# BONE CIRCULATION IN HEMORRHAGIC SHOCK

by

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## ABSTRACT

Bone circulation in Hemorrhagic Shock was studied in 35 male mongrel dogs. The term hemorrhagic shock is defined in this thesis as persistent profound hypotensive syndrome, due to acute hemorrhage of more than one third of blood volume. The method of induction of shock consisted of removal of one third of estimated blood volume (8% of body weight) at a rate of 25 - 50 ml/min, and subsequently dropping the systemic blood pressure in a stepwise manner until the maintaining level of 30 - 35 mmHg is reached. The central venous pressure, pulse and respiratory rates were also recorded.

Bone circulation was studied by (1) recording the blood flow through a cannula inserted into the tibial nutrient vein or artery and (2) recording the intramedullary pressure of tibia.

When one third of estimated blood volume was removed, the bone blood flow through the nutrient vessel decreased to 22·5 $\pm$ 3·4% of control level. The decreased bone blood flow persisted as long as the hemorrhagic shock was maintained for 4 - 18 hours. The decreased bone blood flow was also evidenced by a profound and persistent fall of the intramedullary pressure of bone.

Reinfusion into the animal of lost blood within fifteen minutes to six hours after hemorrhage resulted in a complete or partial recovery of the control systemic blood pressure as well as the control rate of bone blood flow and the control level of intramedullary pressure of bone.

The curve showing relationship between the changes in bone blood flow and the systemic blood pressure is an exponential one with concavity towards the flow axis. This indicates that bone has a vasomotor control mechanism of increasing peripheral resistance during hemorrhagic shock. This was substantiated by the following observations: (1) The severity of decrease in bone blood flow on the side of lumbar sympathectomy was much milder (16% less) compared to the side of the intact sympathetic nerve; (2) Dibenzyline (phenoxybenzamine) a sympatholytic drug or alpha-receptor blocking agent alters the pressure-flow curve of bone circulation in shock to a linear pattern which indicates that the drug blocks the bone vasoconstricting mechanism(s).

It is concluded that bone blood flow decreases in hemorrhagic shock and is not merely due to a decrease in circulatory blood volume, but also due to sympathetic and catecholamine hormonal vasoconstrictor mechanisms.

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# INTRODUCTION AND PURPOSE OF STUDY

Shock is one of the most extensively studied conditions in clinical as well as laboratory medicine, with an almost inexhaustible list of references and many excellent monographs [72, 105, 139, 159] covering this field in depth.

Regional circulation in shock, including evaluation of blood flow, mechanism of control, and functional integrity of various systems and organs such as coronary [26, 42, 112, 123], cerebral [26, 45, 123], pulmonary [70, 108, 123], renal [26, 65, 123, 125, 126], hepatic [26, 123], splanchnic [1, 69, 123, 127] and adrenals [54, 71, 75, 97] have been studied. Available information indicates that there are distinctly different responses of various vascular beds in shock [59].

However, little is known about the bone circulation in shock due to lack of study. A review of literature failed to disclose a previous study on bone circulation in shock.

## Purpose of Study

The aim of this thesis is to find out the answers to basic questions regarding bone circulation in shock, such as fundamental changes in bone hemodynamics, mechanisms whereby such changes are brought about, and comparison with other regional and organ circulations in shock. It is hoped that this study will raise further questions and stimulate future studies.

# REVIEW OF LITERATURE

## BONE CIRCULATION

Anatomy — Vascular supply of bone

Nerve supply of bone

Physiology — Methods of study of bone circulation

Rate of bone blood flow

Rate of entire skeletal blood flow

Control mechanisms

## SHOCK

Definition

Historical aspect

Abnormal physiological aspect

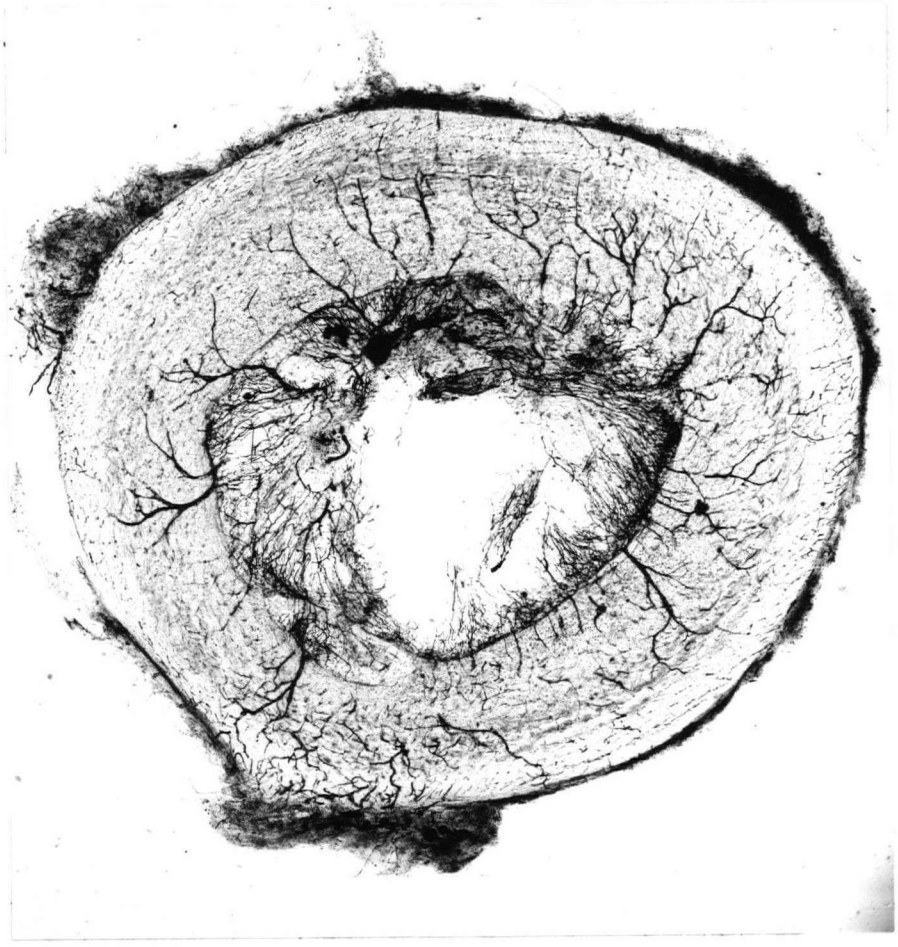
Neural, hormonal, metabolic aspects

Regional circulation in shock

### Vascular Supply of Bone

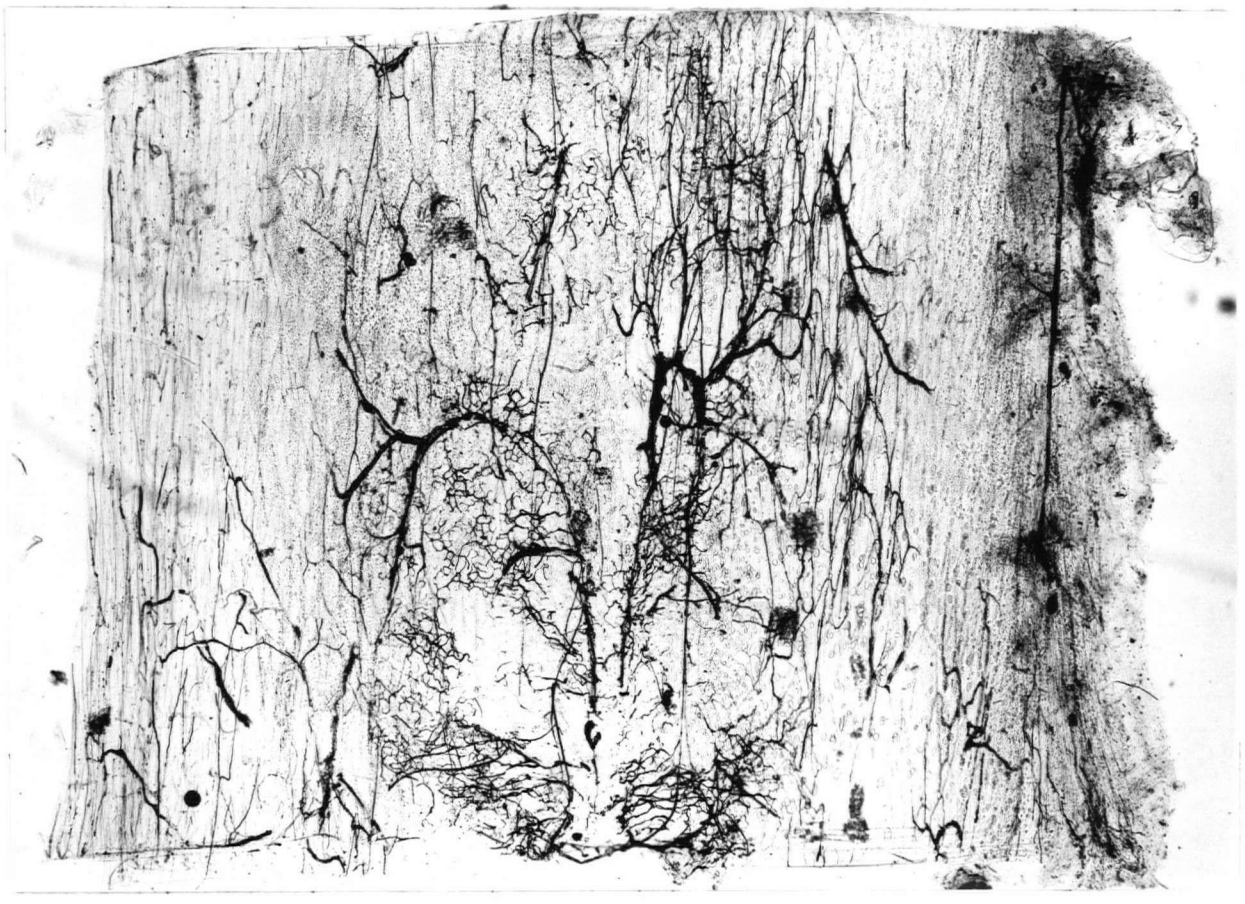
Langer [89] (1876) appeared to be the pioneer in studying the general vascular anatomy of bone. Lexer, Kuliga and Turk (1904), as cited by Laing [87], injected the arterial systems of newborn and adult cadavera with a mercury-turpentine emulsion, followed by stereoscopic radiographs of the specimen, and was able to give a detailed description of the vascular supply of the femur. They found evidence of three main arterial systems supplying all long bones; namely, periosteal, nutrient, and metaphysio-epiphyseal systems.

FIGURE 1



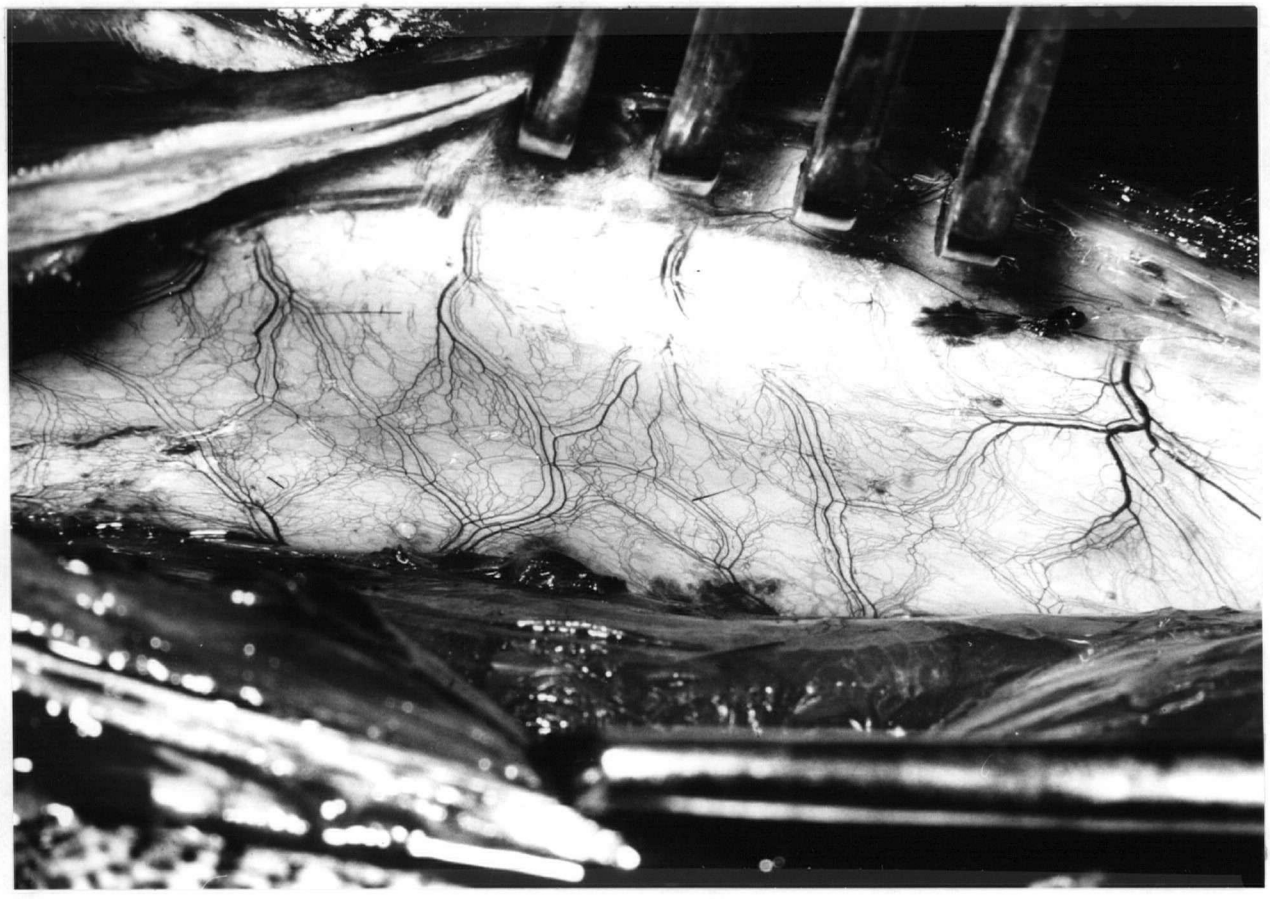
Micrograph of a transverse section of a dog's femur with India Ink injection. It shows the relative contributions of nutrient and periosteal arteries. The bone marrow and inner two thirds are supplied by nutrient artery, and outer third by the periosteal arteries. Note radial arrangement of branches of nutrient artery.

FIGURE 2



Micrograph of sagittal section of a dog's tibia with India Ink injection. It shows distributions of nutrient arterial branches in the marrow cavity (central portion). Note that there are many longitudinal vessels (contained in the Haversian canals) and some transversely running vessels (via Volkmann's canals).

FIGURE 3



Periosteal vessels of a dog's tibia shaft. The dark vessels are veins and lighter ones are arteries. Note that there is a 'trio-arrangement', the artery in the centre and the veins on each side.

Micro angiographic study of bone was carried out by Barclay [4]. Materials, such as vinyl plastic, India ink, Berlin blue, or finely divided barium sulphate (micropaque), are the most commonly used contrast media. This method was used by Trueta and Harrison [149] in studying the vascular anatomy of the femoral head, Haliburton [63] in the talus of man, Nelson [111] in the human tibia, Brookes in tubular bone in rats [16], long bones in the human foetus [17], and in rabbits' femur and tibiofibula [15].

All authors tend to agree that the nutrient artery, after entering the diaphyseal cortex, divides into ascending and descending branches which have further radially oriented branches to the cortex. The ends of the bones are supplied by the epiphyseal and the metaphyseal arteries, which enter the epiphysis and metaphysis through small foramina. After entering the substance of bone, the arteries branch into arterial arcades resembling the arcades of the mesentery of the bowel. These vascular arcades become smaller and smaller, and terminate in small capillary loops beneath the articular cartilage. Such arrangements have been observed by Nelson et al [111] in the human tibia and Rogers and Gladstone [121] in the distil end of the human femur.

The periosteal system forms an abundant vascular network, and this can easily be observed in the periosteum of long bones, such as the tibia. It has been pointed out by Nelson [111], of the prevalence of a trio arrangement of vessels, in which each one of the arterial twigs was accompanied by two veins, in the tibia.

Branemark [14], with a special illumination device, was able to visualize the marrow of fibula of the rabbit, and observed its structure

and function in the microscope without interfering with the normal function of the organ. The vessel caliber was noticed to vary with the functional state of the marrow, and a rough average in a marrow of "ordinary" activity.

| | |
|---|---|
| Arteriole | 10 μ |
| Capillary | 8 μ |
| Sinusoid | 15 - 60 μ |
| Venule | 12 μ |

The flow ratio between the arteriole and the sinusoid was estimated to be approximately 10:1, a figure close to that derived from injection-corrosive preparations of the vascular bed.

