

STUDIES ON THE ROLE OF ARTERIO-
VASCOCONSTRICTION IN THE DEVELOPMENT OF SHOCK

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by
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This thesis is affectionately dedicated to:

My fiancée, Constance English who in the past four years has manifested the major requisite for a scientist's wife -- patience.

My parents, Theophil and Christine, for their whole-hearted moral and material support throughout my academic career.

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Abstract

The major purpose of the present studies was to assess more completely the role of vasoconstriction and of reduced effective regional blood flow in the genesis of shock.

The protection provided by phenoxybenzamine against the development of shock is related to its prolonged and highly specific adrenergic blocking activity. In addition to reducing vasoconstriction due to sympathetic nervous system hyperactivity accompanying shock, phenoxybenzamine may also provide an increased plasma volume and a more favorable distribution of the available blood. Both the reactive β -halogen and an appropriate aromatic group (phenoxyisopropyl, benzyl, etc.) have been shown to be necessary structural components for this activity.

Investigation of the cardiovascular and lethal effects of Escherichia coli endotoxin indicates that peripheral adrenergic mechanisms are intimately involved in these effects. The results support the concept that deleterious effects of endotoxin in shock probably are due to exaggeration of existing vasoconstriction in an already compromised organism.

The rate of total-body uptake of radiocesium (Cs^{137}) is delayed considerably during shock. This is probably due to delay in equilibration between plasma and extracellular fluid. The relation of blood flow to uptake of locally-administered Cs^{137} was investigated in the vascular beds of

hind limbs and ileum in dogs. The flow-uptake relation for Ib^{86} in the vascular beds of the hind limb was affected very little by shock-inducing procedures. However, in the ileum Ib^{86} uptake was considerably depressed even at low flow rates throughout the period of noradrenaline infusion, even prior to the development of shock by these infusions. This decreased uptake also occurred during the normovolemic phase of hemorrhagic shock. These changes indicate that a decreased fraction of the total organ blood flow was traversing vessels from which exchange with tissues occurred readily. The normal flow-uptake relation was maintained throughout in animals pretreated with phenoxymethamine. Thus, a major component on the protection provided by phenoxymethamine may be prevention of the deleterious redistribution of intra-organ blood flow during shock, permitting more effective nutrition of compromised tissues.

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PART I

INTRODUCTION

The state which follows severe trauma undoubtedly has been observed for many thousands of years and probably was associated in the minds of observers with the process of dying. Ambroise Pare' in 1575 is credited with the first archival description of this morbid state as a syndrome consisting of undetectable pulse, feeble heart action, pallor, cold sweat, muscular collapse, and motor and sensory depression. The term "shock" was derived from English translations of the writings of the French surgeon Le Bran who in 1743 used choc to define the actual collision between the trauma-inducing agent and the living organism. Thus, "The Bullet..... thrown by the Gun powder acquires such rapid force that the whole Animal Machine participates in the Shock and Agitation". However, the original meaning was soon lost and the term became incorporated into medical parlance to stand not for the blow itself, but rather, for the effect.

Shock, in its current medical usage, describes a number of apparently similar pathophysiologic states which constitute a general response to injury induced by a wide variety of agents and conditions. In addition to the sequelae of trauma observed by Pare' and Le Bran, later workers were quick to note the appearance of shock or shock-like states in patients suffering from cholera (O'Shaughnessy, 1831), diabetic coma (Paggo, 1874), burns or strangulated hernias (Blum, 1876) and acute infections (Rosberg and Fessler, 1899).

Numerous limited definitions of shock have been proposed. Harkins (1941) lists 24 compiled during the period 1826 to 1940. All have been more or less abandoned because even the best attempts at definition were really only descriptions. Our present inability to arrive at any universally-accepted definition, in fact, reflects the inadequacy of our knowledge and understanding of the problem.

Classifications of shock have been unsatisfactory also because none will satisfy wholly the criteria established by clinicians, pathologists and physiologists. For example, Halsey (1934) proposed a classification based upon the underlying physiologic mechanism.

(a) Hematogenic (oligemic) shock. This includes traumatic and hemorrhagic shock, the characteristic feature being reduction of blood volume.

(b) Neurogenic shock. This includes the so-called "primary" shock associated with pain mentioned in the older literature and shock associated with spinal anesthesia. There is no reduction of blood volume but merely a reduction of blood pressure.

(c) Vasogenic shock. Histamine shock would be typical of this category.

(d) Cardiogenic shock. Here the reduction in blood pressure is due to cardiac inadequacy.

Halsey's scheme meets with three objections. First, it is frequently impossible to determine clinically which mechanism is involved in any given patient suffering from shock. Second, in some forms of shock, for example, those associated with infections, several mechanisms may be combined. Finally, evidence developed in this thesis excludes "neurogenic shock" from consideration as true shock.

Classification also may be based on etiology alone.

(a) Traumatic-hemorrhagic shock. This includes shock due to trauma, burns or hemorrhage.

(b) Toxic-infectious shock. This comprises shock associated with infections, intoxications, ingestion of drugs, for example, histamine shock, anesthetic shock, shock associated with diphtheria or intestinal ob-

struction.

(c) Metabolic shock. In this group would be placed shock due to metabolic disturbances, such as dehydration, diabetic acidosis or anaphylaxis.

This classification is superficial and fails to consider the underlying pathophysiology.

Higgers (1930) attempted a more involved classification based upon three "conditions precedent to shock". These conditions were 1) blood or plasma loss, 2) biological agents and 3) "questionable". However, Higgers' proposal has not been generally accepted. Common agreement among the medical disciplines exists today only at the primitive level of descriptive, etiological differentiation, i.e., traumatic shock, post-operative shock, obstetric shock, burn shock, hemorrhagic shock, etc.

Other factors have contributed to inter- and intradisciplinary confusion regarding shock. The term has been applied to such unrelated conditions such as electric shock, anaphylactic shock, shell shock, and even as synonymous with cerebrovascular accident. Failure to recognize shock as a syndrome by assigning undue diagnostic importance to any one specific clinical sign, e.g., arterial blood pressure, hematocrit, etc. has been another source of contradictions. Grant and Reeve (1931) concluded that the relative importance assigned various signs of shock appears to depend upon the personal views of the attending physician rather than on any generally accepted criteria. Accordingly, shock remains a general concept of a syndrome whose composite signs have been assigned variable and inconsistent positions of diagnostic and prognostic importance. The clinical signs characteristic of shock are presented in Table 1. The order does not imply relative impor-

Table 1

Clinical Signs of Shock

Cold skin
 Pallor
 Tachypnea
 Weak heart sounds
 Tach, rapid pulse
 Hypotension
 Increased time for capillary filling
 Decreased pulse pressure
 Apathy
 Clouded sensorium
 Muscular weakness
 Depressed reflexes
 Oliguria
 Hemocoagulation

tance.

Irrespective of problems of definition, classification and the relative importance of various signs of shock, there is uniform agreement that shock involves the organism as a whole and can be initiated by any form of trauma or stress which causes generalized cardiovascular impairment. The cardinal feature of the syndrome is a precarious state of the circulation resulting in an overall insufficiency of blood flow. When prolonged, this results in variable and inadequate perfusion and nourishment of certain organs and tissues, and ultimately, in their dysfunction. This impairment is characteristically correctable early, but later becomes "irreversible", that is, refractory to current therapeutic measures. The onset of irreversibility is associated with some, at present incompletely understood, event or events leading inexorably to death hours or even days after the traumatic episode. The progressive and generalized nature of the circulatory inadequacy which characterizes shock differentiates it from chronic conditions such as congestive heart failure and from acute and subacute impairment of blood supply to regional vascular beds due to embolism or thrombosis. Acute deaths due to massive hemorrhage or trauma likewise are excluded. It must be remembered, however, that shock may occur secondary to chronic myocardial insufficiency or to local interruption of the circulation and that hemorrhage and trauma may under appropriate circumstances induce the typical picture of shock.

In the past, one serious obstacle to the understanding of shock was the lack of standardized methods for producing it in experimental animals. Within the past 20 years many methods have been developed which induce shock in a reasonably reproducible manner. Efforts have been made to

reproduce every type of shock that is known to occur in man. Among the most widely used of these are withdrawal of blood, total body or local skeletal muscle trauma, prolonged regional ischemia by the application of tourniquets to the extremities, burns, and bacterial infection or injection of toxins. Numerous references also are available to shock produced by exposure and manipulation of the intestines, occlusion of the abdominal aorta or inferior vena cava, embolization of the coronary or cerebral circulation, or by prolonged intravenous infusion of various drugs, such as noradrenaline, adrenaline or histamine. Results from many separate research groups have substantiated the reproducibility of the various shock-inducing techniques. The reliability of shock studies has been correspondingly improved and current research on the shock problem is primarily concerned not with the initiation of the syndrome but with mechanisms responsible for the progressive and seemingly inevitable deterioration during profound and protracted shock. However, interpretations of such studies are necessarily limited to the conditions of the experiments. In the absence of information regarding the basic etiology of shock, these methods merely cause a reasonably reproducible syndrome and it cannot be assumed with any assurance that any one factor or set of factors is involved in the responses of different species to one type of shock-inducing procedure, or is responsible for the lethal tendency in all forms of shock.

Although other factors may operate, a decrease in effective circulating blood volume appears to be a major initiating factor in most forms of clinical and experimental shock. Homeostatic mechanisms activated by this circulatory inadequacy evoke compensatory reactions to maintain arterial blood pressure and cardiac output. These may be considered in two major

categories: 1) Those operating to increase the circulating blood volume, and 2) those concerned with maintaining the blood pressure and redistributing the blood flow.

Mechanisms prominent in the first category include: a) Mobilization of blood from reservoir areas such as the spleen and other visceral or vascular spaces, and b) expansion of plasma volume with fluid from extravascular spaces. Ideal distribution of the available blood volume would assure each organ enough blood for its "survival" and for the minimum level of function requisite for survival of the organism as a whole. Individual requirements of various tissues and organs obviously will vary. In shock, the extent of changes in blood flow is not uniform in different vascular beds, and in any particular area it depends on the relation between the regional arterial pressure or perfusing pressure and the resistance to flow offered by the vascular bed in question. Blood pressure in turn, depends on cardiac output and total peripheral resistance, the resultant of the resistances of all vascular beds. Assuming that the same or very nearly the same arterial blood pressure is available to all parts of the body, the relative participation of any organ or tissue in the generalized vasoconstriction which accompanies shock will be the major determinant of blood flow to that area.

The wide variations in the amount of vasoconstriction occurring in the blood vessels of various tissues in shock probably reflects both differences in the amount of vasomotor innervation and differences in the responsiveness of various vascular beds to neurohumoral vasoactive agents and tissue metabolites. For example, in the skin, where the nerve supply to blood vessels is rich and where wide fluctuations in the amount of vasocon-

striction occur under physiological conditions, vasoconstriction and reduction in blood flow during shock are marked. Severe vasoconstriction accompanied by a marked decrease in blood flow also occurs in certain abdominal viscera, e.g., kidney and intestine. In contrast, there is a relative paucity of sympathetic vasomotor nerves to blood vessels of the heart and brain, and blood flow in these organs is governed largely by metabolic demands rather than by nervous or neurohumoral influences. Thus, there is less vasoconstriction in cerebral and coronary vessels, and blood flow is correspondingly reduced to a lesser degree than in most other areas during shock. The resulting redistribution of the available cardiac output may be regarded teleologically as a response of the body to acute stress. However, selective maintenance of the circulatory integrity of the brain and heart during prolonged periods of reduced effective circulating blood volume may not be entirely advantageous for the preservation of the organism as a whole. In the contrary, failure to maintain a critical minimum oxygenation of certain tissues, particularly the intestines and, less certainly, the liver, appears to contribute to the development of irreversibility.

The concept that severe vasoconstriction which accompanies the development of experimental shock and which is responsible for many of the prominent signs of clinical shock may contribute per se to the causation of the syndrome is not new. Bainbridge and Treves (1917) and Erlanger and Gasser (1919) produced shock in dogs with large doses of adrenaline. Freeman and colleagues, (1933, 1941) reported that the sympathetic nervous system hyperactivity which accompanies decortication (psychic-affective state; "sham rage"), or prolonged infusions of adrenaline caused a marked decrease in circulating blood volume and the development of typical shock. Ergotox-

ine or sympathectomy effectively prevented both the reduction in blood volume and the development of shock. In addition, tolerance to prolonged periods of hemorrhagic hypotension was shown to be considerably greater in sympathectomized than in intact animals (Freeman et al., 1938). Freeman formally hypothesized that vasoconstriction accompanying shock was not merely a concomitant but also a causative factor. Experimental evidence since that time has provided overwhelming support for this concept, but the precise mechanism by which compensatory or otherwise induced vasoconstriction affects total body economy or contributes to the lethal outcome of shock of varying etiology is not known. Recent investigations strongly suggest that prolonged reduction of blood flow in certain viscera, notably the intestine and liver, may be critical to the development of irreversible shock (H.A. Frank et al., 1946; Seligman et al., 1947; Lillchai, 1957; Lillchai and Maclean, 1958; Lillchai et al., 1963). However, the mechanism(s) responsible for the particular vulnerability of the splanchnic vascular bed and the importance of regional vasoconstriction as a determinant of splanchnic blood flow have not been fully clarified.

The major purpose of the present studies was to assess more completely the role of vasoconstriction and of reduced effective regional blood flow in the genesis of shock. Related patterns of investigation were developed with the following specific objectives:

- 1) To evaluate the extent and mechanism of the protection against irreversible shock provided by the adrenergic blocking agent phenoxybenzamine (Dibenzylamine).
- 2) To relate antishock properties of phenoxybenzamine and of congeners of phenoxybenzamine to adrenergic blocking activity and to chemical

reactivity of the drug molecules.

3) To study the interaction of bacterial endotoxins and adrenergic mediators with the hope of incorporating these two shock-promoting factors into a common pattern relating to the development of irreversibility in shock.

4) To determine patterns of regional blood flow during the development of shock and to assess the effects of vasoconstriction and of vasodilation on the intraorgan distribution of blood flow.

PART II

THE CIRCULATION IN CHECK

A SELECTED REVIEW OF THE LITERATURE

Introduction. Shock, regardless of etiology, involves basic cardiovascular malfunction. Thus, consideration of this syndrome necessitates a knowledge and understanding of characteristic cardiovascular changes which occur during its development. The literature review which follows is limited purposely to information pertaining directly to the cardiovascular system in shock. Particular attention has been focused on investigations which have been concerned with the problems of reduced blood flow and vasoconstriction and on other fundamental contributions which have led to major advances in the field of shock. A more detailed discussion of literature which bears directly on the specific objectives of the experimental work presented in this thesis precedes the appropriate sections.

Concept of peripheral resistance. Hemodynamic parlance limits the definition of peripheral to those distal branches of the vascular tree, pulmonary vessels excepted, which are the major determinants of resistance to blood flow, irrespective of their anatomical location.

Resistance to flow includes all factors which impede the flow of blood from the arterial tree and is defined as the ratio between the pressure head ΔP , between any two points, and the resultant flow.

$$R = \frac{\Delta P}{F}$$

Regional, local or territorial resistance is the impedance offered to blood flow through an organ, such as the kidney or intestine, or through any vascular bed supplied by a common artery. Total peripheral resistance (TPR) designates the resistance to flow resulting from all parallel regional resistances derived from the aorta. This term may be a source of confusion

since it implies addition of local resistances. On the contrary, calculation of TPR follows the rule of parallel electrical circuits, namely, the reciprocal of TPR equals the sum of the reciprocals of the parallel regional resistances. Thus, TPR is always less than the resistance through any single local circuit. A large increase in one resistance (kidney, skin) actually may be associated with a decrease in TPR provided that resistances of other areas are reduced simultaneously. Regional or total peripheral resistance may be calculated as peripheral resistance units (PRU) by modifying the basic formula (vide supra):

$$PRU = \frac{\text{mean arterial pressure (mm Hg)}}{\text{flow rate (ml/min)}}$$

where flow rate is either regional blood flow or cardiac output, respectively. Expression of TPR in c.g.s. units is also widely used:

$$TPR \text{ (dynes}\cdot\text{sec/cm}^5\text{)} = \frac{\text{mean arterial pressure (mm Hg)} \times 1332}{\text{cardiac output (ml/sec)}}$$

These expressions for resistance to flow are useful in that they are not dependent on interpretative limitations or assumptions with respect to the flow of viscous liquids through rigid tubes. Such systems adhere to Poiseuille's Law which states that the flow (F) varies inversely as the length of the tube (L) and the viscosity of the fluid (η) and directly as the fourth power of the tube radius (r).

$$F = \frac{\pi r^4}{8 L \eta}$$

The law applies only to Newtonian (viscosity = 1) incompressible liquids during streamlined (not turbulent) flow. Blood always satisfies the second condition and usually the third. However, velocity of blood flow affects viscosity. Therefore, pressure/flow relations are never linear. The dis-

distensibility of blood vessels, which changes $\frac{r^4}{L}$, also makes a major contribution to nonlinearity. Thus, quantitative use of this formula requires knowledge of viscosity and the ratio $\frac{r^4}{L}$ at a particular head of pressure and rate of flow. The importance of Poiseuille's law lies in the feature of flow varying as r^4 . Accordingly, assuming (L) remains constant, a decrease in the $\frac{r^4}{L}$ ratio suggests vasoconstriction. If arterial blood pressure increases concomitantly, or is unchanged, the vasoconstriction is considered to be "active", i.e., not due to passive vessel distensibility. However, if the blood pressure falls, definitive conclusions as to the nature of the vasoconstriction cannot be drawn.

The older concept of the anatomy of the microcirculation depicts capillary beds arranged as a continuation of the main supplying arterioles. The extensive smooth muscle in the walls of arterioles allows for active changes in caliber over a considerable range. Since resistance varies with r^4 , small changes in arteriolar diameter in response to local or nervous influences can induce large changes in regional resistance to flow. However, this anatomical pattern fails to account for the existence of thoroughfare channels or shunts across local vascular beds. Chambers and Zweifach (1944) described in detail an arrangement which is now accepted for many vascular beds. Small arterioles divide into metarterioles which communicate directly with venules (thoroughfare channels). True capillaries arise from the metarterioles. Smooth muscle sphincters guard the entry of blood into the noncontractile capillaries and determine capillary blood flow. Territorial resistance thus may be modified by blood flow through accessory channels as well as by changes in arteriolar diameter.

Blood flow and peripheral resistance in shock. Since a decrease in the

effective circulating blood volume is generally considered to be the major factor initiating the development of shock, it is not surprising that blood flow to most body tissues is diminished. However, the fractional distribution of the cardiac output among the various organs in shock differs markedly from that observed in the normal state. This altered partition of blood flow is due primarily to the existence of relatively uneven patterns of regional resistance, and is responsible for many of the prominent clinical signs of shock (Table I).

A relation between vasoconstriction and the signs of shock was first suggested by Le Bran (1743). Direct experimental evidence originated with the experiments of Halles (1733). He observed that venous pressure rose in animals during the process of arterial hemorrhage and that the decline in arterial blood pressure was not directly related to the amount of blood lost. The general nature of the vasoconstriction observed in shock by Halles and Le Bran and the participation of both arteries and veins in the process has been amply documented in studies too numerous to cite individually. (See Scudder, 1940 for comprehensive bibliography).

Gezell's (1919) measurements of blood flow through a salivary gland under various conditions were a fundamental contribution to the understanding of shock. He noted that hemorrhage or tissue trauma caused a decrease in the basal flow rate through the gland before any fall in blood pressure could be detected. A reduction in blood volume of less than 10 per cent resulted in a 60 per cent fall in blood flow. These results provide conclusive proof of active vasoconstriction in shock. This contribution was instrumental in stimulating subsequent studies on changes in regional blood flow which occur during the development of shock.

1) Total peripheral resistance (TPR). A considerable reduction in cardiac output often precedes any obvious fall in blood pressure during the development of shock. Originally, the compensatory vasoconstriction involved was considered a valuable mechanism for ensuring comparatively better blood flow in vital organs such as the heart and brain. However, the classical work of Osell (1919) suggested that the disproportionate reduction of blood flow in other tissues might, in fact, be a shock-provoking mechanism.

Despite many hypotheses concerning its possible role in shock, the first critical study of generalized vasoconstriction during shock was made by C.J. Wiggers and Verle in 1942. TPR increased somewhat during both the hypovolemic and normovolemic phases of hemorrhagic shock in most of their animals. The increase was never maximal and additional substantial increases in TPR could be obtained by stimulation of sympathetic nerves. H.C. Wiggers and Middleton (1944) found variable trends in the TPR of dogs during the development of hemorrhagic shock. The two groups agreed that TPR changes were not of prognostic value.

The variable response of TPR to hemorrhage is illustrated also by more recent experiments. Gaynell et al. (1955) reported that TPR increased significantly, to 190 per cent of control, following acute hemorrhage in dogs, whereas Fowler and French (1957) observed an average decrease of 16 per cent in TPR, not statistically significant, in dogs following hemorrhage which lowered arterial pressure to 35 mm Hg.

The inconsistent changes in TPR observed during hemorrhagic shock may be related to the cardiovascular changes at the time TPR was measured. In the early stages of experimental hemorrhage compensatory

tendencies, indicated by a rising blood pressure or by an increasing bleeding volume at a constant level of hypotension likely would be manifest as an increase in TFR. Conversely, after prolonged hemorrhage, circulatory decompensation in the form of a decreasing blood pressure or a progressive return of withdrawn blood to maintain a given level of hypotension may be reflected by lower TFR values. Beck and Sontos (1933) noted increased electrical activity in preganglionic sympathetic fibers during the early stages of hemorrhagic shock. Furthermore, the increased TFR reported by Reynell *et al.* (1933), occurred early in hemorrhage (compensatory phase), whereas no consistent changes were evident during protracted hemorrhagic hypotension (decompensation).

However, measured changes in TFR must be evaluated with regard for the parallel arrangement of regional vascular beds. Marked regional changes in resistance can occur and can induce major changes in the distribution of the available blood flow and volume without alteration of TFR.

2) Skin and skeletal muscle. Pallor and cold skin are important and easily recognized signs of clinical shock and reflect extensive vasoconstriction and decreased blood flow in this tissue. Gosell and Moyle (1922) used a simple drop counter to measure the venous drainage from the hind limbs of dogs subjected to graded hemorrhage. The decrease in blood flow was greater than could be accounted for by the fall in blood pressure. Freeman *et al.* (1938) and Schstein *et al.* (1946), using a plethymographic method, also observed a decreased blood flow and vasoconstriction in hind limbs of dogs in hemorrhagic shock. Hind blood flow measured by this same technique was found by Freeman *et al.* (1936) to be markedly reduced in only a small percentage of patients suffering from "clinical shock". However, the presence of an apparently normal blood pressure in

some of these patients may invalidate the clinical diagnosis. Changes in skin blood flow can be inferred indirectly from changes in skin temperature (Green et al., 1949). Paw and ear temperatures of dogs fall progressively during the oligemic phase of hemorrhagic shock, but return to normal after reinfusion of blood. A secondary progressive fall in skin temperature then precedes the decline in arterial pressure which heralds the decompensatory phase of neurotoxic shock (Antes, 1944). A progressive fall in paw and ear temperature was noted also in dogs during shock induced by ischemic compression of limbs (Green et al., 1944a).

