
<td>6. Blood flow to bone with disuse osteoporosis</td>
<td>58</td>
</tr>
<tr>
<td>7. Effect of sciatic nerve section on bone blood flow</td>
<td>64</td>
</tr>
<tr>
<td>8. Effect of fractures of the femoral neck on blood supply to femoral head</td>
<td>70</td>
</tr>
<tr>
<td>9. Effect of epinephrine on bone blood flow</td>
<td>79</td>
</tr>
<tr>
<td>10. Relationship of bone blood flow to blood-bone calcium transfer</td>
<td>84</td>
</tr>
<tr>
<td>V</td>
<td>GENERAL SUMMARY AND CONCLUSIONS</td>
</tr>
<tr>
<td>TABLES</td>
<td>100-115</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>116-129</td>
</tr>
</tbody>
</table>
Chapter I
INTRODUCTION

Bone is a specialized connective tissue in which collagen provides resilience while the crystals of bone mineral impart rigidity. The bones make up the structural framework of the body and protect internal organs. They also provide a vast mineral reservoir, which plays an important part in homeostatic control of the concentration of a number of ions in body fluids—particularly calcium. The blood flow through bone is important in making this reservoir available and also in the general nutrition of the tissue. However, until recently, there was very little information available concerning quantitative aspects of bone blood flow, and this lack of knowledge has hampered progress in bone physiology and in the management of bone diseases and injuries.

The physiological study of bone blood circulation is very difficult and has been limited because of the involved vascular pattern, the rigidity of the tissue and deep location of bone under the soft tissues. The proper interpretation of the results of studies of bone blood circulation is also difficult.

The physiology of bone circulation can be conveniently separated into the following four major aspects:
1. the rate of bone blood flow,
2. the mechanism of regulation of bone blood flow,
3. the factors affecting bone blood flow, and
4. the functions of bone blood flow.

It cannot be emphasized too strongly that a good knowledge of these quantitative and qualitative aspects of the physiology of bone blood circulation and the correlations with bone metabolism is essential for the proper understanding of normal and abnormal behaviour of bone.

Accurate measurement of bone blood flow is extremely difficult. To date, only a few methods of estimation of bone blood flow in animals have been proposed, and all have limitations. There is at present no satisfactory method for the measurement of bone blood flow in man.

Biochemical studies of the arterial and venous blood of bone have been also limited. The metabolic rate of the organic substances of bone is not known. In other words, the amounts of oxygen consumption, carbon dioxide production, hence the respiratory quotient of bone have not been studied. Correlative studies of bone blood flow and mineral metabolism of bone also have been limited. Thus little is known about the quantitative relationship of bone blood flow to the metabolism and behaviour of bone as a living tissue and organ in both health and disease.
This thesis is concerned with the quantitative studies of certain aspects of blood flow to bone in rabbit and dog, including the following:


2. Studies of the normal rates of bone blood flow to various bones in the rabbit and dog.

3. Quantitative studies of factors affecting bone blood flow in the rabbit.

4. Studies of the relationship between blood flow and the blood-bone mineral dynamics in the dog.
Chapter II

REVIEW OF THE LITERATURE

This chapter will review the current concepts of the vascular anatomy of bone, the nerve supply to bone, and the quantitative and qualitative aspects of the physiology of blood circulation in bone.

1. THE VASCULAR ANATOMY OF BONE

Using many methods of gross and micro-angiography, the abundant vascularity of bone, with complex anastomosis of blood vessels in the marrow, cortex and periosteum, has been well demonstrated. Ham (29) emphasized the fact that calcified rigid structure and abundant vascularity are characteristic of bone. The classical studies and findings of Langer (41) in 1876 and Lexer (44) in 1903 have been modified little by subsequent workers, including Johnson (34), Trueta et al. (82, 84), McNab (50), Morgan (54), Haliburton et al. (28) and Kelly et al. (39).

In general, it is agreed that there are three major arterial inlets in long bones—the nutrient, the epi-metaphyseal and the periosteal arteries. Most long bones have one nutrient artery which penetrates the bone in the middle part of the
diaphysis (shaft), few epi-metaphyseal arteries in the regions of each end of bone and multiple periosteal arteries entering the surface of bone.

The nutrient artery divides into two or more main branches toward each end of the bone which divide further into multiple smaller branches. Nelson et al. (55) observed that small arterioles branch radially passing outward toward the cortex in the zone of spongy bone and in the medullary cavity. These vessels further divide into fine branches as they enter the haversian canals. Many haversian canals contain more than one capillary type of vessel. These vessels in the haversian canals make fine anastomosis with the numerous periosteal arteries, many of which reach the interior of the cortex through cross channels which are known as volkmann's canals. As described by Ham (29), the longitudinal (haversian) and the transverse (volkmann) canals provide multiple routes for vascular supply and anastomosis in the cortex.

The metaphyseal arteries which distribute their branches in the end regions of bone anastomose with the terminal twigs of the nutrient artery at about the boundary between shaft and end. The capillaries of the metaphyseal vessels run to the epiphyseal plate in long loops. The epiphyseal arteries, which arise from the joint capsule, do not join the metaphyseal arteries across the epiphyseal plate until bony union is complete.
Thus the three arterial systems in long bones anastomose with each other abundantly and complexly in the cortex and medullary spaces.

While the arterial systems have been described well, little is known about the venous system. In general, however, it is believed that the capillaries drain through venules or sinuses to the large veins which are associated with and parallel to the arteries. It is well known that the large nutrient artery and metaphyseal arteries are accompanied by corresponding veins.

The vascular anatomy of non-tubular cancellous bones has received relatively little study. The head of the femur and the talus are representative examples of such bones. Because of their clinical significance, especially in fractures, the vascular anatomy of the head of the femur was studied recently by Trueta and Harrison (82) and that of talus by Haliburton et al. (28).

The talus is the more appropriate one for study of the general vascular pattern of cancellous bone. Several arteries enter the bone and branch into many arterioles and further into thin walled capillaries or sinuses until they become oriented to each individual marrow space formed by the trabeculae. The main intraosseous arteries are associated with veins which progressively unite to form larger veins and eventually leave the
bone by routes which are almost identical with the arterial inlets.

It cannot be emphasized too strongly that bone of any type is very vascular. There is also an intimate association between the circulation in the marrow cavity and in bone, so that the two cannot be considered separately.

2. NERVE SUPPLY OF BONE

While it is generally believed that bone is abundantly supplied by both sensory and motor nerve fibers, there are few anatomical studies on this subject. Ottolenghi (58) in 1902 studied the nerve supply of bone marrow of man, sheep, dog, rabbit, guinea pig and chicken. In 1964, Sherman (69) observed abundant distribution of nerve fibers in the marrow cavity and space between trabeculae in man. Peterson et al. (59) observed medullated nerve fibers which were associated with the nutrient artery.

Ottolenghi (58) concluded from his findings that: (a) the bone marrow is richly supplied with medullated and non-medullated fibers, (b) these nerves form fine plexuses in the walls of the blood vessels and many ramifications reach the capillaries, (c) in the marrow pulp there are many medullated and non-medullated fibers passing eventually to distant vessels,
and (d) the existence of special nerve endings about independent marrow elements cannot be definitely determined.

These findings indicate that specialized neural reflex mechanisms may be mediated through these nerve fibers. The physiological studies of their possible roles will be reviewed later in this chapter.

3. **THE PHYSIOLOGY OF BONE BLOOD CIRCULATION**

The scope of this subject may be conveniently divided into the following aspects:

A. The rate of bone blood flow
B. The mechanism of regulation
C. The factors affecting bone blood flow
D. The functions of bone blood flow

   General—Maintenance of tissue vitality
       --Nutritional
       --Defensive and reparative
       --Others

   Specific—Mobilization of blood cells
       --Mineral homeostasis

A. **THE RATE OF BONE BLOOD FLOW**

Drinker et al. (23, 24) were perhaps the first group who attempted to measure bone blood flow by a direct method. In 1916 and 1922, they isolated the tibia of the dog and perfused
it through the nutrient artery, using a pump. In their study, the venous outflow was collected in a pan and measured. Obviously, the studies were not made under optimal physiological conditions. They found that the rate of flow ranged from 2.6-15.2 ml/min when perfused at the dog's own circulatory pressure.

In 1955, Fredrickson et al. (26) proposed an indirect and more generally applicable method, based on initial bone clearance of radioactive calcium (Ca⁴⁵). This procedure used the same principle as that employed in measuring the effective renal plasma or blood flow from diodrast or PAH clearance by kidney. "Clearance" is defined as the volume of blood or plasma "cleared" of the substance per minute by uptake or removal by the organ. The validity of the clearance method in measuring of blood flow through an organ depends on the consistency and completeness of removal of the substance concerned by the organ during a single passage. This efficiency of removal of the substance by the organ is expressed by the Extraction Ratio (ER), where ER = (A-V)/A, and A and V are the concentrations of the substance in blood of the artery and vein supplying the organ. The kidney is highly efficient in removing diodrast and PAH from blood, with an ER of about 0.90, so that the renal clearance of these substance gives a useful quantitative measure of effective blood or plasma flow through the kidney. Fredrickson
et al. (26) made the assumption that bone would remove \( \text{Ca}^{45} \) with great efficiency from the blood flowing through bone, by exchange with the very large reservoir of nonradioactive calcium present in bone. In rat tibia, they obtained \( \text{Ca}^{45} \) clearance rates (effective bone blood flow) from 10-30 ml/min/100 g bone. From these data they estimated that the total skeletal blood flow was about 1/5 of the renal blood flow in the rat.

In 1962, Cumming (19, 20) made a study of blood flow through bone marrow in the rabbit by a direct method, collecting venous drainage. He reported that the rate of the femoral marrow blood flow was 0.41-0.51 ml/min/g. In the same year, Post and Shoemaker (61) also measured the blood flow through the femur in dog by collecting from the upper and lower venous efflux systems of the bone following ligation of as many other veins as possible. They obtained an average rate of flow of 11 ml/min through this bone with a range of 4.3-25.1 ml.

In 1963, Copp and Shim (13, 70) reported that the rates of blood flow through various bones in rabbits and dogs were remarkably similar with an average rate of 10 ml/min/100 g. They estimated the skeletal blood flow as about 5-8% of the resting cardiac output. In the same year, Ray et al. (62) and Weinman et al. (85) also measured the bone blood flow in dogs. The rate obtained by Ray et al. was 4.9 ml/min/100 g
and the skeletal blood flow was estimated to be 4-9% of the resting cardiac output. Weinman et al. obtained average rates of 5.6 ml and 7.7 ml/min/100 g in mature and immature dogs respectively. Their estimated skeletal blood flow was about 5-10% of the resting cardiac output. All of these studies used the method of isotope clearance.

In 1964, White et al. (88) proposed a different method of measurement of bone blood flow in which the amount of Cr\textsuperscript{51} tagged blood delivered from the central circulation to bone was measured. This is essentially a measurement of bone blood volume by a dilution technique. In brief, they shut off completely the blood circulation to a limb by a tourniquet and injected Cr\textsuperscript{51} into the systemic circulation. A period of 11 minutes was allowed for equilibration of the concentration of the isotope in the systemic circulation. Then, the isotope concentration per ml blood of the heart, obtained by cardiac puncture, was determined. Finally, the tourniquet placed on the limb was released to allow the circulation to the limb to resume. The radioactivity in the various tissues of the limb, including bone, was analyzed at intervals from 2 seconds to 15 minutes after releasing the tourniquet. By a rather complicated analysis of the curves obtained, they estimated the blood flow through the various tissues, including bone.
They reported an average rate of blood flow through the tibia in rabbits of 0.16 ml/min/g. They also reported the rate of blood flow (ml/min/g) through other tissues measured at the same time as follows: skin, 0.54; skeletal muscle, 0.27; and tendon, 0.10. Since blood flow was measured following a period of absolute ischemia of more than 10 minutes, the hyperemic effect undoubtedly would have magnified their measured rate of flow.

In summary, quantitative measurement of bone blood flow is very difficult and none of the present methods appears to be applicable in man. However, the rates of bone blood flow in animals measured by indirect methods by several groups of workers are remarkably consistent. The range of values reported are as follows: in the rat, 10-30 ml/min/100 g; in the rabbit, 10-16 ml/min/100 g; and in the dog, 5-10 ml/min/100 g. In these studies, the entire skeletal blood flow was estimated to be approximately 5-10% of the resting cardiac output.

B. THE MECHANISMS OF REGULATION OF BONE BLOOD FLOW

Although poorly understood, there is some evidence that the bone blood circulation is controlled by both neural and hormonal mechanisms. In 1916 and 1922, Drinker et al. (23,24), in their experiments perfusing the isolated tibia of the dog through the nutrient artery, found that the outflow of blood
was decreased when they electrically stimulated the nerve fibers of the bone marrow. This was also the case when epinephrine was added to the perfusing blood. In 1952, Bloomenthal et al. (5) observed that the intramedullary pressure of long bone in the dog fell when epinephrine, norepinephrine or pitressin were given. In 1958, Stein et al. (76) reported similar findings. In 1959, Herzig and Root (32) and Weiss and Root (87) found in the cat that stimulation of peripheral ends of various peripheral nerves and administration of epinephrine caused a fall in the intramedullary blood pressure. In 1962 and 1963, Copp and Shim (12, 70) confirmed the above findings in dogs. They found a consistent and persistent fall of the intramedullary blood pressure, and a decrease or cessation of bone bleeding through the drill holes made through the cortex to the medullary cavity during slow intravenous infusion of physiological saline containing a minute amount of epinephrine. Quantitative evidence that epinephrine reduces bone blood flow will be presented in this thesis.