The sinusoids are sometimes spiralled shaped, sometimes more or less hexagonal. They showed a rhythmic function with alternating dilation and emptying. This rhythmic activity is somewhat similar to that described by Knisely [85] in spleen. Foa [47], by measuring changes in volume of bone marrow, suggested that the behavior of the bone marrow was very similar to the spleen; and proposed that the bone marrow circulation may actually be regulated by sphincters similar to those of the venous sinusoids.

In studying the innervation by direct observation of the marrow microcirculation, Branemark commented that the marrow vessels become constricted, and the marrow is emptied of blood as when squeezing a sponge, during adrenaline injection. Another observation was the rich anastomoses and a typical course of marrow capillaries dipping into the compacta, and then turning back again into the marrow sinusoids.

Venous Drainage

Long bones have a central venous sinus. The capacity of the venous system is estimated to be six to eight times that of arterial system [39]. The transverse sinusoids of the marrow drain directly into the central venous sinus or into larger tributaries and then into the central venous sinus [18]. The veins of bone are thin-walled. Hashimoto [66], according to Branemark [14], observed that the nutrient artery pursues a spiral course around the straight central vein and pulsations in the artery were assumed to be a driving force propelling the blood in the thin wall vein, which cannot drive the blood forward by itself.

Intra-osseous phlebography indicated that much of the venous drainage leaves the long bones at the bone ends [31] [145]. Epiphysial ends of long bone are drained by thin-walled vessels, which are parallel to the arteries and leave the bone in very close proximity to the entering arteries. In the cortical bone of human tibia [111], a vein accompanying a radially arranged branch of the nutrient artery has been shown to drain into the central nutrient sinus.

Relative Importance of Three Arterial Systems of Long Bone

Johnson [79], by interfering with two out of the three sources of blood supply of tibia in dog, concluded that the nutrient artery was the most important source, being responsible for the nourishment of the marrow as well as the inner half of the cortex, and was capable of maintaining the viability of the entire shaft. The metaphysial arteries supported the metaphysial regions, and were capable of nourishing partly the area of nutrient artery. The periosteal arteries, being the least important

supplied approximately the outer half of the cortex.

The relative importance of the nutrient artery was also demonstrated by Kistler[84], who observed necrosis of the marrow and some areas of the cortex after induction of embolism with particulate carbon in the nutrient arterial system, by injecting into the femoral artery.

Huggins and Wiege[74] found marrow infarction following ligation of the femoral nutrient artery in the rabbit.

Foster, Kelly and Watts[48] noted, by cutting the nutrient vessels of the femur, together with stripping of its periosteum, it was invariably followed by extensive infarction of bone and of bone marrow in young, rapidly growing rabbits. Impairment in the rate of circumferential growth accompanied cortical infarction, but no delay in longitudinal growth was found. In animals approaching maturity, the operation produces variable results. These workers also emphasized that loss of both endosteal and periosteal blood supply causes a complete infarction of cortical bone. If the source of either one of these blood supplies remained, foci of viable cortex persisted.

By measuring the intramedullary pressure of bone before and after ligation of the nutrient artery of tibia and humerus in dogs, Cuthbertson, Siris and Gilfillan[30] found the intramedullary pressure in these bones fell immediately and profoundly, but in the majority of cases, it returned to pre-occlusion levels within hours to days. Collateral circulations, both extra-osseous, and intra-osseous, were apparently responsible for the restoration of intramedullary pressure.

The role of periosteal arteries was very much disputed. Johnson[79]

believed they supply the outer half of cortex. De Marneffe [99], as cited by Shim [136], believed that in the rat, guinea-pig and rabbit the nutrient artery was mainly responsible for bone marrow nutrition, while the periosteal arterial supply mainly the cortex through the haversian and volkmann canals. On the other extreme, Anseroff [2], Brookes and Harrison [15], MacNab [96] and McAuley [109], believed that the periosteal arterial supply was negligible in normal situations, and that the nutrient artery supplied the whole marrow, and the entire cortex, other than the metaphyses. However, MacNab [96] stated the vital importance of periosteal system under abnormal situation, such as in a fracture, revascularisation from the periosteum help to reinstitute the endisteal circulation.

Trueta and Cavadias [150], by selective interruption of two of the three sources of blood to rabbits' radius, demonstrated that the nutrient artery is the main vessel to supply to the shaft of the radius, and is responsible for at least the irrigation of the whole of the marrow and the inner two-thirds or three-quarters of its cortex. The periosteal vessels supplied the outer part of cortex and kept that part of the bone alive if the nutrient circulation had been suppressed.

The metaphysial vessels alone are incapable of maintaining the marrow and deep half of the cortex alive, but after their union with the epiphysial vascular network following the fusion of the growth plate, enough blood flow is provided to the nutrient artery through its distil branches to maintain the viability of marrow and bone. Simple division of nutrient artery does not cause any significant effect on the viability of the marrow. In the young, the compensatory circulation comes from

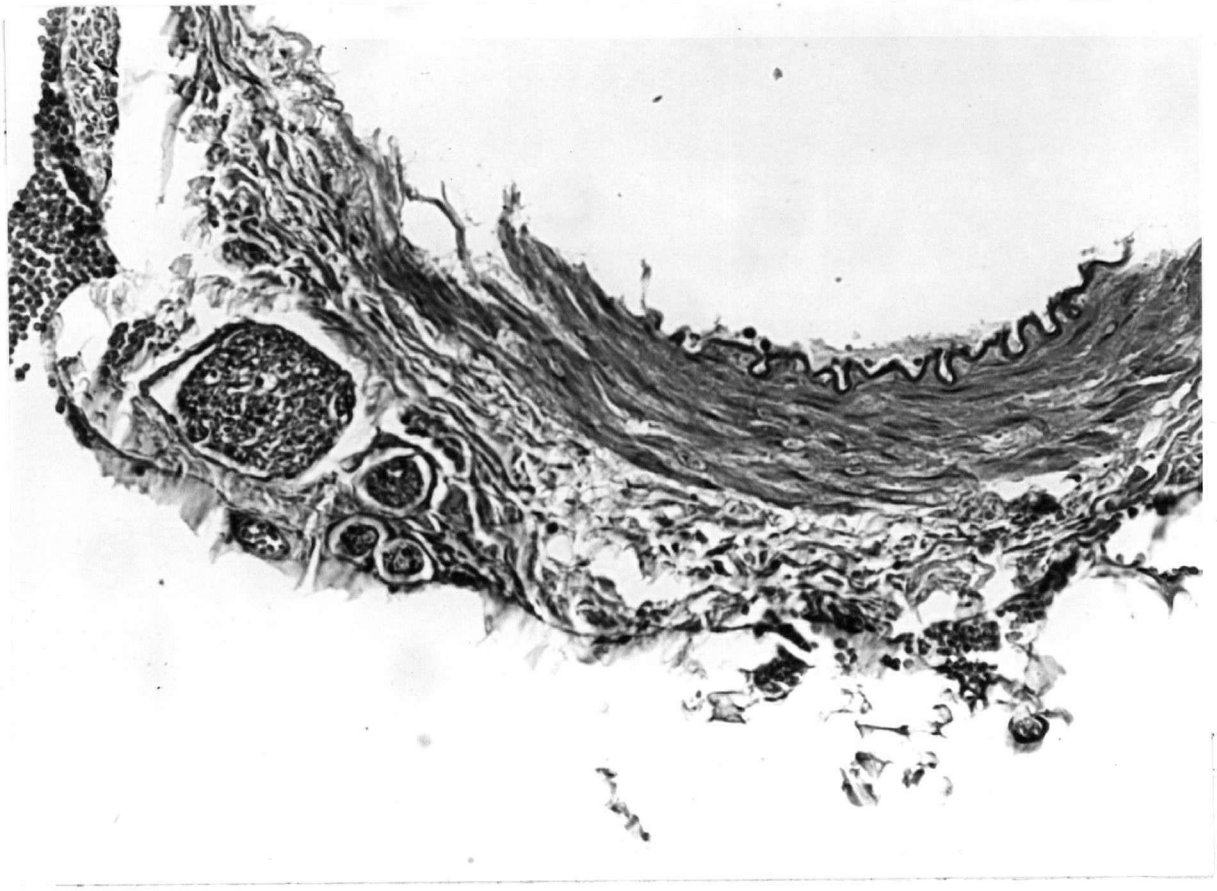
periosteal, while in adults from the metaphysial-epiphysial vascular network. Their findings are essentially the same as those of Johnson [79].

Recently, Shim, Copp and Patterson [136], by using a method of bone clearance of circulatory Strontium-85, studied the rates and regional distributions of the nutrient arterial blood flow as well as the rates of blood supply by the other arterial systems of the femur in the rabbit. The rate of the nutrient arterial blood supply was studied by evaluating the rate of reduction of bone blood flow immediately after ligation of the nutrient artery. They reported a 46% reduction of total blood supply to femur, 37% decrease in upper epiphysial-metaphysial, and 33% decrease in lower epiphysial-metaphysial region, and 71% decrease in the diaphysis, within five minutes after ligation of the nutrient artery. From these data, it was deduced that the nutrient artery supplies about 50% of the total blood supply of the entire femur, about 70% of total blood flow of the shaft, 37% of the total blood flow of the upper epiphysis and metaphysis and 33% of total blood flow of the lower epiphysis and metaphysis of the femur. Their quantitative study corresponded with the qualitative findings of Johnson [79] (1927), Trueta and Cavadias [150] (1964), and many others mentioned above.

Nerve Supply of Bone

According to Drinker and Drinker [36], and Sherman [130], Gros [62], a French anatomist, was the first one to demonstrate the presence of a nerve, which accompanied the nutrient artery into the horse's femur and gave off twigs to the periosteum. With more refined techniques, including staining with gold chloride, osmic acid, or picrocarmine,

FIGURE 4



Micrograph of transverse section of bone, with H & E stain. It shows the presence of nerve bundles, in close proximity to the nutrient vessels of bone.

and then crushing small bits of marrow, Variot and Remy [154] illustrated nerves which varied from 10 - 100 micra in diameter.

Ottolenghi [113] described three main groups of nerve fibres, within the marrow cavity:

1. those which penetrate the walls of the arterioles and form delicate plexiform networks between the adventitia and the media;
2. those which surround the capillaries;
3. those which terminate between the cells of the parenchyma.

The vasomotor nature of nerves of bone is well documented experimentally by various authors [3, 36, 47, 73, 135, 161]. The presence of pain fibres is supported by the common clinical observations that puncture of the bone marrow, many bone tumors, and osteomyelitis cause pain. The detailed histological description as to where and how the nerve endings terminate is little and yet conflicting in the literature. Even though De Castro [34] claimed to have identified sympathetic nerve fibres terminating in a ring on the protoplasm of the osteoblasts in osteoid tissue, and Hurrell [76] believed that nerve fibres extend between bone lamellae, more recent studies by Miller et al [104] using methylene blue immersion technique on thin sections of fresh under-calcified bone, could not substantiate such observations. The latter workers found the epiphysial and metaphysial ends of long bones both in small mammals and in humans to be supplied by small myelinated and unmyelinated nerve fibres from periosteal and joint capsular tissues, and the shaft marrow by fibres entering the bone through the nutrient foramen. Though it is believed that some nerve fibres enter the bone cortex through volkmann's canals, the exact course and disposition of

these fibres has not been determined. Small myelinated fibres wind about the trabeculae of the spongiosa or spread out on the undersurface of the articular cartilage are demonstrated.

Kuntz and Richins [86] and Miller [104] observed the presence of nerve fibres in marrow parenchyma apparently not ending at blood vessels. The former group of workers, by removal of the spinal ganglia of all the nerves contributing to the afferent innervation of one limb three weeks previously, to insure degeneration of the afferent fibres, so that only the sympathetic fibres remained intact, demonstrated that the perivascular plexuses appear to be less abundant and less complex than in sections of the normally innervated marrow. Those nerve fibres in the parenchyma which exhibit no obvious relationship to blood vessels, apparently disappeared, together with the degeneration of the afferent nerve fibres. Sympathetic fibres were exclusively found in tissue incorporated in the vessel wall, although the afferent nerve fibres are also found in close association with the vascular structure. Various types of delicate arborizing structures suggestive of nerve endings have been described [86]. Exact role of each, however, is not completely known yet [104].

## Physiology of Bone Circulation

### Methods of Study

A comprehensive and concise classification of existing methods of studying of bone circulation is given by Shim [137].

1. Quantitative Studies:-

   A. Direct Methods

      (i) Cannulation - collection measurement [81, 29].

      (ii) Application of electromechanical flow meter [167].

B. Indirect Methods

(i) Blood - tissue exchange mechanism.

(a) Fick's Principle

(b) Radioisotope clearance [25, 53, 132, 134]

(ii) Indicator - dilution principle.

(a) Radioisotope ($^{51}Cr$ $^{163}K$ $^{42}Rb$ $^{86}$ $^{148}$)

(b) Dye (Evans blue)

(iii) Venous occlusion plethysmography [40]

2. Qualitative Studies:-

A. Flow Pattern

(i) Vital microscopy [14].

(ii) Bone venography [31, 145].

B. Selective arterial isolation to determine relative importance of arteries

(i) Destruction or occlusion of certain vessels [48, 79, 84, 96, 136, 150].

(a) Study of devitalized area.

(b) Effect on fracture healing or bone growth.

(c) Effect on relative isotope uptake.

(ii) Injection of indicators into an artery to observe the area it sustains [29].

C. Bone Hemodynamics

(i) Direct methods (cannulation).

(a) Assessment of relative flow-volume changes [36, 135].

(b) Study of arteriovenous blood constituent.

(ii) Indirect methods.

(a) Intramedullary blood pressure [3, 67, 91, 144].

(b) Intraosseous thermometry [110].

(c) Oxygen tension of bone [166].

(d) Radioisotope uptake by or clearance from bone [88, 152].

D. Alteration of hemodynamics to stimulate growth, fracture repair, and bone vitality

(i) Sympathectomy [133, 148].

(ii) Arteriovenous fistula [78, 82, 101].

(iii) Periosteal stripping [168].

(iv) Fracture [168].

(v) Ligation of a major vein [77, 82, 116].

(vi) Artery or muscle pedicle transplantation to bone [13, 51].

Of all the currently available quantitative and qualitative methods, there are advantages as well as limitations. The physiological study of bone circulation is difficult due to the deep location and rigid structure, together with the numerous intraosseous as well as extra-osseous vessels forming complex anastomosis.

The cannulation-collection method of measurement of bone blood flow, and the intramedullary pressure as an index of bone hemodynamics, will be further discussed, as these two methods are used in this study in evaluating bone circulation.

The use of direct cannulation of nutrient vessels of bone as an index of bone blood flow dated back to 1916, when Drinker and Drinker [36] perfused an isolated tibia of dog through the nutrient artery with a

pump. They demonstrated the existence of vasomotor nerves to the marrow, evidenced by decreasing blood flow through the cannula following electrical stimulation of the nerve to bone marrow and injection of epinephrine. In 1922, Drinker, Drinker and Lund [37] published the results of extensive experiments using perfusion and dye injection techniques in dogs, cats, guinea-pigs, and rabbits, and confirmed their earlier work. The rate of flow of the perfusing blood from the marrow was recorded, but no attempt was made to relate this to either the weight of the tissue or its haemopoietic activity.

Cumming [29], assuming that the nutrient artery entering the femoral shaft supplied all the marrow except a very small amount in the epiphyses at either end of the bone, estimated the mean rate of blood flow through bone marrow to be 0·51 ml/g wet tissue/min. in the rabbit. He also observed a 20% increase in the rate of blood flow through the bone marrow during the period of rebreathing. Hypoxia had similar effect. Epinephrine decreased venous outflow.

Shim and Patterson [135] cannulated the nutrient vessels of femur and humerus in the rabbit, and tibia in the dog. The outflowing blood through the canula was introduced to a drop counting device connected to a multiple channel electronic-mechanical recorder. They demonstrated the constancy of the bone blood flow by this method, with a standard error of less than 5%. They emphasized this method is very useful for qualitative investigations of the relative changes of the hemodynamics of bone, but not a method of measurement of the total rate of blood flow through a given bone, as it is obvious that the measurement of blood flow through any one or two vessels of a bone would not give a total measurement since there are many arteries and veins other

than the cannulated vessels. They demonstrated the usefulness of their method.

Intramedullary Pressure

Larsen [91], in 1938, inserted a steel cannula into distal femoral metaphysis, while studying diaphyseal necrosis, and observed the intramedullary pressure to be 30 - 40 mmHg. and showed fluctuations related to arterial pulsation.

Bloomenthal et al [12], while studying fat embolism, observed changes in intramedullary pressure with pain stimuli, nervous stimulation, to reflexes, to changes in blood volume, and to a number of drugs. They regarded the intramedullary pressure as measured by the cannula, apparently a fusion of arterial and venous pressure is dependent upon the influx of blood from the artery and its return through the venous drainage.

Miles [103] measured pressure in the femoral heads of over thirty individuals following femoral neck fractures, and noticed fluctuation related to arterial pulsation, and the absence of which was often followed by avascular necrosis of the femoral head.