Green et al. (1944b) reported that a sigmoidal curve convex to the pressure axis accurately related pressure to flow in the hind limbs of dogs, which suggested the presence of an autoregulatory mechanism in the skeletal muscle vasculature. Accordingly, the status of peripheral resistance in hind limbs of dogs in shock can be evaluated accurately only by comparing pressure/flow relations before and after the induction of shock. Comparisons made in this manner have provided firm evidence that hemorrhage causes vasoconstriction which persists throughout the development of shock (Green et al., 1944b). The magnitude and the characteristics of vasoconstriction in muscle during hemorrhagic shock are demonstrated clearly by the experiments of Seikurt and Sothe (1961). An isolated gracilis muscle, connected to the test dog only by its innervation, was perfused at constant pressure from a donor dog during the induction of hemorrhagic shock in the test animal. Vascular resistance in the muscle increased markedly during the early stages of hemorrhagic hypotension and was decreased only slightly by reinfusion of the withdrawn blood. Moreover, resistance remained considerably above the prehemorrhage value throughout the decompensatory phase of neurotoxic shock.

3) Cerebral blood flow. Reduced cerebral blood flow and increased cerebral arterio-venous oxygen difference have been observed generally in both clinical and experimental shock. Stone et al. (1975), utilizing the nitrous oxide technique, observed a 23 per cent reduction in cerebral blood flow and increased cerebral arterio-venous oxygen difference in four normal volunteers following removal of 20 to 30 per cent of their blood volume. Although mental changes, nausea, pallor, cold perspiration, tachypnea and hypotension occurred in these subjects, these signs and symptoms were reversed rapidly by returning the withdrawn blood. Such prompt recovery indicates that the duration of hypovolemia was inadequate to produce true shock and that the experimental condition was probably acute hemorrhage. Similar changes have been observed, however, in patients considered to be in shock (Fazekas et al., 1955).

Experimental investigation of cerebral blood flow in shock has not been extensive. This probably has been due in great part to technical problems in both the direct and indirect methods for measuring flow and in the subsequent interpretation of data obtained by these techniques. E.G. Frank et al. (1956), on the basis of A-V oxygen differences, reported a 64 per cent reduction of blood flow in the superior sagittal sinus of dogs when arterial blood pressure was decreased to 30 mm Hg. However, this flow represents venous drainage from only limited areas of the brain and may not accurately reflect changes in the entire cerebral vascular bed.

More elegant experiments have been devised by Kovač and his colleagues (1958a; 1959) to measure blood flow in dogs' heads during the development of traumatic or hemorrhagic shock. Total carotid arterial blood flow was monitored directly. Prior ligation of the vertebral arter-

ies and veins and the internal jugular veins prevented collateral circulation. However, blood flow to bone, skin, muscle and meninges was not excluded. Shock was produced either by bilateral tourniquet compression of the hind limbs or by hemorrhage. In ischemic shock (Kovach et al., 1959), cardiac output fell but blood flow to the head did not change during the tourniquet period. For a few minutes after release of the tourniquets, cardiac output remained depressed while blood flow rose to 126 per cent of the initial level. Blood flow then fell to 73 to 85 per cent of control and remained at this level until a terminal drop to 35 per cent of control occurred. The fraction of cardiac output perfusing the head doubled during the period of ischemic compression, and until the terminal stages, remained considerably higher than prior to shock. Thus, the fall in cardiac output was not accompanied by a corresponding fall in blood flow to the head, indicating decreased resistance in that area, or at least a smaller increase in resistance relative to other vascular beds. Qualitatively similar changes were observed during hemorrhagic shock (Kovach et al., 1958a). Flow decreased to 73 per cent of control when arterial blood pressure was lowered to 50 to 60 mm Hg and to 27 per cent when blood pressure was reduced to 30 mm Hg. Blood flow increased to 126 per cent of control during the 20 to 30 minute period immediately following reinfusion of blood. Thereafter, blood flow fell progressively, parallel to the decline in blood pressure. Cranial vascular resistance decreased early in the hypovolemic period and gradually returned towards the control level during the latter stages of hypovolemia. Resistance fell from 0.97 to 0.72 immediately after reinfusion but returned to 0.91 shortly thereafter and remained unchanged throughout the period of normovolemic shock. These results in-

dicates that blood flow to the head is maintained fairly well in shock and reductions which occur usually are less than would be expected from the fall in blood pressure.

The relation of diminished cerebral blood flow to the development of irreversibility has not been fully settled. A certain minimum blood flow is required to prevent failure of the cardiovascular and respiratory centers. This requirement is probably satisfied, at least during the early stages of shock, by redistribution of blood from severely vasoconstricted beds. There is good evidence to indicate that normal cerebral perfusion pressure per se cannot prevent shock (Nickerson, 1933; Smith et al., 1937). However, cross-perfusion of the isolated head of dogs subjected to tourniquet or hemorrhagic shock has been reported to increase survival time considerably (Kovach et al., 1938a) and even to improve renal function (Kovach et al., 1938b). The beneficial effects of cross-perfusion could be due to prevention of cerebral hypoxia per se or of generalized vasoconstriction secondary to cerebral ischemia, or to elimination of noxious humoral factors. A compromised cerebral circulation may, therefore, hasten the development of irreversible shock.

4) Coronary blood flow. Extensive studies, particularly in hemorrhagic shock, have demonstrated that coronary blood flow decreases during hypovolemia, returns to normal or even above normal after reinfusion and falls once more shortly before death (Opdyke and Foreman, 1947; Schenck et al., 1948; Edwards et al., 1953, 1954; Samoff et al., 1954; Bachel and Goodale, 1955; Catchpole et al., 1955; E.B. Frank et al., 1956; Sincens et al., 1955). In general, coronary vascular resistance is decreased during all phases of experimental hemorrhagic shock. Therefore,

coronary blood flow in shock represents a larger than normal fraction of the cardiac minute volume.

Deficient myocardial function secondary to diminished coronary blood flow has been implicated as a major factor in the development of irreversibility. Metabolic derangements such as decreased extraction of glucose, of lactate, and of oxygen from coronary blood and a negative pyruvate balance have been reported (Edwards et al., 1934; Michel et al., 1934; Michel and Ceciale, 1935). Ling (1961) concluded that these changes in myocardial metabolism during hemorrhagic shock were not isolated occurrences but followed closely the general pattern of myocardial anoxia resulting from diminished coronary flow. Additional observations suggesting myocardial failure include elevated effective atrial pressure (Varle et al., 1942; Guyton and Crowell, 1961), cardiac dilatation (Kobletsch and Page, 1944), electrocardiographic abnormalities (Isquith and Posternack, 1946) and reduced cardiac output after reinfusion in the presence of normal central venous pressure (C.J. Wiggers, 1945). In addition, Carnoff et al. (1934) reported that the increase in left atrial pressure which occurred in dogs during hemorrhagic shock could be prevented by maintaining normal perfusion of the coronary circulation. However, this salutary effect was seen only in open-chest, artificially-respired preparations and not in intact, spontaneously-breathing dogs, a fact which questions the functional significance of the observation. Lansing et al. (1937; 1938) obtained significant improvement in the survival rates of rats and dogs subjected to hemorrhagic shock when norepinephrine was administered after blood replacement late in shock in rather low dosage over a prolonged period of time. Norepinephrine prevented the fall in cardiac output usually seen in

the postinfusion period. Their conclusion seems justifiable that nor-adrenaline is beneficial when administered after return of the shed blood, and when used in a low dosage sufficient to improve cardiac function without a drastic increase in peripheral vasoconstriction. Gayton and Crowell (1961) demonstrated a relation between survival and oxygen debt accumulated by dogs during the period of hypotension. No deaths occurred in animals whose oxygen debt was less than 100 cc/kg. However, oxygen debt in excess of 100 cc/kg was associated with a sharp increase in mortality and animals whose debt exceeded 130 cc/kg invariably died. Although these authors concluded that the accumulated oxygen debt was secondary to deterioration of myocardial function, they presented no evidence to indicate that the heart was primarily involved.

These metabolic and dynamic signs of myocardial insufficiency have been observed chiefly in the late stages of hemorrhagic shock. Therefore, although impairment of myocardial function, perhaps due to inadequate coronary perfusion, may contribute to the shock process, it probably is not a critical factor in determining the development of irreversibility.

There also is more direct evidence that the heart is not the critical area or limiting factor determining irreversibility. Several investigators have observed, over a wide range of hemorrhagic hypotension, that left ventricular work is reduced considerably more than is coronary flow (Opdyke and Foreman, 1947; Edwards et al., 1954; Michel and Goudale, 1955). These results suggest that blood flow during hypovolemia is adequate to meet the requirements of the reduced work load. Similarly, the prompt response of arterial blood pressure to reinfusion and the relatively high coronary perfusion rates throughout most of the stages of normovolemic shock are appropriate to adequate myocardial function. Finally, as

in the case of the brain, shock can develop even in the presence of normal coronary perfusion pressure (Richerson, 1953a; Smith et al., 1957).

5) Renal blood flow. Renal blood flow is drastically reduced during hemorrhagic hypotension or shock or traumatic shock (Blalock and Levy, 1937; Corcoran and Page, 1943; Corcoran et al., 1943; Leason et al., 1944; Phillips et al., 1946; Selkurt, 1946; Brandfenbrener and Geller, 1952; Heinmann et al., 1953; Foster et al., 1954; Moyer et al., 1954; S.B. Frank et al., 1956; Marson, 1956; Salant et al., 1959).

Indirect measurements during the oligemic phase of experimental hemorrhagic shock by means of inulin and diodrast clearances have shown glomerular filtration rate and renal plasma flow to be disproportionately reduced in relation to blood pressure (Corcoran and Page, 1943). Subsequent reinfusion of blood restored renal blood flow to or even above normal. However, prolonged exposure to profound hypotension compromised the ability of both normal and denervated kidneys to respond to transfusion. Corcoran and Page concluded that renal vasoconstriction during hypovolemia probably was due to sympathetic vasoconstriction of afferent arterioles whereas vasoconstriction sustained despite reinfusion depended on some humoral substance, a possibility which will be discussed further (vide infra). Similar results were reported in cases of human shock by Leason et al. (1944). In addition, these investigators noted that the renal fraction of the cardiac output was materially reduced during shock. They suggested that available blood volume was redistributed to maintain more vital organs, such as the heart and brain.

The limitations of indirect techniques for assessing the renal circulation during shock are obvious and, indeed, were recognized by both groups mentioned above. Direct measurements of renal blood flow have

offered further insight into vascular adjustments which occur in this organ during shock. Selkurt (1943; 1946) found that renal blood flow decreased an average of 42 per cent in dogs immediately after hemorrhage without appreciable change in local resistance. Thereafter, blood flow fell progressively to about 11 per cent of control despite maintenance of the original hypotensive level, indicating a considerable increase in vascular resistance. Simultaneous measurement of paraaminohippurate (PAH) clearance revealed it to be inhibited completely during hypovolemia even when renal arterial flow was as high as 67 ml/minute. The disparity between renal blood flow estimations based on PAH clearance and direct determinations persisted even after reinfusion, averaging 49 and 30 per cent of control, respectively. Selkurt attributed the failure of PAH clearances to correlate well with direct measurements of blood flow during shock to impairment of tubular function by prolonged hypotension. This was evidenced by a reduction in the extraction ratio of PAH and by the presence of mild, diffuse tubular pathology in necropsy specimens.

The increase in renal vascular resistance during the development of hemorrhagic shock is largely due to active renal vasoconstriction. This was demonstrated conclusively by Selkurt (1946). He compared pressure/flow relations obtained by mechanical interference with renal arterial blood flow with those obtained during pressure alterations by hemorrhage. Renal blood flow at comparable renal arterial pressures was much less after hemorrhage than after mechanical interference.

Release of vasoactive substances from ischemic kidney cells during shock has been described. In addition to the classical renin-angiotensin system (Page and Helmer, 1940; Braun-Menendez et al., 1940), certain

other humoral substances have been reported during shock (pressor principle of Corcoran et al., 1943; vaso-excitatory material (VEM) of Chambers and Seelbach, 1947, and sustained pressor principle of Shipley and Heimer, 1948). These vasotropic substances may be involved in the sustained renal vasoconstriction during normovolemic shock (vide supra).

Although the kidneys participate vigorously in the compensatory vasoconstriction which accompanies hemorrhagic or traumatic shock, depressed renal function does not appear to be of primary importance in the development of irreversibility. This should not be interpreted as minimizing the importance of the kidneys in the overall body economy during shock. There is abundant clinical evidence that prolonged ischemia can cause irreparable renal damage leading to the death of patients who survive the initial period of shock. However, under controlled experimental conditions, kidneys removed from animals in shock show only minimal pathological changes (Selkurt, 1945) and do not show significant depression of oxygen consumption or of ATP stores (Russell et al., 1944). In contrast to those of many other organs, kidney cells appear to have the ability to reduce oxygen consumption in proportion to that supplied (Dole et al., 1946). Finally, removal of the kidneys did not alter either the onset of shock or survival time in dogs following hind limb compression (Lobb and Green, 1947) or hemorrhage (Reinhard et al., 1948).

6) Hepatic-splanchnic blood flow. Virtually all investigators agree that the liver and/or intestine are more likely than other organs to be critical in the development of irreversible shock. The liver is partially dependent on the portal venous system for its blood supply. In addition, vascular changes in the liver affect the hemodynamic status of the intestine, and vice versa. Accordingly, these organs will be consid-

ered together in the following discussion.

Hypotension and shock markedly reduce blood flow through the hepato-splanchnic area. Using the method of periodically diverting portal venous flow into a measuring cylinder, S.S. Frank et al. (1936) and Selkurt et al. (1947) noted a drastic reduction to 15 per cent of the control flow during severe hemorrhagic hypotension. Even after moderate hemorrhage, portal flow was reduced by 55 per cent (Blalock and Levy, 1937). Although flows obtained by this technique probably were artificially high due to exclusion of hepatic vascular resistance, these results have been confirmed with other techniques. Weisman et al. (1933), Harrick and Myers (1933) and Heynell et al. (1935) using the bromsulphalein (BSF) extraction technique found that estimated hepatic blood flow of the dog fell 40 to 75 per cent depending upon the severity of the hemorrhage. Harrick and Myers concluded further that blood loss rather than the absolute level of arterial pressure was the major determinant of blood flow reduction. They considered the decline in arterial pressure following a single hemorrhage as a poor index of the degree of circulatory embarrassment of the liver, especially when pronounced peripheral vasoconstriction maintains the arterial blood pressure at a normal or only slightly reduced level. Fine et al. (1947), however, question the validity of the BSF method for estimating hepatic blood flow during shock, since the capacity of the liver to clear this dye may be impaired, resulting in a spuriously low calculated flow. Regardless of this objection, the pronounced reduction in liver blood flow has been confirmed consistently by a variety of methods. These methods include roentgenologic visualization of the portal vein with inferences regarding flow drawn from the vessel caliber (Friedman et al., 1931), microscopic observation of flow in the hepatic

sinusoids (Friedman et al., 1953), thermocouples (Johnson, 1954), and flowmeters placed in appropriate vessels (Pickering, 1950; Hay and Haggins, 1956; Cull et al., 1956).

Mesenteric vascular resistance in dogs was found to increase markedly soon after the beginning of hemorrhage (Selkurt et al., 1957). It declined later in the oligemic period but remained above control levels. In the late stages of hypovolemia, it again rose sharply. Reinfusion of shed blood resulted in only a temporary decline in resistance to control levels or below. These authors also noted a rise in the portal/aortic pressure ratio during hypovolemia and after reinfusion. Similar findings by C.J. Sliggers et al. (1946) suggest hemodynamic relations consistent with pooling of blood in the splanchnic area. Such relations appear to be due to elevation of hepatic and mesenteric resistance and were found to occur during late oligemia, and to an even greater extent, after reinfusion. In contrast, no change or only a slight increase in splanchnic vascular resistance occurred in other experiments during hemorrhagic hypotension induced by somewhat different methods (Pickering, 1950; Reynell et al., 1955; Selkurt and Brocher, 1956; W.M. Levy, 1958). Irrespective of fluctuations in resistance, reduced blood flow caused marked reductions in the oxygen saturation of portal and hepatic venous blood (McMichael, 1957; Smith et al., 1955) and also reduced splanchnic oxygen consumption despite increased extraction (Barwick and Myers, 1955).

The relation of increased hepatic vascular resistance, splanchnic pooling and irreversibility deserves comment. Friedman et al. (1951) using radiographic techniques, showed that there was constriction of the portal and hepatic veins in dogs during hemorrhagic shock and that recovery of normal caliber did not occur within a 6-hour period following

restoration of blood volume. Thus, he concluded that a constriction of the intrahepatic veins accounted for a large part of the increased hepatic vascular resistance reported by Selkurt *et al.* (1947). Reynolds *et al.* (1953), using an I¹³¹ dilution technique, confirmed this observation. Although it is a consistent finding in dogs, splanchnic pooling does not appear to be a critical factor in the development of irreversible shock. H. A. Frank *et al.* (1951b) subjected ACh-fistula dogs to hemorrhagic shock. Survival rates were not raised and bleeding volumes were not altered as compared to unoperated controls, although there was no splanchnic congestion. They concluded that prevention of the sequestration of blood in the splanchnic bed did not prevent the development of irreversibility. It appears probable that irreversibility to transfusion takes place before reinfusion and that congestion of the splanchnic viscera is a sequel rather than a cause.

Early efforts to assess the role of deficient splanchnic blood flow in the genesis of shock were directed mainly towards hepatic blood flow. H. A. Frank *et al.* (1946) maintained dogs at a drastic level of hypotension (30 mm Hg). Animals maintained at this level and receiving arterial blood into the splenic vein by cross-circulation with healthy donor dogs had a 92 per cent survival rate, whereas only 5 per cent of control animals similarly cross-circulated via the jugular vein survived. The results of Seligman and his colleagues (1947) were even more striking. They reported that hemorrhagic shock which had not responded to transfusion and was therefore by definition irreversible, could be treated successfully by cross-circulation with donor blood via the splenic vein (8 of 9 dogs survived), whereas cross-circulation via a systemic vein was not successful (7 of 9 dogs died). Irreversible shock has been prevented

in dogs also by increasing the oxygen content of the portal blood during the shock period by autoperfusion through aortic-portal fistulae (Cohn and Parsons, 1950), femoral artery-portal shunts (Belorse, 1951) and splenic artery-splenic vein anastomoses (Hay and Webb, 1951). When hypotension is limited to the subdiaphragmatic vasculature in dogs the onset and course of shock appears to follow the same pattern as when the entire vascular tree is rendered hypotensive (Richerson, 1955a; Smith *et al.*, 1957). Although these results strongly suggest the importance of the splanchnic circulation in shock, no real distinction can be made between the contribution of the liver and the intestines.

The clearest demonstration of a relation between the intestinal circulation and the development of shock has come from the experiments of Lillehei and his colleagues (1957; 1958; 1962). Dogs were bled to 35 mm Hg and were maintained at this level until maximal bleeding volume was reached (60 to 90 minutes). At this time, a controlled cross-perfusion of the superior mesenteric artery was begun (9 ml/kg/min at a pressure of 140 mm Hg). The animals were maintained in this manner for an additional 3 to 3 1/2 hours at which time the superior mesenteric artery was repaired and the shed blood reinfused. Ninety per cent of dogs so treated survived, compared to 7 per cent of sham-operated dogs receiving no perfusion and 20 per cent when either the aorta distal to the superior mesenteric artery or the inferior vena cava was perfused in like manner. Nine of 10 Sch-fistula dogs that received perfusion via the superior mesenteric artery also survived, thus making it unlikely that increased blood flow to the liver was responsible.

PART III

VASOCONSTRICTION IN THE DEVELOPMENT OF SHOCK: THE PHYSIOLOGICAL
BASIS FOR VASODILATOR THERAPY OF SHOCK

Evidence that vasoconstriction, which occurs in response to various shock-inducing procedures, can make a major contribution to the development of irreversibility by severely restricting blood flow in certain vascular beds, has been discussed in some detail in the immediately preceding sections. The therapeutic use of vasodilating agents is predicated on the concept that reduction in vasoconstriction may improve critical regional blood flows and prevent tissue dysfunction.

Observations which contribute to a theoretical basis for vasodilator therapy of shock have accumulated from many different sources. Several groups of workers have demonstrated decisively the deleterious nature of prolonged vasoconstriction. Bainbridge and Trevan (1917) observed hypotension and shock in dogs following a 20-minute infusion of adrenaline. Associated with the rise in arterial pressure during the infusion was an elevation in portal pressure which persisted after the infusion was discontinued. The latter was thought to be related to the splanchnic congestion accompanying shock. Similar results were obtained by Erlanger and Gasser (1919) who found also that the pathological changes in animals dying following adrenaline infusions were indistinguishable from those of animals dying as a result of traumatic shock.

More recently Freeman (1933) showed in cats that prolonged (2 hour) infusions of much smaller doses of adrenaline or sympathetic nervous system hyperactivity following decortication caused a loss of more than 25 per cent of the circulating blood volume within a short period of time and was associated with a progressive decline in arterial blood pressure followed by death within a few hours. The production of shock in dogs by prolonged infusions of adrenaline (Freeman et al., 1941) or of nor-adrenaline (Yard and Nickerson, 1936) has been reported to be associated

consistently with the same high mortality, hemoconcentration, decreased plasma volume, low peripheral blood flow and pathological findings characteristic of shock produced by other procedures.

Even milder adrenergic vasoconstriction superimposed on hemorrhage or trauma can contribute to the development of shock. Bosington et al. (1933a) established that relatively small amounts of adrenaline or of noradrenaline or increased sympathetic vasoconstriction following section of the splanchnic nerves hastens circulatory failure due to hemorrhage. In groups of animals subjected to a standard hemorrhagic shock procedure, those with evidence of greater initial intrinsic sympathetic nervous system activity, i.e., higher blood pressures and heart rates, have a higher mortality (Cleghorn et al., 1943; Hickerson, 1962). Administration of a small fraction of the usually fatal dose of Escherichia coli endotoxin to rabbits markedly increases mortality from hemorrhage (Schweinburg and Fine, 1939; Salady et al., 1958), an effect presumably due to augmented vasoconstriction (Boquet et al., 1947; Delaunay et al., 1947; Delaunay and Lebrun, 1947; Boquet and Izard, 1950; Zweifach et al., 1956; Thomas, 1956; Ball and Kelly, 1957; Armin and Grant, 1957; Gourzis et al., 1961). Similarly, intravenous infusion of small amounts of metaraminol (Aramine) superimposed on sublethal doses of E. coli endotoxin is highly lethal to dogs (Lillehei and MacLean, 1959).