In 1963, Shaw (67, 68) also confirmed the above findings. In the same year, Trotman and Kelly (80) reported a 27% increase in blood flow to the tibia in the anaesthetized dog on the fourth day after lumbar sympathectomy. Their study was based on the rate of bone uptake of radioactive rubidium (Rb$^{86}$).
The anatomical fact that bone is rich in both medullated and non-medullated nerve fibers will be recalled. Since epinephrine, norepinephrine and pitressin are all naturally occurring substances in the body, the above evidence strongly indicates that the bone blood circulation is controlled by both sympathetic vasomotor nerves and by vasopressor hormones.

C. FACTORS AFFECTING BONE BLOOD FLOW

Since the bone blood circulation is an important part of peripheral circulation and since bone is an organ having many important functions in the body, there must be many factors affecting bone blood circulation. They can be either physiological factors (i.e. respiration) or pathological (i.e. hemorrhage), and either systemic factors (i.e. shock) or local (i.e. fracture). The action therefore could be either direct or indirect, and the effect of such action could be either hyperemia or ischemia of bone. Active hyperemia in bone following acute infection (acute osteomyelitis) is well known clinically. Ischemia following fractures of bone, and hyperemia in the stage of healing of fractures are also well known. Evidence was found in the isolated perfusion experiment of the tibia in the dog by Drinker et al. (24) in 1922, that anoxia would increase the bone blood flow. This effect could be of value since, in hypoxic or anoxic state, the body requires more circulating red
cells which must be mobilized from the bone marrow. Local ischemia of bone, whatever the cause, might result in accumulation of CO₂ and acid metabolites and increase local hydrogen ion concentration, all of which might lead eventually to active hyperemia due to chemical vasodilation (53). Experimental studies on these factors affecting bone blood flow have been limited and observations have been fragmentary. There is need for more experimental studies on both qualitative and quantitative aspects.

D. THE FUNCTIONAL ASPECTS OF BONE BLOOD FLOW

The prime function of blood circulation through any tissues or organs is to maintain the life of cells, tissue and organ. This is certainly true for bone. Ham (29) and Harris and Ham (31) have stressed the importance of the vascular supply to bone in relation to nourishment of bone cells. Avascular or ischemic necrosis of bone by some known (i.e. fracture) or unknown reason is one of the serious problems in orthopedic surgery (2, 66).

Johnson (34) in 1927 made an excellent study of the relative importance of each of the three arterial systems in long bone. Following selective ligation or destruction of two of the three arterial systems of the tibia in the dog he observed specific areas of dead bone. He concluded that the
nutrient artery is responsible for the nourishment of the bone marrow and the inner half or 2/3 of the cortex of the shaft, that metaphyseal arteries supply blood to the metaphyseal areas and that the periosteal arteries supply the outer half or 1/3 of the cortex of the shaft. Subsequent workers have agreed with Johnson although some workers questioned the role of the periosteal arteries. However, Trueta and Caladias (84) in 1964 studied this problem carefully and extensively in the rabbit's radius and confirmed Johnson's findings.

The function of bone blood flow in fracture healing or repair of other injuries is of serious concern to the orthopedic surgeon. Although ischemia is known to hamper the processes of repair of bone injury, the threshold level of quantitative ischemia or harmful level of hyperemia for repair of fracture is not known as has been emphasized by Ray (64).

The function of the bone blood circulation in growth and development of bones is well known (81, 83). Hyperemia in growing stage of bone causes increase in rate of bone growth. This knowledge is clinically applied to correct leg-length discrepancy in growing children. This has been accomplished by surgical creation of an arterio-venous fistula (33, 86), lumbar sympathectomy (4, 30), and surgical stripping of periosteum. Juvenile osteochondrosis and some congenital anomalies (i.e.
congenital hip dislocation in infant) may be due to local defect of vascular development causing local ischemia in the developmental stage of bone.

Little is known quantitatively about the correlation of oxygen consumption of bone to bone metabolism (53, 65), but the problem is now being studied in this laboratory.

Mobilization of circulating blood cells from the bone into blood and transport of hemopoietic materials to bone marrow are important specific functions of bone blood circulation. Another important specific function of bone blood flow is the blood-bone mineral transfer which is essential for calcification of bone and regulation of mineral metabolism. Bone is a major mineral and electrolytes reservoir and plays an important role in calcium homeostasis. These important aspects have been stressed by McLean et al. (48), Neuman et al. (56), Copp et al. (11, 15, 16), and many others.
Chapter III

THE METHOD OF MEASUREMENT OF BONE BLOOD FLOW
DEVELOPED AND USED IN THE STUDY

The indirect method of measurement of bone blood flow which was developed and used in this study will be described in this chapter. The method is based on measurement of bone clearance of radioactive strontium (Sr$^{85}$) in the initial 5 minutes following intravenous injections. Clearance is defined as the volume of blood cleared of the isotope by bone uptake expressed as ml per minute.

The advantages and limitations of the method will be discussed later.

1. THE TECHNIQUES AND PROCEDURES

1. In the anaesthetized animal, the carotid artery is cannulated with a polyethylene tube (PE 60) directed towards the heart.

2. The cannula is connected to a slow constant rate syringe pump as shown in Figure 2. (The syringe pump used was No. 5-8292, American Instrument Co., Silver Spring, Maryland.)

3. A known amount of radiostrontium (Sr$^{85}$Cl$_2$, 5-10 microcuries/kg of animal weight) is injected into an ear vein
or jugular vein. This injected dose is same as that in the standard solution prepared.

4. The pump is turned on immediately following the injection and the carotid arterial blood is withdrawn continuously at the rate of approximately 1.5-2.0 ml/min.

5. At the end of the fifth minute, acute cardiac arrest is induced in the animal to secure instantaneous circulatory arrest. This is done by injection of about 10 ml of 6-10% EDTA solution into the heart through the jugular vein which produces immediate cardiac arrest.

6. The withdrawn blood is gently shaken to mix thoroughly and 1 ml aliquots are pipetted into two or three planchets.

7. The bone(s) for study, are removed quickly, soft tissue is removed and the fresh weight of the bone is determined.

8. The bones are then ashed and dissolved in 1N HNO₃.

9. The average radioactivity in the 1 ml blood aliquots is determined. The isotope uptake by the bones is also determined by measuring the radioactivity in aliquots of bone ash dissolved in HNO₃.

10. The individual bone uptake of radioactivity is divided by the radioactivity per ml blood to obtain the 5 minute clearance of isotope by that bone.
11. This value is divided by 5 to obtain the clearance in ml/min.

2. **PRINCIPLE**

The method is based on Fick Principle, and is essentially the same as that for determining blood flow through kidney by measuring clearance of para-aminohippuric acid (PAH) (9) or diodrast (18).

The Fick Principle states that the blood flow through an organ is equal to the amount of the substance taken up from the blood passing through it divided by the arteriovenous concentration difference of the substance. It is formulated in the following equation: 

\[ F = \frac{Q}{A-V} \]

where 

- \( F \) is the volume of blood flow,
- \( Q \) is the amount of the reference substance taken up (or added) by the organ,
- \( A \) and \( V \) are, respectively, the concentrations of the substance in 1 ml of blood from the artery and vein.

It is easy to determine the Sr\(^{85} \) concentration in blood of the nutrient artery because this will be the same as that obtained by sampling any convenient artery. However, collection of venous blood from bone is extremely difficult because of the very small size of these vessels. However, as Fredrickson et al. (26) pointed out, if the Sr\(^{85} \) is exchanged almost completely for non-radioactive calcium from the vast mineral store in bone
during the period that the blood is flowing through the bone vessels, the venous concentration will be very low \((v \rightarrow 0)\), and the formula becomes \(F = Q/A\). This is the same as the clearance of \(\text{Sr}^{85}\) by bone uptake, given by the formula:

\[
\frac{C_{\text{Sr}^{85}}}{\text{Bone uptake of } \text{Sr}^{85}} = \frac{\text{Sr}^{85} \text{ conc. in } 1 \text{ ml systemic arterial blood}}{\text{Sr}^{85} \text{ conc. in } 1 \text{ ml systemic arterial blood}}
\]

Here, we shall consider some dynamic aspects of the method. Since both the arterial concentration of \(\text{Sr}^{85}\) and bone uptake change continuously, the \(\text{Sr}^{85}\) clearance must be measured on a dynamic basis. Figure 1 illustrates the time-concentration curve for \(\text{Sr}^{85}\) disappearance from the systemic artery constructed with the averaged data from 10 rabbits. In the first five minutes following injection, the \(\text{Sr}^{85}\) concentration changes rapidly, but changes slowly after that time. The bone uptake of \(\text{Sr}^{85}\) \((dU)\), during an infinitely short period of time \((dt)\), under the specific activity or concentration of the isotope in 1 ml blood \((Sdt)\), can be expressed as follows:

\[
dU = C_{\text{Sr}} \times Sdt
\]

where \(C_{\text{Sr}}\) is \(\text{Sr}^{85}\) clearance and presumably is constant during the clearance period. Integration for 5 minutes can be expressed as follows:

\[
\int_0^5 dU = C_{\text{Sr}} \times \int_0^5 Sdt
\]
Figure 1

Time-concentration curve for Sr$^{85}$ disappearance from blood.
Method of Continuous Withdrawal of Systemic Arterial Blood for Integration of Average Isotope Concentration per Ml. Blood.

Method of continuous withdrawal of systemic arterial blood for integration of average isotope concentration per ml blood.
Sr\textsuperscript{85} concentration in 1 ml carotid arterial blood (average for 10 dogs) at 10 seconds and each minute.
Therefore, the 5 minute bone clearance of the isotope would be expressed as:

\[ C_{\text{Sr}} = \frac{\int_0^5 dU}{\int_0^5 Sdt} \]

The average \( \text{Sr}^{85} \) clearance by bone, \( C \), in ml/min can be obtained by dividing the 5 minute clearance by 5. That is:

\[ C = \frac{\int_0^5 dU}{5 \int_0^5 Sdt} \]

The above formula means, essentially, that the average \( \text{Sr}^{85} \) clearance of bone (ml blood/min) can be obtained by dividing the average minute bone uptake of isotope per minute by the average concentration of the isotope in 1 ml of systemic arterial blood collected during the 5 minute clearance period. This may be determined by drawing the arterial blood continuously from the carotid during the clearance period using a constant rate syringe pump. This blood is then mixed well and the average concentration of the isotope in 1 ml of the blood is determined.

3. **THE VALIDITY OF THE METHOD**

In evaluating the validity of the method, the following questions are pertinent:

1. Is bone uptake of the isotope dependent upon bone blood flow?
2. How efficient is bone in removing the isotope from the blood passing through bone?

The answers to these questions are given in detail in the chapter on Experiments I and II respectively. It was proven that the bone uptake of Sr\textsuperscript{85} is entirely dependent on bone blood flow. The ischemic heads of the femur had only 1.5% of the isotope uptake of the normal head.

Secondly, the validity of Sr\textsuperscript{85} clearance as a measure of bone blood flow depends on the consistency and completeness of removal of the isotope by bone during a single passage. This efficiency of bone is expressed by the extraction ratio (ER), where \( ER = \frac{(A-V)}{A} \), and \( A \) and \( V \) are the concentrations of the isotope in arterial and venous blood of bone. Here, it will be recalled that the venous concentration (\( V \)) was taken as zero in the course of derivation of the clearance formula. It is important to know whether it is, in fact, zero or for example, 50% of the arterial concentration, in order to know the efficiency of removal of the isotope by bone and to know how much of actual bone blood flow is measured by clearance. If bone removes the isotope in the bone artery completely, the venous concentration will be zero, and this clearance will equal the actual bone blood flow. In such cases, the extraction ratio would be 1.0.
The average extraction ratio determined for tibia in 10 dogs was 0.75 and was fairly consistent for the first 5 one minute clearance periods and from dog to dog (vide infra, Experiment II). These findings justify the use of initial bone clearance of Sr$^{85}$ as a measure of bone blood flow. The clearance value (ml/min) is referred to as "the effective bone blood flow". Actual bone blood flow can be calculated readily by dividing this value by the extraction ratio, 0.75.

4. **POSSIBLE SOURCES OF ERROR**

The following possible sources of error must be considered.

a. **Errors related to induction of instantaneous circulatory (cardiac) arrest:** As we considered the dynamic aspect of the method with regard to rapid and continuous changes in concentration of the isotope in circulating blood and in bone uptake, any timing error is critical. Therefore, at the end of 5 minute clearance time, the cardiac arrest must be induced accurately and instantaneously. It was proven that injection of EDTA solution (10-15 ml of 6-10%) into the jugular vein caused immediate diastolic cardiac arrest due to cardiac hypocalcemia.

b. **Errors related to withdrawal of carotid arterial blood:** The continuous withdrawal of the carotid arterial blood through a polyethylene tube using a constant rate syringe pump is an
important part of the technique to determine the integrated average concentration of the isotope in 1 ml blood. Any technical failure in this process would result in error in the clearance determination. Timing error, dead space in the tube and mechanical defect of the pump in withdrawing constantly would produce higher or lower average concentration of the isotope in the blood, hence, lower or higher rate of clearance respectively. Careful checking prior to and during the experiments should minimize possible errors arising from this mechanical aspect.

c. Sources of error related to the length of clearance time: It was found that the bone efficiency of isotope uptake, that is, Sr$^{85}$ extraction ratio of bone, gradually decreases as time passes by. Obviously, the longer the period of Sr$^{85}$ accumulation in the skeleton, the higher will be the isotope concentration in the labile exchangeable mineral pool of bone, hence, in the venous blood. In order to minimize the error, a shorter period of clearance (i.e. less than 5 minutes) would be more ideal. The initial 5 minute clearance time was chosen in this study after analyzing the time-concentration curve (Figure 1) for the isotope disappearance from the systemic arterial blood. At the end of 5 minutes following isotope injection, the curve indicates that equilibrium between blood and tissue concentrations of isotope may be approached.
d. **Errors related to determining bone weight:** Muscles, tendons and ligaments attached to bone must be carefully cleaned off prior to weighing the bone. Since the clearance is computed on the basis of bone weight, this must be determined accurately. In general, the weights of bones on both sides (i.e. left and right tibiae) are surprisingly similar if the soft tissues are completely removed. Any discrepancy generally indicates incomplete removal of the soft tissues on either side, unless there is unilateral pathology of bone (i.e. osteoporosis).

e. **Error related to radioisotope counting:** Since the radioisotope is continuously decaying (half life of Sr$^{85}$ is 64-65 days), the bone and blood samples should be counted on the same day. All counting times including that of background must be standardized. Because of the random nature of radioactive decay, if $N$ disintegrations are counted, the standard error of this determination is $\sqrt{N}$. For example, for 100 counts standard deviation is $\sqrt{100}$ or 10 (i.e. 10% of the count). For a count of 10,000, the standard deviation is 100 (i.e. 1% of the count). For this reason, greater precision is obtained with higher counts. However, at very high counting rates, a second error is introduced if two counts are so close together that the detector records them as a single count. This coincidence error is a function of the lag time of detector and the scaling circuit.
Possible errors due to differences in geometry of the counting chamber and samples must be also considered. All must be standardized including the physical condition of the samples (size, shape and density, etc.). Careful preparation of the standard solution of the isotope is of great importance.