Stein et al [143] observed the intramedullary pressure as well as pulse pressure of the diaphysis is significantly greater than the pulse pressure in the epiphysis in the same bone.

Shaw [129], used heated thermocouple to measure bone blood flow, and reported the direct relationship between intramedullary pressure and bone blood flow. The validity of using a thermocouple to measure bone blood flow is, however, open to discussion.

Azuma[3] performed histological studies of bone used for measuring intramedullary pressure, and found that the cannula actually ruptured some venous sinuses, arterials and venules, with the tips emerged in an artificial blood pool, and the intramedullary pressure did not appear to represent pressure of venous sinuses. This view was supported by Hawk and Shim[67], and probably explained the wide range of intramedullary pressure recorded even in the same bone. Hawk and Shim, by measuring bone blood flow by direct cannulation of nutrient vessels, and recorded intramedullary pressure in the same bone, concluded that the intramedullary pressure is bone blood flow dependent and reflects well the changes in the hemodynamics of bone. In this thesis, therefore, the methods used for evaluation of bone circulation in hemorrhagic shock are the above methods of Hawk and Shim.

Rate of Bone Blood Flow

The direct methods by cannulating nutrient vessels of bone are not reliable in measurement of absolute rate of bone blood flow as discussed before. Edholm et al[40] applied venous occlusion plethysmography to measure bone blood flow in Paget's disease, but the validity of their method is very doubtful. This method, of necessity, ignores the rich supply of vessels, other than the main nutrient arteries which contribute to the circulation of the long bones. Thermocouples had been used by McPherson et al[110], but this method only gave qualitative information rather than quantitative. There are many limitations to the use of heated thermocouples to measure blood flow. The thermocouple probe can sample only a limited amount of tissue and may only reflect a purely local change in blood flow. Presence of a clot around the probe decreases its sensitivity. Bill[9], as cited by Kane[80], pointed out "there is no standard type of relationship between the thermal

conductivity and the flow in any tissue into which the probe is blindly introduced".

Matumoto and Mizuno [100], according to Shim [137], developed an indirect method based on clearance of a radiopaque dye; the dye is injected into bone and using a theoretical exponential correlative curve, the clearance rate is converted to blood flow of bone.

The bone seeking characteristics of various isotopes of calcium and strontium have been utilized by many workers [24, 25, 53, 118, 132] as an indicator of bone blood flow.

Shim [132], Copp and Shim [24], described a method for quantitative method using $Sr^{85}$. In 10 dogs they injected a non-diffusible plasma dye (T-1824) and $Sr^{85}$ into the nutrient artery of tibia and in the next five minutes recovered 90% of the plasma dye and only 21% of the $Sr^{85}$ from cannulated femoral vein. This indicated removal of 76% of the $Sr^{85}$ in blood flowing through bone, compared to 90% removal of diodrast by kidney. They concluded that the initial clearance of $Sr^{85}$ should give a useful measure of bone blood flow and found this to be 9 - 12 mm/min/100grm of fresh weight of different bones in adult dogs and rabbits. Their method was considered valid by Ray [118], and other workers [153, 160, 163, 164] gave comparable results, with different methods and isotopes.

Recently Shim et al [138] measured the bone blood flow of various bones in the lower extremity in a 26 year old man just before a high-thigh amputation for osteogenic sarcoma of distil femoral metaphysis, using $^{85}Sr$ clearance technique. The estimated bone blood flow was 2·5 c.c./min./100 gr. of wet bone in human.

## SUMMARY OF LITERATURE REVIEW ON THE RATE OF BONE BLOOD FLOW

| Author | Year | Method | Species | Flow ml/min.-100 gr. |
|---|---|---|---|---|
| Edholm et al | 1945 | Plethysmography | Human | 1·0 |
| Frederizkson et al | 1955 | $^{45}Ca$ | Rat | 10 - 30 |
| Copp | 1957 | $^{45}Ca$ | Dog | 2·5 - 5·8 |
| Cumming | 1960 | Venous Collection | Rabbit (marrow) | 41 - 51 |
| Barnes et al | 1961 | $^{87}Sr$ | Human | 1·25 |
| Holling et al | 1961 | Plethysmography | Dog | 5·8 - 7·7 |
| Shim | 1963 | $^{85}Sr$ | Rabbit | 16·0 |
| Weiman et al | 1963 | $^{47}Ca$ | Dog | 5·6 mature |
| | | | | 7·7 immature |
| Ray | 1964 | $^{45}Ca$ | Dog | 4·9 mature |
| | | | | 6·5 immature |
| Copp and Shim | 1964 | $^{85}Sr$ | Rabbit) Dog ) | 9 - 12 |
| Kane and Grim | 1964 | $^{42}K$, $^{86}Rb$ | Dog | 12 |
| White and Stein | 1965 | $^{51}Cr$ RBC | Rabbit | 16 |
| Copp and Shim | 1965 | $^{85}Sr$ | Rabbit and Dog | 10 |
| Van Dyke et al | 1965 | $^{18}F$ | Rat | 10 |
| Shim et al | 1967 | $^{85}Sr$ | Dog and Rabbit | 13·2/12·5 |
| Shim et al | 1971 | $^{85}Sr$ | Human | 2·43 |

Rate of Entire Skeletal Blood Flow

With the application of indirect method of bone clearance of a circulating bone seeking radioisotope, and assuming the total skeletal weight to be a percentage of total body weight (15% in human [138]), the skeletal blood flow estimated as percentage of resting cardiac output is summarised in the following table:[138]

| Author | Species | Percentage of Resting Cardiac Output |
|---|---|---|
| Van Dyke et al | Rat | 4·3 |
| Shim, Copp and Patterson | Rabbit | 7·1 ± 2·3 |
| Shim, Copp and Patterson | Dog | 7·3 ± 3·0 |
| Ray, Aovadrand Galante | Dog | 3·5 - 9·4 |
| Weinman et al | Dog - mature | 5·0 - 7·0 |
| | - puppy | 8·0 - 10·0 |
| Shim et al | Man | 4·7 - 6·3 |

The Regulation Mechanisms of Bone Blood Flow

Although not completely understood, there is accumulating evidence that bone blood circulation is controlled by neural, hormonal as well as metabolic mechanisms.

Evidence for a Neural Control Mechanism

The presence of nerves in bone have been demonstrated by many workers [62, 113, 130, 154], and their vasomotor nature is also well recognised [3, 36, 47, 73, 135, 161].

Drinker and Drinker [36], by cannulation of the nutrient artery of the isolated tibia of the dog, demonstrated decrease of blood outflow from the bone when the nerve fibres to the bone were stimulated electrically.

Sympathetic nerve trunk stimulation in the rabbit by Shim and Patterson[135] had similar effect. Weiss and Root[161] stimulated the peripheral end of the cut sciatic nerve and observed reduced marrow pressure in the tibia in five cats. Azuma[3] made similar observations in the rabbit.

Using radioactive $K^{42}$ and $Rb^{86}$ to estimate bone blood flow by fractional distribution of radioactive isotopes, Trotman and Kelly[148] demonstrated a 27% increase in blood flow to the tibia in the anaesthetised dog following lumbar sympathectomy. Effect, however, disappeared completely nine weeks later.

Shim et al[133], using $Sr^{85}$ clearance method, demonstrated the rate of bone blood flow in the side of sciatic nerve section was generally increased by five to forty-five percent in the tibia, fibula, talus and calcaneus. All the above direct and indirect methods are suggestive of a neural mechanism of control in bone circulation.

Evidence for a Hormonal Control Mechanism

Drinker and Drinker[36] observed decrease bone blood outflow in the dog's isolated tibia when epinephrine was perfused. Bloomenthal[12], Stein[144], Shaw[129], Hawk and Shim[67], and Azuma[3], also observed a fall in the intramedullary pressure of bone following the administration of epinephrine and norepinephrine in experimental animals. Stein[144] and Shim[131] observed a decrease in, or arrest of, bone bleeding following epinephrine infusion in the dog.

Quantitative estimation by Cumming[29], Shim[132] in the rabbit, and Woodhouse[166] in the dog showed epinephrine reduced bone blood flow.

Shim [132] showed with 2 - 4 microgram/kg/min of intravenous epinephrine infusion, blood flow to tibia and humerus was reduced by 74 - 81%.

Evidence for a Metabolic Control Mechanism

There is strong evidence that bone blood flow is controlled by metabolic factors such as acid metabolites, pH and oxygen and carbon dioxide both at systemic and local levels. Thus with rebreathing of expired air, or a gas mixture low in oxygen and high in carbon dioxide, Cumming [29], Shim and Patterson [135], were able to demonstrate an increase of blood outflow through the nutrient vein in rabbits.

Intravenous or intra-arterial injection of $^{N}/15$ lactic acid, resulted in an increase of nutrient arterial outflow, measured with electromagnetic flowmeter by Woodhouse [167]. Reactive hyperemia of bone after femoral arterial occlusion was unabolished by electrical stimulation of nerve, or exogenous vasopressor drugs, was also reported by Shim and Patterson [135]. Their observations suggest that the metabolic control mechanism maybe the most potent of the three control mechanisms mentioned above.

SHOCK

Definition

It is very frustrating to admit a subject so intensively studied as shock has no universally acknowledged definition. Sometimes the use of the term "shock" has been criticized because of its lack of specificity. The work has been used in a number of different senses - for example, as a clinical description - by Cannon [20] or Weil [158]. The latter referred to shock as a descriptive term used by clinicians to

denote a syndrome characterized by prostration and hypotension, and usually is accompanied by pallor, coldness and moistness of the skin, and collapse of superficial veins, alteration of mental status, and suppression of formation of urine.

The term "shock" has also been used as a description of some underlying disturbance - for example, Blalock, as cited by Reeves [119], defined shock as "peripheral circulatory failure". It has also been used as the causation of some underlying disturbance - for example, hemorrhagic shock, anaphyllactic shock and endotoxin shock, etc., attempting to relate the cause to the condition. Confusion has thus arisen, in that the word shock has been used in a single and consistent sense, but the definition is so loose as to lack clarity. Perhaps "shock" is no more exact than "fever", but it describes a group of clinical symptoms which require immediate attention in order to improve blood flow. The common thread in all form of shock is an inadequate circulation with diminished blood flow to tissue, resulting in cell hypoxia and its seguelae [6]. Hardaway [64] defined shock as "inadequate capillary perfusion due to any of many causes", and is probably the most accepted one at present time, as it describes the final common pathway of the shock syndrome.

No arbitrary limit or single parameter, either clinical, physiological or laboratory, used alone, is adequate to define shock. Where is the line of demarcation between "Hypotension" and "Hemorrhagic shock", or "Toxaemia" and "Endotoxin shock"?

"Shock" will remain a useful term, provided we regard it as a

generic one and use it to define a group or class of conditions having a basic similarity, but differing in important details [142]. Perhaps we can be comforted by the philosophical approach of Cannon [20] (1923), "It seems to me that, in such a complex as shock, definition is not a prime requisite. The important matter is to obtain a careful description of the observed facts".

Historical Aspect

The clinical syndrome, which we call shock, has been given a variety of names, without knowing what exactly it means. According to Simone [141], this corresponds to the Latin word "conlapsus", used by a Roman playwright two thousand years ago, with reference to illness in Conlapsa membra, when Dido, Queen of Carthage, whose love had been thwarted by Aeneas, fell as if lifeless.

The present day medical concept of shock was brought into light by Morris [106], who offered the following terms for the word shock: "Sudden vital depression, great venous depression, final sinking of vitality, nervous shock, and violent mental emotion". He attempted to classify shock into those following surgical operations and injuries, and shock arising from mental causes.

Gross, according to Simone [141], described shock as "a rude unhinging machinery of life", as perhaps the most sagacious definition at that time. In the remainder of the 19th century, the term "shock" was used very loosely. Pain and mental agitation were regarded upon the primary aetiology of shock.

Crile [28] initiated experimental studies in animals in 1899. He

demonstrated that the heart is capable of pumping blood supplied to it, and implicated that dysfunction of the vasomotor centre and the peripheral circulation are the possible physiological explanations in shock.

Henderson[63] in 1910 pointed out the important relationship between venous return, cardiac output and arterial pressure.

Wiggers'[165] renowned monograph: Physiology of Shock, published in 1950, remained the major reference to the accomplishments of that era. The experimental model of hemorrhagic shock he designed, is still a classical model in laboratory study of shock.

Associated with each major war or conflict there was usually more incentive to better care, together with enthusiasm on experimental studies. During World War I, Bayliss and Cannon[7] studied the effect of wound shock following laceration and crushing of muscle in experimental animals. The systemic effects of these injuries were attributed to the circulation of tissue breakdown, without appropriate attention to importance of fluid loss and infection.

Keith[83] developed the method of measuring blood volume by dye dilution technique and began to realise the volume depletion in wound shock.

The interest in studying shock probably declined in between the world wars. The role of local tissue fluid losses into local areas of traumatic injury was realised by Blalock[11]. The possible functional deficiency of the adrenal cortex in shock was investigated.[147]

During the Second World War, there was renewed interest, with emphasis on the importance of volume depletion, infection and renal failure. Shock was explained more in hemodynamic terms, such as blood flow, resistance and effectiveness of perfusion. With the application of cardiac catheterisation, Cournand [27] confirmed a reduction in cardiac output in relation to fluid loss in patients, and thus opened a new era for investigation of shock.

After the Second World War, studies began to focus on evaluation of blood flow and the functional integrity of various systems and organs. The blood supply, mechanism of control of regional circulation, including cerebral, pulmonary, hepatic, renal have been extensively studied. Role of micro circulation, association with slugging, or embolic occlusion was also brought into notice.

In the Korean conflict, the syndrome of oliguric renal failure following shock, was a major cause of death.

In the recent Vietnam conflict, improved therapeutic measures made it possible to maintain life inconsistent with survival only a decade ago. Also with improved diagnostic tools, it became possible to demonstrate respiratory and circulatory function better than ever before. A new syndrome, referred to at various times as the shock lung, non-infectious congestive atelectasis, the adult equivalent of the respiratory distress syndrome of the newborn, the pulmonary equivalent of acute tubular necrosis, or post traumatic lung, was brought into focus [43].

The changes in the subcellular level, such as alterations of mitochondria [35, 102], lysosomal disruption [8], nuclear ribonucleic

acid synthesis [92] of various organs in shock, are current topics of medical research.

Abnormal Physiological Aspects of Shock

Hemorrhagic shock is the experimental model used in this study, therefore our discussion will be mostly on this type of shock. The abnormal changes in shock are innumerable, but we will attempt to discuss them under:

A. Neural Aspects

B. Hormonal Aspects

C. Metabolic Aspects

Knowing that control of bone blood circulation is basically related with neural, hormonal and metabolic mechanisms, we hope to correlate the abnormal physiological aspects in shock with the changes in bone blood circulation in shock.

Neural Aspects

In hemorrhagic shock, hypovolemia, or decreased effective circulating blood volume stimulate the autonomic nervous system. Hypovolemia could be due to external or internal hemorrhage or sequestration of fluid or vascular pooling. Venous return to the heart is decreased, followed by decrease in cardiac output with decreased arterial blood pressure which stimulate baroreceptors and increase heart rate and force of contraction. Thus the sympathetic nervous system is alarmed. Blood flow to the skin, skeletal muscles, kidneys and splanchnic bed is economized by both arterial and venous vasoconstriction in order to redistribute blood to more vital organs, particularly the heart and brain.

It is generally accepted that as blood is lost, the cardiovascular system adjusts to accommodate the smaller volume and that, initially, this adjustment is mediated by the vasomotor nerve aided along by an increase of plasma level of catecholamines, resulting in increase vasomotor tone [115]. Page and Abell [114] studied the caliber of blood vessels through micro windows placed in rabbits' ears and in the mesentery. Vasoconstriction was shown regularly in early stages of shock by various means. Moderate vasodilation only occurs shortly before death. Gernandt [55] demonstrated efferent impulses in the splanchnic nerve of cats increased markedly when the animals were bled just as they did during asphyxia. Denervation eliminates this response. Landgren [90] demonstrated a heavy chemoreceptor discharge due to stagnant hypoxia, and that after hemorrhage, a further drop of arterial pressure with sectioning of the sinus nerves.

Hormonal Aspects

Hormones are biologically active substances discharged by glandular tissues into the circulation and are transported to tissues where they regulate the rates of important metabolic processes. The hormones are structurally polypeptides, aromatic amides, or steroids.