Vasoconstriction is more pronounced in traumatic than in hemorrhagic shock presumably due to the reflex response to nociceptive afferent nervous impulses from the area of trauma. Yang and his colleagues (1947) observed that animals subjected to trauma died with a residual blood volume considerably above that adequate to maintain life in animals

subjected to hemorrhage. However, after dorsal root section to eliminate vasoconstriction reflexly induced by afferent impulses from the traumatized areas, animals subjected to trauma survived until their circulating blood volume had fallen to levels comparable to those lethal to animals subjected to hemorrhage (Yang, 1947). Conversely, although reflex vasoconstriction produced by sciatic nerve stimulation in dogs subjected to hemorrhage caused a significant increase in mean arterial blood pressure, death ensued at circulating blood volumes comparable to those encountered at the time of death in traumatized animals (Overman and Yang, 1947).

Eversole et al. (1944) likewise observed a more favorable response to trauma, longer survival and decreased mortality, following spinal anesthesia or local anesthetic blockade of peripheral nerves to the traumatized extremities. Interruption of afferent fibers and decreased reflex sympathetic stimulation probably was the main factor responsible, but interruption of efferent sympathetic vasoconstrictor pathways probably was also of importance in some of these experiments. Adrenergic vasoconstriction can thus produce irreversible shock per se and also can significantly potentiate the development of shock which is due primarily to other causes (Yang and Overman, 1949).

The deleterious role of vasoconstriction is implicit in the tolerance to hypotension associated with vasodilatation rather than vasoconstriction. Vasodilator hypotension (arterial pressures 50-80 mm Hg) following complete transection of the eighth cervical segment of the spinal cord in dogs (H.C. Figgers et al., 1943; Phemister et al., 1943), or produced by continuous stimulation of the carotid or aortic depressor nerves in rabbits (Phemister, 1943; Phemister et al., 1944), is quite

compatible with prolonged survival if fluid balance is maintained and the subject is positioned to facilitate venous return and cerebral circulation. It was thought by these investigators that survival was most likely correlated with the excellent blood flow through tissues which resulted from the vasodilatation.

A significant increase in the survival rate or survival time of animals subjected to hemorrhagic, traumatic or endotoxin shock has been demonstrated by many workers using many different procedures and drugs which induce vasodilatation or limit vasoconstriction. These include sympatholytic (Freeman, 1938), adrenergic blocking drugs (Schlossberg and Sawyer, 1933; Penner and Bernheim, 1943; H.C. Siggers et al., 1943, 1950; Hemington et al., 1950a and b; Baez et al., 1952; Beck and Lots, 1953; Hershey et al., 1954, 1955; H.E. Levy et al., 1954, Lots et al., 1955; Beck and Redick, 1956; Jacob et al., 1956; Carruthers and Cowley, 1956; Spurr et al., 1956; Overton and DeShay, 1956; Kovach et al., 1957; Baez et al., 1958; Roth et al., 1958; Inglis et al., 1959; Lillchai and MacLean, 1959; Nickerson and Carter, 1959), ganglionic blocking agents, (Glasser and Page, 1948; North and Wells, 1951; Hershey et al., 1954; Spocrel, 1955; Ross and Herszeg, 1956; Beck and Alexander, 1959; Salts, 1960) and direct acting vasodilators (Zingg et al., 1959; Makstian et al., 1961).

These blocking drugs are effective in prolonging survival time or reducing mortality in shock when administered prior to the shock-inducing procedure or shortly thereafter, but are ineffective when used in the later stages of shock. Lots et al. (1955) obtained no protection when phenoxybenzamine was given after 35 minutes of a 90-minute period

of hemorrhagic hypotension, and telazolone and chlorpromazine were also ineffective when used late in the hypotensive period (Gowley et al., 1936; Carruthers and Gowley, 1936). Similarly, R.E. Levy et al. (1934) found no protection from phenylephrine or dihydroergotamine given intravenously or intraperitoneally after the completion of arm trauma, and ganglionic blocking agents have been reported not to protect if administered much after release of tourniquets (Spoerel, 1933; Saltz, 1960).

Although it is generally accepted that agents which inhibit sympathetic vasoconstriction can protect against a fatal outcome in animals subjected to various shock-inducing procedures, interpretation of the significance of these observations is controversial. In many of the reported hemorrhagic shock experiments loss of blood has been less in the treated than in the control series, and it has been suggested by several authors that the observed protection is due only to a reduction in the degree of stress applied in bleeding to a standard pressure, or by loss of a smaller volume of fluid into damaged tissues (Gowley et al., 1936; Jacob et al., 1956; Spoerel, 1933; Saltz, 1960; Page, 1961). However, in many cases differences in bleeding volumes were relatively small while mortality differences were striking, suggesting a beneficial effect of the blocking drugs apart from any effect which they might exert on blood loss. In addition, marked protection without any appreciable change in bleeding volume can be induced by appropriate doses of a blocking agent such as Eibenamine or phenylephrine (Karrington et al., 1930a; Mac et al., 1933), particularly if administered several hours before the animal is subjected to the shock-inducing procedure (Nicherson, 1962). Furthermore, protection has been demonstrated in experiments in which a vasodilator was administered after bleeding had been completed (Kings et al.,

1939; Bakstian et al., 1941). Kovach et al. (1937) and Speerel (1935) found the protective effects of Ribonamine and hexamethonium in tourniquet shock not to be dependent on a reduced loss of fluid into the involved extremities.

It appears safe to conclude that protection against death from shock has been achieved by drugs capable of improving blood flow by inhibiting vasoconstriction. However, interpretation of the mechanisms involved remains controversial.

PART IV

TECHNIQUES FOR THE PRODUCTION
OF HYDROGENIC BRICK IN 1948

Historical. Many techniques have been developed which induce hemorrhagic shock with reasonable certainty. These involve 2 general principles: 1) withdrawal of a fixed percentage of the estimated or measured blood volume irrespective of the change in arterial blood pressure, and 2) maintaining a preselected level of hypotension by bleeding a variable volume.

Many variations of the former have been described. The method of Valcott (1943a) utilizes approximation of the acutely lethal bleeding volume followed by rapid reinfusion of a fixed percentage of the collected blood. Unanesthetized* dogs are bled very rapidly from both femoral arteries until blood flow ceases or respiration fails. Twenty-five per cent of the withdrawn blood is then reinfused rapidly intravenously or intra-arterially. After reinfusion, arterial pressure gradually recovers to about 60 mm Hg until the terminal stages at which time a progressive decline occurs. Average survival time in their series of animals was 3 3/4 hours. Several modifications of this method have been utilized. Arimoto et al. (1945) bled unanesthetized dogs varying percentages of the predetermined blood volume and returned 93 per cent of the withdrawn blood 30 minutes later. The bleeding was repeated 5 times at hourly intervals. Removal of 25 per cent of the blood volume by this method produced irreversible shock in 60 per cent of their animals. In addition, the authors developed a prognostic index consisting of the product of the arterial blood pressure and the arterial pO_2 after the first hemorrhage. T'ang et al. (1958) produced hemorrhagic shock in unanesthetized dogs by rapid arterial bleeding of a predetermined percentage of the blood volume, which was estimated to be 7.7 per cent of body weight. These workers

* No general anesthetic is used. However, the animals are pretreated with morphine and minor surgery is performed under local anesthesia.

also developed a mathematical expression for approximating the survival time of their animals from values for bleeding volume (expressed as per cent of estimated blood volume), arterial pressure immediately after hemorrhage and a constant.

Tosten and his associates (1943) described a 2-step method similar to that of Fang. Initially, 20 to 30 per cent of the estimated blood volume was removed. This was followed 30 minutes later by a second withdrawal of 10 to 15 per cent of the blood volume. Nylon *et al.* (1944) bled dogs on a volume/body weight basis. Hemorrhage was performed every 30 minutes until drastic hypotension was maintained. The fact that all dogs died within an hour strongly indicates that collapse due to acute exsanguination and not true shock was induced.

Techniques utilizing a preselected level of hypotension are exemplified by the procedure employed by C.J. Wiggers and co-workers at Western Reserve, and first described by Verle *et al.* (1942). Their procedure consists of controlled bleeding (30 ml/min) of dogs pretreated with morphine and anesthetized with barbitol, until the arterial pressure falls to 50 mm Hg. This level is maintained for 90 minutes by withdrawal or reinfusion of small amounts of blood. At the end of this period, additional blood is withdrawn to reduce the blood pressure to 30 mm Hg, where it is maintained for another 45 minutes. The withdrawn blood is then reinfused intravenously. Reinfusion restores the blood pressure to near the prebleeding level for periods of 1/2 to 1 1/2 hours. Thereafter arterial pressure declines slowly and death occurs in approximately 50 per cent of animals, generally 3 to 6 hours after reinfusion.

Several modifications of the basic Western Reserve method have been proposed. These have consisted mainly of altering the level or the

duration of hypotension. For example, Sellhart (1946) found the duration of hypotension originally used by Verie and colleagues to be too long for dogs subjected to prior surgical trauma such as laparotomy. Accordingly, he kept to 90 and 45 minute periods but raised the hypotensive levels to 60 and 40 mm Hg, respectively. Similarly, H.C. Wiggers and his associates (1945) found that a single hypotensive period of 45 mm Hg for 90 minutes was adequate to produce irreversible shock in unaneesthetized dogs. On the other hand, H.A. Frank et al. (1951a) bled unaneesthetized dogs to a level of 30 mm Hg and maintained them at this level until 40 per cent of the shed blood was spontaneously "taken up" by the animal to maintain its pressure. The average time for this to occur in their experiments was 4 hours. Eighty per cent of the animals were shocked irreversibly at this time despite reinfusion of the remaining blood.

Some points of comparison between the two general methods for producing hemorrhagic shock may be made here. The development of irreversible shock obviously is not determined by the amount of blood removed (bleeding volume) per se, but by the residual volume which is available to the animal after hemorrhage. Data from many laboratories have demonstrated a wide range of blood volumes in dogs. Pennycastle and Claghorn (1942) reported values of 60 to 167.6 ml/kg in 106 dogs ranging in weight from 4.7 to 19.3 kg. Similarly Walcott (1945b) found a range of 81 to 122 ml/kg in 13 dogs weighing 7.8 to 15.3 kg. It is evident therefore that production of hemorrhagic shock by bleeding a volume determined on the basis of body weight or of blood volume estimated from body weight invariably leads to difficulties. This is further evidenced by the wide range of bleeding volumes, calculated on a body weight basis, necessary to produce shock. In addition, the residual blood volume does not depend on the

pre-existing blood volume alone. Even when the prehemorrhage blood volume has been determined directly, removal of a preselected percentage of this volume does not always give reproducible results. Walcott (1943b) has indicated that the capacity of animals to replace circulating volume lost by hemorrhage with tissue and extracellular fluid and erythrocytes is an important determinant of residual blood volume.

The Western Reserve method and its modifications circumvent the major objections to the standard volume techniques just discussed. The procedure of exposing the animals to a preselected level and duration of hypotension, which can be varied depending upon the existing experimental conditions, would, in theory at least, impose a similar stress upon every animal. Although this probably is not the case, the mortality data obtained with the Western Reserve technique or variations thereof are more consistently reproducible which suggests that a more uniform stress is being applied. This method offers the additional advantage of permitting observations to be made on animals after reinfusion of the withdrawn blood, *i.e.*, during the normovolemic stages of irreversible shock as well as during hypovolemia. Most of the constant-volume methods involve only hypovolemic shock without eventual complete reinfusion of the shed blood.

Certain technical refinements have made the Western Reserve method a more efficient laboratory procedure. Lammson and Refurk (1945) introduced a method for maintaining selected hypotensive levels by using an arterial pressure compensator. Basically, this method consists of attaching the catheter from the artery of the dog to a reservoir suspended by a pulley. Blood flows into the reservoir until the blood pressure of the animal and the hydrostatic pressure within the reservoir are in equilibrium. The arterial pressure of the animal is thus dependent upon



the height of the reservoir. If the arterial pressure of the animal rises, blood flows into the reservoir until equilibrium is once again attained. Conversely, if the blood pressure of the dog tends to fall, blood will enter the animal from the reservoir and pressure is maintained constant.

However, this system has certain limitations. If the level of blood pressure is to be maintained exactly, it becomes necessary to readjust the level of the reservoir as the height of the column of blood in it changes. This is particularly true when a reservoir of small diameter is used to obtain accurate measurements of blood loss. In addition, this method does not permit adequate mixing of blood in the reservoir. Consequently, the formed elements tend to sediment. Particularly after a prolonged period of equilibrium the volume exchanged between the reservoir and the animal may consist of high hematocrit blood. Finally, since the surface area of the reservoir exposed to air is small, the erythrocytes may be inadequately oxygenated during prolonged periods of stasis. Such periods of hypoxia may cause potassium release or otherwise compromise the metabolic integrity of the cellular elements of blood and retransfusion of the animal may actually prove harmful.

Beck (1953) described a modification of the basic Lenson and DeTurk apparatus which operates independently of changes in the hydrostatic pressure of blood within the reservoir and maintains this blood adequately mixed and oxygenated (Figure 1). It depends on establishing equilibrium between the arterial pressure of the dog and a source of oxygen under controlled pressure. The animal is allowed to bleed from a femoral artery into a reservoir which consists of an inverted 1-11/16" cylinder with 10 ml graduations. A flow of oxygen is introduced into the reservoir

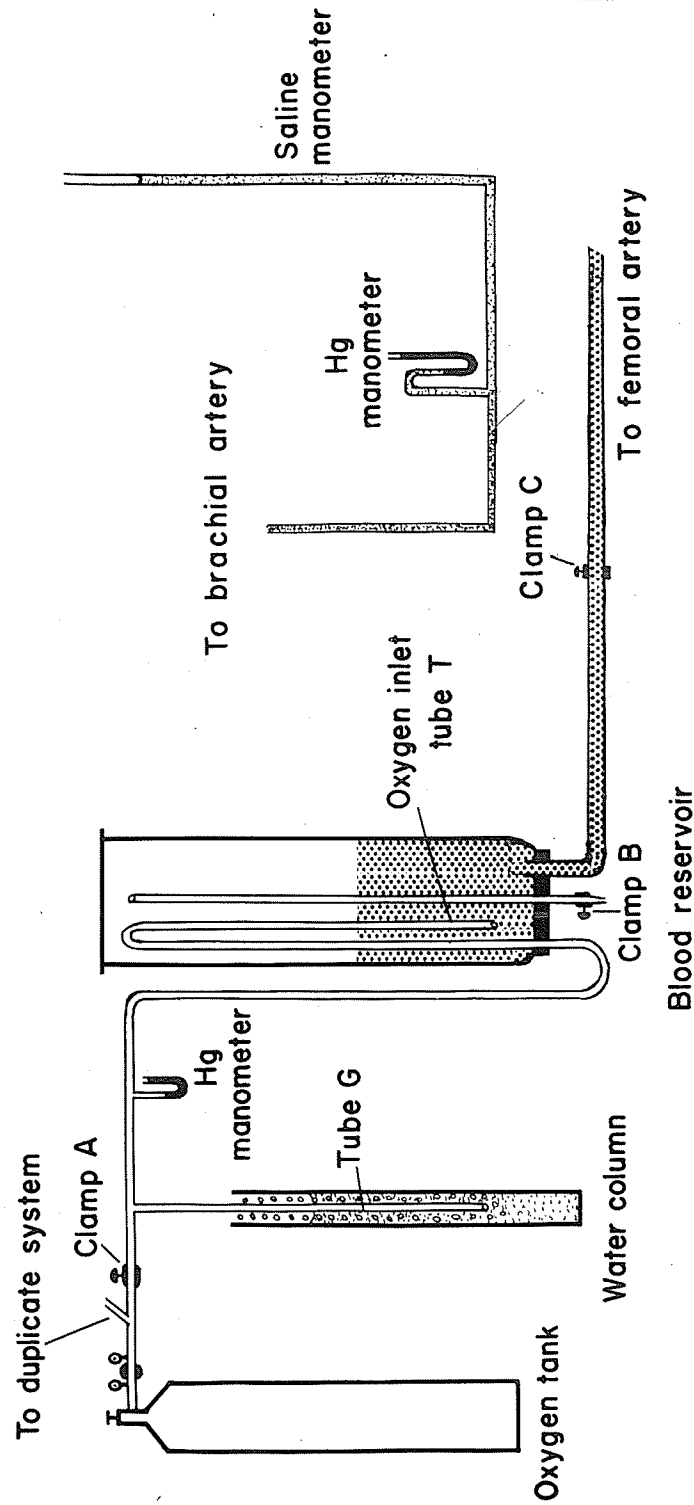


Figure 1
 Apparatus for Maintaining Stable Hypoxemia (Sect. 1955)*

through a glass tube (T). The height of the reservoir is adjusted so that the open end of this tube is approximately at heart level. When blood enters the reservoir, it is kept mixed and oxygenated by the gentle bubbling action of the oxygen. The pressure within the reservoir can be altered in several ways, as follows: 1) Clamp A leading directly from the source of oxygen can be adjusted. However, the changes in pressure produced in this manner are large and not suitable for accurate moment-to-moment control of the intra-reservoir pressure. Therefore, clamp A is adjusted at the beginning of the experiment to maintain a convenient flow of oxygen. At the time of reinfusion of shed blood this clamp is opened somewhat to increase the reservoir pressure and facilitate the return of blood to the animal. 2) Clamp B is attached to a glass tube projecting directly into the reservoir. When the blood level in the reservoir has risen above the inverted arm of tube T, the pressure above the blood will tend to rise with further blood loss. This tendency to rise is countered by a small, continuous flow of oxygen out of the capillary tube controlled by clamp B. This slow leak of oxygen through the capillary tube, and consequently through the inlet tube (T), has the triple function of a) maintaining the pressure above the blood so that this pressure plus the hydrostatic pressure of the blood is always equal to or slightly above the applied pressure, b) keeping the blood well oxygenated, and c) preventing sedimentation of formed elements of the blood. 3) A glass tube (C) is attached to a sidearm from the tubing carrying oxygen to the reservoir. Tube C projects into a column of water to varying depths. With certain limitations due to the rate of gas flow, the pressure in the system between clamp A and the animal is dependent on the depth to which tube C is immersed. A slow, steady escape of gas bubbles from tube C indicates that

the pressure in the system is just in excess of the hydrostatic pressure. Therefore, in order to establish an equilibrium pressure of 35 mm Hg (47.6 cm H₂O) in the arterial system of the dog and in the reservoir system distal to clamp A, tube C is immersed 47.6 cm into the water column. Small changes in arterial pressure can be produced quickly and simply by altering the depth of immersion.

In practice, stabilization of arterial blood pressure during the controlled periods of hypotension is obtained by the combined use of tube C and clamp B. During the period of rapid hemorrhage, clamp B is kept open to permit quick displacement of gas from the reservoir. When arterial pressure reaches the preselected level the rate of entry of blood and displacement of gas from the reservoir decreases and clamp B is partially closed. Measurements of arterial pressure are made from a brachial artery. When the pressure falls below 70 mm Hg measurements are made with a saline manometer, which permits more accurate determination and adjustment of the pressure than does the mercury manometer.

At the beginning of our investigations of the problem of shock our laboratory was faced with the problem of developing a suitable method for producing hemorrhagic shock in dogs. The importance of simplicity and reproducibility were obvious. In addition other criteria had to be met including the following: 1) the hypotensive stress should be variable to permit establishment of zones in the procedure where a) all or a great majority of the animals could be expected to respond favorably to reinfusion of withdrawn blood and would survive, b) about 50 per cent of the animals would be irreversibly shocked, i.e., would die despite reinfusion of their shed blood, and c) the majority of animals would be irreversibly shocked; 2) the technique should permit observations to be made during

simple hypovolemia and, after shock developed, during both the hypo- and normovolemic stages of irreversible shock, and 3) the method should permit observations during the critical period of hypovolemic shock, *i.e.*, when irreversibility intervenes, without the complication of spontaneous or drug-induced uptake of blood by the animal. The Western Reserve method utilizing the Beck modification of the Lenson and De Turk reservoir technique appeared to fulfill the criteria of simplicity and reproducibility. Certain additional modifications with respect to hypotensive levels and duration of hypotension were developed to meet our experimental requirements.

Standardization of technique for the production of hemorrhagic shock in dogs.

Methods. Each experiment was performed on 2 dogs simultaneously. Members of each pair were matched as well as possible with respect to body weight and sex. The animals were free from obvious infection or malnutrition. They were housed in individual cages and kept in a constant environment (temperature range 68 to 70°F) for 1 week prior to the experiment. Their diet consisted of standard Purina chow pellets and water ad libitum. Food was withheld for about 18 hours prior to the experiment. The animals were anesthetized with a standard dose of pentobarbital sodium (33 mg/kg) given intravenously, and after a 10-minute equilibration period, the following surgery was performed: the left brachial artery was exposed and cannulated for measurement of arterial blood pressure, the left femoral artery was cannulated for bleeding and the corresponding femoral vein was exposed for injections. The arterial catheters were of the maximum bore permitted by the vessels and were inserted well up into the artery to avoid resistance from vasospasm during hypovolemia. Mean arterial pressure was

measured by means of mercury and saline manometers connected to the catheter introduced into the subclavian via the brachial artery. The zero reference point was considered to be 3 cm above the animal board, a level approximating that of the right atrium. After bleeding from small vessels at the operative sites had ceased, 3.0 mg/kg heparin sodium was administered intravenously, and an additional 1.0 mg/kg was placed in the reservoir. In the majority of experiments, bleeding was begun in both animals simultaneously. The rate of bleeding was controlled by clamp C on the tube connected to the femoral arterial catheter so that the arterial pressures were reduced to 35 mm Hg over a period of 10 minutes. After a 30-minute period of hypotension at 35 mm Hg, the blood pressure was raised to 60 mm Hg by a small retransfusion of blood and maintained at this level for an additional 30 minutes. At the end of this period, the connection between the animal and the reservoir was clamped for 2 hours. During this period, the animals' blood pressure was uncontrolled except in instances where arterial pressure fell below 40 mm Hg. When this occurred, a small (2 ml/kg) transfusion was given to maintain blood pressure above 40 mm Hg and prevent acute cardiovascular and respiratory collapse. At the end of the 2 hours, the blood in the reservoir was reinfused intra-arterially over a 10-minute period. The catheters were removed, the incisions closed and the animals returned to their cages. Crystalline penicillin G (100,000 units) was administered intramuscularly to combat infection at the wound sites. The animals were observed frequently after reinfusion and postmortem examination was performed shortly after death on a number of animals. Animals alive 48 hours after reinfusion were considered survivors.