5. ADVANTAGES AND LIMITATIONS

This is an indirect method of measurement of bone blood flow based on sound physiological principles. Two important advantages over the direct method must be noted. First, it is generally applicable to any bone and any animal. Second, there is no surgical intervention so that the physiological condition of the circulation is not altered before and during the study. The method developed and used in this study appears to be simple, reliable and generally applicable to any animal or bone.

At present, it is not applicable to man since it requires removal of bones for Sr$^{85}$ assay at the end of the experiment. However, it might be possible to take a suitable bone biopsy for study, or perhaps obtain a bone specimen at amputation.
Chapter IV

EXPERIMENTAL STUDIES

In this chapter, the following series of experimental studies will be reported. In each study, the purpose, the specific method, results and discussion, and the summary of the study will be given.

1. Dependence of bone uptake of Sr$^{85}$ on bone blood flow.
2. Sr$^{85}$ extraction ratio for bone.
3. Normal blood flow to various regions in femur.
4. Normal rates of blood flow through various bones and estimates of total skeletal blood flow.
5. Relative contribution of the three arterial systems in long bone.
7. Effect of sciatic nerve section on bone blood flow.
8. Effect of fractures of femoral neck on blood supply to femoral head.
10. Relationship of bone blood flow to blood-bone calcium transfer.
EXPERIMENT I

DEPENDENCE OF BONE UPTAKE OF Sr\(^{85}\) ON BONE BLOOD FLOW

Ray et al. (63) observed uptake of Sr\(^{90}\) by dead bone which had been reimplanted, and on this basis, questioned whether the isotope uptake was dependent on bone blood flow. In their experiments, the isotope was administered 3 days before bone uptake was measured. Since the method of measuring bone blood flow in this study is based on the uptake and clearance of Sr\(^{85}\) by bone, as described in the preceding chapter, the following experiment was carried out to determine whether immediate uptake of Sr\(^{85}\) by bone (over a 5 minute period) was dependent on an intact circulation.

METHOD

Ten adult white rabbits weighing 2.1-2.6 kg (average 2.4 kg) were used. They were fasted overnight and anaesthetized by intraperitoneal injection of 1 g urethane and 250 mg barbitone/kg body weight. Through a posterior approach, one hip joint in each animal was exposed and the neck of the femur was fractured at the subcapital level (just below the head). Then the ligamentum teres was also cut and the head of the femur was removed completely from the hip joint. Thus the entire blood supply to the femoral head was cut off. The femoral head
then was placed back into the acetabulum, bleeding points were cauterized and the wound was closed. Within a few hours after the surgery, 5-8 micro-curies of carrier free Sr\textsuperscript{85}Cl\textsubscript{2} in 1 ml of normal saline were injected into the systemic circulation through an ear vein. Five minutes after the injection, the animals were sacrificed and the Sr\textsuperscript{85} uptake of the experimental (ischemic) femoral head was compared with that of the control femoral head from the opposite side.

**RESULTS AND DISCUSSION**

The results are summarized in Table I. The significance of the results is quite apparent. The radioisotope uptake in the initial 5 minutes following systemic injection was 28 and 1850 count per minute in the completely ischemic and normal femoral heads respectively. The results clearly indicate that the Sr\textsuperscript{85} uptake by bone in the initial 5 minutes following the isotope injection is entirely dependent on intact blood flow.

Over long periods, it is possible that avascular bone can take up radioisotope to some extent by diffusion. Ray et al. (63) removed the rat radius, killed the cells by boiling and reimplemented the dead bone into healthy muscles. A dose of Sr\textsuperscript{90} was injected, and the uptake of isotope by dead bone 3 days later was found to be the same as the uptake by the normal bone on the opposite side. Boyd et al. (7) also observed uptake of
p³² by a dead femoral head in man several hours after the isotope had been injected. It seems probable that this represents isotope which has been carried to the bone by diffusion and exchanged with calcium in the crystals of bone mineral. It is significant that Ray et al. (63) observed more removal of the deposited isotope in normal bone associated with bone remodelling.

SUMMARY AND CONCLUSION

The radiostrontium uptake by the normal and completely ischemic femoral heads in 10 rabbits in the initial 5 minutes following intravenous injection was studied.

The uptake by the completely ischemic femoral heads was 1.5 ± 0.5% of the normal. It is concluded that the Sr⁸⁵ uptake and clearance of bone in the initial 5 minutes following intravenous injection is entirely dependent on bone blood flow.
EXPERIMENT II

Sr\textsuperscript{85} EXTRACTION RATIO FOR BONE

The validity of the method of estimating blood flow through certain organs such as kidney and bone by measuring the clearance of certain substances depends on the organ's efficiency of removal of the substance from the circulating blood. This efficiency is expressed by the extraction ratio (ER), \( ER = \frac{(A-V)}{A} \), where \( A \) and \( V \) are the concentrations of the substance concerned in its artery and vein respectively. The kidney is highly efficient in removing PAH and diodrast with an extraction ratio about 0.9 for these substances.

To assess the validity of bone clearance of Sr\textsuperscript{85} as a measure of bone blood flow, the Sr\textsuperscript{85} extraction ratio of bone was determined in the following experiments.

METHOD

The study was carried out on 10 adult mongrel dogs (18-25 kg) which were anaesthetized with pentobarbital (nembutal, 30 mg/kg). The Sr\textsuperscript{85} extraction ratio of a representative bone, the tibia, was studied by injecting 1 ml of solution containing about 5 micro-curies of Sr\textsuperscript{85} and 3.75 mg of Evans blue (T-1824) into the nutrient artery and analyzing the blood collected from the ipsilateral femoral vein in the 5 minutes following the injection.
The nutrient artery was exposed as follows. In contrast to man, the nutrient artery in dog normally arises from the anterior tibial artery rather than the posterior tibial. A longitudinal incision was made along the lateral border of the tibia from a point two inches proximal to the head of the fibula to the junction of the middle and lower one third of the leg. The superficial fascia was incised, the peroneal muscles were retracted laterally and the extensors of the foot medially.

The artery was dissected free, and the muscular branches in the upper portion were ligated. In most dogs, the nutrient artery comes off the anterior tibial artery within the first 2-3 inches distal to the point where it passes through the interosseous membrane. It is then directed downward and posteriorly to enter the nutrient foramen at the posterolateral aspect of the tibia. Great care was taken in handling the vessels to avoid injury and spasm.

The femoral vein on the same side was exposed in the femoral triangle and prepared for cannulation. The animal was then given 1 mg heparin/kg i.v. and the vein was cannulated. The control blood was collected for few minutes. The anterior tibial artery was then ligated immediately distal to the origin of the nutrient artery. The prepared solution, as mentioned above, was injected into the nutrient artery slowly (over a one minute period)
using a 23 gauge needle. Beginning immediately after the start of injection, the femoral vein blood was collected for 5 minutes in the first group of 5 dogs and in each of 5 minutes in the second group of 5 dogs. The per cent of the injected dose of Sr$^{85}$ and of Evans blue in this blood was determined. The former was done by measuring the radioactivity in suitable aliquots using a scintillation detector. The latter was determined on plasma using a Klett-Summerson photoelectric colorimeter. The hematocrit reading on this blood was also determined. The per cent dose of Evans blue collected gave a measure of the fraction of the tagged blood entering the nutrient artery which was ultimately recovered in the femoral vein.

At the end of the 5 minute collection period, acute cardiac arrest was induced in each animal as described previously and the tibia was speedily removed for Sr$^{85}$ analysis. The bone was cleaned, dried and ashed at $600^\circ$ C ($1110^\circ$ F) for 24 hours and then dissolved in 1N HNO$_3$.

**CALCULATIONS**

**Symbols:**

- % dose Eb = percentage of the dose of Evans blue injected into the nutrient artery.

- % dose Sr$^{85}$ = percentage of the dose of Sr$^{85}$ injected into the nutrient artery.

- $(% \text{ dose Eb})_{fv} = \% \text{ Eb collected from femoral vein.}$
\[ (% \text{dose } \text{Sr}_{85})_{fv} = \% \text{Sr}_{85} \text{ collected from femoral vein.} \]

\[ U_t = \% \text{Sr}_{85} \text{ taken up by the tibia in } t \text{ minutes.} \]

\[ S = \text{concentration of Sr}_{85} \text{ in blood expressed as } \% \text{Sr}_{85}/\text{ml.} \]

\[ \text{ER} = \text{Extraction Ratio for Sr}_{85} = \frac{(A - V)}{A} \]

\[ = \frac{(Q_A - Q_V)}{Q_A} \text{ where } A \text{ and } V \text{ are the arterial and venous concentrations of Sr}_{85} \text{ in blood entering and leaving bone, and } Q_A \text{ and } Q_V \text{ represent the quantity of Sr}_{85} \text{ as } \% \text{dose Sr}_{85} \text{ in the blood entering and leaving bone during the clearance period.} \]

**Extraction Ratio**

If all the blood containing Evans blue and Sr\textsubscript{85} which entered the nutrient artery was subsequently collected from the femoral vein, \((% \text{dose Eb})_{fv}\) should equal 100%; \(Q_A\) would be 100% and \(Q_V\) would equal \((% \text{dose Sr}_{85})_{fv}\). The extraction ratio would be given by the formula:

\[
(1) \quad \text{ER} = \frac{100\% - (% \text{dose Sr}_{85})_{fv}}{100\%}
\]

However, less than 100\% of the dose of Evans blue was in fact collected from the femoral vein. The small difference (13\%) probably represents blood which had by-passed the femoral vein and has returned by other venous channels. The labelled blood entering the nutrient artery which was ultimately
collected from the femoral vein should be reduced by this amount, and the proportion would be represented by \( (\% \text{ dose Eb})_{fv} \). In this case, \( Q_A = (\% \text{ dose Eb})_{fv} \) and the formula for extraction ratio becomes:

\[
(2) \quad ER = \frac{(\% \text{ dose Eb})_{fv} - (\% \text{ dose Sr}^{85})_{fv}}{(\% \text{ dose Eb})_{fv}}
\]

This formula has been used in calculating the data presented in this thesis.

**Recovery of Sr\(^{85}\)**

The "recovery" of the injected dose of Sr\(^{85}\) was calculated on the basis of the 5 minute uptake by the tibia plus the Sr\(^{85}\) collected from the femoral vein corrected for the additional Sr\(^{85}\) which by-passed this vein. The formula used was:

\[
(3) \quad \text{Recovery of Sr}^{85} = U_5 + \frac{(\% \text{ dose Sr}^{85})_{fv}}{ER}
\]

**RESULTS AND DISCUSSION**

The results are summarized in Figure 4 and Tables II and III. Nearly 90% of the injected dose of Evans blue was recovered in the femoral vein whereas only about 20% of the isotope was recovered in the same blood. The average extraction ratio was \(0.764 \pm 0.066\) (S.E.) with the range 0.63 - 0.80.

This compares favorably with values \((0.34 \pm 0.12)\) reported for removal of bromsulfonphthalein (BSP) by liver (73), but is not as satisfactory as those obtained for extraction ratio of
Diagrammatic illustration of determination of Sr$^{85}$ extraction ratio of tibia
diodrast (18), 0.79-0.96, and PAH (9), 0.81-0.96 by the kidney. This is not surprising in view of the possible blood flow through shunts and in bone marrow. However, the data for bone are remarkably consistent, and in view of this, the initial 5 minute Sr$^{85}$ clearance should give a valid and useful measure of the effective bone blood flow through those regions in bone where active mineral exchange is taking place. The clearance value would be the minimal value. It would give a measure of 75% average of the actual bone blood flow and the latter could be readily estimated by dividing the clearance value by the extraction ratio.

Similar studies on the bone efficiency of Ca$^{45}$ and Ca$^{47}$ uptake were carried out by Ray et al. (62), and Weinman et al. (85). Both groups studied the femur in dogs. Ray et al. found the Ca$^{45}$ extraction ratio was 0.53 for the first 5 minutes, and 0.48 for the first 10 minutes. Weinman et al. using Ca$^{47}$ obtained an extraction ratio of 0.55 for the first 10 minutes. Although these data are somewhat lower than the data obtained in the present study, they are quite comparable.