Catecholamines

The 'resting secretion' of adrenal medulla of dogs, under anaesthesia, recovered from anaesthesia and twenty-four hours later, was estimated by Walker et al [155], by cannulation of adrenal vein and collecting the adrenal venous blood. Catecholamines were measured by photofluorometric method. The level in dogs twenty-four hours after operation, more likely to represent values to be expected in the normal intact dogs, are in the order of 0·001 μg/kg/min. Immobilization

alone has very little effect on secretion, though the output was increased, when complicated by excitement and struggling, or when pain or discomfort was involved. Barbiturate anaesthesia lowered catecholamines secretion. Tissue trauma,including fracture of the long bones, increases secretion of the catecholamines. Blood loss produced an immediate and marked increase in concentration of catecholamines in the adrenal vein blood. The increase was due primarily and initially to an increase in concentration rather than norepinephrine in the adrenal venous blood. Early retransfusion of the lost blood or blood substitute immediately and drastically reduced the catecholamines output [156]. When 1/4 to 1/3 of blood volume was depleted, the output of epinephrine was 0·14 - 0·88 µg/kg/min and norepinephrine was 0·04 - 0·12 µg/kg/min [156]. Other workers [98] reported that with 1/3 of the total blood volume decrease, the average concentration of epinephrine increased from 1·0 to 7·8 µg/litre of plasma, and norepinephrine increased from 2·5 to 3·6 µg/litre of plasma.

Adrenal Corticosteroid Secretion

Graded hemorrhage resulted in depletion of adrenal ascorbic acid in rats, suggestive of corticoid steroid secretion from adrenal may have occurred [95]. Many workers [52, 54, 75] reported hemorrhage accompanied by moderate arterial hypotension have resulted in increased secretion of 17- Hydroxycorticosteroid. Some workers [50, 157] reported secretion of corticoid steroid unchanged or decreased. Such discrepancy is explained by the fact that the latter group of workers [50, 157] subjected the animals to a profound degree of hypotension, with very marked decrease of adrenal blood flow. The calculated secretion rate is lowered even though there is increase of steroids in adrenal venous

blood [157]. Hume and Nelson [75] showed that the adrenal cortex is capable of maintaining high levels of corticoid secretion even in severe shock, in spite of market reduced adrenal blood flow, but when the mean systolic blood pressure is reduced below 35 mmHg, the adrenal blood flow may become so low that the minute corticoid output is reduced. Reinfusion of lost blood resulted in rapid return of secretion of corticoid steroids to control levels [54, 75]. Herman et al [71] suggested shunting of blood from the adrenal cortex directly to the adrenal medulla, and thus accentuate the already poor cortical perfusion. Their study suggested that a flow of greater than 1 ml/min allows adequate perfusion of the adrenal cortex to prevent such shunting and to protect against functional damage. Mack et al [97] suggested that the sensitivity of the adrenal cortex to adrenocorticotrophic hormone in hemorrhagic shock was not altered, based on their studies in hypophysectomised dogs, given exogenous ACTH, and then subjected to hemorrhage. In such hypophysectomised animals, there was a decrease of adrenal corticosteroid secretions, which could be restored to normal levels with systemic or local infusion of saline into lumboadrenal artery. From such studies, it would appear the integrity of the hypothalamus, with intact ACTH secretion, is important for the increase of corticosteroid secretion in shock.

Aldosterone

Aldosterone acts primarily on the transport of sodium in cells of the renal tubules and sweat glands. Sodium reabsorption is increased with an exchange of potassium for sodium in the distal tubules. Sodium is retained and potassium secretion in urine is increased. This hormone in fact regulates cardiac output by increasing the end diastolic volume

and consequently the stroke volume. In addition, aldosterone potentiates the vasoconstrictor activity of norepinephrine and increases peripheral resistance. Increase of aldosterone output in dogs and man in acute hemorrhage have been demonstrated [44, 46]. Mulrow and Ganong [107] confirmed this, and demonstrated in hypophysectomised dogs, a mechanism independent of the pituitary stimulation is present in aldosterone secretion in hemorrhage.

Angiotensin

Angiotensin, secreted in response to release of renin from the juxta-glomerular cells of the kidneys, produces increased aldosterone secretion. Antidiuretic hormone, released by posterior pituitary, reabsorbs water in excess of solute by distil convoluted tubules. The secretions of both hormones in shock are believed to be increased [6, 159].

Metabolic Aspects [6, 159]

There is a general pattern of metabolic changes, involving almost all metabolites so far studied, characteristic of the shock syndrome, but not specific to it. In recent years the biochemical alterations that occur as shock progresses, are often ascribed to hypoxia, resulting from decrease and inadequate tissue perfusion. Cell hypoxia, decreased aerobic oxidation through the Kreb's tricarboxylic acid cycle and the electron transport system and an increase in anaerobic glycolysis by the Embden - Meyerhoff pathway, is observed. Lactate and pyruvate both increase initially, but later lactate increases more than pyruvate. Increased acid metabolites produce metabolic acidosis. Blood pH and carbon dioxide content fall and $p^{CO}2$ may be decreased by pulmonary ventilation. Later, in more profound shock, decrease in pulmonary function may result in respiratory acidosis, superimposed on a metabolic acidosis. Early

development of azotemia reflects an increased metabolic turnover of certain tissue proteins with an increased tissue breakdown, and a decrease in urine output. Fall in serum sodium chloride, a rise in serum potassium, and a reduced urinary excretion of sodium, chloride and water are characteristic.

## Regional Circulation in Shock

### Total Peripheral Resistance

$$\text{Total peripheral resistance} = \frac{\text{Mean Arterial Pressure}}{\text{Cardiac Output}}$$

Fowler and Franch [49] showed questionable decrease of total peripheral resistance, by bleeding dogs at 50 ml/min. to systemic blood pressure of 35 mmHg. and measuring cardiac output by Fick's Principle. Reynell et al [120], however, showed the total peripheral resistance increased by 190%. Variability of findings was noted by Wiggers [165].

### Coronary Circulation

The coronary flow in humans may be estimated with reasonable accuracy by the use of nitrous oxide inhalation method [10, 38, 60, 122]. Application of Fick's Principle with radioisotopes such as $K^{42}$ or $Rb^{86}$ uptake by myocardium is another method of accuracy.

Standardised oligemic shock in dogs is characterised during the hypotensive phase by a decrease in cardiac output, systemic blood pressure, stroke volume, and by an increase in heart rate. Coronary flow and coronary resistance are greatly decreased, though the coronary flow fraction of cardiac output is increased [42]. Coronary flow is generally greater, and the resistance generally less than can be accounted for, by a simple decline in arterial blood pressure [112]. With the use of electromagnetic flowmeters which were chronically

implanted on the left coronary artery as well as the aorta and various systemic arteries [61], the experiments confirmed previous findings - the coronary circulation shows a decreased vascular resistance during hemorrhagic shock.

Cerebral Circulation in Shock

Stone et al [146] measured cerebral blood flow in volunteers subjected to hemorrhage, using nitrous oxide method. Hemorrhage of 20 - 38% of blood volume, resulted in decrease of cerebral blood flow, but cerebral vascular resistance also decreased. Hyperventilation led to respiratory alkalosis, decrease in arterial $Co_2$ and cerebral vasoconstriction. Intravenous morphia improved cerebral blood flow, by depressing respiratory and restored $Co_2$ tension to normal levels. Fazekas et al [45] found the cerebral vascular resistance was not significantly altered in patients in shock . Rutherford et al [123], with the use of labelled microspheres, demonstrated a 56% decrease of cerebral vascular resistance in early shock, and a 16% increase in late shock. Corday and Williams [26], with photoelectric dropmeter in dogs, demonstrated an increase of cerebral vascular resistance as blood pressure was lowered. The considerable differences of opinion in the literature are probably due to difference in techniques employed, and partly reflect the variations in the experimental conditions and different species of test animals [26].

Renal Circulation in Shock

Renal blood flow estimated by PAH clearances differed from those obtained by a direct method [124, 126]. In dogs bleeding to drop the arterial blood pressure to 60 mmHg. direct renal flow measurement was 41% of control, while clearance was zero, because of anuria

present at this level of blood pressure. Direct measurement by Selkurt [124] suggested that the reduction in renal blood flow in a graded hemorrhage was greater than produced by reduced head of arterial pressure, which also suggests that active vasoconstriction has occurred. Results of Corday and Williams [26] also demonstrated marked increase of renal resistance in shock. Green and Kepchar [59] stated that blood is shunted away from the kidneys more than any other organs in shock. The kidneys are highly reactive and also normally receive a high share (20%) of cardiac output. Rutherford [123] demonstrated minimal change of vascular resistance in early stages of shock, and an 80% increase in late hemorrhagic shock in dogs. Selkurt [125] plotted the response of blood flow to progressive decrement of effective perfusion pressure by lowering the arterial pressure applying aortic compression, in a study of "pressure-flow" relationship. This was concave to the pressure axis in a range of 14 to 117 mmHg. Results were essentially the same in the intact as in the denervated kidney. Hemorrhage appeared to abolish the concavity of the pressure flow relationship. This suggests that the renal hemodynamic is largely controlled by circulating blood volume and humoral factors rather than by neural mechanism.

Splanchnic Circulation in Shock

Considerable controversy exists in the literature on this subject. Using Bristle flowmeter in a standardised hemorrhagic shock, Selkurt et al [127] observed that the splanchnic vascular resistance did not increase significantly during hypotension, and that following transfusion, there was a phase of marked reduction, particularly in the mesenteric component. Levy [94] demonstrated no increase of

splanchnic resistance during hemorrhage, though infusion of norepinephrine during hemorrhage resulted in double the resistance. Henly et al [69], with radioisotope technique, demonstrated a 38·4% decrease in portal blood flow in Wiggers' graduated hemorrhage. Reynell et al [120] reported that, after an acute hemorrhage, splanchnic blood flow decreased in proportion to cardiac output, and that splanchnic vascular resistance rose only 24% above control, whereas the total peripheral resistance rose 90% above control. Rutherford et al [123], with labelled microsphere, demonstrated a marked increase of splanchnic (portal vein) resistance, though the hepatic (arterial) resistance actually decreased.

Skin and Muscle Circulation in Shock

Green, Cosby and Lewis [57] reported skin circulation decreased before arterial pressure changed, and skin blood flow stopped when aortic mean pressure fell to 60 - 80 mmHg. The increase of skin vascular resistance is due partially to augmented sympathetic discharge. However, under similar experimental conditions, the muscle artery lumen was often dilated. Information about muscle circulation in shock is fragmentary. Dale and Richards [32, 33] showed small doses of epinephrine caused vasodilation in the denervated muscles of the cat's hind limb. The action of epinephrine in a piece of smooth muscle can be biphasic [19]. It is probable that both vasodilation and vasoconstriction phases of the initial transient vasodilation are due to a direct biphasic action of epinephrine on the smooth muscle coat of the arteriole of the skeletal muscle. Vasodilation invariably comes before vasoconstriction, and in any given infusion of epinephrine, the degrees of the vasodilation and vaso-

constriction are usually equal. Norepinephrine given intra-arterially, in animals or man, constricts muscle vessels in all effective doses [162]. If it is given intravenously in animals, the constrictor action may be overcome by use of systemic blood pressure [23], or by reflex vasodilation of the sympathetic nerve origin [5]. In skin circulation, both epinephrine and norepinephrine given subcutaneously or by slow intravenous infusion, caused severe blood flow reduction [5, 41]. It is reasonable to assume that skin circulation and muscle circulation may behave differently in shock. Rutherford et al [123], with labelled microsphere, demonstrated that vascular resistance of lower extremity markedly increased in shock, with 186% and 108% in early and late stages of shock respectively. This, however, would represent the overall change in vascular resistance in the skin, muscle and bone in the lower extremity.

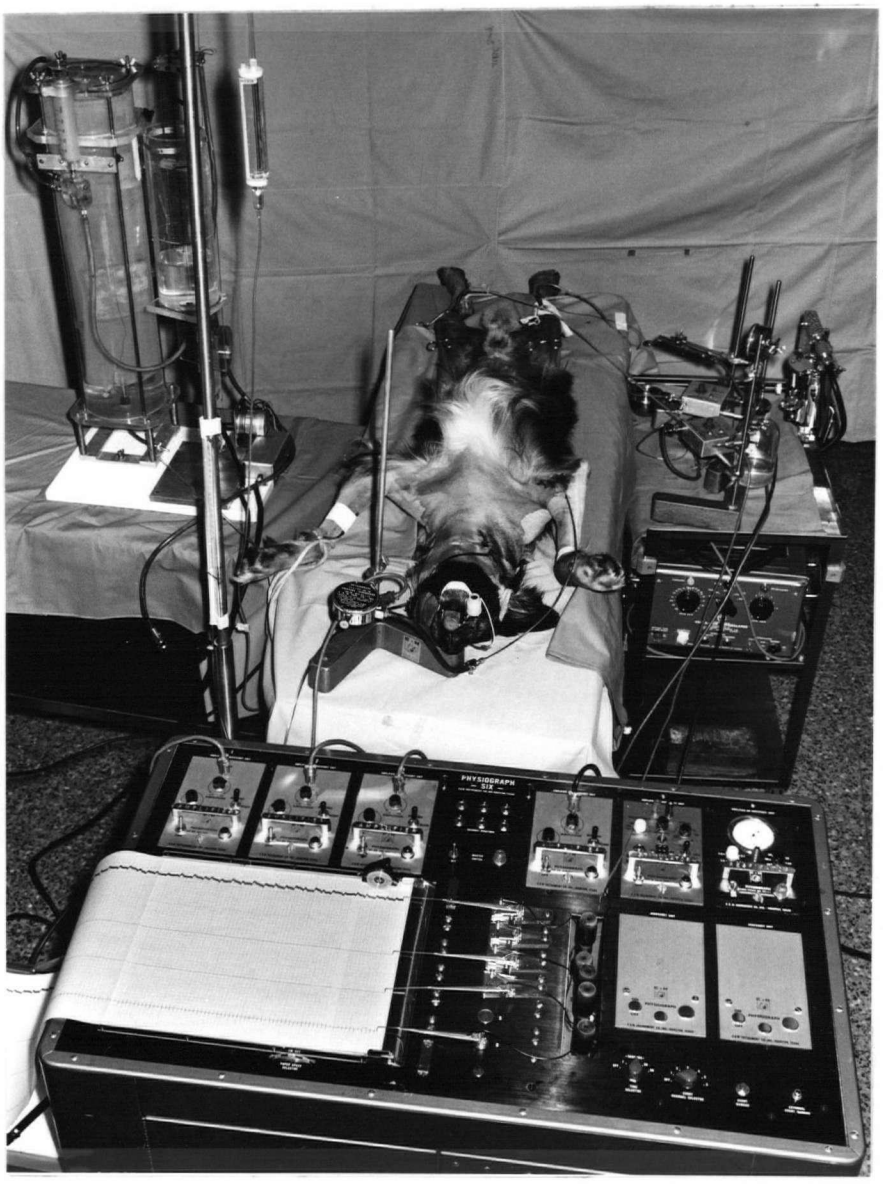
To sum up, there is evidence that various organs' vascular beds behave distinctly different in shock, and neurohormonal and metabolic factors play important roles. Much controversy still exists, though it is generally agreed that blood is shunted in shock to the myocardium and brain from other regions such as kidneys, skin and splanchnic circuits.