Arterial blood pressures, bleeding volumes and heart and respiratory rates were recorded at 5-minute intervals throughout the shock-induc-

ing procedure and for a 15- to 30-minute period after reinfusion of the shed blood. The volume of blood withdrawn to reach the 35 mm Hg level was recorded as the initial bleeding volume. The maximal amount of blood withdrawn at any stage was referred to as the maximal bleeding volume. In almost all experiments the maximal bleeding volume was reached during the final minutes of severe hypotension. The bleeding volume at the beginning of the clamp-off period was also recorded. The procedure described above was designated as the standard hemorrhagic shock procedure.

The degree of hemorrhagic stress was varied in 3 series of experimental animals.

Series 1: These animals were maintained at a level of 35 mm Hg for a period of 30 minutes. At the end of this time, all the withdrawn blood was returned intra-arterially over a 10-minute period.

Series 2: After a 30-minute period at 35 mm Hg, the dogs' blood pressure was raised to 60 mm Hg by a small intra-arterial blood transfusion and maintained at this level for 30 minutes. The remaining blood in the reservoir was then reinfused intra-arterially over a 10-minute period.

Series 3: These animals were subjected to the standard hemorrhagic shock procedure.

Results

Data on mortality, blood pressures, bleeding volumes and heart and respiratory rates for the 3 series are shown in Tables 2 to 3. Total reinfusion after 30 minutes at 35 mm Hg (series 1) resulted in a survival rate of 89 per cent (8 of 9 dogs) (Table 2). If the animals were reinfused completely at the end of the 60 mm Hg period (series 2), only 58 per cent (7 of 12 dogs) survived (Table 3). Thus, a significant number of

animals became irreversible to blood transfusion within this 30-minute period. The standard hemorrhagic shock procedure (series 3) allowed only 23 per cent survival (23 of 30 dogs)(Table 4).

Bleeding volumes are remarkably similar in all 3 series. Of particular interest is the fact that the stress imposed by the standard hemorrhagic shock procedure did not vary seasonally as reported previously by Hemington *et al.* (1950a), and as was our experience when dogs were not kept in a constant environment for several days before the experiment. Table 3 shows that the mortality rate as well as the measured cardiovascular and respiratory parameters did not differ significantly between "summer" and "winter" experimental groups.

One striking observation, which is particularly obvious in the dogs of series 3, deserves comment. Control blood pressures and heart rates, which may be regarded as gross indices of sympathetic nervous system activity, were significantly higher ($P < 0.01$) in the fatalities than in the survivors. This observation once more emphasizes the deleterious effect of increased sympathetic nervous system tone in the shock process.

Table 2
Cardiovascular and Respiratory Parameters in Dogs during
Sublethal Hemorrhagic Hypotension

Arterial Blood Pressure mm Hg	Control	Beatha (1 dog)	Survivors (8 dogs)
	After reinfusion		
Bleeding Volume ml/kg	137*	36.6	34.8 ± 1.8
	137**	57.6	55.7 ± 3.1
Heart Rate beats/min	Control	138	165 ± 12
	Maximum	243	207 ± 14
	After reinfusion	148	159 ± 13
Respiratory Rate resp/min	Control	6	13 ± 3
	Maximum	16	44 ± 5
	After reinfusion	10	31 ± 4

* IBV - initial bleeding volume

** MBV - maximal bleeding volume

All figures are mean ± std. error

Table 3
Cardiovascular and Respiratory Parameters in Dogs during
Moderate Hemorrhagic Shock

		Deaths (3 dogs)		Survivors (7 dogs)	
		Control	After reinfusion	Control	After reinfusion
Arterial Blood Pressure mm Hg					
Bleeding Volumes ml/kg					
Heart Rates beats/min					
Respiratory Rates resp/min					

* IIV - initial bleeding volume

** IIV - maximal bleeding volume

All figures are mean $\pm$ std. error

Table 4

Cardiovascular and Respiratory Parameters in Dogs during

the Standard Hemorrhagic Shock Procedure

		Deaths (23 dogs)	Survivors (7 dogs)
Arterial Blood Pressure mm Hg	Control	140 ± 3	123 ± 3
	After reinfusion	112 ± 4	121 ± 4
	IMV*	41.6 ± 2.0	38.3 ± 1.6
	MMV**	57.0 ± 1.5	55.6 ± 3.6
Bleeding Volume ml/kg	At clamp-off	51.8 ± 1.9	52.2 ± 4.1
	Control	164 ± 6	129 ± 5
	Maximum	242 ± 7	249 ± 8
	After reinfusion	190 ± 7	162 ± 7
Heart Rates beats/min	Control	11 ± 1	7 ± 1
	Maximum	34 ± 3	40 ± 6
	After reinfusion	28 ± 3	35 ± 7
Respiratory rates resp/min	Control	11 ± 1	7 ± 1
	Maximum	34 ± 3	40 ± 6
	After reinfusion	28 ± 3	35 ± 7

* IMV = Initial bleeding volume

** MMV = maximal bleeding volume

All figures are mean ± std. error

Table 5

Cardiovascular and Respiratory Parameters in Dogs during the Standard Hemorrhagic Shock

Procedure Performed in "Summer" or "Winter"

		Deaths		Survivors	
		"Summer" (12 dogs)	"Winter" (11 dogs)	"Summer" (3 dogs)	"Winter" (4 dogs)
Arterial Blood Pressure mm Hg	Control	143 ± 4	138 ± 3	126 ± 9	121 ± 8
	After reinfusion	117 ± 4	107 ± 7	128 ± 7	113 ± 7
Bleeding Volumes ml/kg	1st INV ^a	44.3 ± 2.4	38.5 ± 3.2	36.4 ± 4.6	37.3 ± 4.2
	2nd INV ^a	58.1 ± 2.1	53.8 ± 2.6	53.8 ± 5.0	57.0 ± 4.7
	At clamp-off	53.5 ± 2.6	49.8 ± 2.8	50.8 ± 3.6	53.3 ± 3.6
Heart Rates beats/min	Control	172 ± 12	155 ± 9	141 ± 12	120 ± 10
	Maximum	238 ± 8	246 ± 12	230 ± 15	249 ± 20
	After reinfusion	196 ± 10	182 ± 10	157 ± 18	166 ± 17
Respiratory Rates resp/min	Control	10 ± 2	12 ± 2	9 ± 3	6 ± 2
	Maximum	39 ± 4	29 ± 3	41 ± 5	39 ± 10
	After reinfusion	33 ± 5	23 ± 2	41 ± 8	30 ± 10

^a INV = initial bleeding volume^b INV = maximal bleeding volume

All figures are mean ± std. error

PART V

CHEMICAL STRUCTURE AND BIOLOGICAL
ACTIVITY AMONG COMPOUNDS RELATED
TO PREDNISONEMINE

The protection provided by adrenergic blocking agents and other drugs which induce vasodilation against hemorrhagic and traumatic shock has been interpreted as due to prevention of the deleterious effects of vasoconstriction (Remington *et al.*, 1950a; E. Z. Levy *et al.*, 1954; Nickerson, 1955b; Jacob *et al.*, 1956; Nickerson and Carter, 1959).

However, the validity of this interpretation was questioned by the report of Hess *et al.*, (1956) that *N*-hexahydrobenzyl-*N*-ethyl- β -chloroethylamine (GB-131), a congener of dibenzamine without adrenergic blocking activity, effectively prevented certain metabolic derangements and reduced the mortality attendant to hemorrhagic and traumatic shock in rats. Nickerson and Gump (1949) showed that two major requirements for the highly specific adrenergic blocking activity of the haloalkylamine series of compounds are: a) an appropriate aromatic substituent, and b) a β -alkylhalide group capable of forming an ethyleniminium intermediate, the molecular species directly responsible for the blockade. GB-131 is an entirely saturated molecule which lacks adrenergic blocking activity, but retains the high chemical reactivity conferred by the β -haloalkyl moiety.

In order to evaluate the relative importance of the reactive β -alkylhalide and of adrenergic blockade *per se* in protecting against experimental hemorrhagic shock, we have compared activities of 5 members of this chemical series: Phenoxybenzamine (a potent adrenergic blocking agent with high chemical reactivity), GB-131 (a compound with no adrenergic blocking activity but with high chemical reactivity), *N*-phenoxyisopropyl-*N*-ethyl-aminooethanol (SKF-3331, weak adrenergic blocking agent without a chemically reactive β -halogen) and *N,N*-dibenzylaminooethanol (GB-12) and *N*-phenoxyisopropyl-*N*-benzylaminooethanol (SKF-1010) (hydroly-

as products of dibenzamine and phenoxybenzamine respectively, with neither adrenergic blocking activity nor a chemically reactive (β -halogen).

Methods

Cardiovascular studies: Preliminary experiments were performed in dogs anesthetized with pentobarbital sodium. Blood pressure was recorded from a femoral artery with a pressure transducer and oscillograph. Graded doses of 1-adrenaline bitartrate were administered intravenously as saline solutions, before and at selected intervals after intravenous administration of phenoxybenzamine, GB-131, SRP-5331 and GB-12.

Hemorrhagic shock experiments: Mongrel dogs were subjected to hemorrhagic shock according to the standard technique described previously (Part IV). The drugs were administered intravenously at selected intervals prior to hemorrhage. The pretreatment intervals for agents with relatively transient actions were adjusted so that the effects would be maximal during the period of severe hypovolemia. The prolonged action of phenoxybenzamine permitted testing 18 hours after administration.

Results

Cardiovascular studies: The effects of phenoxybenzamine on arterial blood pressure were measured in 3 dogs for a short period after administration and 18 hours later in the same animals. Slow intravenous administration of 0.125, 0.25 or 1.0 mg/kg caused an immediate slight (5 to 15 per cent) reduction in mean arterial pressure. Eighteen hours later arterial blood pressure was not appreciably different from the control. Particular attention was focused on the degree of adrenergic blockade present 18 hours

after administration of phenoxybenzamine since this was the pretreatment interval selected for the shock experiments. Pressor responses to all of 4 doses of adrenaline (0.75, 1.5, 3.0 and 6.0 $\mu\text{g/kg}$) obtained 18 hours following these doses of phenoxybenzamine were reduced in every animal, and reversed in 2 of 3 dogs which had received 1.0 mg/kg . The mean pressor responses to these doses of adrenaline after 0.125 or 0.25 mg/kg phenoxybenzamine averaged 30 and 31 per cent, respectively, of the control pressor responses. The duration of the pressor responses to adrenaline after phenoxybenzamine was considerably shorter than that observed prior to administration of this agent, which suggests that they represent the unblocked myocardial responses to the injected adrenaline.

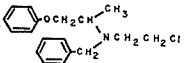
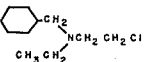
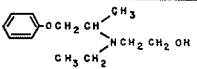
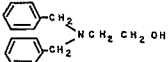
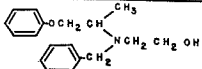
GB-131 in doses of 5.0 and 10 mg/kg intravenously caused a progressive and sustained rise in blood pressure ranging from 12 to 37 mm Hg in 4 dogs. Peak pressures generally occurred within 15 to 30 minutes after administration and blood pressure returned to control levels within 2 hours. Pressor responses to adrenaline were unaltered.

MB-3331 (10 mg/kg intravenously) reduced the pressor responses to adrenaline slightly for about 1 hour after administration in 2 dogs. Changes in blood pressure were not remarkable.

GB-12 (10 mg/kg intravenously) caused no changes in blood pressure or pressor responses to adrenaline in 2 dogs.

Hemorrhagic shock experiments: Data on pretreatment schedules, blood pressures, bleeding volumes and survival rates are presented in Table 6.

Phenoxybenzamine (1.0 mg/kg) administered to dogs 18 hours prior to bleeding did not alter significantly the initial blood pressures or the initial or maximal bleeding volumes, but produced a striking and highly

Drug	Pretreatment Interval	Dose mg/kg	Results +	No.	Percent Survival	Initial B.P. mm Hg	IBV ⁺⁺ ml/kg	MBV ⁺⁺⁺ ml/kg
Control	-----	---	S	7	23	137 ± 3	38.3 ± 1.6	55.6 ± 3.6
			D	23			41.6 ± 2.0	57.0 ± 1.5
 Phenoxybenzamine (Dibenzylamine)	18 hours	0.125	S	8	47	133 ± 4	38.0 ± 2.0	57.1 ± 3.1
			D	9			34.0 ± 3.9	47.0 ± 3.0
		0.25	S	4	44	131 ± 9	34.5 ± 5.4	53.6 ± 5.7
			D	5			31.7 ± 3.5	42.4 ± 4.0
		1.0	S	7	88	129 ± 7	39.5 ± 2.1	52.0 ± 2.8
			D	1			34.4	41.3
 GD-131	3 hours	5.0	S	4	50	138 ± 8	30.4 ± 2.3	40.9 ± 2.7
			D	4			38.8 ± 2.6	47.8 ± 4.1
 SKF-5331	45 min.	10.0	S	3	27	150 ± 5	37.7 ± 3.7	57.2 ± 8.2
			D	8			43.2 ± 2.6	51.5 ± 3.4
 GD-12	30 min.	10.0	S	0	0	138 ± 12	----	----
			D	5			37.4 ± 4.2	52.4 ± 3.0
 SKF-1010	30 min.	10.0	S	0	0	147 ± 11	----	----
			D	4			41.0 ± 6.0	48.9 ± 7.1

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significant ($P < 0.01$) increase in survival. Smaller doses of phenoxybenzamine (0.125 and 0.25 mg/kg) approximately doubled the percentage survival. However, these differences were not statistically significant for the individual groups ($P > 0.1$ and $P > 0.2$ respectively). The bleeding volumes of control and treated animals did not differ significantly.

Administration of GB-131 (5.0 mg/kg) 3 hours prior to bleeding increased the survival rate from 23 to 50 per cent. The difference was not statistically significant, and was probably related to the marked and statistically significant ($P < 0.01$) reduction in the initial and maximal bleeding volumes of dogs receiving this agent. Lower doses did not alter survival rate or bleeding volumes. Higher doses produced severe central nervous system depression. Gross ataxia, proportional to the dose, appeared within 1 hour after administration of 5.0 to 15 mg/kg of the drug and persisted until the animals were anesthetized 2 1/2 hours after injection.

SEP-3331 was administered only 45 minutes prior to bleeding because its weak adrenergic blocking activity is relatively transient. A dose of 10 mg/kg did not alter appreciably either the survival rate or the bleeding volumes of the animals tested. Larger doses caused gross central nervous system stimulation and were not investigated for their effects on the shock process.

GB-12 and SEP-1010, compounds possessing neither adrenergic blocking activity nor a reactive β -halogen, provided no protection against death resulting from the hemorrhagic shock procedure, and may have increased the mortality rate. Bleeding volumes and blood pressures are not altered significantly by either compound.

Discussion

Of the agents tested in the present experiments, only phenylbenzamine provided significant protection against the development of irreversible hemorrhagic shock in dogs. This effect appeared to require doses of the drug which provided a considerable degree of adrenergic blockade throughout the period of hypovolemic stress and was more marked following a dose of 1.0 mg/kg than after 0.125 or 0.25 mg/kg, which produced only minimal blockade.

The discrepancy between our results with GB-131 and those of Bazz et al. (1956) may involve a species difference. These authors did not mention cardiovascular or central nervous system effects due to GB-131 in rats nor did they find a reduction in bleeding volume. However, we observed signs of central nervous system depression and reduced bleeding volumes in dogs following administration of GB-131. In addition, GB-131 caused a rise in arterial blood pressure in dogs. This effect may indicate increased sympathetic nervous system activity, known to increase the sensitivity to shock.

The failure of SKF-5331 to protect against hemorrhagic shock probably can be attributed to the fact that the dose employed produced only a slight and transient adrenergic blockade, as indicated by depression of the pressor response to adrenaline.

These results strongly suggest that adrenergic blockade is an important requirement for antishock activity. The intimate mechanism of the protection afforded by the effective adrenergic blocking agents such as phenylbenzamine and dibenzamine has not yet been elucidated. Several investigators have suggested that reduced bleeding volume, and thus reduced stress,

is an important factor (Cowley et al., 1956; Jacob et al., 1956; Spoerel, 1958; Salts, 1960; Page, 1961). However, in the present study the initial and maximal bleeding volumes of the phenoxybenzamine-protected and control animals did not differ significantly. That the apparent slight reductions in maximal bleeding volume were not responsible for the improved survival is indicated by the fact that the minor differences in dogs pretreated with 1.0 mg/kg phenoxybenzamine were no greater than those observed with smaller doses of this agent, which provided only limited protection. In addition, the reductions in bleeding volumes were more marked among the fatalities than among the survivors. Remington et al. (1930a) and Hess et al. (1938) likewise observed protection without reduced bleeding volumes in animals pretreated with suitable doses of blocking drugs.

The experiments of Williams and Redford (1960) may be of importance in explaining the protection provided by this agent. Phenoxybenzamine was found to produce an increase in plasma volume of 25 per cent in chicks associated with a corresponding fall in venous hematocrit. This suggests that even at equal bleeding volumes, animals pretreated with phenoxybenzamine may have larger residual blood volumes, which may contribute to the more adequate circulation in these animals than in the untreated controls. In addition, hematocrit per se may be of importance. Crowell et al. (1938) have shown an increased susceptibility to hemorrhagic shock in dogs with pre-shock hematocrits below 30 or above 43 per cent.

The relation of adrenergic blockade to antishock activity becomes even more apparent when one considers that, in addition to decreasing tissue perfusion, vasoconstriction may contribute to the etiology of

shock by causing a decrease in the circulating blood volume. Thus, Gibson et al. (1947) noted a reduction in circulating volume of dogs subjected to hemorrhagic or traumatic shock which was greater than could be accounted for by measured external blood loss. In addition, Freeman (1933) found that severe vasoconstriction and shock induced by decortication or infusions of adrenaline were associated with a marked decrease in circulating blood volume.

In view of the protection provided by the adrenergic blocking agent phenoxybenzamine against experimental shock, and the relative ineffectiveness of its congeners in this regard, we were interested in determining the extent to which antishock activity might be related to alterations in blood volume.

Methods

Phenoxybenzamine: Experiments were performed on normal dogs, either unanesthetized or anesthetized with barbital sodium (300 mg/kg intravenously).

1) Unanesthetized dogs: Circulating red cell volume (CRCV) was determined directly in 3 dogs with Cr⁵¹-labelled autologous red blood cells by a modification of the method of Gray and Sterling (1950). Phenoxybenzamine (1.0 mg/kg) was then administered slowly intravenously to 2 of these animals; the third served as control. Measurement of CRCV was repeated 1 1/2 hours and 18 hours after phenoxybenzamine administration. Blood from the brachial vein was withdrawn for microhematocrit determinations before and at the above-mentioned times after injection of phenoxybenzamine.

2) Anesthetized dogs: Following induction of anesthesia, a femoral artery and vein were exposed for measurement of arterial blood pressure and for injections and blood sampling. Plasma volume was measured

directly by the isotope dilution principle in 3 dogs using Radio-iodinated Human Serum Albumin (RIHSA). Phenoxybenzamine (1.0 mg/kg) was then administered slowly intravenously and measurement of plasma volume was repeated at intervals thereafter for 1 1/2 to 3 hours. Plasma volume and CMCV were measured simultaneously in 3 additional dogs before and at 3 hourly intervals after the slow intravenous injection of 1.0 mg/kg of phenoxybenzamine. Plasma protein concentration (Lowry et al., 1951) and femoral venous hematocrit also were determined in these animals before and after phenoxybenzamine administration.

GP-131, GP-5331 and GP-12: Experiments in dogs were limited to measurement of femoral venous hematocrit before and during the 3-hour interval following intravenous administration of these compounds. In order to determine possible species differences, GP-131 was administered also to rats intravenously. Arterial blood pressure and venous hematocrit were then measured.

Results

In vitro tagging of autologous erythrocytes with Cr⁵¹: Early attempts to tag canine erythrocytes by the technique of Gray and Sterling (1950) revealed a considerable incidence of poorly labelled samples. In some instances, whole blood incubated for 1 hour at room temperature with Acid-Citrate-Dextrose solution (ACD) plus Cr⁵¹ picked up only 6 to 10 per cent of the available radioactivity. It was finally determined that dog serum contained an unknown reducing substance(s) which reduced tagging chromate to the ineffective chromic form. Accordingly, a method was developed which resulted in a 95 to 97 per cent uptake of tracer following a 30-minute in-

incubation at room temperature. The modified technique consisted of:

- 1) Centrifugation of the blood sample (10 ml) and removal of supernatant plasma.
- 2) Resuspension of erythrocytes in an equal volume of 0.9 per cent sodium chloride solution, followed by a second centrifugation and removal of supernatant saline.
- 3) Final suspension of erythrocytes in 10 ml ACD containing Cr^{51} plus 0.03 ml of 5 per cent $\text{K}_3\text{Fe}(\text{CN})_6$ to neutralize the effect of any residual reducing substances.

Effect of phenoxybenzamine on measured CBCV: Figure 2 represents the combined data from 2 unanesthetized dogs. Plasma volume was calculated from the measured CBCV and hematocrit. CBCV was essentially unchanged after phenoxybenzamine. However, a marked fall in hematocrit occurred, giving a calculated increase in plasma volume of almost 40 per cent. This effect still was quite prominent after 18 hours. No alterations in measured CBCV, hematocrit or plasma volume were observed in the untreated control. The magnitude of the calculated expansion of plasma volume prompted us to study this change by direct measurement.

Effect of phenoxybenzamine on measured plasma volume. The data for 5 unanesthetized dogs are summarized in Table 7. Administration of phenoxybenzamine resulted in a prompt, sustained fall in hematocrit during the post-injection observation period. This was accompanied by an increase in measured plasma volume averaging 18 per cent. However, only about one-half of the decrease in hematocrit could be accounted for by expansion of the plasma volume. This became apparent when we calculated plasma volume as in the previous experiments (vide supra) from the hematocrit and a CBCV which

Figure 11

Schematic representation of the experimental set-up employed to measure ^{86}Rb uptake in regional vascular beds. The tracer was administered by injection into the main artery leading to the vascular bed. The venous return passed through a coil set in a well-type scintillation counter which monitored the level of radioactivity in the blood, then through a bubble flow meter and was returned to the animal intravenously. Constant-rate sampling was begun immediately upon the appearance of injected tracer in the venous blood and was continued until the level of radioactivity returned to baseline. In cross-perfusion experiments, blood from the donor animal is administered by a Mark-Zabakay peristaltic pump. Mechanical restriction of blood flow was accomplished by pressure regulators on the artery or vein.