Ray et al. (62) found that the bone efficiency of removal of Ca$^{45}$ decreased with time. The data shown in Table III also indicate decreasing extraction efficiency, from first minute to third, 0.80, 0.74 and 0.63 respectively. This might well be due
to gradual accumulation of the isotope introduced in the labile mineral pool. The rapidly exchangeable or labile calcium pool of bone in man has been estimated as approximately 40-80 mg Ca/kg body weight. Obviously, the longer the period of isotope accumulation in the bone, the higher will be the isotope concentration in this pool, and in the venous blood in equilibrium with it. From data on Sr$^{85}$ accumulation in bone, it is estimated that the error introduced by this would be less than 5% at 5 minutes. Shorter clearance periods would minimize this error.

The fact that 87% of Evans blue injected into the nutrient artery was recovered in the ipsilateral femoral venous blood at the level of the femoral triangle indicates that most of the venous drainage from the tibia takes this route. The remaining 13% of the dye could have by-passed the femoral vein, or possibly be retained in the bone. In any case, the values are in good agreement with the data obtained by Post and Shoemaker (61). Although extensive surgical procedures were involved in their method, they collected 73 ± 12% of the blood tagged with Cr$^{51}$ and Evans blue in the upper and lower venous efflux systems of the femur following injection into the nutrient artery of the femur in dogs. These findings indicate that Evans blue is a very useful non-diffusible dye in such studies.
SUMMARY AND CONCLUSION

The extraction ratio for removal of Sr$^{85}$ by a representative bone, the tibia, was measured in 10 dogs. It was determined by injecting a non-diffusible plasma dye, Evans blue, and Sr$^{85}$ in a mixed solution into the nutrient artery of the tibia and analyzing the recovery of these in the femoral venous blood. Eighty-seven per cent of the dye was recovered in this blood in the next 5 minutes, whereas only 21% of injected Sr$^{85}$ was so recovered. By dividing the difference by 87, the extraction ratio was obtained.

The average value for the extraction ratio in the 10 dogs was $0.764 \pm 0.066$ (S.E.). This high value (comparable to the ER of 0.90 for PAH extraction by kidney) would appear to justify use of initial Sr$^{85}$ clearance as a measure of effective bone blood flow.
EXPERIMENT III
NORMAL BLOOD FLOW TO VARIOUS REGIONS IN FEMUR

Anatomical studies and clinical experience indicate that certain regions (i.e. metaphyses) are more vascularized than other areas in the same long bone. However, no quantitative study of the relative regional blood flow in long bones have been made to date. It was studied in the following experiments.

METHOD

The studies were carried out on 50 adult white rabbits weighing 2.1-3.6 kg (average weight 2.7 kg). They were fasted overnight and anaesthetized by intraperitoneal injection of 1 g urethane and 250 mg barbitone. Carrier-free Sr$^{85}$ was injected as described above for determination of bone clearance. In each case, the animal was killed 5 minutes after the injection and the femur was removed from one side. The bone was then divided into 4 segments by cutting with a fine electric saw just below the head; just below the lesser trochanter and just above the condyle. These 4 segments (head, trochanter, shaft and condyle) were cleaned, weighed and analyzed for Sr$^{85}$. The effective bone blood flow through each was determined on the basis of initial Sr$^{85}$ clearance, and is expressed as ml/min/100 g fresh weight.
RESULTS AND DISCUSSION

The results are summarized in Table IV. There were statistically significant differences in the relative blood flow through these various regions. The greatest flow was observed in the head \((18.67 \pm 0.52 \, \text{ml/min/100 g})\) and the lowest flow was through the shaft \((7.50 \pm 0.16 \, \text{ml/min/100 g})\).

These data indicate that the areas containing a large proportion of cancellous spongey bone have a higher rate of blood flow than those which are predominantly composed of compact bone. These observations agree with the anatomical finding and the clinical experience that the metaphyseal regions are more vascularized than the shaft. The data also support the clinical experience that fractures in the metaphyseal regions, in general, heal faster than those in the shaft.

An interesting question arises as to why the femoral head has such a high rate of blood flow compared with other regions in the same bone. Is it because the femoral head is a small bulbous bone which has to withstand tremendous weight bearing stress and hence requires higher rate of metabolism? If this is true, the head of the humerus should have a similar high rate of blood flow since in quadrupeds like the rabbit, the humeral head is also subjected to weight bearing stress. It was found, in fact, that the blood flow to the head of the
humerus in 22 rabbits studied was as high as that to the femoral head, with an average flow of 17 ml/min/100 g bone.

Calandruccio and his associates (8) made correlative studies between the "trochanter-head ratio", (T/H ratio), of P32 uptake and the prognosis of healing of the femoral neck fracture and viability of the femoral head. The T/H ratio would be 1 if the rates of isotope uptake by the trochanter and head of the femur were the same. If the uptake of the femoral head is higher than that of the trochanter, the T/H ratio would be less than 1. This is the normal case in man as well as in the rabbit. The lowest T/H ratio which was found by Calandruccio et al. (8) in fracture of the femoral neck with good prognosis was 0.4-0.5, which means that the isotope uptake of the femoral head was twice as high as that of the trochanter. The data obtained in this study in the rabbit agree with the data for man. As Table IV indicates, the Sr85 clearance by the trochanter was 10.38 and by the head, 18.78, which gives a T/H ratio of 0.55. A high ratio indicates relative reduction in Sr85 uptake and blood flow to the femoral head, and is in general associated with poor prognosis for healing of a fracture of the femoral neck.
SUMMARY AND CONCLUSION

The relative blood flow through the head, trochanter, shaft and condylar regions was studied in femurs from 50 rabbits.

The blood flow through these regions were significantly different with highest flow to the femoral head and the least to the shaft. It is suggested that this may be due to a higher proportion of cancellous bone in the head. The blood flow to the femoral head was twice as high as that to the trochanter giving a normal T/H ratio of 0.55, comparable to that observed in man.
EXPERIMENT IV
NORMAL RATES OF BLOOD FLOW THROUGH VARIOUS BONES
AND ESTIMATES OF TOTAL SKELETAL BLOOD FLOW

Studies of blood flow through various bones have been difficult and limited. Drinker et al. (23, 24), Post and Shoemaker (61), and Cumming (19, 20) attempted to measure the blood flow through the tibia or femur in dog or rabbit by collecting the venous outflow from these bones. Fredrickson et al. (26) estimated bone blood flow in the rat by an indirect method based on measurement of the initial bone clearance of a radioactive calcium (Ca$^{45}$). By the same method, but using different isotopes, Copp and Shim (13, 14, 70), Weinman et al. (85, 86), and Ray et al. (62) measured blood flow in rabbits and dogs. White et al. (88) used a dilution technique employing Cr$^{51}$ tagged red cells.

An extensive study of blood flow to femur, tibia, humerus, talus, calcaneus and the vertebra in the rabbit and the dog was made in the following experiments.

METHOD

The animals used were white adult rabbits weighing 1.8-3.5 kg (average weight 2.5 kg), and adult mongrel dogs weighing 9-25 kg (average weight 18 kg). They were anaesthetized as
previously described. The blood flow through various bones were measured by the method described in Chapter III.

RESULTS AND DISCUSSION

As shown in Table V, the rates of blood flow were remarkably similar for the various bones in rabbits and dogs. The average rates were $10.15 \pm 4.12 \text{ (S.D.) ml/min/100 g wet weight}$ in 10 dogs for 46 bones, and $9.60 \pm 3.28 \text{ (S.D.) ml/min/100 g wet weight}$ in 80 rabbits for 270 bones. These were the average rates for initial Sr$^{85}$ clearance which has been defined as "effective bone blood flow". The average rates of total blood flow, corrected for the extraction ratio (0.75), were $13.20 \pm 5.35 \text{ (S.D.) and } 12.48 \pm 4.26 \text{ (S.D.) ml/min/100 g wet weight, in dog and rabbit respectively.}$ From these data, and by taking the skeletal weight as 10% of the body weight, and the resting cardiac output as 182 ml/min/kg in the dog (72), and 175 ml/min/kg in the rabbit (25), the rate of the skeletal blood flow was estimated as $238 \pm 96 \text{ ml/min or } 7.3 \pm 3.0\%$ of the resting cardiac output in the dog, and $31 \pm 10 \text{ ml/min or } 7.1 \pm 2.3\%$ of the resting cardiac output in the rabbit. These values are in general agreement with those of Weinman et al. (85) who reported a skeletal blood flow equivalent to 5-7% of the resting cardiac output in dogs, and Ray et al. (62) who obtained values of 3.5-9.0%.
Based on effective bone blood flow per 100 g fresh weight, the values reported here are somewhat higher than those obtained by Weinman et al. (5.6-7.7 ml/min/100 g) and by Ray et al. (average value 4.9 ml/min/100 g) and are somewhat lower than the figure of 16 ml/min/100 g given by White et al. (88). However, these latter studies involved longer clearance periods, and accumulation of Sr\(^{85}\) in exchangeable bone pool would tend to reduce the Sr\(^{85}\) clearance.

Bone blood flow appears high relative to the metabolic needs of bone, which contains so much inert material. However, this may be related to its important homeostatic function in mineral metabolism. Whatever the reason, the data obtained in this study indicate quantitatively that the blood circulation through the skeleton is an important part of the peripheral circulation.

**SUMMARY AND CONCLUSIONS**

The rates of blood flow through various bones in the rabbit and the dog were studied. The average rate for 270 bones from 80 rabbits was 12.48 ± 4.26 (S.D.), and for 46 bones from 10 dogs was 13.20 ± 5.35 (S.D.) ml/min/100 g wet weight. Skeletal blood flow was estimated to be 7.3 ± 3.0 (S.D.) % of the resting cardiac output in the dog and 7.1 ± 2.3 (S.D.) % of the resting cardiac output in the rabbit.
EXPERIMENT V

RELATIVE CONTRIBUTION OF THE THREE ARTERIAL SYSTEMS
IN LONG BONE

A typical long bone receives its blood supply from three arterial systems with abundant anastomoses: the nutrient, epi-metaphyseal and periosteal vessels. This has been firmly established by many workers since the classical study made in 1903 by Lexer (44).

The relative physiological importance of each of the three arterial systems was studied by observing the effects of selective suppression of one or more sources of blood supply. Johnson (34) in 1927 was the first to study this problem and his findings provide a basis of our understanding of this subject. By interfering with two of the three sources of blood supply and observing the extent of the necrotic bone produced, he concluded that the nutrient artery of the tibia in the dog is responsible for the nourishment of the bone marrow and the inner half of the cortex of the shaft; the metaphyseal vessels supply the metaphyses and are able to supply the area of the nutrient artery, while the periosteal arteries supply the outer half of the cortex. Thus, he found that the nutrient artery system is the most important, the metaphyseal is the intermediate, and the periosteal
is the least important. Several workers have subsequently confirmed the findings of Johnson (34), although McNab (50) and McAuley (46) both felt that the periosteal system played only a minor role. Trueta and Caladias (84) in 1964 confirmed Johnson's findings in a series of critical studies on the radius of the rabbit using similar methods. Cuthbertson et al. (21) recently reported that ligation of the nutrient artery of the tibia and humerus in the dog caused an immediate and profound fall of the intramedullary pressure in these bones.

However, to date, there has been no quantitative study of the relative contribution of each of the three arterial systems to the blood supply to long bone. This was investigated in the following experiments.

**METHOD**

The study was carried out on 13 adult white rabbits weighing 2.4-3.2 kg (average weight 2.8 kg). They were anaesthetized as described previously and the areas of the thigh and groin were shaved. The nutrient artery of one femur in each animal was exposed through an anterior approach. This vessel originates from the deep femoral artery and passes obliquely downward and inferiorly along the iliopsoas muscle to reach the nutrient foramen at the area antero-inferior to the lesser trochanter. The proximal end of the nutrient artery was carefully dissected free from
the nutrient vein and the other adjacent tissues. A ligature loop was placed around it so that it could be ligated readily. The nutrient artery on the opposite side (control) was merely exposed in a sham operation. Then, the carotid artery was cannulated preparatory to measurement of Sr\textsuperscript{85} clearance of bone as described in Chapter III. The nutrient artery was ligated and the solution of Sr\textsuperscript{85} was immediately injected into an ear vein. At the end of 5 minutes, the animal was sacrificed and both femurs were quickly removed. The bones were cleaned and weighed. They were then divided into three segments by transverse cuts at the level of inferior margin of the lesser trochanter and at the supracondylar level using a fine electric saw. Each of these segments was weighed and the Sr\textsuperscript{85} clearance of these segments was determined as described above.

RESULTS AND DISCUSSION

The results are summarized in Table VIII and Figure 5. Ligation of the nutrient artery reduced the rate of blood flow to the shaft by 71%, to the upper end by 37%, and to the lower end by 33%. The normal rates of blood flow through the upper segment, shaft and lower segment were 10.8, 8.5 and 10.8 ml/min/100 g wet weight, respectively. Ligation of the nutrient artery reduced these rates to 6.8, 2.5 and 7.2 ml/min/100 g wet weight, respectively.
Figure 5

Effect of Ligation of Nutrient Artery of Femur on Sr$^{85}$ Clearance in 13 Rabbits.

( ml / min. / 100 gm. )

Effect of ligation of nutrient artery of femur on Sr$^{85}$ clearance in 13 rabbits.

(ml/min/100 g)
The observed reduction of blood flow following ligation of the nutrient artery should provide a direct indication of the relative contribution of this vessel to the supply of these bone segments, provided that there has been no compensatory flow through other anastomotic channels. Since such anastomotic flow, if it occurred, would increase the apparent blood flow, the figures presented must be considered minimal reductions, and it is possible that the contribution of the nutrient artery to blood supply to the shaft may be considerably higher than 71%.