## MATERIALS AND METHODS

### General Set Up (Figure 5)

35 male mongrel dogs weighing 8 - 33 kilograms were used in this study. Sodium pentobarbitol (nembutal) 30 mg/kg was given intravenously for anaesthesia. The animals were all intubated but allowed to breath spontaneously. Heparin 300 I.U/kg was given, and

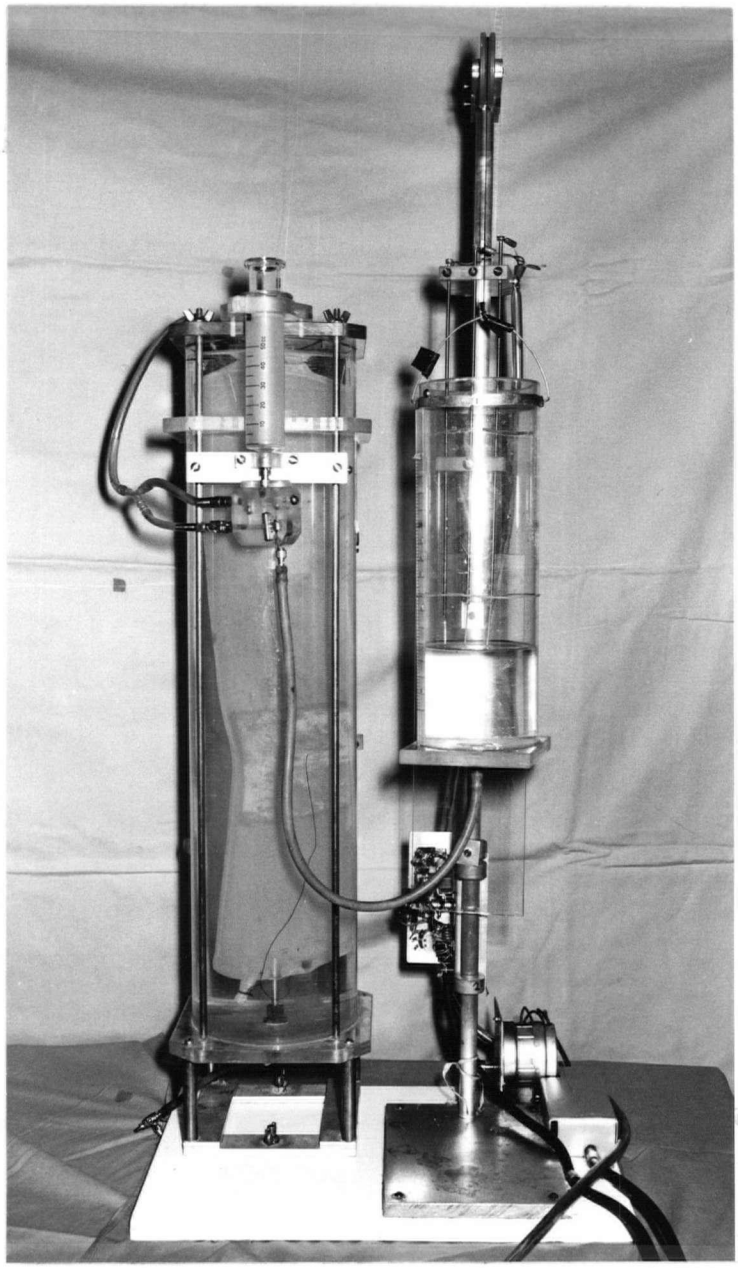
FIGURE 5




General Set up in Experiment. The dog was under nembutal anaesthesia. The right brachial artery was cannulated to measure systemic blood pressure. Tibial nutrient artery or vein was cannulated to measure bone blood flow. Cannula inserted into the tibia to measure the intramedullary pressure of bone. Results were recorded in the multichannel Physiograph. The right common carotid artery was cannulated and connected to the "Bleeding Reservoir".

repeated every two to three hours. The extremities, anterior chest, and the abdomen were shaved. The right brachial artery was cannulated with a large polyethylene tube and connected to a pressure transducer, and the blood pressure was continuously recorded in the multi-channel physiograph. The right external jugular vein was cannulated to monitor the central venous pressure continuously. The right common carotid artery was also cannulated with a polyethylene tube and was connected to the bleeding reservoir. (Figure 6). It has a two-litre plastic bag, containing 200 ml heparinised saline, and connected to the venous system via rubber tubing with a three-way tap. The plastic bag is placed in a water-cylinder and any inflow of blood into the plastic bag will displace water in the cylinder and amount of water displacement is recorded in another graduated cylinder. Thus the amount of bleeding volume can be read in this graduated cylinder. The hydrostatic pressure in the plastic bag is controlled by the level of water in the graduated cylinder. Three electrodes, the level of which are adjustable, are so designed that their tips just dip into the water in the graduated cylinder, and any further changes in level of water will result in automatic adjustment of the water height of the graduated cylinder by an electric motor. Sodium nitrite is added to saturate the water in the graduated cylinder, for facilitation of conduction of electricity in the solution. With this apparatus the animals can be bled to a predetermined level of blood pressure, and artificially maintained at such level for a considerable time. Intravenous medications and fluid replacement were given via left forearm vein. During surgical procedure, slow I.V. saline and Dextran replacement were given to maintain a constant central venous pressure.

FIGURE 6



The "Bleeding Reservoir" used in Experiment. Blood volume lost was measured by changes in the volume in the graduated cylinder. The level of systemic blood pressure in the animal was controlled by the height of the electrodes. The height of the graduated cylinder was continously adjusted by an electric motor, to maintain the tips of the electrodes just emerged into the sodium nitrite solution in the graduated cylinder.

Study of Bone Blood Flow

The bone blood flow was studied by cannulating the tibial nutrient vessels, as described by Shim and Patterson[135]. Skin incision extended from three inches below the knee to two inches above the ankle in the anterolateral aspect of leg was made. The interval between tibialis anterior muscle and the anterolateral surface of the shaft was held open by a self-retaining retractor. The nutrient artery usually arises from the anterior tibial artery above the middle of the shaft of tibia. The nutrient vein can usually be found in the same area. Muscular branches of the anterior tibial vessels were all ligated, and the main vessel was then cannulated with a polyethylene catheter (PE 50 to 90). Thus the nutrient arterial or venous outflow of the tibia was then measured by a drop counting device, and continuously recorded in the physiograph.

Bone Marrow Cavity Pressure

The subcutaneous anteromedial surface of the tibia was chosen for insertion of cannula. A steel drill, with a diameter of 0·093 in, was inserted from this anteromedial surface near the diaphysis, followed by a No. 13 gauge steel cannula inserted into the medullary cavity. The cannula was connected to a pressure transducer with a polyethylene tube filled with heparinised saline. The pressure was continuously recorded in the physiograph. It is important to prevent any gas bubble in the tubings, in order to have sensitive recording.

Transperitoneal Lumbar Sympathectomy

In five dogs, the right lumbar sympathetic trunk was identified via a midline incision, with transperitoneal approach. Electrical stimulation with voltage 12, and frequency 200/sec., was applied in each case to

confirm the anatomy, by observation of the effect of electrical stimulation on the bone circulation of the ipsilateral tibia. In these five dogs, bilateral cannulation of tibial nutrient vessels was carried out, and subsequently subjected to the shock procedure to observe the effect of sympathectomy.

Use of Dibenzyline (Phenoxybenzamine)[22, 56]

This alpha-receptor blocking agent, was given in four dogs, intravenously at a dosage of 2 mg/kg over a period of at least one hour. The alpha-receptor blocking effect was tested by epinephrine infusion, at a dosage of 0·3 - 1 μg/kg/min, and by lumbar sympathetic chain electric stimulation in each animal before the animal was subjected to hemorrhagic shock.

Induction and Sustaining of Hemorrhagic Shock

Prior to shocking the animals, the systemic blood pressure, pulse rate, respiratory rate, central venous pressure, bone blood flow as measured by number of drops per unit time, and intramedullary pressure were all recorded. They served as the control values. Induction of hemorrhage was done by bleeding the animals into the reservoir, adjusted to about 25 - 5- ml/min., and to one third of estimated blood volume, estimated as 8% of the total body weight of the individual animals[169]. All the parameters recorded in the control phase were repeatedly recorded at this stage of experimentation.

After one third of blood volume was bled, the systemic blood pressure dropped significantly. At this stage, further bleeding was inducted, and the levels of the electrodes in the bleeding reservoir were adjusted to lower the systemic blood pressure step by step, 10 - 15 mmHg. each time.

Eventually, the systemic blood pressure was lowered and maintained at about 30 - 35 mmHg., until the animal died.

## RESULTS

### I Acute Hemorrhage (Figure 7)

When the dogs were bled at a rate of 25 - 50 ml/min., there was a gradual fall of the systemic blood pressure and corresponding decrease in bone blood flow. The central venous pressure also gradually fell and so did the intramedullary pressure.

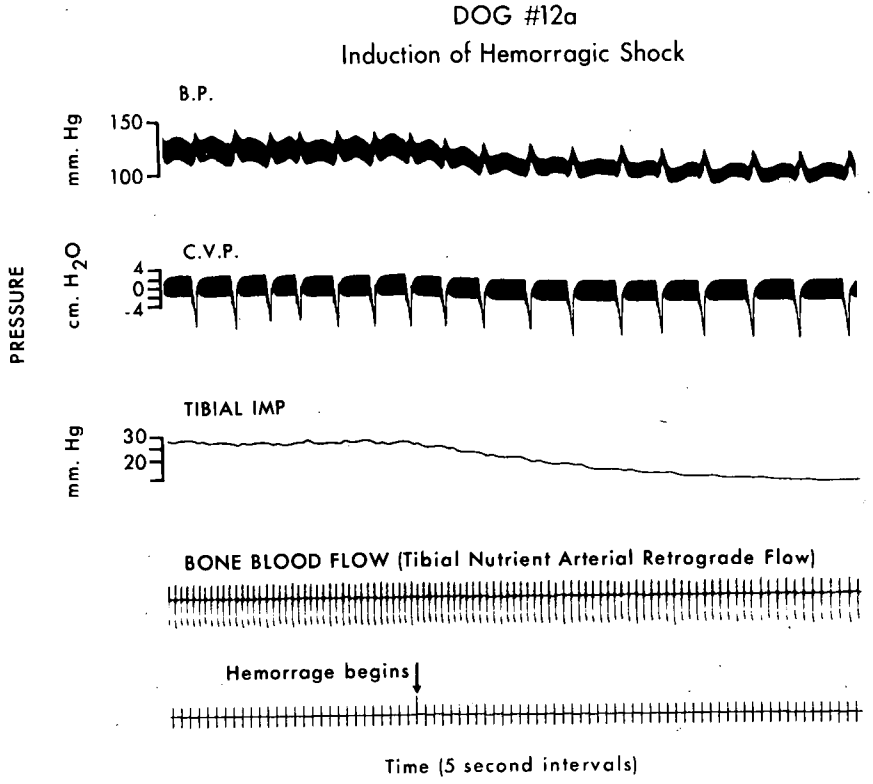
### II Effect of One Third of Estimated Blood Volume Loss (Figure 8)

| | Control | One Third Blood Volume Loss |
|---|---|---|
| Systemic B.P. | 100% (150.5 ± 5.8 mmHg.) standard error | 55·2 ± 5·1% standard error (range 30 - 80%) |
| Bone Blood Flow | 100% | 22·5 ± 3·4% standard error (range 8 - 45%) |
| Intramedullary Pressure | 55 ± 8·2 mmHg. (25 - 130 mmHg.) | Not recordable |
| Pulse Rate | 88 ± 9·6/min. standard error | 112 ± 10·2min. standard error |
| Respiratory Rate | 3·8 ± 1·2/min. standard error | 8·2 ± 1·5/min. standard error |
| Central Venous Pressure | 4·2 ± 0·8 cm $H_2O$ standard error | - 1·2 ± 0·3 cm $H_2O$ standard error (range - 3 to + 1 cm $H_2O$) |

### III Effect of Prolonged Hemorrhage

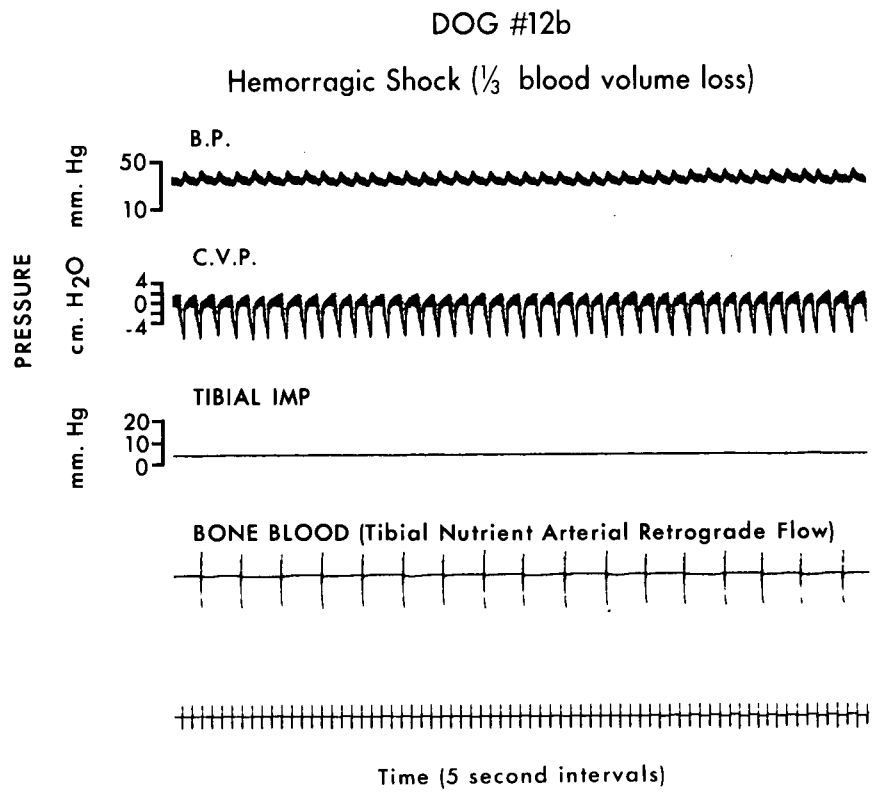
In all dogs, the systemic blood pressure was brought down stepwise by 10 - 15 mmHg., and kept steady for ¼ - ½ hour, and eventually maintained around 30 - 35 mmHg., until eventually all the animals exsanguinated. The duration of experiment varied from four hours to eighteen hours. During this time, bone blood flow remained decreased and the intramedullary

FIGURE 7




The Induction of Hemorrhagic Shock. Note the fall of systemic blood pressure, gradual decline in central venous pressure, tibial intramedullary pressure and tibial nutrient arterial retrograde flow, which was measured by a mechanical dropmeter. Each vertical stroke represents one drop of blood.

FIGURE 8




The Effects of Acute Blood Loss with one third of estimated blood volume removed. Note the fall of systemic blood pressure and central venous pressure. The tibial intramedullary pressure fell to unrecordable level. Bone blood flow, measured by tibial nutrient arterial retrograde flow, also decreased.

pressure of bone fell to unrecordable level.

IV Effect of Re-infusion of Lost Blood (Figure 9)

When lost blood was re-infused into the dogs, fifteen minutes to six hours after induction of hemorrhage, at a rate of about 50 - 100 ml/min., the systemic blood pressure, central venous pressure, tibial intramedullary pressure and bone blood flow were fully or partially returned to control values. The longer the duration in which the animal was in shock, the less complete was the recovery observed.

V Relationship between Bone Blood Flow and Systemic Arterial Pressure (Figure 10)

In ten dogs, the percentage fall of systemic blood pressure was plotted against the percentage decrease of bone blood flow. The curve is not a straight line, but rather an exponential one with concavity towards the flow axis. The curve intercepts the pressure axis at 15%.

VI Effect of Electrical Stimulation of Lumbar Sympathetic Chain (Figure 11)

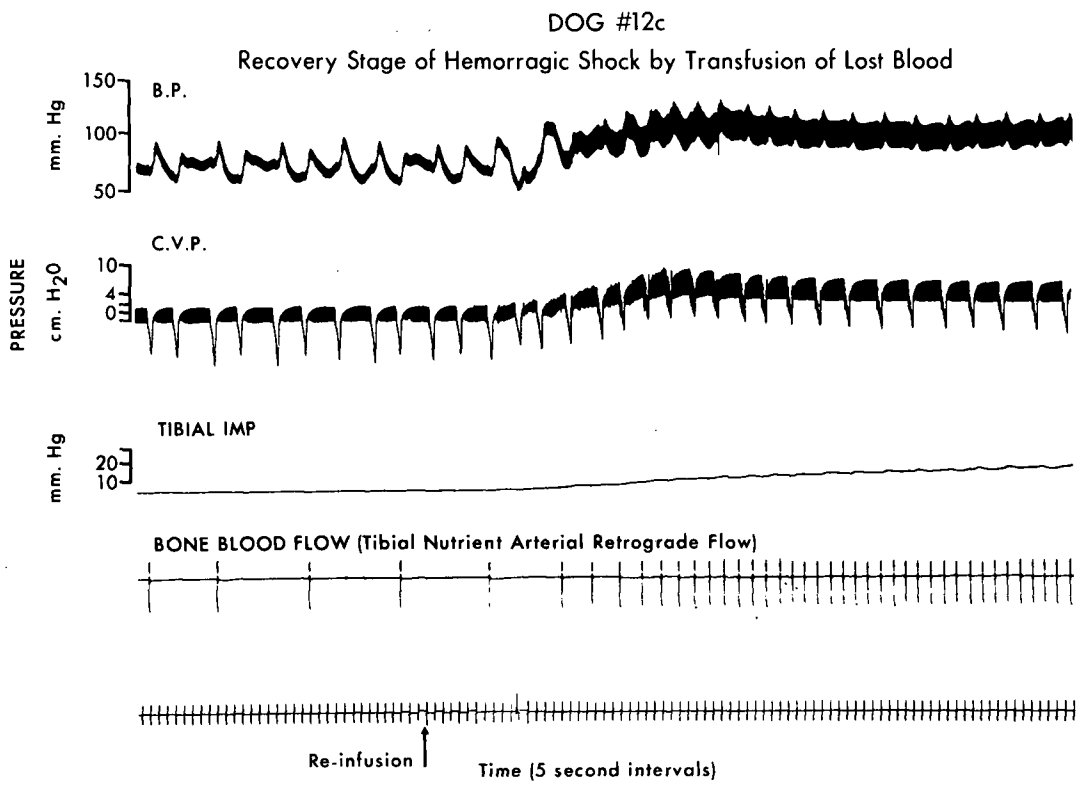
Right lumbar sympathetic chain of five dogs was identified, and electrical stimulation with voltage of 12v, and frequency of 200 per second was applied in each. An abrupt and immediate drop of tibial intramedullary pressure of the same side, coupled with decrease of bone blood flow were observed repeatedly in all five dogs.