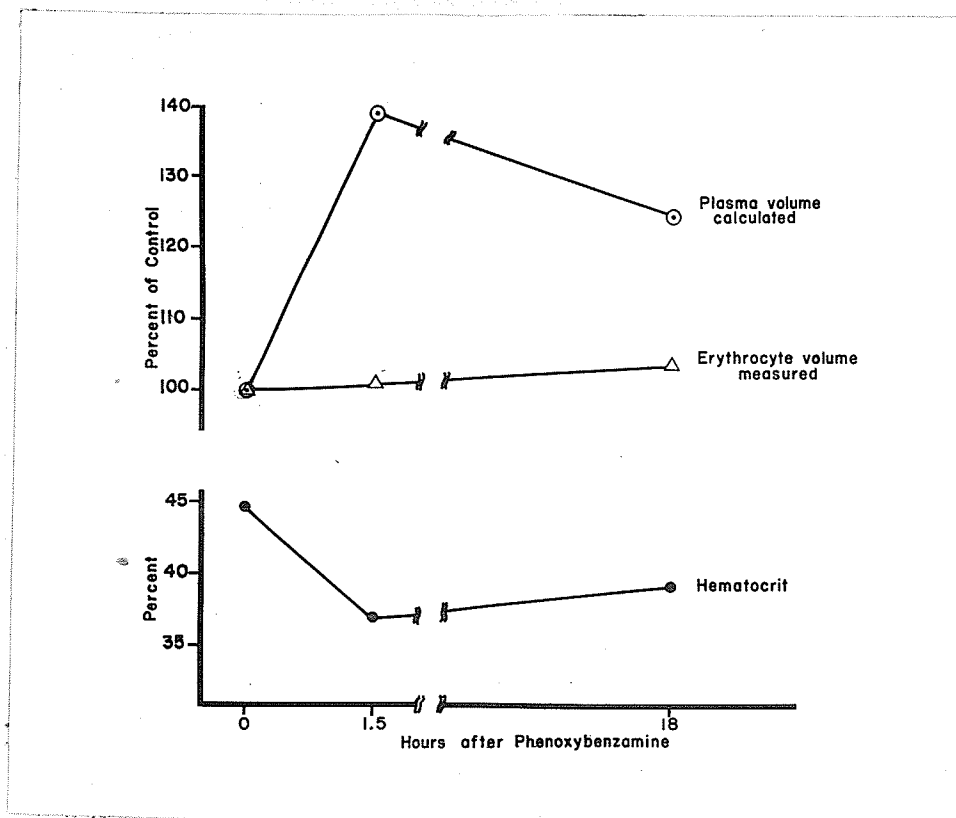


Figure 2

Mean measured erythrocyte volume and femoral venous hematocrit in 2 dogs before and after intravenous administration of phenoxybenzamine. Plasma volume calculated from erythrocyte volume and hematocrit.

Table 7

Measured Plasma Volume and Calculated Venous Hematocrits and Calculated

CNCV^a in Dogs Before and After Intravenous Administration of Phenylbenzamine.

| Dog # | Measured Plasma Volume (ml) | | | | | | Hematocrit | | | | | | CNCV ^a , ml |
|-------|-----------------------------|-------------------------------|------|------|------|------|------------|-------------------------------|------|------|------|-----|------------------------|
| | Cont. | Minutes after Phenylbenzamine | | | | | Cont. | Minutes after Phenylbenzamine | | | | | |
| | | 30 | 60 | 90 | 120 | 180 | | 30 | 60 | 90 | 120 | 180 | |
| PT-1 | 796 | 905 | 905 | 920 | 920 | 920 | 41.8 | 41.0 | 41.0 | 40.3 | 37.3 | 646 | |
| PT-2 | 870 | 955 | 955 | 1130 | 1130 | 1130 | 43.5 | 36.6 | 36.6 | 35.9 | 34.8 | 723 | |
| PT-3 | 1068 | 1105 | 1105 | 1148 | 1148 | 1148 | 40.4 | 37.5 | 37.5 | 36.8 | 37.1 | 763 | |
| PT-4 | 1048 | 1100 | 1100 | 1192 | 1192 | 1192 | 42.2 | 40.8 | 40.8 | 37.3 | 37.3 | 763 | |
| PT-5 | 950 | 1135 | 1135 | 1225 | 1225 | 1225 | 45.8 | 37.8 | 37.8 | 37.3 | 37.3 | 883 | |

^a CNCV calculated from the control plasma volumes and hematocrits, and are assumed to remain relatively constant throughout the experiment.

was assumed to remain constant on the basis of the results of the previous experiments. The increase in plasma volume determined in this manner averaged 36 per cent. Figure 3 shows the results of one of these experiments and illustrates the differences between measured and calculated plasma volume.

Effect of phenoxybenzamine on plasma volume, CBCV and plasma proteins measured simultaneously: The data for 3 dogs are summarized in Table 5, and the results for 1 of these illustrated in Figure 4. During the 2-hour period following phenoxybenzamine, CBCV remained unchanged. Plasma volume increased an average of 18 per cent, accompanied by a proportionate decrease in plasma protein concentration. Total body hematocrit (TBH) was determined from the ratio of measured CBCV to the measured total blood volume. In the control, TBH was less than the femoral venous hematocrit. After phenoxybenzamine both hematocrit values fell. However, femoral venous hematocrit decreased more and approached TBH. Thus, the altered relation of venous to total body hematocrit explains the discrepant results obtained when only a single direct measurement was made.

Effect of GB-131, SKF-5331 and GB-12 on venous hematocrit: GB-131 in doses of 5.0 and 10.0 mg/kg intravenously caused a progressive, sustained and apparently dose-related increase in venous hematocrit in 4 dogs. The hematocrit rose from 43 to 51 per cent following 5 mg/kg GB-131 and from 50 to 65 per cent after 10 mg/kg. The increase in hematocrit paralleled the previously described rise in blood pressure but, in contrast to blood pressure, showed no tendency to return toward the control level during the 3-hour observation period. GB-131 was administered to 3 rats intravenously in a dose of 2 mg/kg, which was the largest dose utilized by Racz et al. (1956), and

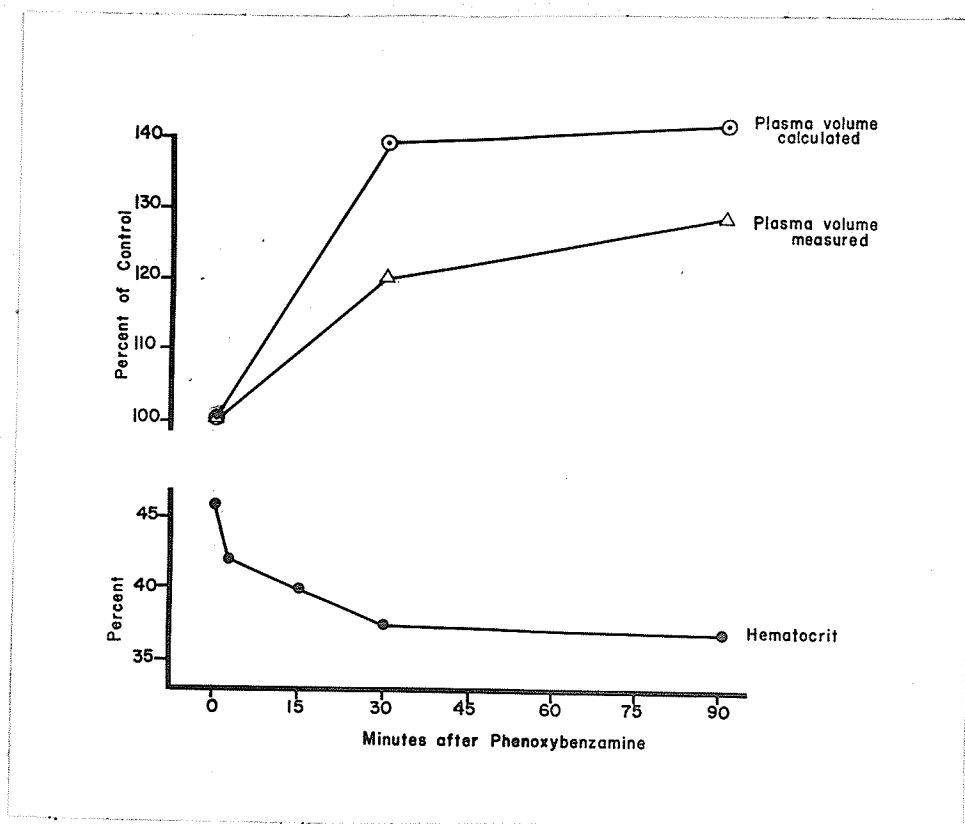


Figure 3

Measured plasma volume and femoral venous hematocrit in a dog before and after intravenous administration of phenoxybenzamine. The calculated plasma volume is derived from the hematocrit and an assumed constant erythrocyte mass.

Table 3

Measured Plasma and Erythrocyte Volume, Plasma Protein Concentrations, and Total Body and Total Body Hematocrit in Dogs Before and After the Intravenous Administration of Phosphorybous.

| Dog # | Control | | Plasma Vol., ml | | Hem. Vol., ml | | Total Body Lit. | | Plasma Prot., g/ml | |
|-----------|---------|---------------------------|-----------------|---------------------------|---------------|---------------------------|-----------------|---------------------------|--------------------|---------------------------|
| | Cont. | Hrs. after Phosphorybous. | Cont. | Hrs. after Phosphorybous. | Cont. | Hrs. after Phosphorybous. | Cont. | Hrs. after Phosphorybous. | Cont. | Hrs. after Phosphorybous. |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| PK-1007-1 | 580 | 515 | 520 | 975 | 42.3 | 34.0 | 37.9 | 34.4 | 108 | 93 |
| PK-1007-2 | 585 | 600 | 946 | 1067 | 42.0 | 36.5 | 38.2 | 36.0 | 78 | 68 |
| PK-1007-3 | 492 | 471 | 701 | 637 | 44.3 | 39.0 | 39.0 | 36.0 | 84 | 68 |

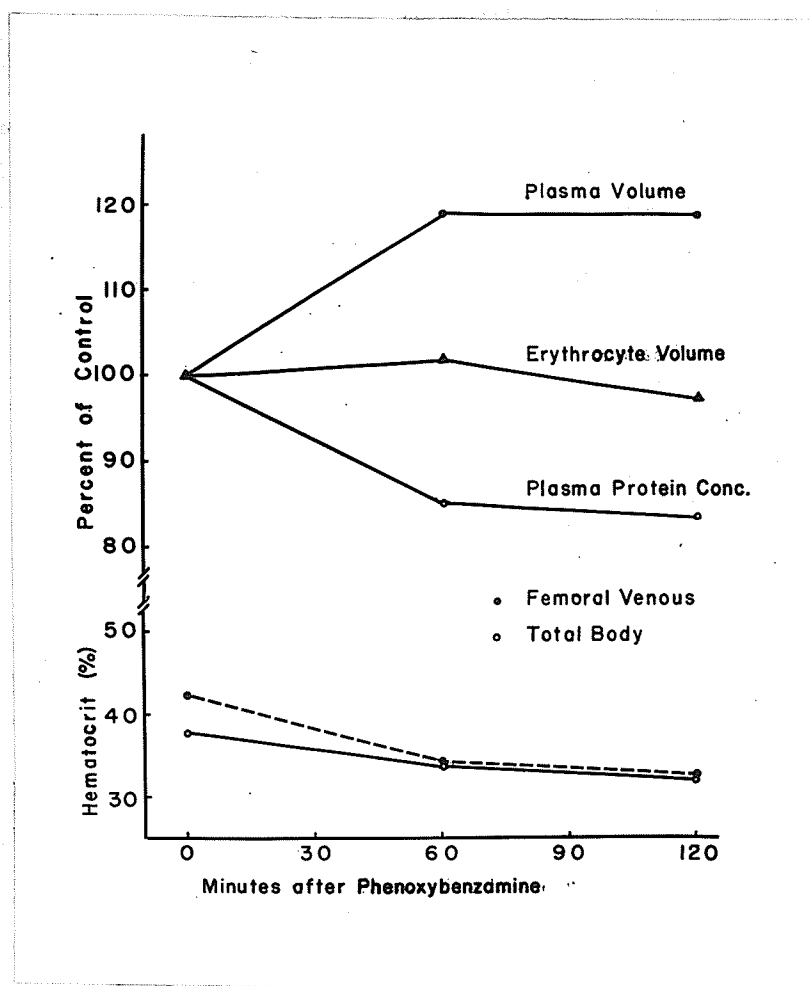


Figure 4

Measured plasma and erythrocyte volumes, plasma protein concentration and femoral venous hematocrit in a dog before and after intravenous administration of phenoxybenzamine. Total body hematocrit calculated from measured values for plasma and erythrocyte volumes.

which provided striking protection against shock in their studies. The effect on arterial pressure was not remarkable. However, venous hematocrit fell from a mean of 47 to 38 per cent during the 3-hour observation period.

SEP-5331 (10 mg/kg intravenously) increased venous hematocrit from 43 to 51 per cent in 2 dogs and this elevation was maintained during the 3-hour observation period.

GD-12 (10 mg/kg intravenously) caused no changes in hematocrit in 2 dogs.

Discussion

Administration of phenoxybenzamine to normal dogs resulted in a prompt and sustained decrease in femoral venous hematocrit. Only about one-half of this reduction could be accounted for by expansion of plasma volume with extracellular fluid.

Peripheral venous hematocrit is usually higher than TBM. This difference apparently is due to hemodynamic factors which permit more rapid passage of the central core of erythrocytes through blood vessels with laminar flow and favor plasma skimming in the microcirculation. After phenoxybenzamine, both femoral and total body hematocrits of dogs decreased. However, femoral venous hematocrit decreased more and approached TBM, which suggests that vasodilatation produced by phenoxybenzamine facilitated the entry of erythrocytes into areas of the microcirculation which previously were relatively cell-free. This could explain the additional decrease in hematocrit seen in the present experiments which could not be accounted for by expansion of plasma volume with extracellular fluid.

The discrepant effects of GD-131 in dogs and rats appear to in-

clude more than those on bleeding volume and central nervous system activity. GB-131 raised the arterial blood pressure and increased the venous hematocrit markedly in dogs. Both effects suggest increased sympathetic nervous system activity, known to increase sensitivity to shock, and in addition, increased hematocrit per se in the range observed has been shown to reduce tolerance to shock (Crowell et al., 1953). In contrast, a considerable fall in venous hematocrit occurred in rats following administration of GB-131. This apparent hemodilution may have played a prominent role in the protection against hemorrhagic and traumatic shock in this species reported by Koss et al. (1956).

Two factors may explain the failure of SEP-5031 to protect against hemorrhagic shock: 1) The weak and transient adrenergic blockade mentioned previously, and 2) the observed increase in hematocrit following its administration.

Summary

Phenoxybenzamine and several of its congeners were investigated in an attempt to correlate ability to protect against hemorrhagic shock in dogs with pharmacological activity and chemical structure, with particular reference to the roles of adrenergic blocking activity (present in phenoxybenzamine, minimal in SEP-5031 and absent in GB-131, GB-12 and SEP-1010) and of a reactive β -haloalkylamine grouping (present in phenoxybenzamine and GB-131 and absent in the other agents tested).

Phenoxybenzamine, in doses providing a considerable degree of adrenergic blockade during the period of hemorrhagic stress, offered considerable protection against the development of irreversible shock without significantly reducing bleeding volumes. Dogs pretreated with phenoxybenz-

asine were found to have larger blood volumes due to expansion of plasma volume with extracellular fluid, and lower hematocrits. Two factors appear to be responsible for the lowered hematocrits 1) selective expansion of plasma volume with extracellular fluid, and 2) equalization of total body and venous hematocrits, presumably by the entry of erythrocytes into areas of the microcirculation which previously were relatively cell-free.

Some reduction in mortality was observed in dogs pretreated with GD-131. However, this was accompanied by a significant decrease in bleeding volumes and evidence of central nervous system depression. Elevated blood pressure and hemoconcentration following GD-131 suggest that increased sympathetic nervous system activity may have been an additional factor contributing to mortality.

GD-5331, GD-12 and GD-1010 did not provide protection in the doses employed.

These results indicate that the protection provided by phenoxybenzamine is related to its prolonged and highly specific adrenergic blocking activity. In addition to reducing vasoconstriction due to sympathetic nervous system hyperactivity accompanying shock, phenoxybenzamine may also provide an increased plasma volume and a more favorable distribution of the available blood. Both the reactive β -halogen and an appropriate aromatic group (phenoxyisopropyl, benzyl, etc.) have been shown to be necessary structural components for this activity. The reactive β -halogen or the phenoxyisopropyl and/or benzyl groupings alone are not adequate to account for the protection provided against hemorrhagic shock.

PART VI

ADRENERGIC MECHANISMS INVOLVED IN
THE EFFECTS OF BACTERIAL ENDOTOXINS;
A CONTRIBUTION TO THE BACTERIAL AND
TOXIN THEORIES OF SHOCK

Introduction

The immunological and cardiovascular effects of bacterial endotoxins have aroused considerable laboratory and clinical interest and have been the subject of several recent reviews (Sennett and Cluff, 1937; Gilbert, 1960; Rosen, 1961).

Endotoxins are characteristic of Gram-negative bacteria, particularly of coliform and related bacilli (Zahl and Hutner, 1943; Co Tri and Schrift, 1944; Frebey and Pittman, 1945). Endotoxin-like materials have been demonstrated also in an occasional Gram-positive organism (Singer *et al.*, 1951), in a fungus (Harkness *et al.*, 1950) and in lysates of hemolytic streptococci (Stetson, 1956). However, these exceptions do not alter the basic fact that Gram-negative bacteria are by far the predominant source of endotoxins.

The endotoxins produced by Gram-negative bacilli differ in their antigenic properties but all which have been isolated are similar in structure, being complex polysaccharides or lipopolysaccharides. All produce the same physiological changes in man and experimental animals (Sennett and Cluff, 1937; Rosen, 1961).

Considerable evidence has been presented to suggest that bacterial endotoxins may be an important causative factor in the genesis of irreversible hemorrhagic or traumatic shock (Fine *et al.*, 1959). Although the mechanisms by which endotoxins may produce deleterious effects in shock have not been clearly delineated, several observations suggest that certain responses characteristic of endotoxins per se may involve the sympathetic nervous system, (Boquet *et al.*, 1947; Boquet and Izard, 1950; Thomas, 1956; Zweifach *et al.*, 1956; Ball and Kelly, 1957). In view of evidence indicat-

ing an adverse role of adrenergic vasoconstriction in the development of shock, a relationship between the "adrenergic" effects of endotoxins and the development of hemorrhagic or traumatic shock is strongly suggested.

Evidence of involvement of toxin in the genesis of shock. The earliest theory of shock being due to a toxemia appeared in the writings of Larrey (1812), who stated that the cause of shock lies in the injured part and secondarily affects the internal organs. However, it remained for World War I and the alarming incidence of shock associated with combat wound infections to lend the necessary impetus to investigations in this field.

Subsequent experiments by Cannon (1923) suggested that toxic products arising from a traumatized area were important factors in the genesis of shock and led to his formal proposal of a toxemia theory. When a hind limb of a cat was subjected to a selected degree of trauma, he observed a persistent decline in arterial blood pressure commencing within 20 minutes of the trauma. Exclusion of the traumatized limb from the systemic circulation by means of a tight tourniquet prevented the development of shock until the removal of the tourniquet. Factors emanating from the shocking procedure per se such as accumulation of lactic acid in the injured area or central nervous system stimulation did not appear to be directly responsible for the development of shock. In addition, the escape of fluid into the traumatized limb by hemorrhage and transudation was deemed inadequate to explain the resulting circulatory failure. Cannon therefore concluded that an unidentified toxic substance(s) from the traumatized area entered the general circulation and contributed to the development of irreversible shock.

Experiments designed to test the validity of this concept were encouraging at first. Halper and Saggart (1933), utilizing cross-circulation

techniques reported the induction of shock in normal recipient cats perfused with the blood of donor animals whose hind limbs had been traumatized. However, differences between the blood pressure of donor and recipient animals raise a doubt as to the validity of interpretations based on data from such experiments. The blood pressure of the donor animal is usually lower due to imposition of trauma. Under these conditions, free cross-circulation would result in a net efflux of blood from the normal to the traumatized cats. The consequent hypovolemia could very well be directly responsible for the development of shock in the initially normal animal. However, similar results were obtained in dogs at a later date and this objection was partially overcome by interposing a pump to maintain equivalent transfusion volumes between the normal dog and the traumatized animal (Best and Jelaudt, 1941).

Since then, and particularly during the past 20 years, such experimental effort has been directed toward identification of toxic substances in the blood of animals subjected to traumatic, hemorrhagic or burn shock. Hypotheses have been conceived to equate the shock syndrome with the effects produced by substances liberated from traumatized tissues into the blood stream or by abnormal blood and tissue levels of certain metabolites. In the broadest sense, the toxics which have been proposed fall into the categories of non-bacterial or of bacterial origin.

The former include histamine (Dale et al., 1919), vasodepressor material (VDM, ferritin) elaborated by the hypoxic liver (Shorr et al., 1931), autolysates of muscle (Turck, 1918), and products of cellular disintegration (Harrison and Blalock, 1932). However, the bulk of experimental evidence appears to negate a specific causal relationship between any one

of these and the irreversibility of shock. Important differences between histamine-induced hypotension and hemorrhagic or traumatic shock argue against a common pathway for these conditions (O'Shaughnessy and Stone, 1935). VBM has been found to be present only under specific conditions of anesthesia and blood levels of VBM (injected in the form of ferritin) which were considerably higher than those observed in hemorrhagic shock did not affect blood pressure or survival of intact or hepatectomized animals (H.A. Frank, 1952). Results with injured tissues are inconclusive at best since these tissues act as non-specific irritants which evoke large effusions at the site of implantation and thus loss of circulating plasma volume. This, in addition to electrolyte depletion, rather than generalized toxic reactions might cause a shock-like state. The advent of World War II found the toxin theory essentially unchanged from that propounded by Cannon in 1923. The possibility that the toxin might be of bacterial origin had not been investigated.

Evidence of the bacterial nature of toxin in the blood of shocked animals.

The onset of World War II gave renewed impetus to the problem of shock following bacterial infections. Attention was directed to the role of endogenous bacterial toxins in the production of experimental shock (Aub *et al.*, 1945; Pope *et al.*, 1945, Nathanson *et al.*, 1945). They analyzed the drainage fluid of isolated canine gastrocnemius preparations for the presence of organisms under strict aseptic conditions. Large numbers of bacteria, particularly *Clostridia*, were found to be present normally. Following a 5-hour period of ischemia and restoration of blood flow, the muscle exudate contained large amounts of lecithinase, an exotoxin of *Clostridia*. In addition, the preparatory surgical wounds were found to be contaminated

with Clostridia welchii. Injection of this post-ischemic exudate into normal dogs resulted in the appearance of typical signs of shock. The anaerobic nature of Clostridia led the authors to conclude that the multiplication of these organisms and the formation of toxins is facilitated by prolonged ischemia and that absorption of exotoxins via the lymphatic vessels may be a major factor in the genesis of shock.