The remaining blood supply (29% of control flow) to the shaft after ligation of the nutrient artery must be from periosteal vessels and the epi-metaphyseal system supplying the bone ends. The same applies to the residual blood flow to the upper (63%) and lower (67%) ends. It is, unfortunately, not possible to differentiate the contribution of the epi-metaphyseal system from that of the periosteal vessels in these experiments, although from the work of others, it appears probable that periosteal contribution is not great.

The profound reduction of blood flow through the shaft and through each end of the bone following the ligation of the nutrient artery confirms quantitatively the findings of Johnson (34), Trueta and Caladias (84), Cuthbertson et al. (21) and many others who studied qualitatively the relative importance of the
Regional Blood Supply of Normal Femur.

- 63% by upper Epi-metaphyseal Artery
- 37% by Nutrient Artery

- 71% by Nutrient Artery
- 29% by Periosteal Arteries (+ epi-metaphyseal artery)

- 67% by Lower Epi-metaphyseal Artery
- 33% by Nutrient Artery

Relative contribution of the various vascular systems to blood supply to femur.
routes of blood supply in long bone. The 71% reduction in blood flow to the shaft after ligation of the nutrient artery agrees with Johnson's finding that the nutrient artery is responsible for the blood supply to the marrow space and much of the shaft cortex. It also supports the finding of Cuthbertson et al. (21) that ligation of the nutrient artery caused an immediate and profound fall in the intramedullary pressure of the dog femur.

SUMMARY AND CONCLUSIONS

The relative contribution of the three arterial systems to blood supply of femur was studied in 13 adult rabbits. The nutrient artery was ligated and the resulting changes of the rates of blood flow through the upper epi-metaphyseal area, the shaft and the lower metaphyseal areas were determined 5 minutes after ligation. The data indicated that the nutrient artery was responsible for at least 71% of the blood supply to the shaft, 37% of the flow to the upper end and 33% of the flow to the lower end. The remaining blood supply to each area could be attributed to the respective regional arterial systems.
EXPERIMENT VI

BLOOD FLOW TO BONE WITH DISUSE OSTEOPOROSIS

Osteoporosis is a common metabolic bone disease characterized by decrease in the mass of bone without significant change in the ratio of the organic (matrix) and inorganic (mineral) components. It can be produced in laboratory animals by various methods. Osteoporosis may be localized or generalized. It is often found in various clinical conditions such as Cushing's disease and syndrome, rheumatoid arthritis, scurvy, hyperparathyroidism, senility and following immobilization, i.e., typically in a plaster cast.

However, the pathogenesis of osteoporosis is poorly understood. The classical theory of Albright (1) attributes the condition to a lack of formation of organic matrix. Many subsequent studies did not entirely support the Albright hypothesis. It is known that the osteoporosis which occurs in osteitis fibrosa generalisata resulting from hyperparathyroidism is in fact due to increased bone resorption rather than reduced bone formation. More recently some workers, particularly Nordin (57) and Jowsey et al. (36), have expressed the opinion that osteoporosis is due to prolonged negative calcium balance. Jowsey and Gershon-Cohen (36) reported in 1964 that osteoporosis could be produced by a prolonged feeding of calcium deficient diet to adult cats.
and that it could be cured by a normal or high calcium diet. McLean and Urist (48) state that osteoporosis is a type of atrophy occurring in bone. At present, there are two main theories as to the cause of osteoporosis. The first suggests that it is due to reduced bone formation, the second proposes that it is due to increased bone resorption, often secondary to calcium deficiency. It is possible that both factors contribute to senile osteoporosis. It has also been suggested that reduced blood supply may be responsible for the bone atrophy. There is little quantitative data on this point, so it was decided to use the methods for measurement of bone blood flow described in Chapter III to determine whether the disuse osteoporosis as a result of prolonged immobilization in a cast, might be associated with changes in blood flow.

**METHOD**

The study was made using 22 adult rabbits weighing 2.0-3.3 kg (average weight 2.5 kg). A long leg cast of plaster was applied to one hind leg in each animal with the knee in partial flexion. Care was taken to avoid undue pressure and circulatory embarrassment from the plaster cast. The animals were then divided into two groups of equal number. One group of 11 animals was used to study the effects of a short term (1-2 weeks) immobilization and the second group of 11 animals was used for the
long term (2 months) experiments. At the end of the period of immobilization, the blood flow through the calcaneus, tibia and femur on both limbs was measured by the method described in Chapter III. Roentgenographs were made of the tibia from the long term group, and ash/dry weight ratios were measured.

**RESULTS AND DISCUSSION**

The rates of blood flow are summarized in Table IX, and the values for fresh, dry and ash weight of the bones are summarized in Table X. There were no significant changes in bone weight after 1-2 weeks of immobilization, but the blood flow to the calcaneus and tibia was significantly reduced. In the second group, in which the limb was immobilized for 2 months, there was definite evidence of local osteoporosis, particularly in the calcaneus and talus. The blood flow to the entire bone was not changed appreciably, but because of the reduced mass of the bones from the immobilized side, blood flow per 100 g fresh weight was actually increased significantly in the case of tibia and calcaneus.

Although the initial effect of immobilization appeared to be a reduction in blood flow, this was not true after the prolonged immobilization when actual osteoporosis had developed. For this reason, it is difficult to believe that the osteoporosis is due primarily to reduced blood flow. It is interesting
that the most marked effect was observed in the calcaneus, while there was little effect on femur. As shown in Figure 7, there was definite roentgenographic evidence of osteoporosis in the tibia, with the most striking effect on the cancellous bone of the metaphysis. This is in agreement with the clinical observation that cancellous bone is more affected by this condition. In the tibia, such changes were apparent, even though the average loss of bone mineral was only 16%.

**SUMMARY AND CONCLUSIONS**

A preliminary study was made of quantitative changes in bone blood flow in disuse osteoporosis in the rabbit. The bones of the limb which had been immobilized in a long leg plaster cast for 1-2 weeks had a considerable decrease in the rate of bone blood flow without significant change in the bone weight. The bones in the limbs which were immobilized for 2 months became osteoporotic as demonstrated by roentgenographs and ash weight analyses. These osteoporotic bones, however, did not show any change in blood flow on the basis of each individual bone compared to the corresponding bone in the control limb. However, when the rate of blood flow to tibia and calcaneus was computed on the basis of bone weight, it was increased significantly because of the marked decrease in bone weight.
Roentgenograph of tibia (E) from limb immobilized in plaster cast for 2 months and control bone (C) from the opposite side.
The results suggest that a decrease in the bone blood flow occurs in the early stages of immobilization. However, after osteoporosis has developed, the blood flow calculated on a weight basis was normal or even increased, so that it is difficult to attribute this condition to reduced blood flow.
There is considerable evidence for the control of bone blood circulation by both sympathetic vasomotor nerve and vaso-pressor hormones. Anatomical studies of nerve supply to bone by Ottolenghi (58) and Sherman (69) disclosed that the bone is abundantly supplied by both myelinated and unmyelinated nerve fibers. Trotman and Kelly (80) reported a 27% increase in bone blood flow following lumbar sympathectomy in the dog. Weiss and Root (87) observed in the cat that electrical stimulation of the distal cut ends of certain peripheral nerves in the limb caused a fall in the intramedullary blood pressure. Bloomenthal et al. (5), Stein et al. (76), Copp and Shim (12, 70) and Shaw (67, 68) all observed falls in intramedullary blood pressure following administration of epinephrine, norepinephrine or pitressin.

In order to study the effect of complete sciatic nerve injury (section) on bone blood flow in the limb, the following experiments were carried out in the rabbit.

**METHOD**

The studies were made in 10 adult rabbits weighing 1.9-2.7 kg (average weight 2.4 kg). The animals were lightly anaesthetized with ether. The gluteal region was shaved. The sciatic
nerve on one side was exposed behind the hip joint through a posterior approach by splitting the gluteus maximus. The nerve was then identified at the inferior border of the pyriformis muscle and was cut completely with a knife or scissors under direct vision. The wound was closed with black silk. Postoperatively, the animals received 30,000 units of procaine penicillin. The wound healed by the 7th day. The rates of blood flow through the leg bones (tibia-fibula) and the representative foot bones (talus-calcaneus) in both normal and experimental sides in all animals were studied by the method described in Chapter III between 8th and 14th day after the operation.

RESULTS AND DISCUSSION

The results are summarized in Table XI. There was no significant change in the weights of the bones in the paralyzed limb. The average rate of blood flow in the normal tibia-fibula was $9.89 \pm 1.70$ ml/min/100 g wet weight while, on the paralyzed side, it was $12.87 \pm 1.81$ ml/min/100 g wet weight. The increase in the paralyzed side was $2.98 \pm 2.42$ ml which was not statistically significant. The rate of blood flow to the foot bones was $7.57 \pm 1.52$ ml/min/100 wet weight in the control limb as compared to $13.52 \pm 1.86$ ml/min/100 g wet weight on the side of sciatic nerve section. The rate of increase in this case was $5.95 \pm 2.48$ ml/min/100 g which was statistically significant ($0.05 > p > 0.025$).
The results indicate that sciatic nerve section may cause a considerable increase in bone blood flow in the leg and foot. The effect is more marked in the foot bones than in the leg bones. The sciatic nerve, the longest somatic nerve in the body, contains three elements: the somatic motor, sensory and sympathetic vasomotor. The increase in the bone blood flow could be explained by the fact that the section of the sciatic nerve interrupts the sympathetic outflow to the blood vessels in the leg and foot including the vessels in bones. The sympathetic vasomotor nerve supply below the knee is almost entirely conveyed by the sciatic nerve (40, 92).

A knowledge of the basic pattern of the sympathetic nerve supply to the blood vessels in the limbs is essential to understand the results of this study. This subject was studied by Kramer and Todd (40) and Woolard (92). It was also illustrated well by Lockhart et al. (45). There are two distinct patterns: the proximal and the distal.

The pattern of the proximal vascular innervation does not extend beyond the larger vessels of the limbs and is limited to the proximal portions of the femoral and brachial arteries. In the case of the upper limb, the vascular nerve supply originates from the middle and lower cervical ganglia of the cervical sympathetic chain, and is then conveyed to the subclavian and axillary
arteries. The proximal vascular nerve supply to the lower limb is derived from the aortic plexes in the abdomen and conveyed in a plexiform manner along the common and external iliac arteries.

The distal pattern of vascular nerve supply originates from the spinal nerve trunks and is carried to the peripheral vessels by the somatic peripheral nerves. The upper limits are the proximal portions of the brachial and femoral arteries, and the peripheral limits are the levels of the arterioles and capillaries in the hand and foot. The peripheral sympathetic fibers in the limbs, therefore, travel exclusively with the somatic nerve trunks and thus the vasoconstrictor impulses are conveyed to the minute vessels of the limbs. Hence the section of a peripheral nerve, such as the sciatic nerve, causes complete denervation of the sympathetic vasoconstrictors in the area of its distribution.

The femoral artery is innervated by the sympathetic vasoconstrictor fibers conveyed within the femoral and obturator nerves and the popliteal artery by the vasomotor fibers carried by the femoral and saphenous nerves. However, the sympathetic supply below the knee is conveyed entirely by the sciatic nerve. It then divides along the medial and lateral popliteal nerves, the anterior and posterior tibial nerves, and the plantar nerves
to supply their corresponding arteries at various levels including the terminal arterioles and precapillary sphincters.

The tendency for an increase in bone blood flow in the limb following section of the sciatic nerve noted in this study supports the finding of Trotman and Kelly (80), who observed that lumbar sympathectomy in dog caused a 27% increase in bone blood flow in the leg. The fact that electrical stimulation of the peripheral cut ends of certain nerves in the limb caused a fall in the intramedullary pressure of bone, as observed by Weiss and Root (87), could well be due to the vasoconstriction and decrease in bone blood flow due to increased sympathetic tone locally.

This knowledge could be applied to clinical conditions in which increased bone blood flow is desirable. Such increased flow might promote fracture healing and prevent non-union in regions of low vascularity, such as the junction of the middle and lower third of tibia. Blocking the peroneal or sciatic nerve should increase bone blood flow in this area and in the foot.

**SUMMARY AND CONCLUSION**

The effect of the section of the sciatic nerve on the blood flow of bones in the limb was studied in the rabbit. The effect was evaluated between 1-2 weeks after complete surgical section of the sciatic nerve. This procedure resulted in a tendency
towards increased blood flow to the leg bones and a significant increase in flow to the foot bones. It is suggested that this increase in bone blood flow is due to interruption of the sympathetic vasomotor outflow to the leg and foot as a result of cutting the sciatic nerve. The results of the study support the view that sympathectomy should increase bone blood flow in the limb, and agrees with the findings of Trotman and Kelly (80).
EXPERIMENT VIII

EFFECT OF FRACTURES OF FEMORAL NECK ON BLOOD SUPPLY TO FEMORAL HEAD

No matter how treated, the fractures of the neck of the femur are frequently complicated by nonunion, degenerative arthritis or aseptic necrosis of the femoral head (2). The impairment of the blood supply to the femoral head is generally known to be an important cause of these complications. Because of the unique anatomy of the vascular supply to the neck and head of the femur, the fracture of the neck alone may well be serious enough at the time the fracture occurs to interrupt blood supply to the head. In addition, however, there are many other factors which may aggravate the degree of impairment of blood supply to the head. Fracture high in the neck, displacement of the fragments, rotation of the proximal fragment (71) and increased intraarticular pressure (74) are a few examples.