VII Effect of Lumbar Sympathectomy

A. Before Induction of Hemorrhagic Shock (Figure 12)

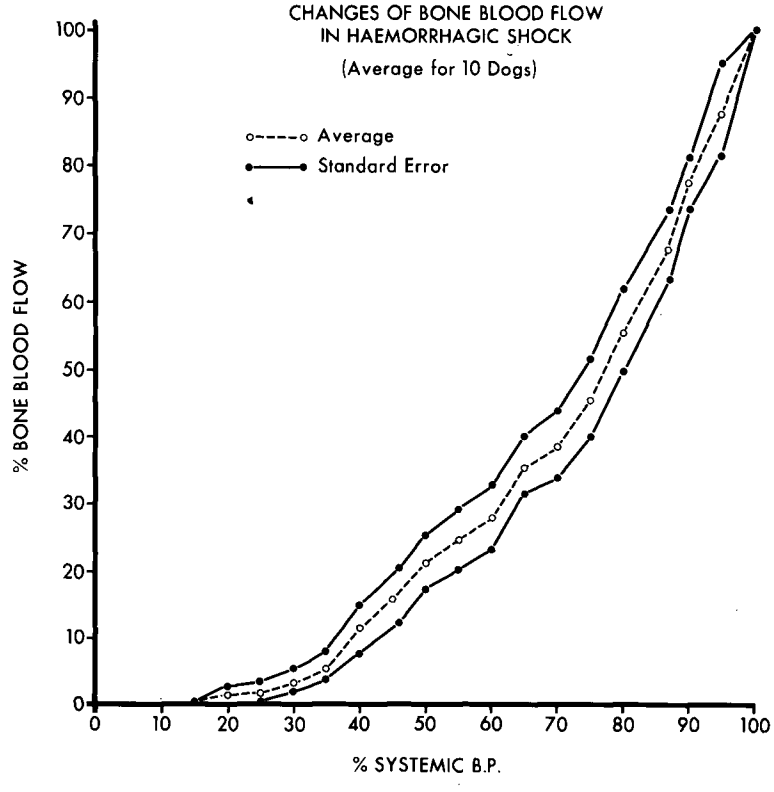
Sympathectomy of right lumbar trunk was performed in five dogs, in which bilateral cannulation of nutrient vessels was carried out. During the surgical procedure, compression of the inferior vena cava caused a rise of intramedullary pressure of both left and right tibia,

FIGURE 9



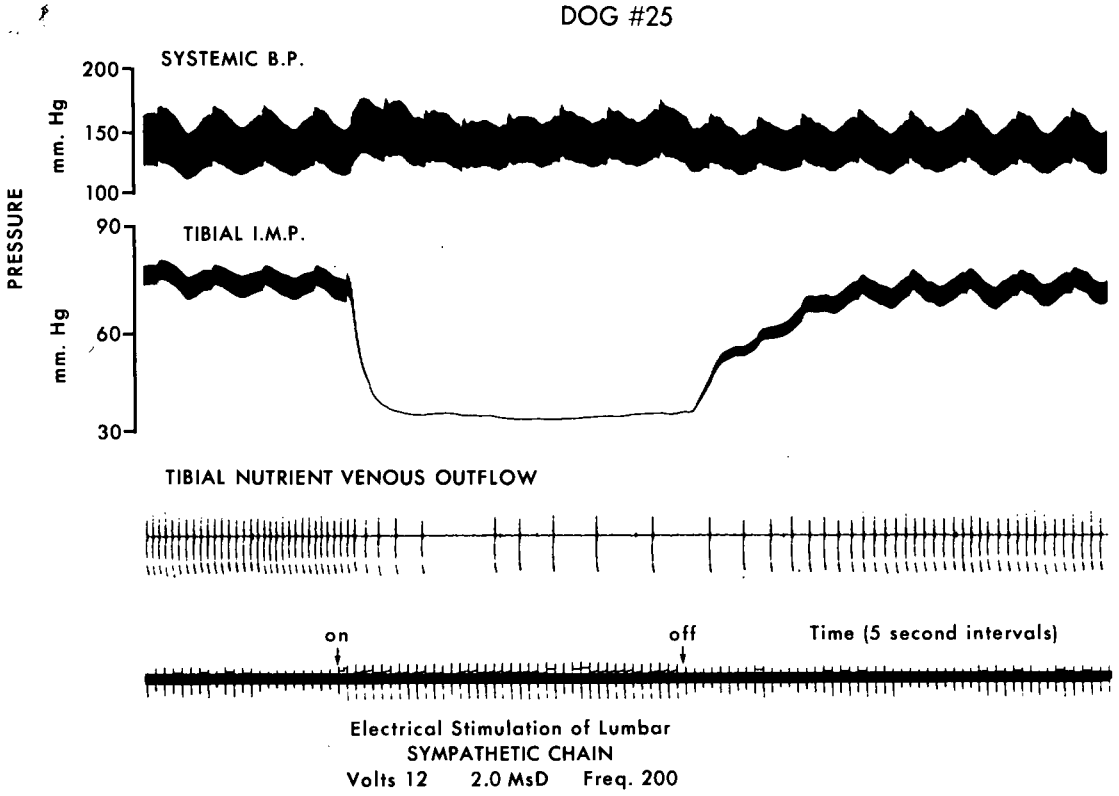

The Effects of Re-infusion of Lost Blood, 6 hours after hemorrhagic shock. Note the recovery of systemic blood pressure, central venous pressure, tibial intramedullary pressure, and bone blood flow.

FIGURE 10



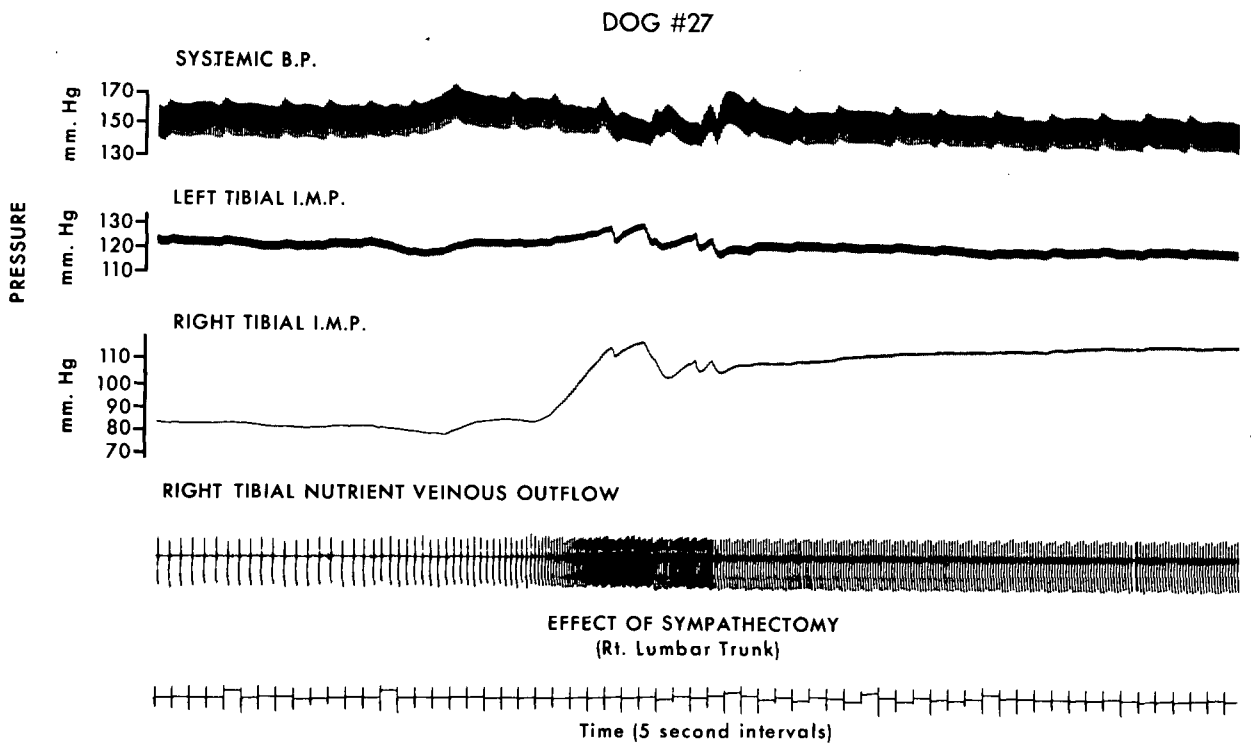

Graph to Show the Percentage Changes of Bone Blood Flow, with respect to percentage changes in systemic blood pressure in hemorrhagic shock.

FIGURE 11




The Effects of Electrical Stimulation of Lumbar Sympathetic Chain. An abrupt decrease of tibial intramedullary pressure, and tibial nutrient venous outflow were demonstrated.

FIGURE 12



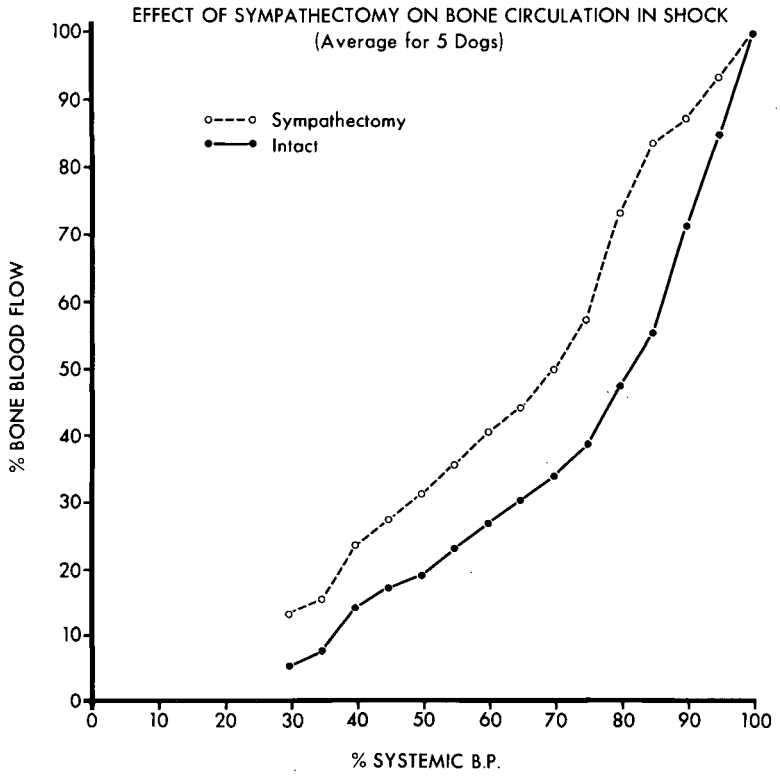

The Effects of Sympathectomy of Right Lumbar Trunk on Bone Blood Flow. Compression on the inferior vena cava during surgical procedure, resulted in venous congestion, with increase of intramedullary pressure of both tibia, and right tibial nutrient venous outflow. Note the increase of right tibial intramedullary pressure, as opposed to the left, which remained unchanged, after right lumbar sympathectomy. The right tibial nutrient venous outflow was also significantly increased after sympathectomy.

but it was coupled with an increase of nutrient venous outflow indicating bone venous congestion. This was purely a mechanical effect. After sympathectomy of right lumbar trunk, obvious increase of right tibial nutrient venous outflow, ranging from 15 - 110 percent, was observed, with an increase of intramedullary pressure of right tibia in four of the five dogs.

B. After Induction of Hemorrhagic Shock

In the same five dogs, bone blood flow was studied after induction of hemorrhagic shock. The bone blood flow as measured by direct cannulation on both sides was compared and plotted as percentage change in respect to percentage change of systemic blood pressure (Figure 13). In all five dogs, there was significantly less decrease in percentage of bone blood flow in the sympathectomised side as compared to the control side which has intact lumbar sympathetic nerve. This effect was already obvious when systemic blood pressure fell to the 90% level of the control pressure, and persisted until the systemic blood pressure fell to 30% level of the control. The difference in percentage of bone blood flow between sympathectomised and control side ranged from 10 - 30 percent with an average of 16 percent. Control studies were carried out in two dogs, in which bilateral cannulation of nutrient vessels was performed, without sympathectomy of either side, and subsequently underwent the same hemorrhage procedure. The percentage change of bone blood flow in the right and left side in these two animals was practically the same on both sides, well within 5% range of difference from each other.

FIGURE 13




The Effect of Sympathectomy on Bone Blood Flow in Shock.
The upper curve represents the bone blood flow in the sympathectomised side, with the control (nerve intact) side represented by the lower curve. There is less decrease of bone blood flow in the sympathectomised side compared with the control.

VIII Effect of Epinephrine and Norepinephrine

Infusion of epinephrine at a rate of 0·3 μg/kg/min. resulted in slight increase of systolic blood pressure and slight increase of pulse pressure, abrupt fall in tibial intramedullary pressure, and a persistent decrease of tibial nutrient venous outflow by about 30%. The above parameters returned to control levels within half to two minutes after the infusion was stopped (Figure 14).

Infusion of norepinephrine at a rate of 0·3 μg/kg/min. resulted in quite similar effects (Figure 15).

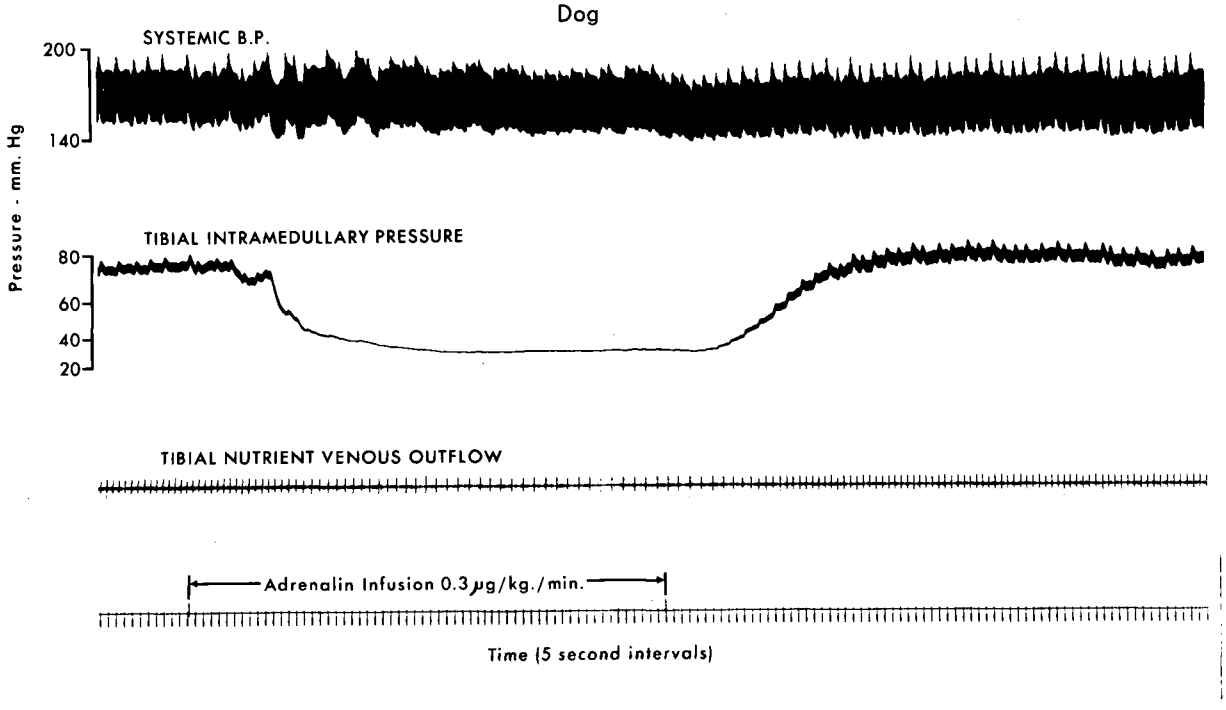
IX Effect of Dibenzyline (Phenoxybenzamine)

In four dogs, dibenzyline (phenoxybenzamine) was given with the dosage of 2 mg/kg body weight intravenously over a period of over one hour. The alpha-receptor blocking effect in each animal was confirmed by epinephrine infusion at a rate of 0·3 μg/kg/min., in which case the tibial nutrient venous outflow did not show any decrease.

On electrical stimulation of the lumbar sympathetic trunk, the ipsilateral tibial intramedullary pressure and bone blood flow decreased to a lesser extent, than would be expected if dibenzyline were not given, indicating a partial blockage of the sympathetic discharge with this dosage of the drug.