Priametal and his associates (1944) utilizing a similar experimental design came to the same conclusions. Their procedure consisted of removing some muscle tissue from dogs, crushing it and returning it under sterile conditions. Local or systemic treatment with sulfamerazine, immediate application of a plaster cast or debridement of the wound within 17 hours prevented shock; otherwise, irreversible shock developed. However, Clostridial toxin does not appear to be necessary for the development of shock. Both traumatic and hemorrhagic shock can be produced in dogs in the absence of these organisms (Mylon and Winternitz, 1945), and in rats and rabbits, which do not harbor Clostridia during life (Schweinburg and Sylvester, 1953; Friedman et al., 1957). In addition, dogs immunized with polyvalent gas bacillus antitoxin are not protected against traumatic (Mylon and Winternitz, 1945) or hemorrhagic shock (Schweinburg et al., 1954).

By far the most provocative contributions to the bacterial toxin theory of shock have come from the work of Fine and his associates. Initial investigations showed that prior bowel sterilization by rigorous treatment with oral chlortetracycline (Aureomycin) or non-absorbable antibiotics was reported to provide a high degree of protection to dogs subjected to hemorrhagic or septic shock (S.A. Frank et al., 1952; Jacob et al., 1954; S.B. Frank et al., 1955). These results would appear to indicate either that intestinal bacteria were invading the blood stream in

greater numbers during shock and/or resistance to them or their products was greatly reduced. Failure to obtain positive blood cultures during shock was strong evidence against the former (Fine, 1958). However, Fine's group was able to detect a marked decrease in the antibacterial defenses of animals during hemorrhagic shock. They found a decrease in the capacity of the reticuloendothelial system to clear the bloodstream of injected bacteria (Schweinsburg *et al.*, 1954), a thousand-fold increase in sensitivity to the lethal effects of exogenous bacterial endotoxin (Schweinsburg and Fine, 1955) and an impairment of serum factors which are involved in the overall response to tissue injury (M.D. Frank *et al.*, 1955a; Schweinsburg *et al.*, 1956; Huttenberg and Fine, 1956). The possibility that the observed breakdown of antibacterial defenses would permit the demonstration of a circulating toxin was confirmed in 1957 by Schweinsburg and his colleagues. These investigators reported the presence of a "toxin" in the blood of animals in advanced stages of hemorrhagic shock. "Shock blood" from such animals caused death when administered to sublethally hemorrhaged animals but, in contrast to the results obtained from earlier cross-perfusion experiments (Melver and Haggart, 1923; Best and Solandt, 1941), had no obvious deleterious effects on normal animals. Havin *et al.* (1958a and b), isolated this material, which had the biological properties of a bacterial endotoxin. Accordingly, it was suggested that absorption of Gram-negative bacterial endotoxin from the ischemic intestine may contribute to irreversibility by overwhelming body defenses, specifically the reticuloendothelial system, already compromised by hemorrhage. Support for this hypothesis was provided by evidence from several sources. The critical nature of the intestinal vascular bed in the general shock situation was emphasized by the work of Lillehei (1957), who showed that prevention of ischemia of the intestine

by controlled perfusion of the superior mesenteric artery during the period of hemorrhagic hypotension markedly reduces mortality. Ravin et al. (1960) reported detectable levels of P^{32} -labelled endotoxin in the blood of irreversibly shocked animals after its oral administration. Furthermore, sub-lethal hemorrhage and blockade of the reticuloendothelial system with thorius dioxide were found to produce comparable marked increases in susceptibility of animals to the lethal effects of exogenous endotoxin (Schweinburg and Pine, 1955; Schweinburg et al., 1956; Pine et al., 1959). Conversely, induced tolerance to endotoxin increased resistance to hemorrhagic and traumatic shock considerably (Smiddy and Pine, 1957; Zweifach and Thomas, 1957). As a corollary of the above observations, Smiddy et al. (1958) implied that the salutary effects of Dibenzamine in hemorrhagic shock might be attributed to a direct anti-endotoxic action.

In contrast to the observations outlined above, Hardy et al. (1954) and Vivaldi (1954) could not demonstrate protection against hemorrhagic shock by prior administration of antibiotics to dogs and Sanford and Noyes (1958) observed no increased absorption of orally administered Cr^{51} -labelled endotoxin from the bowel of dogs subjected to irreversible hemorrhagic shock. In addition, the latter investigators could not demonstrate an increased sensitivity of their hemorrhaged animals to the lethal effects of exogenous endotoxin, and Greisman (1960) felt that the increased sensitivity he observed could be attributed to factors ancillary to the actual shock procedure.

Purpose and scope of the investigation. Irrespective of the specific relation of bacterial endotoxin to the causation of irreversibility in hemorrhagic shock, present evidence permits two important conclusions. First, a

toxin may be present in the blood of animals subjected to irreversible hemorrhagic shock. Available information indicates that this toxin is derived from the lipopolysaccharide fraction of the cell wall of Gram-negative bacteria. Indirect evidence (Zweifach and Thomas, 1957) suggests that a similar toxin is present in animals subjected to traumatic shock. Second, administration of adequate doses of endotoxin or infection with Gram-negative organisms (Gilbert et al., 1954; Lillshel and MacLean, 1958) can itself induce a shock syndrome comparable to that produced by prolonged hemorrhagic hypotension.

Several observations suggest that the deleterious effects of bacterial endotoxin may involve the sympathetic nervous system. Responses to exogenous endotoxin include increased vasconstriction, which can be inhibited by dibenamine or dihydroergotamine (Bequet et al., 1947; Bequet and Izard, 1950; Zweifach et al., 1956) and the Shwartzman phenomenon, peculiar to endotoxin, which also appear to have an important adrenergic component (Thomas, 1956; Hall and Kelly, 1957).

In view of a priori evidence indicating an adverse role of adrenergic vasoconstriction in the development of shock, it may reasonably be postulated that the deleterious effects of endotoxin are not, as Fine has proposed, due to a direct toxic effect on tissues concerned with antibacterial defenses whose activity is depressed by shock, but rather are secondary to an increased responsiveness to adrenergic stimuli, which compromises blood flow to critical vascular beds still further. In order to assess more completely the role of adrenergic factors in the effects of bacterial endotoxins we have investigated the effects of bacterial endotoxin on responses of intact animals and of isolated vascular smooth muscle to adrenaline and to noradrenaline, and the effects of pretreatment

with reserpine or phenoxybenzamine on the acute lethality of bacterial endotoxin.

Methods

Pressor responses to adrenaline and noradrenaline. Twelve female albino rabbits weighing 1.5 to 2.5 kg were given 10 mg (total dose) of crude E. coli endotoxin (Group I) or 60 µg/kg of purified E. coli endotoxin (Bifco Laboratories 0127:85) (Group II) intravenously. Preliminary experiments demonstrated that these doses were adequate to prepare rabbits for the generalized Shwartzman reaction^{*}. Eighteen to 24 hours later, the animals were anesthetized with barbital sodium (350 mg/kg intraperitoneally). Blood pressure was recorded from a femoral artery by means of a pressure transducer and oscillograph. Graded doses of adrenaline and noradrenaline were administered intravenously as saline solutions of the bitartrate salts. An equal number of similar, but untreated animals served as controls.

In vitro responses of isolated vascular smooth muscle. Spirally-sectioned strips of aortas from freshly-killed rabbits were suspended in Krebs-Ringer solution alone or containing, 10, 20 or 50 per cent autologous whole blood or 10 per cent cells or plasma. The bath was kept at 37°C and was aerated with 5 per cent CO₂-95 per cent O₂. Isotonic contractions were recorded under appropriate loading. After a stable response to a standard concentration of adrenaline (5×10^{-7} g/ml) was obtained, a six-point adrenaline dose-response curve was determined using a range of concentrations from $3 \times$

^{*} Preparing and provoking doses of endotoxin were administered intravenously, usually 18 hours apart. The reaction which follows the provoking dose consists of extensive hemorrhagic necrosis of internal organs, the most characteristic of which is bilateral cortical necrosis of the kidneys (Shwartzman, 1937).

10^{-5} to 1×10^{-6} g/ml. The strips then were exposed to 1×10^{-6} g/ml purified E. coli endotoxin in the same medium as that employed during the control period. After a 20-minute exposure to endotoxin, the dose-response curve for adrenaline was redetermined. The strips were washed between exposures to adrenaline and endotoxin was present in the bath fluid between and during tests. In other experiments the response to a single selected dose of adrenaline was determined following a 20-minute exposure to endotoxin (1×10^{-6} to 8×10^{-6} g/ml) and was compared with control responses obtained before exposure to and 20 minutes after removal of the endotoxin.

Acute lethality of endotoxin. Female albino rabbits (1.2 to 2.5 kg) and Swiss albino mice (18 to 24 g) were used throughout. All agents were administered intraperitoneally to mice and intravenously to rabbits. Where possible (see Results), the LD₅₀ was determined according to the method of Litchfield and Wilcoxon (1949).

Reserpine pretreatment. Reserpine was administered to 44 rabbits and 20 mice in doses of 3.0 mg/kg and 1.0 mg/kg, respectively. Twenty-four hours later the rabbits were divided into 8 groups, and the mice into 5 groups each of which received one of a series of graded doses of purified E. coli endotoxin. Control animals to which the reserpine vehicle had been administered previously were injected with endotoxin at the same time.

Phenoxybenzamine pretreatment. Fifteen rabbits were injected with 3.0 mg/kg phenoxybenzamine and an equal number received the vehicle as control. One hour later groups of 5 animals each received one of a series of graded doses of purified E. coli endotoxin. Twenty-four mice were injected with 6.0 mg/kg and 45 with 18 mg/kg of phenoxybenzamine. Twenty-four hours

later, each series was divided into 4 groups, each of which received one of a series of graded doses of purified E. coli endotoxin. Thirty and 45 mice, respectively, received the phenoxybenzamine vehicle and served as controls for these experiments.

Incubation experiments in vitro. The reaction mixture contained: 22.5 mg of phenoxybenzamine and 22.5 mg of purified E. coli endotoxin in 3.75 ml of 50 per cent ethanol-water. Sodium bicarbonate (27.5 mg) was added to keep the medium slightly alkaline, and thus permit rapid transformation of phenoxybenzamine to the reactive ethyleniminonium (Harvey and Nickerson, 1953). The mixture was stirred slowly at room temperature for 18 hours, at which time little or no active phenoxybenzamine could remain (Harvey and Nickerson, 1953). At the end of the incubation period, 4 groups of 6 mice each were injected with graded doses of the solution. Equal numbers of animals were injected with the same doses of control solution prepared and incubated as above except that phenoxybenzamine was omitted.

Results

Pressor responses to adrenaline and noradrenaline. Pressor responses of Group I control and endotoxin-pretreated animals to adrenaline and noradrenaline are shown in Figure 3. Responses of the endotoxin-pretreated rabbits were significantly greater ($0.02 > P > 0.01$) than those of the controls at each dose level of both agents. However, a significant difference ($0.05 > P > 0.04$) in the basal blood pressures of control and test rabbits (79 ± 6 vs. 60 ± 6 mm Hg, respectively) raised the question of a possible causal relationship between the lower blood pressure in the experimental animals and the greater pressor responses to adrenaline and

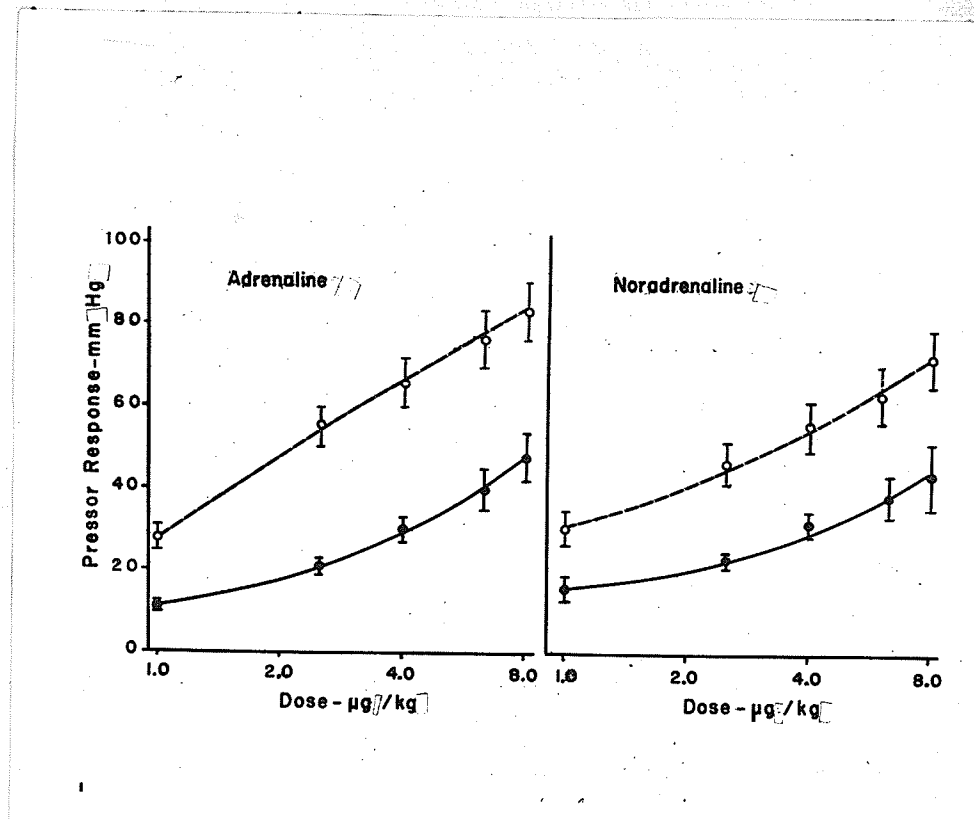


Figure 5

Pressor responses to adrenaline and noradrenaline in rabbits. Each point represents the mean and standard error of the responses of 6 control animals (solid circles) or of 6 animals pretreated with crude E. coli endotoxin (open circles).

noradrenaline. Although analysis of data for individual experiments revealed no correlation between these variables, the experiment was repeated in 12 additional rabbits (Group II), using purified rather than crude endotoxin. In this series, the animals were tested with adrenaline only. The results (Figure 6) show the pressor responses again to be significantly increased ($0.05 > P > 0.04$) over those of the controls. The basal blood pressures of experimental and control animals in Group II were essentially the same (62 ± 3 and 61 ± 5 mm Hg, respectively), confirming that the different pressor responses recorded from animals in Group I were not due simply to the different basal pressures.

In vitro responses of isolated vascular smooth muscle. Exposure of isolated strips of rabbit aorta to 1×10^{-6} g/ml purified *E. coli* endotoxin in Krebs-Ringer solution for periods of up to a total of 4 hours did not alter the adrenaline dose-response curve. Increasing the concentration of endotoxin to 2.5×10^{-5} g/ml and prolonging the exposure to 2 hours between tests also failed to alter the response.

Responses to adrenaline were somewhat reduced when 10 per cent or more of whole blood or blood cells was added to the Krebs-Ringer solution. Addition of 1×10^{-6} g/ml purified *E. coli* endotoxin to either of these media resulted in a definite increase in responses to adrenaline over most or all of the dose-response curve (Figure 7). The differences are more obvious in experiments employing 10 per cent blood cells and in both, the differences are somewhat greater with the higher doses of adrenaline. Addition of 10 per cent plasma, with or without endotoxin, to the Krebs-Ringer solution did not alter the responses to adrenaline.

The effect of endotoxin in a blood-containing medium was more clearly defined in experiments in which the response to a single concentra-

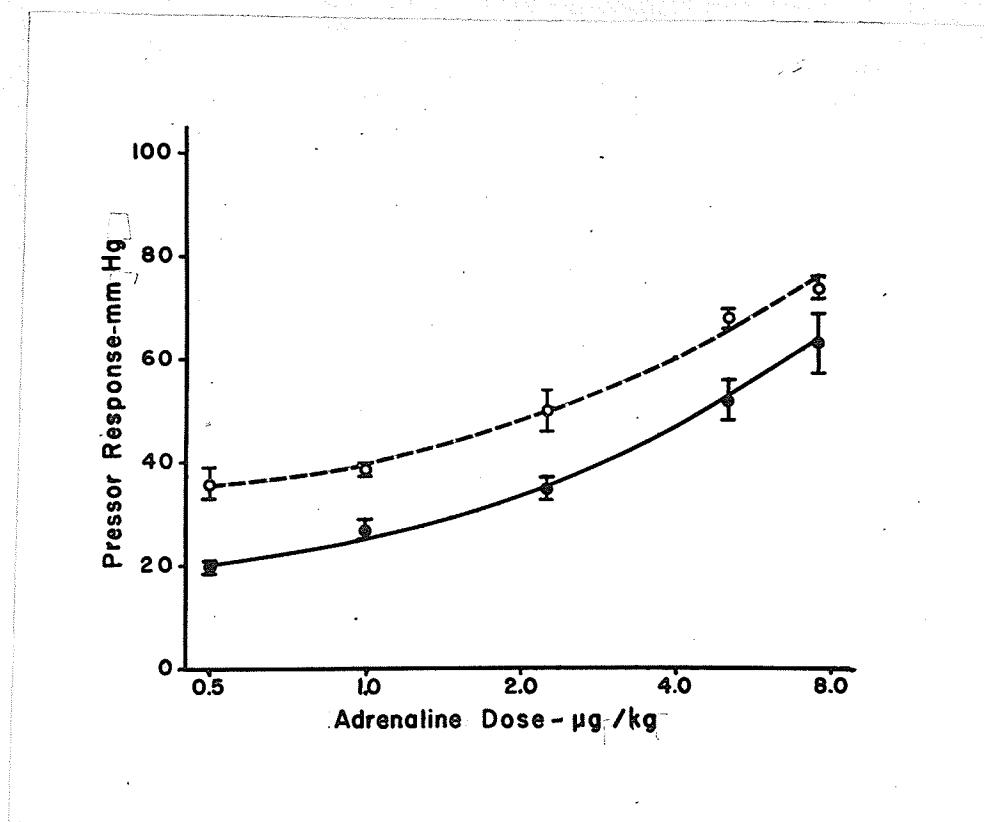


Figure 6

Pressor responses to adrenaline in rabbits. Each point represents the mean and standard error of the responses of 6 control animals (solid circles) or of 6 animals pretreated with purified *B. coli* endotoxin (open circles).

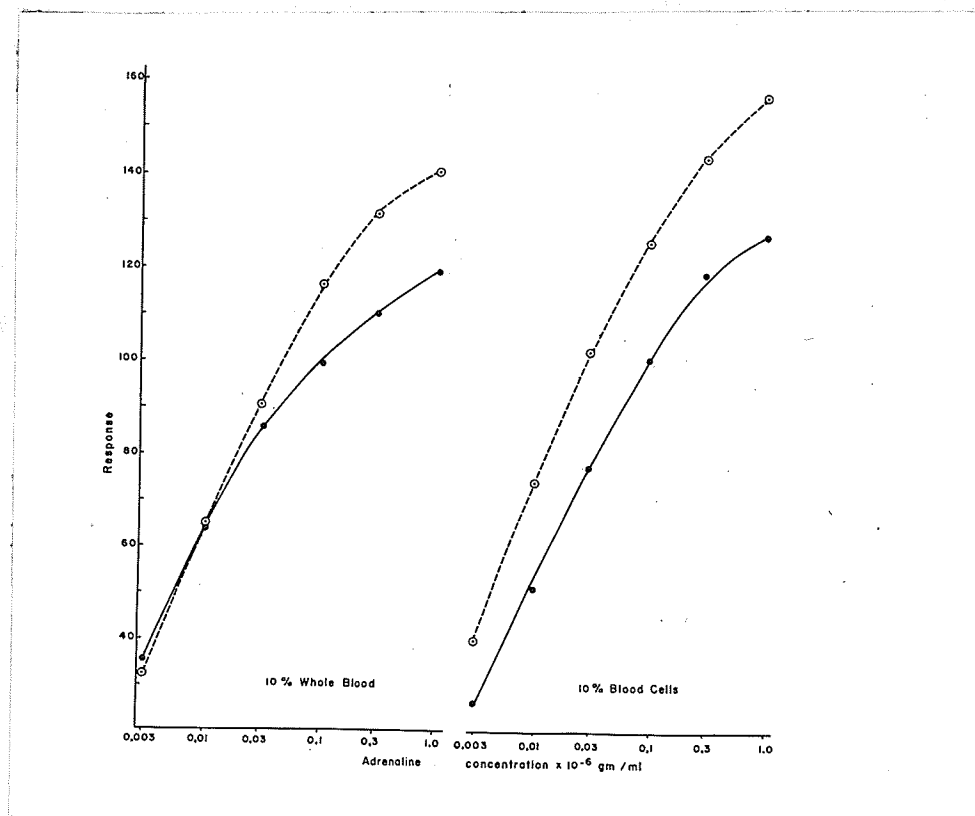


Figure 7

Dose-response curves of isolated rabbit aorta strips to adrenaline in Krebs-Binger solution containing 10 per cent autologous whole blood or 10 per cent blood cells, with (open circles) or without (solid circles) 1×10^{-6} g/ml purified *E. coli* endotoxin. Each point represents the mean of determinations on 5 separate strips. All responses expressed in arbitrary units relative to the control responses of each strip.

tion of adrenaline in the presence of endotoxin was compared directly with immediately preceding and following control responses. In every one of 30 tests on strips from 10 animals the response in the presence of endotoxin was greater ($P < 0.001$). The increase due to endotoxin averaged 18 per cent and ranged from 6 to 46 per cent. Within the ranges studied, 1 to 8×10^{-6} g/ml endotoxin and 10 to 90 per cent blood, there appeared to be no correlation between the concentrations of these materials and the magnitude of the augmentation by endotoxin.

Acute lethality of endotoxin. Mortalities after 48 hours in the various experimental and control groups are recorded in Tables 9 to 13. In 2 of the series (Tables 9 and 10) mortalities tended toward a bimodal distribution, which precluded comparison of the control and treated groups on the basis of LD₅₀ values calculated from dose-response curves. However, the relatively even distribution of animals between experimental and control groups at comparable doses of endotoxin made it possible to employ a Chi-square analysis of the composite data. (The large group of control rabbits given 200 µg/kg of endotoxin in the experiments summarized in Table 9 would weigh against the significance of a difference).