The normal anatomy of the vascular supply to the femoral head in man was studied sufficiently by Trueta and Harrison (82) that their description has been a standard reference on this subject. There are at least four sources of blood supply: the lateral and medial (foveal) epiphyseal arteries, and the superior and inferior metaphyseal arteries. However, the relative importance of these vessels has not been critically studied to date.
The injuries of these vessels in femoral neck fractures and their significance in blood supply to the femoral head is a matter of serious concern to the orthopedic surgeon in connection with prevention and prediction of avascular necrosis and viability of the femoral head. Boyd et al. (6, 7) measured $P^{32}$ uptake by the femoral head following fracture of the femoral neck. Miles (51) observed the intramedullary pressure of the femoral head; Woodhouse (89) studied the presence or absence of dissolved oxygen in the medullary space in the femoral head using an oxy­meter, and Milch (52) determined the distribution of tetracycline in the femoral head following its administration. Smith (71) investigated the effect of rotation and valgus malposition of the proximal fragment on the blood supply to the femoral head. He also made important observations on the role of the medial (foveal) epiphyseal artery following the transcervical fracture in the blood supply to the femoral head. He observed decrease or arrest of bleeding from the proximal surface of the fracture when the proximal fragment was rotated.

To date, however, the normal volume or the normal rate of blood flow to the femoral head and its quantitative changes following transcervical fractures have not been studied. This was investigated in the following experiments. In a number of dissections in both living and dead rabbits, with the aid of a
dissecting microscope, it was found that the vascular anatomy of the femoral head and neck in the rabbit is very similar to that in man. Therefore, it was felt that measurement of normal blood supply to the femoral head and a study of the changes produced in the experimental transcervical fractures of the femoral neck in this animal might provide valuable information on this serious condition in man.

METHOD

The study was made in 80 adult rabbits weighing 2.2-3.5 kg (average weight 2.7 kg). They were divided into 4 groups. The first and second groups (10 rabbits in each) served as controls. In each animal, the femur on the opposite (unoperated) side served as a control.

The animals were anaesthetized by intraperitoneal injection of urethane (1 g/kg) and barbitone (250 mg/kg). The gluteal regions were shaved. The hip joint was exposed through a posterior approach by splitting the gluteus maximus. Then, the joint capsule was opened at the posterior aspect. During the operation, the bleeding points were cauterized. In the first group of 10 animals, the effect of the posterior capsulotomy on the blood supply to the femoral head was studied 1-2 hours after the operation. In the second group of 10 animals, the effect of a combination of subcapital fracture and section of the ligamentum
teres was studied. In the third group of 20 animals, the effect of subcapital fracture on the blood supply to the femoral head was determined. In the fourth group of 40 rabbits, the effect of non-subcapital fractures was investigated. Fractures were induced under direct vision using a bone cutting forceps or sharp pointed scissors. The proximal fragment was not rotated. The wound was closed without closing the joint capsule. One or two hours after the operation, the rates of blood flow through the femoral head were measured by the method described in Chapter III.

RESULTS AND DISCUSSION

The results are summarized in Table XII and Figures 8 and 9. Simple posterior capsulotomy of the hip joint did not affect the blood supply to the femoral head. On the other hand, the blood supply to the head was completely cut off when two procedures of subcapital fracture and section of the teres ligament were combined. Subcapital fracture alone reduced the blood supply to the femoral head by an average of 83% (range 72-90%), leaving only 17% of the normal blood supply. This presumably came through the ligamentum teres. On the other hand, non-subcapital fractures (oblique fracture with a calcar beak, and fractures through the base of the neck) reduced the blood supply to the femoral head by only 52% (range 23-70%).
Effect of Subcapital Fracture on Blood Supply to Femoral Head. (20 Rabbits)

Control: 15.6 ± 0.9  
Fractured: 2.9 ± 0.8

( ml Blood / Min. / 100 gm. fresh Bone )

Effect of subcapital fracture on blood supply to femoral head. (20 rabbits)
Effect of non-subcapital fractures of femoral neck on blood supply to head. (40 rabbits)

Control: 20.6 ± 0.7
Fractured: 9.7 ± 0.5

( ml Blood / Min. / 100 gm. fresh Bone )

- 52% (23-70)
The results indicate that any type of complete transcervical fractures in the hip joint causes a marked impairment of blood supply to the femoral head. Subcapital fracture reduces the blood supply to the femoral head far more than those at other sites. The data indicate that the femoral head with the subcapital fracture receives only 17% of the normal blood supply presumably via the ligamentum teres, which indicates a critical reduction of the blood supply to the head in this case. The rate of reduction ranged from 72 to 90% which indicates a fairly consistent pattern. On the other hand, an oblique fracture with a calcar beak and basal neck fracture impaired the blood supply to the femoral head to an extent which varied from 23-70% with the average reduction of 52%. It suggests that these types of fracture cause vascular injury to a varying extent. It must be noted that the neck of the femur in the rabbit is relatively short and it was difficult to produce similar type of fracture even when made under direct vision. It was only possible to make two distinct types of fractures, subcapital and non-subcapital.

The consistent and great reduction of blood supply to the femoral head in the case of subcapital fracture could be due to injuries of all blood vessels except the foveal or medial epiphyseal artery which is associated with the ligamentum teres.
This is substantiated by the fact that there was no blood supply to the femoral head at all when the subcapital fracture and section of the ligamentum teres were combined, as shown in Table XII. It should be noted, however, that bleeding from the ligamentum teres was not observed when it was cut. This was probably due to vascular spasm caused by cutting ligament.

The results in this study support the clinical observation that subcapital type of femoral neck fracture causes nonunion of the fracture and aseptic necrosis of the femoral head more often than non-subcapital types do. The fact that the non-subcapital types reduce blood supply to the femoral head less severely than the subcapital type, could well be due to lesser injury to the blood vessels in the neck and at the base of the femoral head which were contained in the elastic retinaculum (or internal folding of the capsule) on the surface of the femoral neck. Although many blood vessels were observed in the antero-superior part of the retinaculum which was still connected with the upper fragment, it was not possible to determine whether or not they were functioning.

Active bleeding from the distal surface of the fracture was easily observed. But it was the rule, rather than the exception, that there was no active bleeding from the proximal surface of the fracture. It was also difficult to observe bleeding from the proximal surface under the microscope.
These results have important implications with regard to the serious orthopedic problem of fractures in this region.

SUMMARY AND CONCLUSION

1. The effects of the experimental fractures of the femoral neck on the blood supply to the femoral head were studied in adult rabbits.

2. The average rate of normal blood supply to the femoral head in 80 rabbits was about 18 ml/min/100 g wet weight.

3. Subcapital fracture in 20 rabbits reduced blood supply to the femoral head by 83% (range 72-90%).

4. The non-subcapital types of fracture in 40 rabbits reduced the blood supply to the femoral head by an average of 52% (range 23-70%).

5. There was essentially no blood supply to the femoral head when subcapital fracture and section of the ligamentum teres were combined.

6. Posterior capsulotomy of the hip joint had no effect on the blood supply to the femoral head.

7. The medial or foveal epiphyseal artery in the ligamentum teres appears to be responsible for about 17% of the blood supply of the femoral head in the adult rabbit.
EXPERIMENT IX

EFFECT OF EPINEPHRINE ON BONE BLOOD FLOW

Evidence that epinephrine reduces bone blood flow has been accumulated through various studies. Drinker et al. (23, 24) in their experiments on perfusion of isolated tibia through the nutrient artery in the dog found that outflow of the blood from the bone was reduced when epinephrine was added to the inflowing blood. Bloomenthal et al. (5) observed a fall in the intramedullary blood pressure of long bone when epinephrine was given to the dog. Similar observations were made by Stein et al. (76), Herzig and Root (32), Copp and Shim (12, 70) and Shaw (67, 68). In addition, Stein et al. and Copp and Shim also observed that epinephrine reduced and/or stopped bone bleeding through drill holes made in the cortex of bone.

Thus various qualitative studies provide evidence that epinephrine reduces bone blood circulation presumably through its vasoconstrictor action on bone blood vessels. However, to date, this effect has not been demonstrated by quantitative measurement of changes in bone blood flow. This was investigated in the following experiment.

METHOD

The animals used were 20 white rabbits weighing 1.4-2.6 kg (average weight 1.90 kg). They were divided into two groups.
The animals were fasted overnight and anaesthetized by intraperitoneal injection of 1 g of urethane and 250 mg barbitone per kg body weight. The first group of 10 animals served as control and the second 10 rabbits for study of the effects of epinephrine. The blood flow was measured in two representative bones (the humerus and tibia-fibula) using the method described in Chapter III. Epinephrine, about 2-4 micro-gram/kg/min in 1:100,000 or 1:150,000 dilution of epinephrine hydrochloride, was slowly infused into an ear vein with a constant rate syringe pump. It was given for the 5 minutes of the Sr$^{85}$ clearance period.

RESULTS AND DISCUSSION

The results are summarized in Table XIII and Figure 10. Epinephrine reduced the rate of blood flow to the humerus and tibia-fibula by 74 and 81%, respectively. The average effective bone blood flow with standard error in the normal (control) group was $16.43 \pm 1.02$ ml/min/100 g fresh weight and was $3.69 \pm 0.35$ ml/min/100 g fresh weight in the group which received epinephrine. The reduction in blood flow ($12.74 \pm 1.04$) was highly significant ($p < 0.0001$). The data demonstrate that epinephrine greatly reduces bone blood flow. They demonstrate the effect of epinephrine on bone blood circulation quantitatively and confirm the evidence obtained by various qualitative methods of study. The results support the observations of Drinker et al. (24) who
Initial 5 minutes bone clearance of Sr$^{85}$ in rabbits

(ml blood/min/100 gm. bone)

Control
16.43 ± 1.02 (S.E.)
4.59 (S.D.)

Epinephrine
3.69 ± 0.35 (S.E.)
1.57 (S.D.)

Initial 5 minutes bone clearance of Sr$^{85}$ in rabbits

(ml blood/min/100 g bone)
found that outflow of blood from perfused bone in the dog was decreased when epinephrine was added to the inflowing blood. It also explains the reasons for the fall of intramedullary pressure of bone following administration of epinephrine in the dog observed by Bloomenthal et al. (5) and many others. In a sense, the medullary cavity of bone is a space inside a rigid closed box. The blood pressure in the medullary space should fall if blood flow into the space is decreased, and vice versa. The results also explain the fact that bleeding from bone was reduced or even stopped when epinephrine was given to the experimental animals. This was observed by Stein et al. (76) and Copp and Shim (12, 70). The reduced bone blood flow could well be due to constriction of intraosseous vessels.

**SUMMARY AND CONCLUSION**

The effect of epinephrine on the rate of bone blood flow in the rabbit was studied. The blood flow through representative bones in the front and hind limbs (humerus and the tibia-fibula), was reduced to 1/4 of the rate of flow in the normal animals when 2-4 micro-gram/kg/min of epinephrine hydrochloride was given intravenously. The average rate of bone blood flow with the standard error in the control group was $16.43 \pm 1.02$ ml/min/100 g wet weight as compared to $3.69 \pm 0.35$ ml/min/100 g wet weight in the group which received epinephrine.
The results indicate that vessels of the skeleton, like those of the splanchnic area and skin, are constricted by epinephrine, and may be considered expendable in emergency situations.
EXPERIMENT X

RELATIONSHIP OF BONE BLOOD FLOW TO
BLOOD-BONE CALCIUM TRANSFER

It is well known that bone functions as an important mineral reservoir (11, 16, 48, 56). The movement or transfer of mineral between the two compartments, blood and bone, must depend on the circulation through bone. It is reasonable to assume that there is a quantitative correlation between bone blood flow and blood-bone mineral transfer. In the immediately preceding chapter, it was shown that epinephrine greatly reduced bone blood flow. It was felt that it would be interesting to determine whether epinephrine also reduced calcium movement between the blood and bone compartments. This was studied in the following experiments.

METHOD

The experiments were carried out on 20 adult mongrel dogs weighing 12-29 kg (average weight 19 kg) which had been fasted overnight. In 10 dogs, epinephrine was infused for one hour at the rate of 2-4 micro-gram/kg/min and the effect on plasma calcium and inorganic phosphate was determined. In two of these animals, blood sugar was also determined by the method of Folin and Wu, through the courtesy of the Clinical Investigation Unit at Shaughnessy Hospital. In a second group of 5 dogs, calcium
(as CaCl₂ in isotonic solution) was infused intravenously for 1 hour, using a constant rate syringe pump. The dose administered was equivalent to 9.0 mg Ca/Kg per one hour period. The plasma calcium returned to the control level within a few hours, and the calcium infusion was then repeated, but with the additional infusion of 2-4 micro-gram epinephrine/kg/min. Plasma calcium and inorganic phosphate was determined on samples drawn from the jugular vein at 15-30 minute intervals. In the third group of 5 dogs, plasma calcium was lowered by i.v. infusion of a chelating agent, ethylenediaminetetraacetate (EDTA), for one hour in a dose sufficient to chelate 8.5 mg Ca/kg. Plasma calcium returned to the control level within a few hours, and the infusion was then repeated with simultaneous infusion of 2-4 micro-gram epinephrine/kg/min for the one hour period.

Plasma calcium was determined by a semiautomatic microtitration with EDTA, using the method of Copp (17). Inorganic phosphate was determined by the Taussky and Shorr (79) modification of the method of Fiske and Subbarow.