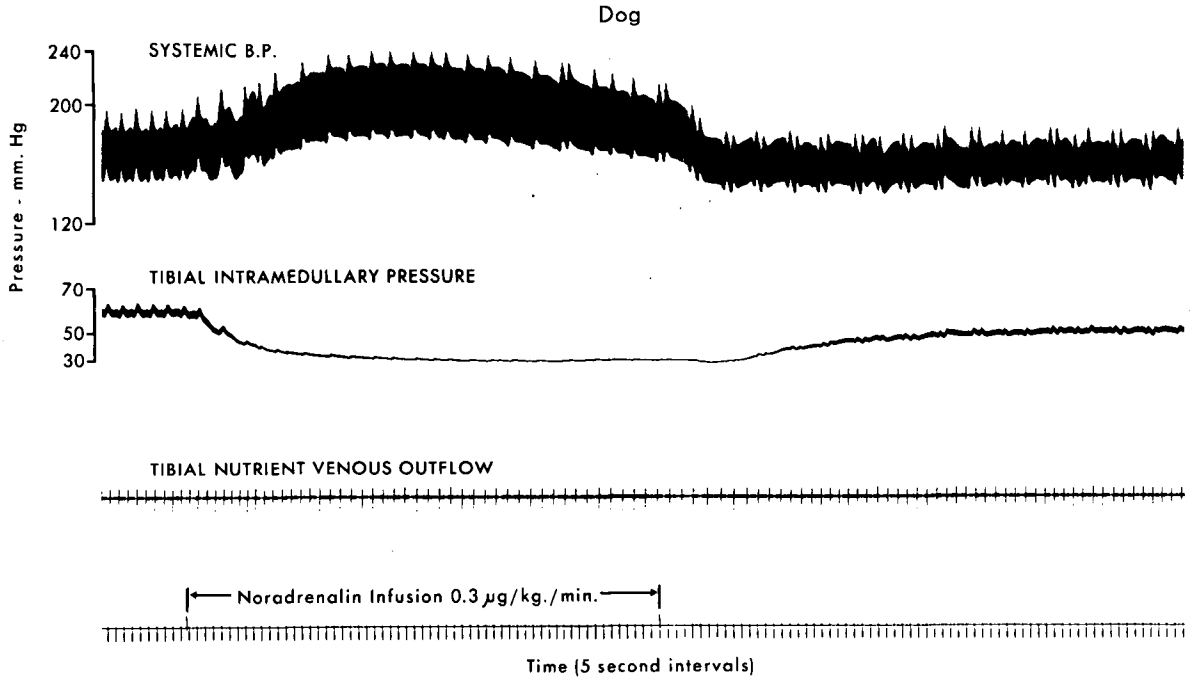
The four dogs were subsequently subjected to hemorrhage as described above. The percentage change of bone blood flow and systemic blood pressure were plotted (Figure 16). A linear relationship between bone blood flow and systemic blood pressure was demonstrated. This indicates that neural control of bone vasomotor mechanisms has been blocked by dibenzyline.

FIGURE 14



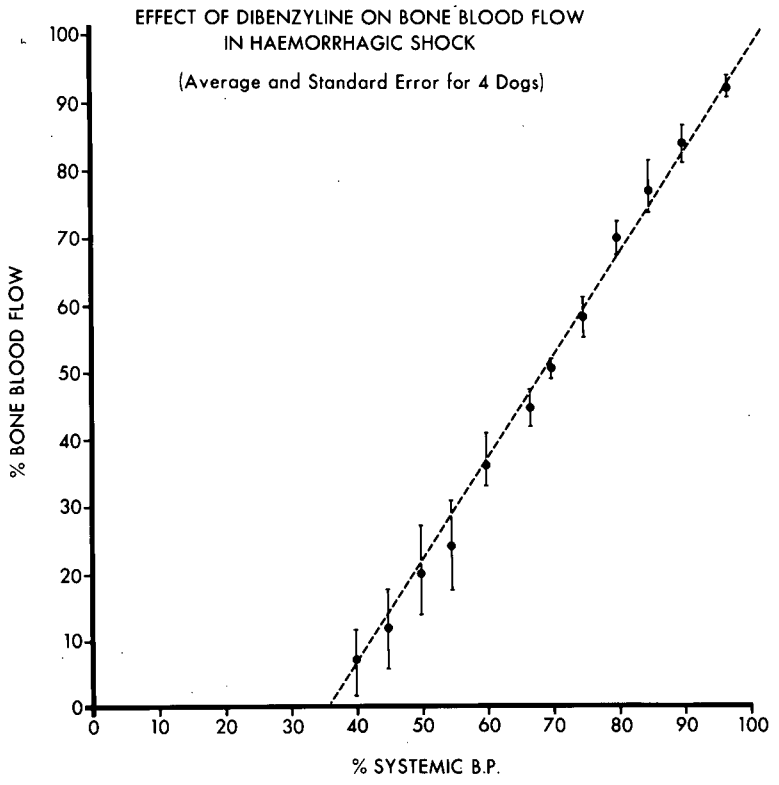

The Effects of Epinephrine (adrenalin) Infusion on Bone Blood Flow. Note the abrupt fall in tibial intramedullary pressure, and decrease of tibial nutrient venous outflow. Prompt return to control levels occurred when infusion was stopped.

FIGURE 15




The Effects of Norepinephrine (noradrenalin) Infusion. Systemic blood pressure increased because of generalized vasoconstriction. Note the fall of tibial intramedullary pressure and slight decrease of tibial nutrient venous outflow.

FIGURE 16




The Effect of Dibenzyline (phenoxybenzamine) on Bone Circulation in Shock. Note the linear relationship between the systemic blood pressure and bone blood flow.

## DISCUSSION

The purpose of this study, as mentioned before, is to find out the fundamental changes in bone hemodynamics, mechanisms whereby such changes are brought about, and to compare with other regional circulation in shock. Trueta [151] commented upon profuse bleeding produced the collapse of intraosseous pressure, and this was interpreted as being caused by emptying of the sinusoids and veins to replace the loss in the systemic circulation. However, little is known or studied on bone circulation in shock. In our survey, no previous study on bone circulation in shock was found in the literature, and the purpose of the present study is to carry out such a work.

The literature on bone circulation has been reviewed with special emphasis on the anatomical aspect of blood supply of bone, its innervation, methods of study of bone blood flow, both quantitatively and qualitatively, and the control mechanisms involved in bone circulation. The nerve supply of bone has been demonstrated by many workers [62, 113, 130, 154] and the vasomotor nature is well known [3, 36, 47, 73, 135, 161]. Humoral mechanisms involved in bone circulation are also supported by the studies of many workers [3, 12, 36, 129, 132, 144, 166]. Metabolic factors influencing bone circulation are also demonstrated [29, 135, 167].

The current concept of shock was reviewed. It would appear that decreased capillary perfusion of tissue suggested by Hardaway [64] is perhaps the generally accepted description of the term 'shock', in the light of our present knowledge. Decreased tissue perfusion is the final outcome pathway of the shock syndrome, and is the fundamental

pathophysiology in the pathogenesis of shock. The neural aspect of alarming the sympathetic nervous system for redistribution of blood by arterial and venous constriction to the central circulation, particularly the heart and brain, is discussed. Evidence for increase vasomotor tone is reviewed [55, 90, 114]. Hormonal output, involving catecholamines [98, 156], adrenal corticosteroid [52, 54, 75], aldosterone [44, 46, 107] and renin-angiotensin secretion [6, 159], have been reported. The metabolic aspects secondary to cellular hypoxia in shock, include metabolic acidosis, lactate and pyruvate acid accummulation, azotemia, hyponatraemia and hyperkalemia, are recognised.

The regional and various organs' blood flow in shock are reviewed. Coronary circulation [42, 61, 112] and cerebral circulation [123, 147] are preferentially perfused, and their vascular resistance usually decreases in shock as opposed to renal circulation [26, 59, 123, 124] and skin circulation [57]. In other vascular beds, notably splanchnic circulation [69, 94, 120, 123, 127], there is considerable controversy in the literature. Pooling of blood in the splanchnic bed with marked distention of the liver is a prominent feature in the dog and the rat in shock. A muscular sphincter like structure is present in the region of the effluent hepatic vein which is highly sensitive to vasoactive product, and this structure is thought to be responsible for congestion of liver during shock [21].

Knowing that neural, hormonal and metabolic changes occur in shock, and knowing that bone circulation is affected by neural, hormonal and metabolic factors, it is most interesting to find out the changes of hemodynamics in bone circulation in shock, and evaluation of various mechanisms by which such changes are effected.

## Validity of Experimental Methods

### I Parameters Used in Measuring Bone Circulation

#### A. Direct Cannulation - Collection Method

Cannulation of nutrient vessel, either vein or artery of tibia as described by Shim and Patterson [135], was used in this experiment. Knowing the vascular anatomy of bone, with the three separate systems of the nutrient, periosteal and epiphysial-metaphysial vessels with numerous intraosseous and extraosseous anastomoses, this method cannot be used to measure the total blood flow of a given bone. However, it is useful for qualitative investigations of the relative changes of the hemodynamics of bone with reasonable degree of accuracy [135], since the nutrient vascular system is widely distributed throughout the long bone - a representative vascular system of long bone.

#### B. Intramedullary Pressure as an Index of Bone Blood Flow

Since Larsen [91] started to measure intramedullary pressure in dogs' distal femoral metaphysis, its close relationship with bone hemodynamics has been implied by many workers [3, 12, 103, 129]. Clinically intramedullary pressure has been used to correlate with viability of femoral head after femoral neck fracture [103]. Hawk and Shim [67], by measuring intramedullary pressure and bone blood flow simultaneously, concluded that the intramedullary pressure is bone blood flow dependent, and reflects well the changes in the hemodynamics of bone. The volume of blood inside the bone is also reflected by intramedullary pressure. Therefore, intramedullary pressure is a valid parameter in evaluation of bone hemodynamics.

### II Validity of the Hemorrhagic Shock Model

From the point of view of obtaining valid data, the animal preparation

should mimic the conditions encountered in human situations. Experimental models generally fall into two categories: (a) those in which a fixed volume of blood is withdrawn, regardless of changes in blood pressure, and (b) those in which enough blood is withdrawn to lower the mean arterial pressure to certain predetermined levels, either once or repeatedly in stepwise fashion. After bleeding a percentage of predetermined blood volumes, it was found that the residual volume varies considerably [128]. Such a method does not generally produce predictable and consistent results. The Western Reserve method, or Wiggers' method [165], consists of lowering the arterial pressure to 50 mmHg. for ninety minutes, then at 30 mmHg. for forty-five minutes, followed by re-infusion at a rate of 50 ml/min. Arbitrary aspects of the method as noted in various modifications of this method, are the levels of hypotension (35 - 70 mmHg.) and the percentage take-up from the reservoir to the animal accepted as end point (15 - 30% of blood volume [128]). Shoemaker, Walker and Moore [140] commented on the advantage of Wiggers' method as that a relatively stable hemorrhaged preparation is provided for a period of several hours and can be reproducible in any laboratory. However, one of the disadvantages of the Wigger method is that the animal is suddenly plunged into late or irreversible shock; this state is then artificially prolonged by the gradual return of the shed-blood at a rate sufficient to maintain the blood pressure at a predetermined value. This experimental situation differs remarkably from the usual course of hemorrhagic shock.

The method we adopted consisted of removal of one third of estimated blood volume (8% of body weight) at a rate of 25 - 50 ml/min. This generally lowers the systemic blood pressure to $55{\cdot}2 \pm 5{\cdot}1\%$ of the initial

blood pressure, considerably higher than that of standard Wiggers' procedure. We then brought the systemic blood pressure down further in a stepwise manner, and maintaining a relatively stable blood pressure at each step for ¼ - ½ hour, until the systemic B.P. fell to 30 - 35 mmHg.

We attempted to show the events occurring from the very beginning of hemorrhage until the animals exsanguinated. This model appears more mimic to clinical situations, and bears similarity to the model used by Shoemaker et al [140]. The preparation can be reproducible as all the procedures were specified.

DISCUSSION ON RESULTS

I Acute Hemorrhage

By hemorrhaging at the rate specified, we observed the gradual fall of intramedullary, with gradual obliteration of the normal fluctuation associated with arterial pulsation. Nutrient venous or arterial outflow decreased. Both parameters are qualitative measures of bone hemodynamics, indicating a decrease of bone circulation in hemorrhage. Trueta [151] observed a profuse bleeding was associated with a marked fall of intramedullary pressure. He thought that the sinusoids and veins of bone are emptied with the blood being removed to replace the loss by the systemic circulation. The skeleton, in addition to being hemopoietic organs, also acts as stores of blood. Structures such as spleen and liver in the dog and cat add to the active circulation as much as 30% of existing blood volume [21]. Foa [47], by measuring changes in volume of bone marrow, suggested that the behaviour of the bone marrow was very similar to the spleen. Branemark [14] actually observed the living marrow in the fibula of the

rabbit, and reported a rhythmic activity with alternating dilation and emptying of the marrow sinusoids, very similar to those described by Knisely [85] in the spleen. During epinephrine injection, Branemark [14] observed the marrow vessels became constricted and the marrow emptied of blood as though it was squeezed, similar to that of a sponge when squeezed. With the present knowledge of nature of intramedullary pressure [3, 12, 103, 129] the decrease observed during shock is most likely associated with decrease of the blood volume inside the marrow due to vasoconstriction in early stages and followed by a decreased blood supply to bone. This is quite contrary to pooling of blood in the splanchnic circulation observed in the dog and rat in shock. The total skeletal blood flow was estimated in dogs as 7·3 ± 3·0% of the resting cardiac output by Shim, Copp and Patterson [132]. Little knowledge of the volume of blood inside the bone marrow is available. Knowing that there are 206 bones in the human body, the volume may be considerable. Further studies in this area are required before any quantitative estimation is justified.

II Effect of One Third Blood Volume Loss

When one third of the estimated blood volume is lost, the systemic blood pressure fell to 55·2 ± 5·1 % (range 30 - 80%) of the control level. Manger et al [98] reported after approximately one third of total blood volume loss in dogs, the mean arterial pressure fell by a range of 56 - 81%. The rate of bleeding was varied from extremely rapid (138 - 217 ml/min.) to slow (5 - 12 ml/min.).

Bone blood flow decreased to 22·5 ± 3·4% of control level. Intramedullary pressure invariably fell to unrecordable level. The bone circulation decreased markedly with the specific amount of hemorrhage.

It is difficult to compare quantitatively the changes of bone circulation with other regional circulation in shock, because the methods of inducing shock and the methods of assessing blood flow are different.

However, Rutherford et al [123], immediately after bleeding in dogs to lower the systemic blood pressure to 40 mmHg., measured regional blood flow of various organs by labelled microspheres, as percentage of control groups' values: myocardium 70%, cerebral 86%, renal 41%, hepatic (arterial) 64%, splanchnic (portal vein) 29%, bronchial 26%, and lower extremity 13%. In the coronary and cerebral circulation, the shares of cardiac output in early shock were actually found to have increased, though the absolute amount of flow was decreased with a proportional decrease in vascular resistance. Similar observations were made in coronary [42, 61, 112] and cerebral [147] circulations by others.

On the other hand, a marked decrease of blood flow, out of proportion to reduction of arterial pressure, was noticed in renal [26, 124], and skin [57] circulation. It would appear that bone circulation in shock behaves more like the latter group of regional circuit.

III Prolonged Hemorrhage

All the parameters remained low during prolonged hemorrhage, and the duration lasted four hours to eighteen hours, until the dogs eventually exsanguinated.

IV Re-infusion of Lost Blood

This varied from fifteen minutes to six hours after induction of hemorrhage. Re-infusion of lost blood resulted in partial or complete

recovery of the control levels of systemic blood pressure, tibial intramedullary pressure, and bone blood flow. It was observed that the longer the duration of the shock, the less complete was the recovery observed.

V Relationship between Bone Blood Flow and Systemic B.P. (Figure 10)

The curve obtained was an exponential one with concavity towards the flow axis. It must be noted that the systemic blood pressure is not exactly the perfusing pressure at the level of regional circulation of bone, although the systemic blood pressure is closely related or proportional to the perfusing pressure.

In order to interpret the curve of flow and pressure relationship, it is necessary to understand the resistance and capacitance phenomena in vascular beds. Green et al [58] expressed the relationship between blood flow and pressure in a circulatory bed as

$$F = c \times P^{n}$$

where

F = flow in ml/min.

c = a constant

n = an exponent having a value between 1 and 3

The lowest value of n and highest value of c were found at "low vasomotor tone" and vice versa. Levy and Share [93] have confirmed these findings and demonstrated that with maximal dilation induced by a ten minute period of ischemia and subsequent perfusion with hypoxic blood in the dog's hind limb, a linear relationship between F and P was obtained, indicating the value of n is 1.

By local perfusion of nutrient artery in canine tibia, without shocking the animals and maintaining a constant systemic blood pressure, Kato et al[81] demonstrated a linear relationship between perfusion pressure and measured bone blood flow rate. This was interpreted as no significant change in the peripheral resistance under such experimental conditions.

The flow and pressure curve in our study would suggest an increase of vascular resistance as judged by the shape of the curve.

The method to calculate the value of n is as follows:

$$F = c\ P^n$$

therefore $\log F = \log c + n \log P$.

By plotting the graph of log F against log P, a straight line is obtained, and the slope represents n. In our study, n was found to be 2·4, indicating presence of vasomotor bone.