Pretreatment with reserpine provided significant ($P < 0.01$) protection against the lethal effects of *E. coli* endotoxin in rabbits (Table 9) and increased somewhat the survival rate in mice (Table 10), although the difference in this species was not statistically significant ($0.1 > P > 0.05$).

Phenoxylbenzamine afforded significant protection ($0.05 > P > 0.04$) in rabbits (Table II). Pretreatment with 6.0 mg/kg did not protect mice against endotoxin administered 24 hours later. However, 18 mg/kg

Table 9

Effect of Reserpine Pretreatment on Lethality of
E. Coli Endotoxin in Rabbits

| Dose Endotoxin - $\mu\text{g/kg}$ | Deaths/Total No. Injected | |
|-----------------------------------|---------------------------|------------|
| | Control | Pretreated |
| 60 | 2/5 | 0/5 |
| 90 | 0/5 | 1/5 |
| 110 | 2/5 | 0/5 |
| 135 | 3/5 | 0/5 |
| 200 | 1/13 | - - |
| 250 | 4/10 | 2/5 |
| 315 | 5/10 | 4/9 |
| 400 | 5/5 | 0/5 |
| 500 | 5/5 | 0/5 |
| Total | 27/63 | 7/44 |

Table 10

Effect of Reserpine Pretreatment on Lethality of
E. coli Endotoxin in Mice

| Dose Endotoxin - mg/kg | Deaths/Total No. Injected | |
|------------------------|---------------------------|------------|
| | Control | Pretreated |
| 10 | 3/6 | 3/6 |
| 12.5 | 3/6 | 1/6 |
| 20.0 | 4/6 | 1/6 |
| 32.5 | 4/6 | 2/6 |
| 50.0 | 6/6 | 6/6 |
| Total | 20/30 | 13/30 |

doubled (44.3 vs. 22.3 mg/kg) the LD₅₀ of E. coli endotoxin in this species ($P < 0.001$) (Table 12).

The LD₅₀ of the incubated phenoxybenzamine-endotoxin mixture in mice was not different from that of the control endotoxin solution, indicating that phenoxybenzamine did not inactivate E. coli endotoxin by any reaction demonstrable in vitro (Table 13).

Discussion

The results reported above demonstrate that appropriate pretreatment with E. coli endotoxin can increase the pressor response to adrenaline and noradrenaline in intact rabbits, and that exposure to endotoxin in a medium containing whole blood or the cellular elements of blood can augment the response of rabbit aorta strips to adrenaline in vitro. Zweifach et al. (1956) demonstrated previously an increased sensitivity of blood vessels in the rat mesenteric to topically-applied adrenaline following administration of endotoxin. These results and the previously mentioned observation that adrenergic blocking agents effectively inhibit the increased vasodilation induced by endotoxin (Boquet et al., 1947; Boquet and Izard, 1950; Zweifach et al., 1956) and the Sherrington phenomena (Thomas, 1936; Hall and Kelly, 1957) strongly suggest that these effects of endotoxin are mediated through or are highly dependent on peripheral adrenergic mechanisms.

It is of interest that the addition of endotoxin to a bath fluid containing whole blood or blood cells increases responses of rabbit aorta strips to adrenaline, whereas it was entirely without effect when added to plain Krebs-Ringer solution, or to solution containing plasma. These results may help to explain the previously reported failure to dem-

Table II

Effect of Phenoxybenzamine Pretreatment on Lethality of
E. coli Endotoxin in Rabbits

| Dose Endotoxin - $\mu\text{g/kg}$ | Deaths/Total No. Injected | |
|-----------------------------------|---------------------------|------------|
| | Control | Pretreated |
| 315 | 9/10 | 1/5 |
| 400 | 5/5 | 2/5 |
| 500 | 3/5 | 2/5 |
| Total | 17/20 | 5/15 |

Table 12

Effect of Phenoxybenzamine Pretreatment on Lethality of

E. coli Endotoxin in Mice

| Dose Endotoxin - mg/kg | Deaths/Total No. Injected | | |
|--------------------------|---------------------------|---------------|---------------|
| | Control | Pretreated | |
| | | 6 mg/kg | 18 mg/kg |
| 10.0 | 2/12 | 2/6 | 0/6 |
| 12.5 | 3/12 | 0/6 | 1/6 |
| 20.0 | 7/12 | 3/6 | 0/6 |
| 30.0 | 7/12 | 3/6 | 0/6 |
| 40.0 | ----- | ----- | 5/6 |
| 53.0 | ----- | ----- | 4/6 |
| 70.0 | ----- | ----- | 5/6 |
| LD ₅₀ - mg/kg | 22.5 | 18.0 | 44.3 |
| (95% Conf. Limits) | (17.1 - 29.7) | (12.0 - 27.0) | (34.7 - 56.5) |

Table 13

Effect of In Vitro Incubation with Phenoxylbenzamine on
Letthality of E. Coli Endotoxin in Mice

| Dose Endotoxin - mg/kg | Deaths/Total No. Injected | |
|--------------------------|---------------------------|----------------------------------|
| | Control | Incubated with Phenoxylbenzamine |
| 10.0 | 1/6 | 0/6 |
| 12.5 | 4/6 | 1/6 |
| 15.0 | 3/6 | 3/6 |
| 30.0 | 3/6 | 6/6 |
| LD ₅₀ - mg/kg | 20.0 | 22.0 |
| (95% Conf. Limits) | (12.1 - 33.2) | (16.3 - 39.7) |

enhancement of responses to adrenaline by endotoxin in vitro in a simple salt medium (Vargas and Bach, 1957), and are entirely compatible with the observation of Hinchaw et al. (1957), who found that the isolated canine lung failed to respond to endotoxin when perfused with gelatin or dextran solutions, but did so during whole-blood perfusion. The nature of the interaction between endotoxin and blood elements to enhance responses to adrenergic stimuli is unknown, but the dependence of increased reactivity on multiple factors may explain the failure of others to demonstrate vasoconstriction or increased reactivity to adrenaline in isolated limbs of dogs, (Gilbert and Gordon, 1956; Hinchaw et al., 1958), and the contradictory results obtained with perfused rabbit ears (Zemifach et al., 1956; Meyer and Ballin, 1959).

Several workers have observed that adrenergic blocking agents may protect against endotoxin toxicity. Lillchaj and Maclean (1958) found that phenoxybenzamine or chlorpromazine markedly increased the survival rate of dogs given E. coli endotoxin, and ergotamine has been reported to protect dogs against many of the deleterious effects of Shigella endotoxin (Fenner and Bernheim, 1943). The failure of Hayes et al. (1956) to demonstrate protection by phenoxybenzamine against lethal doses of E. coli endotoxin in mice probably can be attributed to the relatively small doses administered intraperitoneally, which were inadequate to maintain a substantial adrenergic blockade after the time interval employed. Conversely, administration of the pressor drug metaraminol (Aramine) has been found to potentiate shock due to E. coli endotoxin (Lillchaj and Maclean, 1959). It is of particular interest in this regard that reserpine has been reported to increase the toxicity of endotoxin administered 1 hour later (Tauber and Carson, 1958), at a time when the reserpine-

induced release of catecholamines is near its peak.

Protection against endotoxin toxicity by pretreatment with reserpine or phenoxybenzamine appears to be attributable to alteration of contributing adrenergic mechanisms rather than to any direct anti-endotoxic action. Reserpine disappears rapidly from body fluids (Sheppard et al., 1955) and phenoxybenzamine is known to react rapidly at body pH to form inactive products (Harvey and Nickerson, 1953). Consequently, it is unlikely that appreciable amounts of either of these agents were present 24 hours after their administration, at the time of challenge with endotoxin. However, with the doses employed, inhibition of responses to adrenergic nerve activity due to peripheral catecholamine depletion or α -receptor blockade would persist. In addition, phenoxybenzamine failed to alter the toxicity of endotoxin under conditions of in vitro incubation favorable for its reaction with materials found in biological systems (Harvey and Nickerson, 1954).

Although the effects of reserpine on adrenergic mechanisms are more diffuse, phenoxybenzamine very specifically inhibits adrenergic responses subserved by α -receptors (Nickerson and Chan, 1956). The only such response which is of major importance to the body economy is vasoconstriction, and it is reasonable to assume that inhibition of endotoxin-potentiated adrenergic vasoconstriction is involved in the observed protection.

Summary

The possible involvement of adrenergic mechanisms in the effects of Escherichia coli endotoxin was investigated in several preparations. Appropriate pretreatment of rabbits with E. coli endotoxin significantly increased pressor responses to adrenaline and noradrenaline as compared

to untreated controls. Exposure of isolated rabbit aorta strips to E. coli endotoxin in a medium containing whole blood or cellular constituents of blood significantly increased the response to adrenaline. Endotoxin had no effect on responses to adrenaline in vitro when plain Krebs-Hinger solution was used.

Pretreatment with reserpine or phenoxybenzamine protected rabbits and mice against the acute lethal effects of E. coli endotoxin. The time period intervening between reserpine or phenoxybenzamine administration and challenge by endotoxin precluded a direct anti-endotoxic action of these agents. In addition, incubation of phenoxybenzamine with endotoxin in vitro, under conditions which would favor reaction, did not decrease the toxicity of the latter.

These results indicate that peripheral adrenergic mechanisms are intimately involved in the effects of E. coli endotoxin and support the concept that deleterious effects of endotoxin in shock probably are due to exaggeration of existing vasoconstriction in an already compromised organism.

PART VII

TOTAL BODY AND REGIONAL UPTAKE
OF RADIOACTIVE IODINE ADMINISTERED SINCE
INDUCED IN 1955 BY HYPERTHYROIDISM AND
BY PROLONGED INFUSIONS OF TRIAMTERALINE

Introduction

The concept that reduced effective circulating blood volume complicated by excessive vasoconstriction collaborate to produce a critical reduction in effective blood flow through certain important body organs during the development of shock has been the principal tenet of this thesis. Review of the literature has emphasized the considerable effort which has been made to elucidate the circulatory derangements accompanying shock. Measurement of blood flow per se has been complicated by technical problems which have clouded the significance of conclusions obtained from such experiments. Introduction of rotameters, bubble flowmeters and bristle flowmeters into the circulation can disrupt cardiovascular homeostasis considerably.

During the development of our program, a method of blood flow measurement was reported which appeared to offer a new and promising approach to the problem of blood flow measurement. This technique, first described by Sapirstein in 1956 and later expanded by this investigator (1958), depends on the rapid uptake of radioactive potassium or rubidium by body tissues.

A foreign substance, after a single intravenous administration, will be distributed initially to the organs in proportion to their blood flow. The substance will then be carried away from the organs by their venous drainage. However, if uptake of the material by the tissues approaches 100 per cent, the venous drainage will be small relative to the arterial delivery for a certain period of time. During this time, the fractional distribution of the material among the organs will correspond to the fractional distribution of the cardiac output among them. The time

will be greatest for substances which are most completely transferred from the vascular system to the tissues and which have a large volume of distribution within the tissues. Radioactive potassium (K^{42}) is such a substance.

Sapirstein (1936) proceeded to show that K^{42} is cleared from the blood of rats within seconds after administration. The fraction of the tracer present in most tissues (brain excluded) remained constant between 5 and 60 seconds after injection. He concluded that the early distribution of K^{42} reflected the distribution of the cardiac output.

This method offered the attractive possibility of measuring total organ blood flow in animals during the development of shock without the extensive surgery which the use of mechanical flow-meters requires. Our plan initially was to measure total body blood flow in unshocked animals and to compare the distribution of the cardiac output in these animals with that in animals subjected to progressive degrees of hemorrhagic stress. In our experiments, the more convenient rubidium (Rb^{86}) was substituted for K^{42} . Previous investigators (Sapirstein, 1936 and Love et al., 1934) have demonstrated that Rb^{86} exchanges freely with intracellular potassium in a manner very similar to that of K^{42} .

Methods

1) Controls: Four dogs, anesthetized with pentobarbital sodium, were prepared according to our standard procedure for producing hemorrhagic shock (Part IV). Before bleeding was started, $Rb^{86} Cl^*$ was injected rapidly

* Radioactive rubidium (Rb^{86}) was obtained from Abbott Pharmaceuticals, Oak Ridge, Tennessee, as a sterile solution of the chloride salt. The material was of high specific activity (257 to 611 millicuries/gram). Appropriate dilutions of the stock material were made with 0.9 per cent sodium chloride solution.

through a PE 60 polyethylene catheter passed into the inferior vena cava via a femoral vein. The dose of tracer was accurately determined and varied between 5 and 10 microcuries. During the ensuing 2 minute period, serial femoral arterial samples were collected through a PE 260 polyethylene catheter 15 to 20 cm in length for determination of cardiac output by the isotope dilution method. The blood passed into a sample collector which permitted 30 collections per minute. The bleeding rate was adjusted to provide approximately 1 ml/sample. At the end of this time, the animals were killed rapidly by an intravenous injection of saturated KCl which produced cardiac arrest within 3 seconds. The viscera, heart and samples of muscle and skin were removed and rinsed with 0.9 per cent NaCl to remove excess blood. The organs were weighed, placed in individual Mason jars and digested in 6N H_2SO_4 under 20 psi steam pressure for 3 hours. Five ml aliquots of each organ digest were analyzed in a well-type scintillation counter. One-half ml aliquots of the blood samples were counted for the determination of cardiac output.

2) Experimental: Four dogs, anesthetized with pentobarbital sodium were subjected to varying portions of our standard hemorrhagic shock procedure. Two animals underwent the entire shock procedure. One hour after reinfusion of the shed blood (normovolemic shock), ^{86}Cl was administered intravenously. Two dogs were carried into the "clamp-off" period. When their blood pressure fell below 45 mm Hg, a small sustaining blood transfusion (2 ml/kg) was administered. At this point of initial cardiovascular collapse, ^{86}Cl was administered intravenously. The procedures following administration of the tracer in these animals were the same as those outlined for the controls.

3) Calculation of cardiac output and organ blood flow. The curves employed in the calculation of cardiac output were obtained from the amounts of radioactivity contained in each of the successive blood samples removed during the 2-minute period after the injection of indicator. The observed concentrations of indicator in the arterial blood were summed up to the time of first recirculation (identified as the first recognizable deviation from rectilinearity on a semi-logarithmic plot of concentration). To this value was added the sum of the converging geometric series of values extrapolated from the rectilinear descending limb of the curve. The sum of the determined and extrapolated concentration values was used as the denominator in the usual equation for the calculation of the cardiac output.

The indicator content of dog organs was determined from an aliquot of a digest of the organ and expressed as a fraction of injected radioactivity. Blood flow (ml blood/minute/gram of tissue) was then calculated from the product of the percentage of injected tracer and cardiac output divided by the weight of the organ.

Results

1) Control: The distribution of the cardiac output in the 4 animals (Table 14) paralleled very closely that reported by Sapirostein (1936). Cardiac index averaged 3.93 ± 0.25 l./sq M/min which was somewhat higher than a previously reported mean value (Spector, 1936), but was within the wide range of values reported in this compendium (1.97-4.18 l./sq M/min). Total skin and muscle ^{86}Sr uptake was calculated on the basis of previously reported values for the total mass of these tissues in dogs (Kelton,

Table 14
⁸⁶
 Fractionation of Rubidium and Organ Blood Flow in
 Intact, Anesthetized Dogs

| Organ | Per cent of
Injected ⁸⁶ Rb | Blood Flow
ml/g/min |
|-------------------|--|------------------------|
| Skin | 17.9 ± 3.8 | 0.20 ± 0.03 |
| Spleen | 1.08 ± 0.09 | 0.65 ± 0.11 |
| Pancreas | 1.04 ± 0.18 | 0.87 ± 0.05 |
| Stomach | 3.17 ± 0.62 | 0.67 ± 0.11 |
| Duodenum | 1.76 ± 0.24 | 0.96 ± 0.11 |
| Ileum and Jejunum | 9.25 ± 0.62 | 0.96 ± 0.07 |
| Colon | 2.24 ± 0.26 | 1.00 ± 0.12 |
| Kidneys | 11.27 ± 1.7 | 3.62 ± 0.69 |
| Liver | 8.79 ± 1.3 | 0.49 ± 0.06 |
| Heart | 4.30 ± 0.49 | 1.13 ± 0.06 |
| Skeletal muscle | 19.45 ± 5.3 | 0.08 ± 0.02 |

1937). The former was taken as 15.11 per cent and the latter as 42.84 per cent of body weight. The tracer was cleared rapidly from the blood stream (Figure 8) and only negligible amounts were present in the blood at the time of sacrifice.

3) Experimental: In the shocked animals, especially those in hypovolemic shock, tissue content of Rb^{86} was low and clearance of the isotope was delayed (Figure 8), a considerable amount of the injected total being present in the bloodstream at the time of sacrifice. Under these circumstances calculation of absolute values for the fractionation of the injected tracer and for organ blood flow was impossible. The isotope content of the tissues of these dogs, although probably a function of blood flow, nevertheless was a measure of only relative extractions. The relative values for various organs are presented in Figure 9. The heights of these columns represent the relative effective blood flows of the various organs during hypovolemic and normovolemic shock, expressed as a percentage of the blood flow for each organ in control dogs (see Table 14). Quantitative comparisons are possible only between the organs of any one animal. The values for spleen, pancreas and gastrointestinal tract have been combined under the heading of "viscera".

During hypovolemic shock cardiac output was only 25 per cent of the control value. Coronary blood flow was maintained at a relatively higher level than that of other organs. Flows to the skin, kidneys and viscera were most markedly reduced. Restoration of blood volume improved both cardiac output and blood flow. However, the former remained about 45 per cent below the control value. Liver blood flow appeared to increase much more than that to other tissues. However, this increase may be more

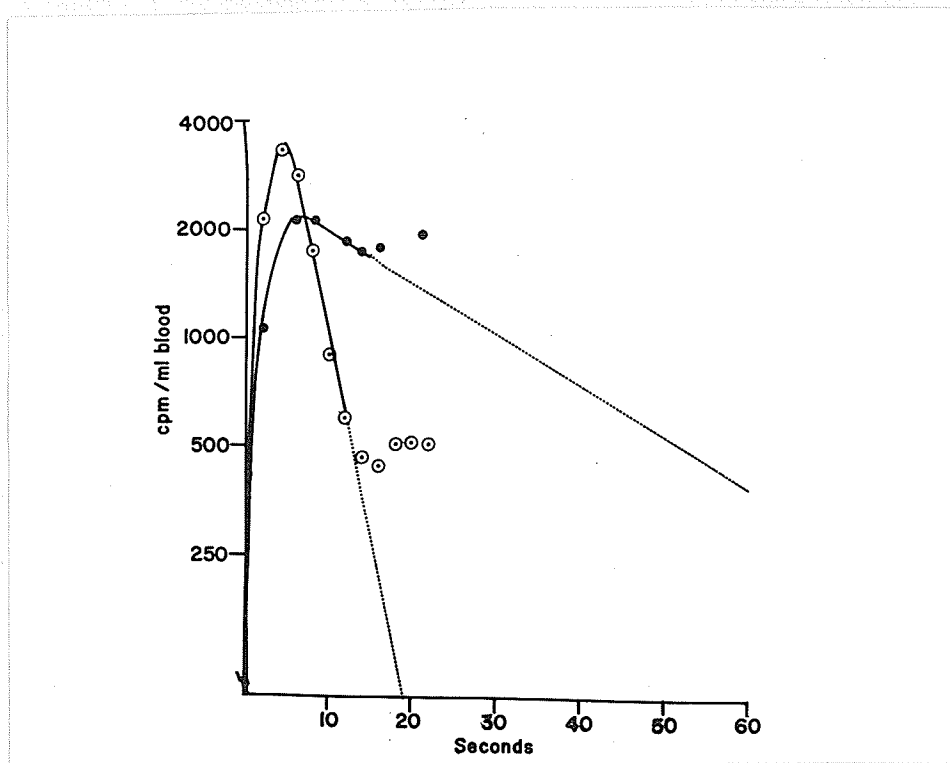


Figure 2

Semi-logarithmic plots of the arterial concentrations of ^{51}Cr following intravenous administration in a normal dog (open circles) and in a dog during severe hypovolemic shock (closed circles). The tracer appeared in the arterial blood of the normal dog 5 seconds after injection, whereas its appearance was delayed to 21 seconds in the shocked animal.

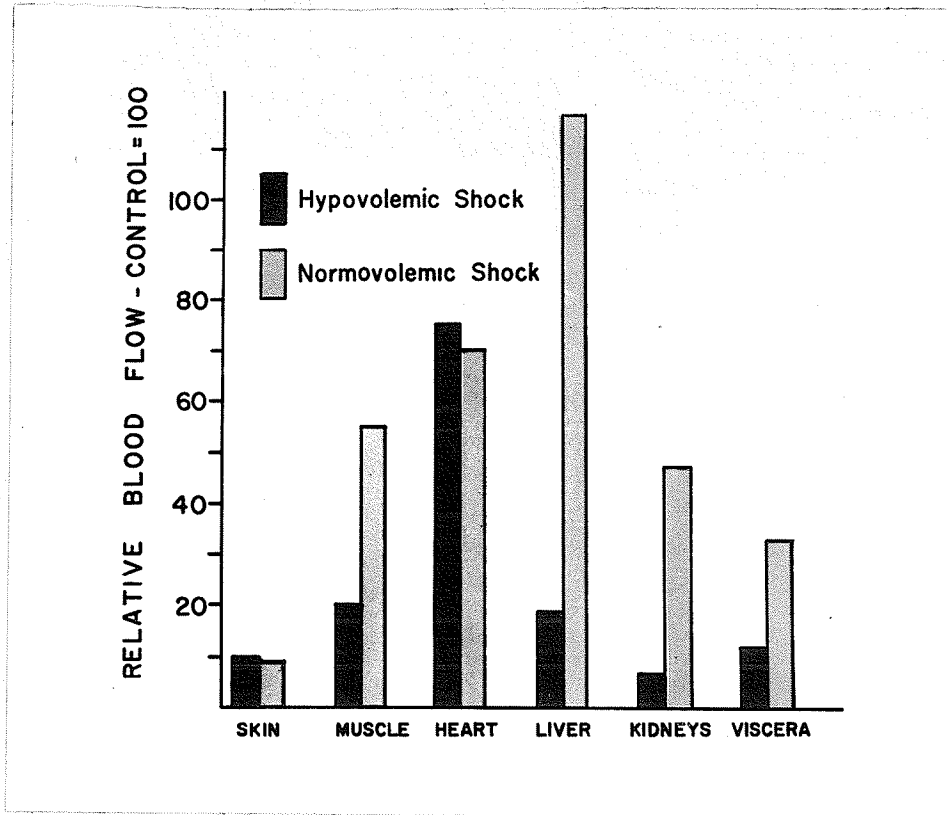


Figure 9

Relative extraction of Rb^{86} by various organs during severe hypovolemic and normovolemic shock. Relative blood flow expressed in terms of normal values for organ blood flow.

apparent than real, a point which will be discussed further. Coronary blood flow did not change appreciably after reinfusion.