**RESULTS AND DISCUSSION**

The results are summarized in Tables XIV-XVI, and are illustrated in Figures 11-14. There was no significant effect of epinephrine on plasma calcium, but the inorganic phosphate level in plasma fell from 5.72 to 3.10 mg-%. In the two dogs
Figure 11

Effect of epinephrine infusion on calcium and inorganic phosphate levels in plasma.
Effect of epinephrine infusion on levels of plasma calcium, inorganic phosphate and glucose.
in which blood glucose was determined, the infusion resulted in a 2-3 fold increase from the fasting level. It seems probable that the fall in plasma inorganic phosphate is the result of increased utilization in the phosphorylation processes involved in glycogenolysis and enhanced metabolic activity due to epinephrine. It is significant that the fall in inorganic phosphate is not associated with any change in plasma calcium. Albright and Reifenstein (1) have suggested that there is a reciprocal relationship between the concentrations of these two ions in blood, and that the rise in plasma calcium following parathormone administration is secondary to the fall in inorganic phosphate. The above results refute this claim, for the fall in inorganic phosphate induced by epinephrine had no effect on the plasma calcium level.

**Calcium Infusions**

As shown in Table XV and Figure 13, infusion of calcium for one hour raised the level of plasma calcium 2.12 mg-%. The latter returned to the original control level 2 1/2 hours later. When the calcium infusion was repeated with simultaneous administration of epinephrine, the rise in plasma calcium at the end of the hour was almost twice as great (+3.94 mg-%). The average total urinary excretion during the first infusion was 1.85 mg, and
Effect of epinephrine on the hypercalcemia produced by calcium infusion.
during the second one-hour infusion with epinephrine, it was 1.07 mg. The reduced excretion in the latter, despite higher plasma calcium levels, may be due to vasoconstriction and reduced blood flow to kidney. However, the total excretion was in both cases less than 1% of the infused calcium, so that this cannot account for the difference observed.

EDTA Infusion

As shown in Table XVI and Figure 14, infusion of EDTA for an hour resulted in a fall of 1.76 mg-% in the plasma calcium level. Within a few hours, plasma calcium had returned to the original level, presumably as a result of calcium mobilization from bone. When the EDTA infusion was repeated with simultaneous infusion of epinephrine, the fall in plasma calcium was almost twice as great (-3.30 mg-%). The dotted line in Figure 14 indicates the hypothetical fall in plasma calcium which would have occurred if the same quantity of calcium had been removed from the extracellular fluid calcium pool, with no contribution from bone. The difference between this and the observed curve may be attributed to calcium mobilization from the skeleton. It is significant that the curve obtained when epinephrine was infused along with the EDTA approached the hypothetical fall (dotted line) which would have been expected if the calcium had been removed only from the extracellular calcium pool with
Effect of epinephrine on the hypocalcemia produced by i.v. infusion of EDTA. The sloping dotted lines indicate the fall in plasma calcium that might have been expected if the calcium had been removed from the extracellular pool alone, without any contribution from bone, and is the curve that would be expected if bone blood flow was zero.
no contribution from bone. This is consistent with the view that profound vasoconstriction and reduction in bone blood flow as a result of the epinephrine infusion also sharply reduces the availability of bone calcium for homeostatic control of hypocalcemia.

It has been postulated that there is an equilibrium between plasma calcium and the calcium situated on the surface of the crystals of bone salt accessible to the circulation (11, 16). Such an equilibrium is illustrated diagrammatically in Figure 15. Experiments with radioactive calcium and strontium have indicated that 0.3-0.6% of the calcium in bone is readily exchangeable. This would correspond to the calcium in equilibrium with blood calcium mentioned above, and would constitute a labile calcium storage pool in which calcium might be stored, or from which it might be withdrawn. The possible manner in which this might occur is illustrated in Figures 16 and 17. When calcium is infused, raising the plasma calcium level, there will be a concentration difference and calcium will flow into the labile bone pool. If the plasma calcium level is lowered with EDTA, the opposite will occur. It will be noted in the model that the rate of transfer will be dependent on the capacity of the pipe connecting the two pools (blood and bone) which will correspond to the bone blood flow. As illustrated in Figure 17,
Model indicating hypothetical bone-blood calcium equilibrium.
Figure 16

Model illustrating the possible role of bone blood flow in the exchange of calcium between blood and the labile calcium storage pool in bone.
Model illustrating the possible effect of epinephrine induced vasoconstriction on mineral transfer between blood and the labile calcium storage pool in bone.
constriction of the bone vessels by epinephrine should sharply reduce the communication between the two pools and impair the "buffering" effect of the labile bone pool. This effect was clearly demonstrated in the experiments described above.

**SUMMARY AND CONCLUSIONS**

Intravenous infusion of 9.0 mg Ca/kg over a 1 hour period raised the plasma Ca 2.12 mg-%. When the infusion was repeated in the same animals with simultaneous infusion of 2-4 microgram epinephrine/kg/min, the calcium rose 3.94 mg-%. The difference was attributed to epinephrine-induced vasoconstriction and reduced blood flow, which in turn would reduce the rate at which calcium could be stored in bone. Similarly, i.v. infusion of EDTA sufficient to chelate and remove 8.5 mg Ca/kg over a 1 hour period reduced the plasma calcium 1.79 mg-%; similar infusion with simultaneous epinephrine infusion resulted in a fall of 3.30 mg-%. The difference is also attributed to vasoconstriction and reduced blood flow, which would interfere with mobilization of calcium from the skeleton. These experiments emphasize the importance of bone blood flow in the homeostatic role of the skeleton in regulating the plasma calcium level.
Chapter V

GENERAL SUMMARY AND CONCLUSIONS

1. An indirect method for measuring bone blood flow has been developed. It is based on the initial (0-5 min) bone clearance of radioactive strontium (Sr\textsuperscript{85}), a bone-seeking radioisotope. The method appears to be valid, generally applicable and useful for studying quantitatively some of the factors affecting bone blood flow.

2. Bone uptake of radiostrontium in the first 5 minutes following intravenous injection is entirely dependent on intact bone blood flow.

3. The efficiency of removal of Sr\textsuperscript{85} in blood passing through bone is determined by the extraction ratio (ER). A method was developed for determining this value. In determinations on tibias from 10 dogs, the mean value for the extraction ratio was 0.764 ± 0.066 (SE).

4. This high value would appear to justify use of initial bone clearance of Sr\textsuperscript{85} as a measure of effective bone blood flow. Total bone blood flow can be estimated by dividing this value by the extraction ratio.

5. For 270 bones from 80 rabbits, the mean effective bone blood flow was 9.60 ± 0.19 (SE) ml/min/100 g fresh weight. For 46
bones from 10 dogs, the effective bone blood flow was 10.15 ± 0.61 (SE) ml/min/100 g fresh weight. Values for individual bones were remarkably consistent.

6. Total skeletal blood flow was estimated to be 7.1 ± 0.25 (SE) % of the resting cardiac output in the rabbits studied, and 7.30 ± 0.95 (SE) % of the resting cardiac output in the dogs.

7. Blood flow in ml/min/100 g fresh weight was significantly higher in the head of femur (18.67 ± 0.52) than in the shaft (7.50 ± 0.16).

8. In rabbit femur, the nutrient artery appears to supply 70% of the blood flow to the shaft and 1/3 of the flow to the two ends.

9. Immobilization of one leg in a long plaster cast for 2 weeks resulted in a small decrease in blood flow to the foot bones; immobilization for 2 months resulted in disuse osteoporosis of tibia and calcaneus, and a significant increase in relative bone blood flow per unit weight.

10. Complete section of the sciatic nerve increased blood flow to tibia and foot bones, presumably due to interruption of sympathetic vasoconstrictor fibers.

11. Fractures of the neck of femur reduced blood flow to the femoral head by 52-83%, the greatest reduction occurring
with subcapital fractures. When the latter was combined with section of the ligamentum teres, there was essentially no blood flow to the head of femur.

12. Continuous infusion of epinephrine (2-4 micro-gram/kg/min) reduced bone blood flow to 20-25% of normal, presumably due to constriction of the vessels supplying bone.

13. Continuous i.v. infusion of epinephrine (2-4 micro-gram/kg/min) in the dog raised the blood glucose level and lowered the plasma inorganic phosphate, but had no effect on plasma calcium. However, epinephrine infusion did seriously interfere with calcium storage in or mobilization from bone, indicating the importance of bone blood flow in homeostatic control of plasma calcium.
Table I

Sr$^{85}$ uptake by normal and ischemic femoral heads
(5 minutes after i.v. injection)

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Normal CPM</th>
<th>Ischemic CPM</th>
<th>% of normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2432</td>
<td>50</td>
<td>2.0</td>
</tr>
<tr>
<td>2</td>
<td>2315</td>
<td>38</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>2656</td>
<td>67</td>
<td>2.5</td>
</tr>
<tr>
<td>4</td>
<td>2040</td>
<td>28</td>
<td>1.4</td>
</tr>
<tr>
<td>5</td>
<td>1365</td>
<td>8</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>1402</td>
<td>14</td>
<td>1.0</td>
</tr>
<tr>
<td>7</td>
<td>2206</td>
<td>12</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>1160</td>
<td>28</td>
<td>2.4</td>
</tr>
<tr>
<td>9</td>
<td>1608</td>
<td>22</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>1305</td>
<td>13</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Average $\pm$ SE 1850 $\pm$ 13.4  28 $\pm$ 6  1.5 $\pm$ 0.16

CPM: Counts per minute corrected for background.
(Note that there is essentially no uptake by ischemic bone.)
### Table II

Extraction ratio for uptake of Sr$^{85}$ by tibia

(initial 5 min)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dogs</td>
<td>10</td>
</tr>
<tr>
<td>Evans blue collected (a), %</td>
<td>87.3 ± 2.9</td>
</tr>
<tr>
<td>Sr$^{85}$ collected (b), %</td>
<td>20.6 ± 1.6</td>
</tr>
<tr>
<td>Sr$^{85}$ extraction ratio, a-b/a</td>
<td>0.754 ± 0.066</td>
</tr>
<tr>
<td>Actual bone uptake of Sr$^{85}$, %</td>
<td>70.9 ± 1.1</td>
</tr>
<tr>
<td>Total Sr$^{85}$ recovery, %</td>
<td>94.6 ± 1.6</td>
</tr>
</tbody>
</table>

* Averages ± standard error
Table III

Sr$^{85}$ extraction ratio at 1 minute intervals *

<table>
<thead>
<tr>
<th>Interval (in minutes)</th>
<th>% Evans dye collected (a)</th>
<th>% Sr$^{85}$ collected (b)</th>
<th>Extraction ratio, a-b/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>63.2</td>
<td>12.8</td>
<td>0.80</td>
</tr>
<tr>
<td>1 - 2</td>
<td>18.4</td>
<td>5.5</td>
<td>0.74</td>
</tr>
<tr>
<td>2 - 3</td>
<td>4.9</td>
<td>2.0</td>
<td>0.63</td>
</tr>
<tr>
<td>3 - 4</td>
<td>4.1</td>
<td>1.1</td>
<td>0.76</td>
</tr>
<tr>
<td>4 - 5</td>
<td>2.0</td>
<td>0.6</td>
<td>0.65</td>
</tr>
<tr>
<td>0 - 5</td>
<td>92.6</td>
<td>22.0</td>
<td>0.75</td>
</tr>
</tbody>
</table>

* Average for 5 dogs.
Table IV

Effective bone blood flow ($\text{Sr}^{85}$ clearance) in various regions of rabbit femur

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of animals</th>
<th>Average $\pm$ SE ml/min/100 g fresh weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head</td>
<td>50</td>
<td>$18.67 \pm 0.52$</td>
</tr>
<tr>
<td>Trochanter</td>
<td>50</td>
<td>$10.38 \pm 0.46$</td>
</tr>
<tr>
<td>Shaft</td>
<td>50</td>
<td>$7.50 \pm 0.16$</td>
</tr>
<tr>
<td>Condyle</td>
<td>50</td>
<td>$12.06 \pm 0.54$</td>
</tr>
</tbody>
</table>
### Table V

Effective blood flow (Sr\(^{85}\) clearance) in various bones expressed as ml/min/100 g fresh weight

<table>
<thead>
<tr>
<th>Bone</th>
<th>DOGS (10)</th>
<th>RABBITS (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Average ± SE</td>
</tr>
<tr>
<td>Femur</td>
<td>8</td>
<td>9.28 ± 1.28</td>
</tr>
<tr>
<td>Tibia</td>
<td>10</td>
<td>10.48 ± 2.20</td>
</tr>
<tr>
<td>Humerus</td>
<td>10</td>
<td>10.14 ± 1.46</td>
</tr>
<tr>
<td>Talus</td>
<td>9</td>
<td>10.30 ± 2.27</td>
</tr>
<tr>
<td>Calcaneus</td>
<td>9</td>
<td>11.00 ± 2.66</td>
</tr>
<tr>
<td>Vertebra</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Average ± SE | 46 | 10.15 ± 0.61 | 270 | 9.60 ± 0.19 |

Blood flow corrected for extraction ratio

<table>
<thead>
<tr>
<th></th>
<th>DOGS (10)</th>
<th>RABBITS (80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood flow</td>
<td>13.20 ± 0.79</td>
<td>12.48 ± 0.26</td>
</tr>
</tbody>
</table>
Table VI

Estimate of total skeletal blood flow
in dogs and rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Dogs</th>
<th>Rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals studied</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>Number of bones</td>
<td>46</td>
<td>270</td>
</tr>
<tr>
<td>Effective bone blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Sr\textsuperscript{85} clearance)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml/min/100 g fresh bone</td>
<td>10.15 ± 4.12</td>
<td>9.60 ± 3.28</td>
</tr>
<tr>
<td>Bone blood flow corrected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>for extraction ratio of 0.75</td>
<td>13.20 ± 5.35</td>
<td>12.48 ± 4.26</td>
</tr>
<tr>
<td>Total skeletal blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ml/min (skeleton estimated as 10% of body weight)</td>
<td>238 ± 14</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>Total skeletal blood flow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>as % of resting cardiac output</td>
<td>7.3 ± 3.0</td>
<td>7.1 ± 2.3</td>
</tr>
</tbody>
</table>