VI Effect of Electrical Stimulation of Lumbar Sympathetic Chain

An abrupt and immediate drop of tibial intramedullary pressure, together with a marked decrease of bone blood flow, was observed, indicating decrease of bone circulation due to an increase of sympathetic activity. The presence of nerves in the bone has been documented by various workers[113, 130, 154], and their vasomotor nature was also well documented[3, 36, 47, 73, 135, 161].

VII Effect of Lumbar Sympathectomy

A. Before Induction of Hemorrhage

Sympathectomy of right lumbar trunk, performed in five dogs, resulted in an increase of nutrient venous outflow ranging from 15 - 110 percent above control value in acute experiment. Trotman and Kelly[148]

demonstrated a 27% increase in bone blood flow to the tibia in the anaesthetised dog following lumbar sympathectomy, while Shim et al[133] showed a 5 - 45 percent increase in blood flow of bones in the leg and foot in rabbits after sciatic nerve section, which carries most of the sympathetic fibres below the knee. The increase of bone blood flow after sympathectomy, which was also observed in this study, indicates the presence of vasomotor control of bone.

B. Effect of Lumbar Sympathectomy in Shock

In five dogs, after lumbar sympathectomy on right side was performed and leaving the left side intact, hemorrhagic shock was induced. The decrease of bone blood flow on the sympathectomised side was correspondingly less than the control (Figure 13). The difference was apparent, and maximal when the systemic blood pressure was between 75 - 85%, indicating the presence of more vasoconstriction in the control side, occurring already in the early stage of hemorrhage. This implies that the sympathetic discharge occurs rapidly to loss of blood volume. The average difference in the percentage of bone blood flow between sympathectomised and control was 16·1%.

To compare the bone blood flow in the right and left sides without sympathectomy, two experiments were performed which showed less than 5% difference throughout the whole range of systemic blood pressure. Such experiments are statistically not significant, because only two were performed, but the results agree with the work of Trotman and Kelly[148], who demonstrated no significant difference in bone blood flow in both sides in six dogs used as control.

VIII Effect of Catecholamines (Figures 14 and 15)

The 'resting level' of catecholamine was estimated by Walker et al [155] to be in the order of 0·001 μg/kg/min. Blood loss produced an immediate and marked increase in the plasma concentration of catecholamine, primarily of epinephrine with less norepinephrine. When ¼ to 1/3 of the blood volume is depleted, the output of epinephrine was 0.14 - 0·88 μg/kg/min. and norepinephrine was 0·04 - 0·12 mg/kg/min. [156]. Manger [98] reported the level of epinephrine increased from 1·0 to 7·8 μg/L of plasma, and norepinephrine increased from 2·5 - 3·6 μg/L of plasma.

When epinephrine at the dosage of 0·3 μg/kg/min. was infused, an abrupt fall in tibial intramedullary pressure and decrease of bone blood flow were observed. The above dosage of epinephrine corresponds to the range of epinephrine level in blood when 1/3 of estimated blood volume was removed by other workers [98, 156]. This suggests for an evidence that bone blood flow is also affected by epinephrine in shock. Norepinephrine at the dose of 0·3 μg/kg/min. resulted in elevation of systemic blood pressure due to its generalized vasoconstriction effect, fall of intramedullary pressure, and a less obvious decrease in bone blood flow. With smaller doses of norepinephrine, the effect on bone circulation was not consistent.

IX Effect of Dibenzyline on Bone Blood Flow in Hemorrhagic Shock

Dibenzyline (phenoxybenzamine) [22, 56] produces a prolonged and effective blockage of alpha-adrenergic receptors. It does neither produce the characteristic blockage by altering the function of adrenergic nerves nor the basic response mechanisms of effector cells, but rather it appears to act specifically for catecholamines and closely related

compounds and is considered to be an interaction with specific tissue receptors. Responses to circulating catecholamines are inhibited more effectively than those to mediator released locally at nerve endings. Blockage effect is relatively slow and mild in first one to two hours after drug administration, but the effect is very persistent. When dibenzyline was administered intravenously at the dosage of 2 mg/kg over a period of one hour, the systemic blood pressure dropped from control 140 mmHg. to 105 mmHg. (average for four dogs). This seems partly due to the drug action of lifting off the vasomotor tone. Under the dibenzyline medication, the hemorrhagic shock induction did not produce concave bone blood flow-pressure relation curve; instead, the relation was a linear one (Figure 16). This was interpreted as no significant increase of vascular resistance after dibenzyline was administered, and the decrease of bone blood flow became proportional to decrease of the perfusing pressure.

Similar observations were made by Levy and Share [93]. The hind limb vascular bed of the dog was maximally dilated by ten minute period of ischaemia and subsequent perfusion with hypoxic blood, and this produced a linear flow-pressure relationship. Kato et al [81] also demonstrated a linear relationship between flow and perfusing pressure by locally perfusing the canine tibia, without exciting the neurohormonal mechanisms and thus maintaining a constant peripheral resistance. All the above information supports our interpretation that catecholamine reduces bone blood flow in hemorrhagic shock through vasoconstriction action.

## SUGGESTED FUTURE STUDIES

### 1. Bone and Marrow Blood Volume

In our present study we deduced decrease of volume of blood inside the marrow, coincided with decrease of intramedullary pressure and decrease of bone circulation in shock. As Trueta[151] interpreted, the blood in the sinusoids was removed away to replace the loss by the systemic circulation. This was also the impression of Foa[47] and Branemark[14]. However, little quantitative studies of the volume of blood inside the bone marrow is available, and to assess the contribution of blood from bone circulation to the effective circulatory volume in shock is a pure conjecture. Future study is necessary.

### 2. Ischemic Effect on Bone Marrow in Shock

The injurious effect of shock on various organs - such as acute tubular neurosis in kidneys, myocardial infarction, Sheehan's syndrome in pituitary, are well recognised. We demonstrated the significant decrease in bone blood flow in shock. Can similar injurious effect occur in the bone? The answer to this question has to be given by more refined histological or biochemical techniques with future studies in this area.

### 3. Pathogenesis of Fat Embolism

Trauma and hypovolemic shock have been implicated in the pathogenesis of fat embolism[117]. Bone marrow is rich in fat. By studying the composition of nutrient venous outflow in shock, it is possible to bring more light to the possible association of pathogenesis of fat embolism to shock.

4. Metabolic Aspect of Bone Circulation in Shock

Metabolic factors, such as changes in pH, hypoxia, hypercapnia, and accummulation of different metabolites, such as lactic and pyruvatic acids, are present in hemorrhagic shock. Even though the effect of some factors influencing bone circulation has been studied. Hypercapnia and hypoxia increase bone blood flow [29, 135], parental lactic acid also increases nutrient arterial outflow [167], and reactive hyperemia of bone after femoral arterial occlusion was unabolished by electrical stimulation or exogenous vasopressin [135]. But quantitative studies of a combination of the various metabolic factors in shock are not available.

## SUMMARY

Bone circulation in hemorrhagic shock was studied in thirty-five male mongrel dogs. The term shock is defined in this thesis as persistent profound hypotensive syndrome, due to acute hemorrhage of more than one third of blood volume.

The method of induction of shock consisted of removal of one third of estimated blood volume (8% of body weight) at a rate of 25 - 50 ml/min., and subsequently dropping the systemic pressure in a stepwise manner until the maintaining level of 30 - 35 mmHg. was reached. The central venous pressure, pulse and respiratory rates were also recorded.

Bone circulation was studied by (1) recording the blood flow through a cannula inserted into the tibial nutrient vein or artery, and (2) recording the bone marrow cavity pressure of tibia. When one third of the estimated blood volume was removed, the bone blood flow decreased to 22·4 $\pm$ 3·4 % of control level.

The duration of hemorrhagic shock varied from four hours to eighteen hours, and bone blood flow was decreased persistently. Intramedullary pressure of tibia invariably fell to unrecordable level after one third of blood volume was removed.

Re-infusion of lost blood, fifteen minutes to six hours, after hemorrhage resulted in partial or complete recovery of the control levels of systemic blood pressure as well as bone blood flow and intramedullary pressure of bone.

The curve showing relationship between changes in bone blood flow and systemic blood pressure was an exponential one with concavity towards the flow axis. The presence of increased peripheral resistance of bone during hemorrhagic shock was deduced.

Bilateral cannulation of tibial nutrient artery or vein with lumbar sympathectomy on one side showed a correspondingly less decrease (average 16·1%) of bone blood flow on the sympathectomised side in hemorrhagic shock.

Dibenzyline (phenoxybenzamine) altered the pressure-flow relation curve to a linear pattern in bone circulation in shock.

These observations indicate that the bone circulation decreased in hemorrhagic shock, and apart from the decreased circulating blood volume, there are active vasomotor control mechanisms responsible for the reduction in bone blood flow. These mechanisms are neural (sympathetic) and hormonal (catecholamine).

## CONCLUSION

1. Hemorrhagic shock in dogs decreases bone circulation, as measured by bone blood flow and intradmedullary pressure.

2. The decrease of bone circulation persists as long as eighteen hours and recovers partially or completely the normal rate of flow if the lost blood is re-infused.

3. The relationship of changes in bone blood flow and systemic blood pressure indicates that vasomotor control mechanisms play a role in shock to increase bone vascular resistance.

4. The role of nervous control of bone circulation in shock is demonstrated: sympathetic stimulation decreases bone circulation and sympathectomy decreases the vasomotor response in bone circulation in shock.

5. The role of catecholamines on bone circulation in shock is demonstrated: epinephrine and norepinephrine infusion decreases bone circulation.

6. Dibenzyline (phenoxybenzamine), an alpha-receptor blocking drug, blocks the bone vasoconstriction action in shock. This indicates the bone vasomotor system has alpha-receptors.

7. Bone circulation decreases in shock, not only due to decreased perfusing pressure and circulating blood volume, but also due to neural and hormonal active vasomotor control mechanisms.

TABLE I

## RELATIONSHIP BETWEEN PERCENTAGE CHANGE IN SYSTEMIC BLOOD PRESSURE AND BONE BLOOD FLOW

| Dogs | % Bone Blood Flow | % Systemic B.P. 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 35 | 30 | 25 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 100 | 88.5 | 78 | 66 | 55 | 44 | 32.5 | 24 | 18 | 17 | 16.5 | - | - | - | - | - | - |
| 6 | 100 | 92 | 84 | 76.5 | 68.5 | 61 | 54 | 50.5 | 47.5 | 42.5 | 35 | 25.5 | 12 | 2 | - | - | - |
| 8 | 100 | 92 | 83.5 | 76.5 | 68 | 59 | 50.5 | 42.5 | 36.5 | 31 | 26.5 | 22.5 | 15 | 9 | 5 | - | - |
| 10 | 100 | 95 | 83 | 74.5 | 66 | 54.5 | 40 | 29 | 25 | 22 | 17.5 | 13 | 9.5 | 7 | 5 | - | - |
| 12 | 100 | 80 | 65 | 61 | 54 | 51 | 46 | 41 | 36 | 30.5 | 25 | 25 | 23 | 20 | 16 | 13 | 10 |
| 14 | 100 | 91.5 | 83.5 | 74 | 68.5 | 61.5 | 55 | 38.5 | 36 | 32.5 | 28.5 | 22.5 | 16 | 10 | 4 | - | - |
| 15 | 100 | 86 | 72.5 | 60 | 24.5 | 10 | 8 | 4.5 | 4 | 3.5 | 3 | 2 | 1.5 | 1 | - | - | - |
| 28 | 100 | 77 | 58.5 | 41.5 | 24 | 14.6 | 9 | 6 | 5 | 4 | - | - | - | - | - | - | - |
| 32 | 100 | 98 | 96 | 87.5 | 72.5 | 57.1 | 51 | 45.5 | 43 | 40 | 38 | 32.5 | 25 | - | - | - | - |
| 34 | 100 | 76 | 68 | 59 | 51.5 | 42 | 36.5 | 30 | 23 | 18.5 | 14 | 10.5 | 8 | - | - | - | - |
| Mean | | 87.6 | 77.2 | 67.6 | 55.2 | 45.5 | 38.2 | 35.2 | 27.4 | 24.1 | 20.4 | 15.3 | 11 | 4.9 | 3.0 | 1.3 | |
| S.E. | | 5.7 | 3.6 | 4.1 | 5.6 | 5.9 | 5.5 | 4.2 | 4.7 | 4.3 | 3.9 | 3.8 | 3.0 | 1.9 | 1.6 | 1.3 | |
| Mean ± S.E. | | 87.6 ±5.7 | 77.2 ±3.6 | 67.6 ±4.1 | 55.2 ±5.6 | 45.5 ±5.9 | 38.2 ±5.5 | 35.2 ±4.2 | 27.4 ±4.7 | 24.1 ±4.3 | 20.4 ±3.9 | 15.3 ±3.8 | 11 ±3.0 | 4.9 ±1.9 | 3.0 ±1.6 | | |

TABLE II

## EFFECT OF LUMBAR SYMPATHECTOMY ON BONE BLOOD FLOW IN SHOCK

| | | % Systemic Blood Pressure | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 35 | 30 |
| % Bone Blood Flow | Nerve Intact | 100 | 75 | 58 | 40 | 25 | 14.5 | 9.5 | 6.5 | 5 | 4 | - | - | - | - | - |
| | | 100 | 98 | 96 | 88 | 72.5 | 57 | 51 | 45 | 43 | 40.5 | 38 | 33 | 25.5 | - | - |
| | | 100 | 88 | 78 | 67 | 56 | 45 | 36 | 29 | 22.5 | 18 | 14 | 10.5 | 8 | 5.5 | - |
| | | 100 | 82 | 62 | 40 | 39.5 | 38.5 | 37.5 | 36.5 | 36.5 | 36.5 | 36.5 | 36.5 | 36.5 | 31.5 | 26.5 |
| | | 100 | 82 | 64 | 43.5 | 41.5 | 39 | 36 | 34 | 27 | 14 | 7 | 3 | 0 | - | - |
| | Mean ± S.E. | 100 | 85.0 ± 3.9 | 71.6 ± 7.3 | 55.5 ± 3.1 | 46.9 ± 8.0 | 38.8 ± 6.9 | 34 ± 6.7 | 30.2 ± 6.4 | 26.8 ± 4.5 | 22.6 ± 7.2 | 19.1 ± 7.7 | 16.6 ± 7.6 | 14.0 ± 7.3 | 7.4 ± 6.2 | 5.3 ± 5.3 |
| | Sympath-ectomised | 100 | 93 | 86 | 78 | 58 | 17.5 | 12 | 8.5 | 6.5 | 4.5 | - | - | - | - | - |
| | | 100 | 97 | 94.5 | 90 | 84 | 78 | 69 | 60 | 57 | 53 | 50 | 43 | 36.5 | - | - |
| | | 100 | 90 | 80 | 71 | 62 | 53 | 47 | 42 | 37 | 31 | 24 | 19.5 | 18.5 | 17.5 | 9.5 |
| | | 100 | 94 | 87 | 84.5 | 73 | 68 | 57 | 51 | 51 | 51 | 51 | 51 | 50 | 53 | 53 |
| | | 100 | 94 | 89 | 85 | 77 | 71 | 65 | 59 | 51 | 38.5 | 31 | 22 | 12 | 6.5 | - |
| | Mean ± S.E. | 100 | 93.6 ± 1.1 | 87.3 ± 2.3 | 81.7 ± 3.3 | 70.8 ± 4.8 | 57.5 ±10.8 | 50.0 ±10.2 | 44.1 ± 9.4 | 40.5 ± 9.1 | 35.6 ± 8.7 | 31.2 ± 9.4 | 27.1 ± 9.0 | 23.4 ± 9.1 | 15.4 ± 9.6 | 13.0 ±10.1 |

TABLE III

EFFECT OF DIBENZYLINE ON BONE CIRCULATION IN SHOCK

| | % Systemic Blood Pressure | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 100 | 95 | 90 | 85 | 80 | 75 | 70 | 65 | 60 | 55 | 50 | 45 | 40 | 35 | 30 |
| % Bone Blood Flow | 100 | 95 | 90.5 | 85.5 | 76.5 | 63 | 50 | 36.5 | 34 | 8 | 5 | 0 | 0 | 0 | |
| | 100 | 92 | 84 | 76 | 68 | 60 | 51 | 45 | 38 | 28 | 25 | 12 | 4 | 0 | |
| | 100 | 88 | 78 | 72 | 66 | 53 | 48 | 44 | 28 | 19 | 14 | 5 | 0 | 0 | |
| | 100 | 91 | 82 | 73 | 66 | 59.5 | 53.5 | 46.5 | 42 | 39 | 35.3 | 29 | 21 | 0 | |
| Mean | 100 | 91.5 | 83.6 | 76.1 | 69.1 | 57.4 | 49.6 | 44.0 | 35.5 | 23.5 | 19.8 | 11.5 | 6.2 | 0 | |
| S.E. | ± 0 | ± 1.4 | ± 2.9 | ± 2.8 | ± 2.7 | ± 2.9 | ± 1.5 | ± 2.6 | ± 2.9 | ± 6.6 | ± 6.5 | ± 6.3 | ± 5.0 | | |

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