* The values are average ± standard deviation.
Table VII

Estimated bone blood flow in dog femur

<table>
<thead>
<tr>
<th>Bone Weight (g)</th>
<th>Whole Femur Flow (ml/min)</th>
<th>Flow per 100 g fresh weight (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>4.2</td>
<td>11.3</td>
</tr>
<tr>
<td>62</td>
<td>4.9</td>
<td>7.8</td>
</tr>
<tr>
<td>63</td>
<td>6.3</td>
<td>10.1</td>
</tr>
<tr>
<td>105</td>
<td>9.7</td>
<td>9.1</td>
</tr>
<tr>
<td>110</td>
<td>14.7</td>
<td>13.3</td>
</tr>
<tr>
<td>110</td>
<td>21.8</td>
<td>19.6</td>
</tr>
<tr>
<td>120</td>
<td>13.6</td>
<td>11.2</td>
</tr>
<tr>
<td>120</td>
<td>25.2</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Average $\pm$ SE

91 $\pm$ 11 $\quad$ 12.55 $\pm$ 2.76 $\quad$ 12.92 $\pm$ 1.71
**Table VIII**

Effect of ligation of nutrient artery on effective blood flow to rabbit femur

<table>
<thead>
<tr>
<th>Regions in femur</th>
<th>ml blood/min/100 g*</th>
<th>Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Control</td>
</tr>
<tr>
<td>Upper metaphysis</td>
<td>13</td>
<td>10.8 ± 0.8</td>
</tr>
<tr>
<td>Diaphysis (shaft)</td>
<td>13</td>
<td>8.5 ± 0.9</td>
</tr>
<tr>
<td>Lower metaphysis</td>
<td>13</td>
<td>10.8 ± 1.0</td>
</tr>
</tbody>
</table>

* Averages ± SE
Table IX

Effect of immobilization of limb on effective bone blood flow in rabbit

<table>
<thead>
<tr>
<th>Bone</th>
<th>n</th>
<th>Control Side</th>
<th>Immobilized Side</th>
<th>Difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcaneus</td>
<td>11</td>
<td>9.36 ± 0.15</td>
<td>7.43 ± 0.15</td>
<td>-1.93 ± 0.21</td>
<td>9.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>Tibia</td>
<td>11</td>
<td>9.33 ± 0.11</td>
<td>8.19 ± 0.14</td>
<td>-1.14 ± 0.18</td>
<td>6.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Femur</td>
<td>11</td>
<td>9.71 ± 0.10</td>
<td>9.27 ± 0.13</td>
<td>-0.44 ± 0.16</td>
<td>2.7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Group I. 1-2 weeks immobilization in long leg cast.

Effective blood flow in ml/min/100 g fresh weight*

<table>
<thead>
<tr>
<th>Bone</th>
<th>n</th>
<th>Control Side</th>
<th>Immobilized Side</th>
<th>Difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcaneus</td>
<td>8</td>
<td>8.40 ± 0.13</td>
<td>11.79 ± 0.14</td>
<td>+3.39 ± 0.19</td>
<td>17.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Tibia</td>
<td>8</td>
<td>9.65 ± 0.13</td>
<td>11.29 ± 0.19</td>
<td>+1.64 ± 0.22</td>
<td>7.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Femur</td>
<td>8</td>
<td>10.31 ± 0.23</td>
<td>10.70 ± 0.21</td>
<td>+0.39 ± 0.31</td>
<td>1.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Group II. 2 months immobilization in long leg cast.

Effective blood flow in ml/min/100 g fresh weight*

Effective blood flow in ml/min/whole bone*

<table>
<thead>
<tr>
<th>Bone</th>
<th>n</th>
<th>Control Side</th>
<th>Immobilized Side</th>
<th>Difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcaneus</td>
<td>8</td>
<td>0.096 ± 0.010</td>
<td>0.099 ± 0.012</td>
<td>+0.003 ± 0.016</td>
<td>0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Tibia</td>
<td>8</td>
<td>0.743 ± 0.101</td>
<td>0.769 ± 0.135</td>
<td>+0.026 ± 0.169</td>
<td>0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Femur</td>
<td>8</td>
<td>0.963 ± 0.113</td>
<td>0.979 ± 0.109</td>
<td>+0.016 ± 0.156</td>
<td>0.10</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Averages ± SE

| t    | "Fisher" test ratio = difference/SE
| p    | probability that difference is due to chance
| NS   | not statistically significant
Table X

Bone weight changes in disuse osteoporosis*  
(8 rabbits)

<table>
<thead>
<tr>
<th>Bones</th>
<th>Wet wt.</th>
<th>Dry wt.</th>
<th>Ash wt.</th>
<th>Ash wt. x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>CALCANEUS</td>
<td>Control</td>
<td>1.16</td>
<td>0.83</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>Exp'ntl</td>
<td>0.83</td>
<td>0.52</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>-%</td>
<td>-28%</td>
<td>-38%</td>
<td>-38%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.6%</td>
</tr>
<tr>
<td>TIBIA</td>
<td>Control</td>
<td>9.81</td>
<td>5.44</td>
<td>3.03</td>
</tr>
<tr>
<td></td>
<td>Exp'ntl</td>
<td>6.88</td>
<td>4.65</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>-%</td>
<td>-12%</td>
<td>-14%</td>
<td>-16%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.3%</td>
</tr>
<tr>
<td>FEMUR</td>
<td>Control</td>
<td>9.17</td>
<td>5.66</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>Exp'ntl</td>
<td>8.73</td>
<td>5.08</td>
<td>2.75</td>
</tr>
<tr>
<td></td>
<td>-%</td>
<td>-9%</td>
<td>-10%</td>
<td>-13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-1.7%</td>
</tr>
</tbody>
</table>

* Disuse osteoporosis due to immobilization for 2 months in a long leg cast.
Table XI

Effect of sciatic nerve section on bone blood flow in the limb

<table>
<thead>
<tr>
<th>Bones</th>
<th>Control (ml/min/100 g wet weight)</th>
<th>Experimental</th>
<th>Dif. ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg bones*</td>
<td>9.89 ± 1.70</td>
<td>12.87 ± 1.81</td>
<td>2.98 ± 2.42</td>
</tr>
<tr>
<td>Foot bones**</td>
<td>7.91 ± 1.52</td>
<td>13.52 ± 1.86</td>
<td>5.95 ± 2.48</td>
</tr>
</tbody>
</table>

* Tibia-fibula

** Talus-calcaneus

The difference in the foot bones, with standard error, is significant with 0.05 > P > 0.025. The values are average for 20 bones in 10 rabbits.
Table XII
Effect of various procedures on effective blood flow to femoral head

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of Rabbits</th>
<th>ml blood/min/100 g fresh bone</th>
<th>Control</th>
<th>Exp'ntl</th>
<th>Diff. ± SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior capsulotomy</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21.1 ± 1.48</td>
<td>21.7 ± 1.71</td>
<td>+0.6 ± 2.26</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcapital fracture + section of ligamentum teres</td>
<td>10</td>
<td>17.5 ± 1.00</td>
<td>0.3 ± 0.1</td>
<td>-17.2 ± 1.01 (98.5%)</td>
<td>17.0</td>
<td>p 0.0001</td>
<td></td>
</tr>
<tr>
<td>Subcapital fracture of neck</td>
<td>20</td>
<td>15.6 ± 0.99</td>
<td>2.9 ± 0.87</td>
<td>-12.7 ± 1.31 (-83%)</td>
<td>9.7</td>
<td>p 0.0001</td>
<td></td>
</tr>
<tr>
<td>Non-subcapital fracture of neck</td>
<td>40</td>
<td>20.6 ± 0.71</td>
<td>9.7 ± 0.51</td>
<td>-10.9 ± 0.87 (-52%)</td>
<td>12.5</td>
<td>p 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

\[ t = \text{test ratio (Fisher)} \]

\[ p = \text{probability that difference occurred by chance.} \]

\[ * = \text{average ± standard error.} \]
Table XIII

Effect of epinephrine on effective bone blood flow

(Sr$^{85}$ clearance in ml/min/100 g fresh weight)

<table>
<thead>
<tr>
<th></th>
<th>Humerus</th>
<th>Tibia + Fibula</th>
<th>Both Bones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of bones</td>
<td>10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Control group</td>
<td>16.95±1.43</td>
<td>15.91±1.41</td>
<td>16.43±1.02</td>
</tr>
<tr>
<td>(mean ± SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine infused</td>
<td>4.42±0.49</td>
<td>2.96±0.51</td>
<td>3.69±0.35</td>
</tr>
<tr>
<td>(mean ± SE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td>-12.53±1.52</td>
<td>-12.95±1.50</td>
<td>-12.74±1.04</td>
</tr>
<tr>
<td>% decrease</td>
<td>74%</td>
<td>81%</td>
<td>78%</td>
</tr>
<tr>
<td>&quot;Fisher&quot; test ratio, $t$</td>
<td>8.3</td>
<td>8.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Probability that difference is due to chance, $p$</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Table XIV

Effect of i.v. infusion of epinephrine on plasma calcium and inorganic phosphate

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>Ca (10 dogs)</th>
<th>P (6 dogs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9.85 ± 0.29</td>
<td>5.12 ± 0.48</td>
</tr>
<tr>
<td>1/2</td>
<td>9.84 ± 0.27</td>
<td>5.43 ± 0.51</td>
</tr>
<tr>
<td>1</td>
<td>9.82 ± 0.27</td>
<td>5.72 ± 0.43</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Epinephrine infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
</tr>
<tr>
<td>3/4</td>
</tr>
<tr>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1 1/2</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>
Table XV

Effect of i.v. calcium infusion with and without simultaneous epinephrine infusion on plasma calcium and phosphate in 5 dogs

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>mg/100 ml plasma*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ca</td>
<td>P</td>
</tr>
<tr>
<td>Control period</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.23 ± 0.14</td>
<td>4.66 ± 0.17</td>
</tr>
<tr>
<td>1/4</td>
<td>10.25 ± 0.03</td>
<td>5.21 ± 0.24</td>
</tr>
<tr>
<td>1/2</td>
<td>10.31 ± 0.01</td>
<td>5.38 ± 0.18</td>
</tr>
<tr>
<td>Calcium infusion, 9.0 mg/kg/hour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>11.82 ± 0.27</td>
<td>5.81 ± 0.41</td>
</tr>
<tr>
<td>3/4</td>
<td>12.35 ± 0.29</td>
<td>5.75 ± 0.10</td>
</tr>
<tr>
<td>1</td>
<td>12.43 ± 0.31</td>
<td>5.94 ± 0.19</td>
</tr>
<tr>
<td>Infusion stopped</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>11.94 ± 0.15</td>
<td>5.45 ± 0.17</td>
</tr>
<tr>
<td>1 1/2</td>
<td>11.12 ± 0.10</td>
<td>5.31 ± 0.22</td>
</tr>
<tr>
<td>2 1/2</td>
<td>10.49 ± 0.20</td>
<td>5.33 ± 0.15</td>
</tr>
<tr>
<td>Calcium infusion, 9.0 mg/kg/hour plus epinephrine infusion, 2-4 micro-g/kg/min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>12.69 ± 0.12</td>
<td>3.73 ± 0.52</td>
</tr>
<tr>
<td>3/4</td>
<td>13.77 ± 0.13</td>
<td>3.16 ± 0.50</td>
</tr>
<tr>
<td>1</td>
<td>14.43 ± 0.13</td>
<td>3.07 ± 0.61</td>
</tr>
<tr>
<td>Infusion stopped</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>13.03 ± 0.23</td>
<td>4.49 ± 0.53</td>
</tr>
<tr>
<td>1 1/2</td>
<td>12.12 ± 0.10</td>
<td>4.00 ± 0.15</td>
</tr>
<tr>
<td>3 1/2</td>
<td>10.48 ± 0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

* Average values ± standard error
Table XVI

Effect of EDTA infusion (i.v.) without and with simultaneous infusion of epinephrine on plasma calcium and inorganic phosphate in 5 dogs

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>mg/100 ml plasma*</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control period</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.04 ± 0.15</td>
<td>5.25 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>1/4</td>
<td>10.09 ± 0.16</td>
<td>5.40 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>10.05 ± 0.15</td>
<td>5.51 ± 0.10</td>
<td></td>
</tr>
<tr>
<td><strong>EDTA infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>9.01 ± 0.23</td>
<td>5.35 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>8.60 ± 0.17</td>
<td>5.30 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>8.26 ± 0.14</td>
<td>5.03 ± 0.11</td>
<td></td>
</tr>
<tr>
<td><strong>Infusion stopped</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>9.32 ± 0.10</td>
<td>5.16 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>1 1/2</td>
<td>10.06 ± 0.12</td>
<td>5.39 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>2 1/2</td>
<td>10.17 ± 0.10</td>
<td>5.72 ± 0.12</td>
<td></td>
</tr>
<tr>
<td><strong>EDTA infusion with epinephrine infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>8.04 ± 0.14</td>
<td>3.58 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>3/4</td>
<td>7.41 ± 0.11</td>
<td>3.03 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>6.87 ± 0.17</td>
<td>2.35 ± 0.15</td>
<td></td>
</tr>
<tr>
<td><strong>Infusion stopped</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>8.42 ± 0.12</td>
<td>2.91 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>1 1/2</td>
<td>9.80 ± 0.11</td>
<td>4.17 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>2 1/2</td>
<td>10.14 ± 0.12</td>
<td>4.59 ± 0.25</td>
<td></td>
</tr>
</tbody>
</table>

* Average values ± standard error