Discussion

The Sapirstein method for measuring the distribution of the cardiac output is based on the fact that the uptake of an indicator by an organ can be described as the product its blood flow, the integrated arterial concentration of the tracer, and the organ's extraction ratio for the indicator. As used here, the extraction ratio is defined as the ratio between the integrated arteriovenous concentration difference and the integrated arterial concentration from the moment of injection of tracer to the moment under consideration. Since the arterial blood concentrations which supply each organ are essentially equal it follows that organ uptake is related to whole body uptake as is organ blood flow to cardiac output, provided that every organ has the same extraction ratio.

If indicators which did not recirculate at all were available, the extraction ratio of every organ would be 1.0 and the uptake of each organ would describe its blood flow exactly. At the present time no such indicator is known. For indicators which do recirculate, with each recirculation, those organs which have higher extraction ratios accumulate tracer, while those which have lower extraction ratios accumulate more slowly or lose indicator. The fact that organs (other than brain) showed a constant K^{42} content over the period of 30-120 seconds after administration of the tracer was interpreted by Sapirstein (1956) to indicate that no recirculation of indicator occurred and that the extraction ratios approximated 1.0. One observation in particular makes this interpretation untenable. The lin-

ear extrapolation of the descending limb of the arterial concentration curve (Figure 8) describes the arterial concentration in the absence of recirculation. Displacement of the true curve above this extrapolated line must be due to recirculating isotope. The amount of isotope recirculating can be estimated by multiplying the cardiac output by the area enclosed between the true and extrapolated curves. The data of Sagirstein (1936) in fact, show that 33 to 46 per cent of the injected dose undergoes arterial recirculation. This suggests incomplete extraction of K^{42} or the presence of anatomical shunts in at least some organs. Tracer which was not retained by tissues during the first passage is returned to the arterial system and redistributed.

Under these circumstances, organs with higher than average extraction ratios would accumulate tracer in excess of their fraction of the cardiac output, while those with lower extraction ratios would have a correspondingly lower uptake. However, two observations suggest that the extraction ratios of various organs (brain excluded) are very similar. Sagirstein (1936, 1938) observed that the K^{42} or Hb^{86} content of various organs reached a plateau within 30 seconds after administration which persisted for at least an additional 90 seconds. If interorgan extraction ratios were unequal, a progressive rise in Hb^{86} or K^{42} content would have occurred during this period in organs with higher and a progressive fall in those with lower extraction ratios. However, these observations do not reflect events during the first few recirculations of isotope. The period 30-120 seconds after tracer administration probably reflects mainly the exchange of Hb^{86} or K^{42} accumulated by the cells with potassium in interstitial fluid. In view of the small amount of tracer relative to the large reex-

voir of intracellular potassium, the absolute interorgan shift of tracer may have been too small to produce gains or losses which could be measured accurately by this technique. A second argument for similar extraction ratios is found in the work of Sheppard *et al.* (1953), who found that 1 minute after the injection of K^{42} in dogs, the integrated venous concentration of the renal, femoral and hepatic veins differed from each other by less than 10 per cent of the integrated arteriovenous concentration differences at the same time. This implies that the clearance ratios of these vascular beds must differ from each other only slightly.

Thus, in the absence of direct measurements of the relative extraction ratios of various vascular beds, one can only assume that the interorgan distribution of injected Rb^{86} reflects the fractionation of the cardiac output.

The question of relative interorgan extraction ratios becomes more important when our data for shocked animals are considered. In these animals, the clearance of injected Rb^{86} from blood was delayed considerably. This observation per se is important in evaluating cardiovascular dynamics during shock. Two hypotheses could explain this finding. There might be cellular damage which prevented the usual rapid exchange of tracer between extra- and intracellular loci, or alternatively, there might be a defect in the delivery of Rb^{86} to various organs or parts of organs so that the tracer available for exchange with intracellular potassium per unit time was reduced. In order to differentiate between these possibilities, the disappearance of Rb^{86} from the bloodstream was compared with the rate of equilibration of sodium thiocyanate, a substance which equilibrates with extracellular fluid but does not enter cells to an appreciable degree.

Methods

Nineteen dogs, anesthetized with pentobarbital sodium, were subjected to the following progressive degrees of hemorrhagic shock.

Group I - (4 dogs) - control

Group II - (3 dogs) Bled until the blood pressure fell to 35 mm Hg and maintained at this level for 30 minutes prior to testing.

Group III - (3 dogs) Similar to Group II except that all shed blood was reinfused at the end of the 30-minute period of hypovolemia. They were tested 1 hour after reinfusion.

Group IV - (3 dogs) After the 30-minute period at 35 mm Hg, the blood pressure was raised to 60 mm Hg by a small transfusion. The animals were maintained at this level for 30 minutes prior to testing.

Group V - (3 dogs) Similar to Group IV except that the shed blood was reinfused at the end of the 30-minute period at 60 mm Hg and the animals tested 1 hour later.

Group VI - (2 dogs) After the 30-minute period at 35 mm Hg and 10-minutes at 60 mm Hg, the connection between the animals and the bleeding reservoir was clamped. The animals were then maintained for 2 hours, and tested at the end of this period.

Group VII - (2 dogs) Similar to Group VI except that all the shed blood was reinfused at the end of the "clamp-off" period. The test was performed 1 hour after reinfusion.

At the appropriate times, Narcan (200 to 300 mg) and ⁸⁶Rb Cl

(5 to 10 μ c) were administered simultaneously. Serial arterial blood samples were then collected from a femoral artery for a period of 15 minutes. One half ml aliquots of the blood samples were analyzed for their content of the isotope as previously described. The remaining blood was centrifuged and 1.0 ml aliquots of plasma were treated according to the method described Mier (1931) for the colorimetric determination of thiocyanate. The disappearance curves for the two substances were then plotted.

Results

The disappearance curves for thiocyanate and Rb^{86} for the various groups of animals are shown in Figure 10.

In the controls, clearance of the Rb^{86} was complete after 3 minutes and its disappearance corresponded closely to the major inflection of the thiocyanate curve (Figure 10A).

After exposure to a 30-minute period of hypovolemia (sublethal shock), there was a considerable delay in the equilibration of thiocyanate. Clearance of Rb^{86} also was slowed and its disappearance from the bloodstream again corresponded to the major inflection of the thiocyanate curve (Figure 10B). However, reinfusion of shed blood to such animals restored both the thiocyanate equilibration and Rb^{86} disappearance curves to normal (Figure 10C).

Increasing the severity of shock aggravated the delay in the disappearance of both thiocyanate and Rb^{86} (Figure 10D) and under these circumstances (lethal to approximately 30 per cent of animals) return of the shed blood did not restore the normal clearance patterns (Figure 10E). The curves for the severely shocked animals (Figures 10F and 10G) show further changes in the same direction.

Figure 10

Mean disappearance curves for arterial Ba^{55} (closed circles) and thio-
cyanate (open circles) in unshocked controls and in dogs subjected to
progressive degrees of hemorrhagic shock. The percentages indicate the
approximate lethality of each shocking procedure.

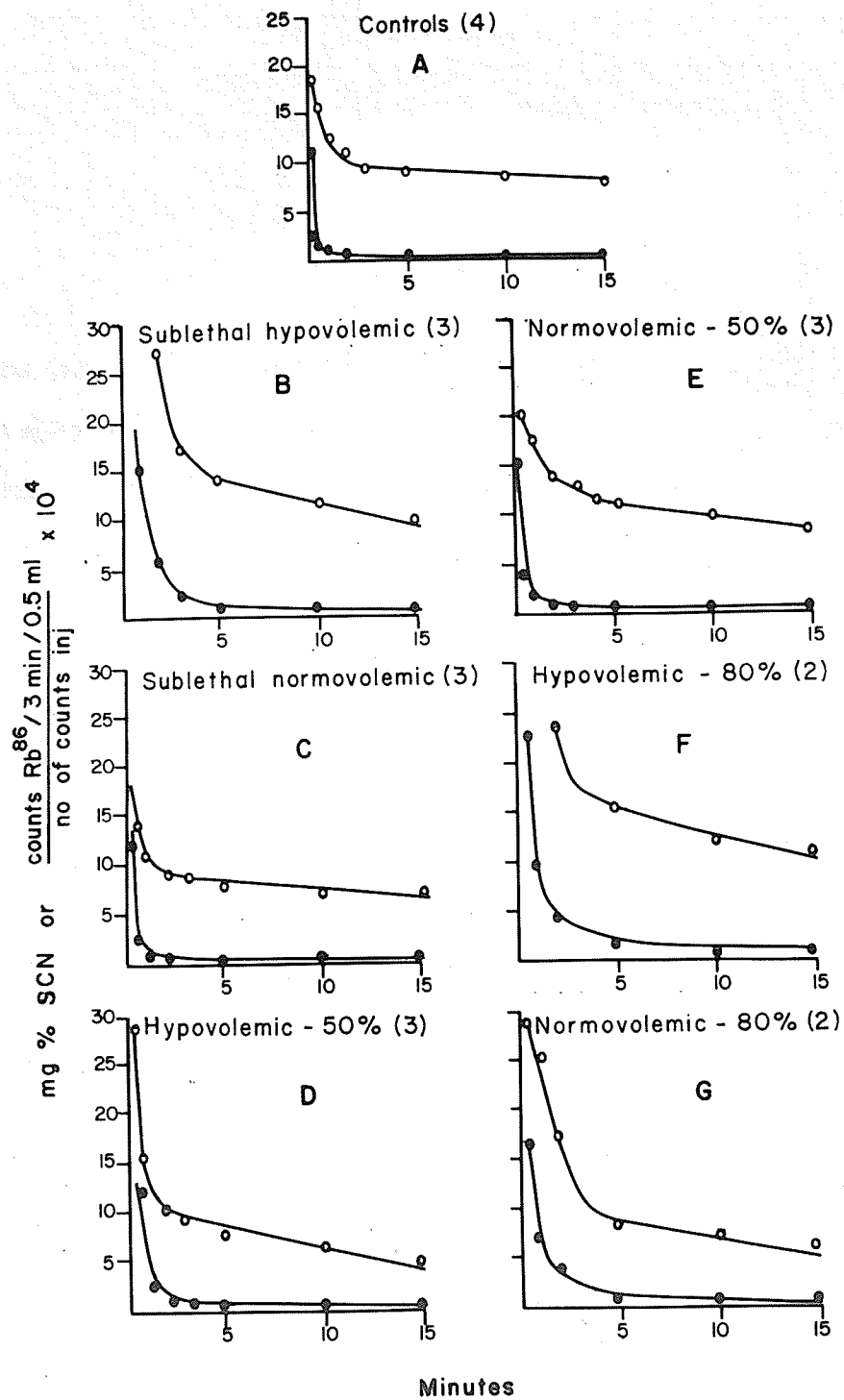


Figure 10

Discussion

Two mechanisms may be responsible for the low tracer content of tissues during shock. The first is a distribution defect which results in improper perfusion of tissues. The second is cellular and might be considered as an increased barrier to the diffusion of Hb^{56} at the cell membrane. The above experiments provide no evidence for decreased membrane permeability. Cell damage, by destroying selectivity might be expected actually to result in an increased rate of exchange. The isotope content of the tissues of animals studied during shock is a function only of blood flow which readily equilibrates with the perfused tissues, the effective nutrient blood flow. The persistence of the tracers in the circulation could reflect a considerable flow through channels which do not permit effective exchange. This possibility is further strengthened by the very high values for "apparent" liver blood flow shown in Figure 9, which could be explained by uptake of Hb^{56} shunted across the portal bed. The magnitude, location and mechanism of shunting may be an important consideration in the overall picture of shock.

Although shown in the above experiments not to be applicable to the measurement of total organ blood flow during shock, Hb^{56} clearance could be an effective tool for measuring exchange between blood and tissue cells in individual vascular beds during hemorrhagic shock. Clearance of this tracer from blood is a function of the diffusion gradient across the capillary membrane and the rate of exchange between interstitial fluid and cells; uptake of tracer, determined by venous sampling after intra-arterial injection should be a function of the distribution of total organ blood flow between "nutrient" capillaries, i.e., those which ex-

change with tissue cells occurs readily, and other vascular channels.

Accordingly, ^{56}Fe uptake was measured in the vascular beds of the hind limb and of the terminal ileum of dogs subjected to hemorrhagic shock. The hind limb is an anatomically convenient skin and muscle bed and served as a basis for comparing subsequent data obtained using the intestine, a critical region during the development of shock.

Methods

Preparation of the animals. Healthy mongrel dogs of 12 to 25 kg weight, lightly anesthetized with pentobarbital, were used throughout. Routine procedures in all animals included cannulation of the right femoral artery for bleeding and of the left brachial artery for measurement of blood pressure during the induction of hemorrhagic shock. Depending on the vascular bed to be investigated, the following surgical procedures were performed.

Experiments on the hind limb. The femoral vessels of the left leg were exposed for a distance of about 3 inches. The femoral vein was dissected free and branches draining the upper thigh were ligated. A polyethylene catheter was inserted into the femoral artery through a small branch. A tourniquet was then passed beneath the femoral vessels and tightened around the limb to minimize collateral circulation. After heparinization, a large polyethylene catheter was inserted distally into the femoral vein. The venous return of the hind limb passed first through a polyethylene coil inserted into a well-type scintillation counting chamber, and then through an automatic recording bubble flowmeter. Blood was returned to the animal via the right femoral vein. The extracorporeal volume was about 30 ml, and was filled with donor blood (Figure 11).

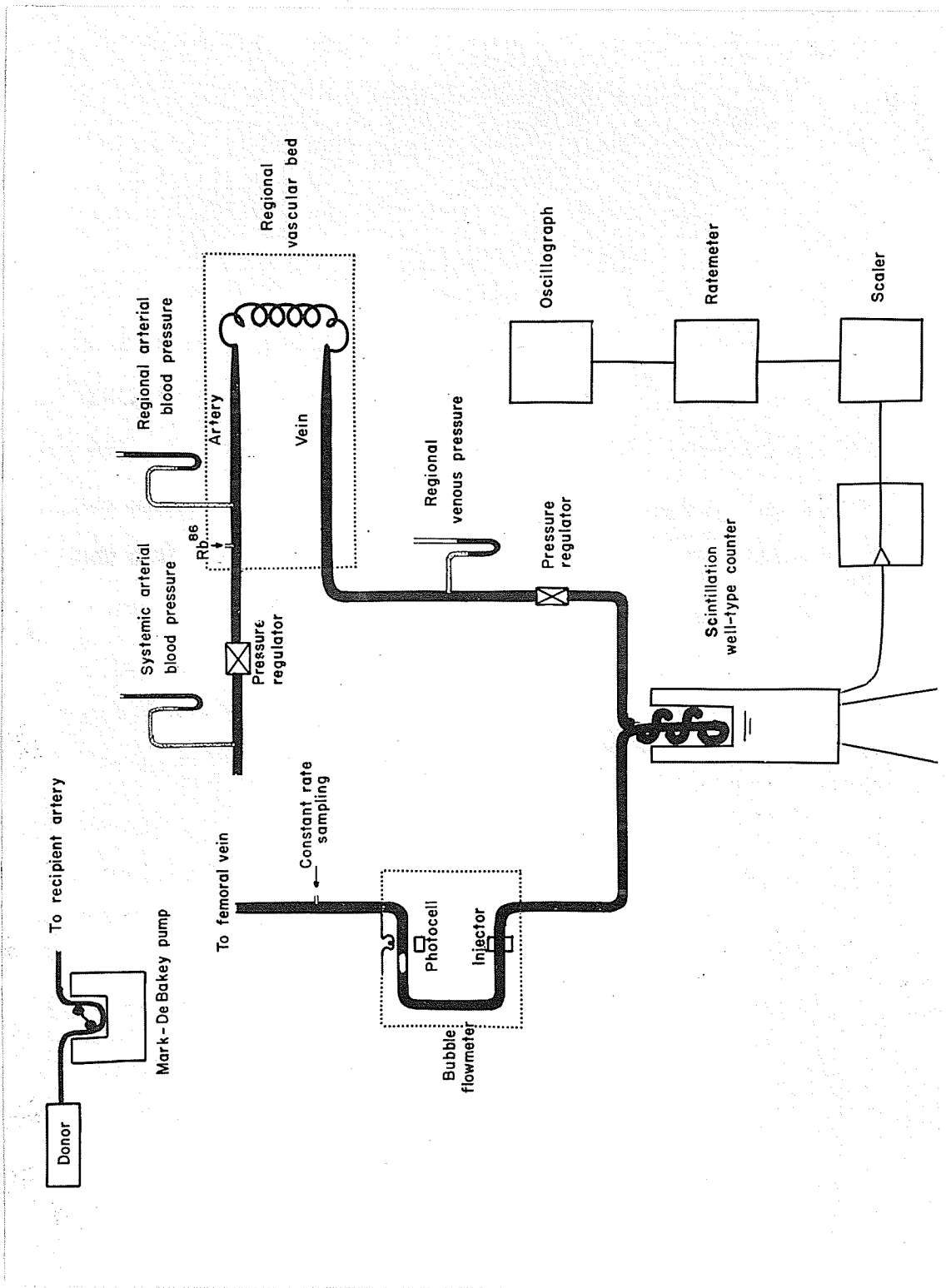


Figure 11

Experiments on segments of ileum. The terminal ileum was exposed through a midline abdominal incision. The last 12 to 14 inches of ileum are drained by 3 or 4 venous arcades which unite to empty into a large mesenteric vein. This vein was carefully dissected from the surrounding mesentery and lymph nodes. The superior mesenteric artery was located in the same region, the branch supplying the arcade immediately rostral to those under investigation was isolated, and a polyethylene catheter inserted and passed into the main arterial trunk. After ligation of the anastomotic circulation between the arcades under investigation and those immediately adjacent, a large polyethylene catheter was inserted into the vein draining the isolated arcades. The venous flow passed through the coil inserted in the well-type scintillation counter, through the flowmeter and was returned to the animal via the right femoral vein (Figure 11). This procedure permitted study of the circulation in a representative segment of intestine with minimal involvement of the remainder of the portal bed.

Measurement of blood flow. Venous outflow was recorded by means of our modification of the automatic bubble flowmeter developed by Dr. C.E. Nash of the Department of Pharmacology, University of Alberta. It combines the desirable feature of low resistance (Figure 12) with adequate accuracy and simplicity of design and operation. A photo-electric cell sensing the passage of the air bubble activates a solenoid which automatically injects a new bubble. The passage time of the bubble through the meter was recorded on an ink-writing oscillograph.

Measurement of Tracer Uptake. ⁸⁶Cl was administered intra-arterially in 0.9 per cent NaCl solution. The volumes injected did not exceed 0.10

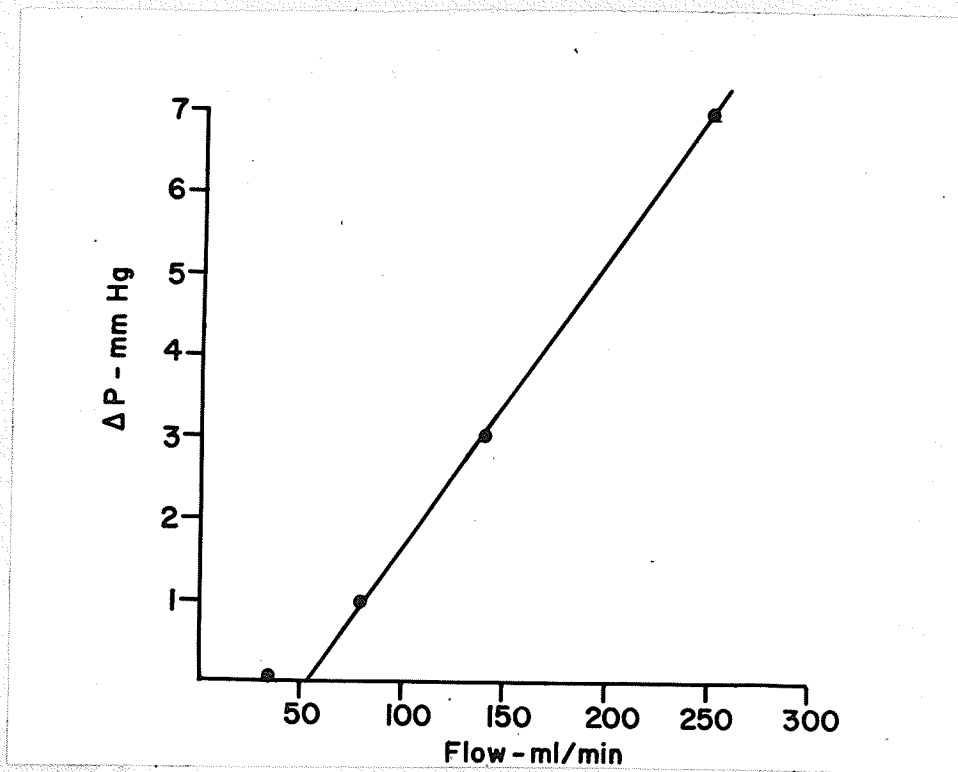


Figure 12

Resistance to blood flow in the bubble flow meter. At the largest flow encountered in our experiments (104 ml/min), the pressure drop was less than 2.0 mm Hg.

ml in the hind-limb experiments or 0.20 ml in the intestine experiments. The average doses were 1.35×10^5 cpm in the case of the intestine and 8×10^4 cpm in experiments on the hind limb. Sampling of the venous blood from a point just distal to the flowmeter by means of Harvard Model 500-000 constant-rate withdrawal pump was begun immediately upon appearance of tracer in the coil lying in the well-type scintillation counter (Figure 11) and continued as long as detectable amounts of radioactivity remained in the venous blood. The per cent uptake of ^{86}Rb following each injection was calculated as follows:

$$\text{Uptake} = 100 - \frac{\text{cpm in sample} \times \frac{\text{total flow during sampling period}}{\text{volume of sample}}}{\text{cpm injected}} \times 100$$

This calculation is valid only if blood flow during the sampling period remains constant, and we have made measurements only during periods of relatively stable flow (variation not greater than ± 5 per cent).

A second method of measuring uptake, used in early experiments when the constant-rate withdrawal pump was not available, utilized the area of the curve described by the oscillograph in response to the presence of the tracer in the venous outflow. Since the counting efficiency varies inversely with the flow rate (i.e., the greater the flow rate, the fewer counts recorded per unit of radioactivity passing through the system), it was necessary to plot flow vs. counting rate with the aid of a model system (Figure 13). This curve permitted calculation of the total number of counts present in the flow as a percentage of the number of counts actually recorded. By applying this factor to the area under the curve inscribed by the oscillograph (measured by planimetry), a close approximation of the total number of counts in the venous outflow could be made. For example,

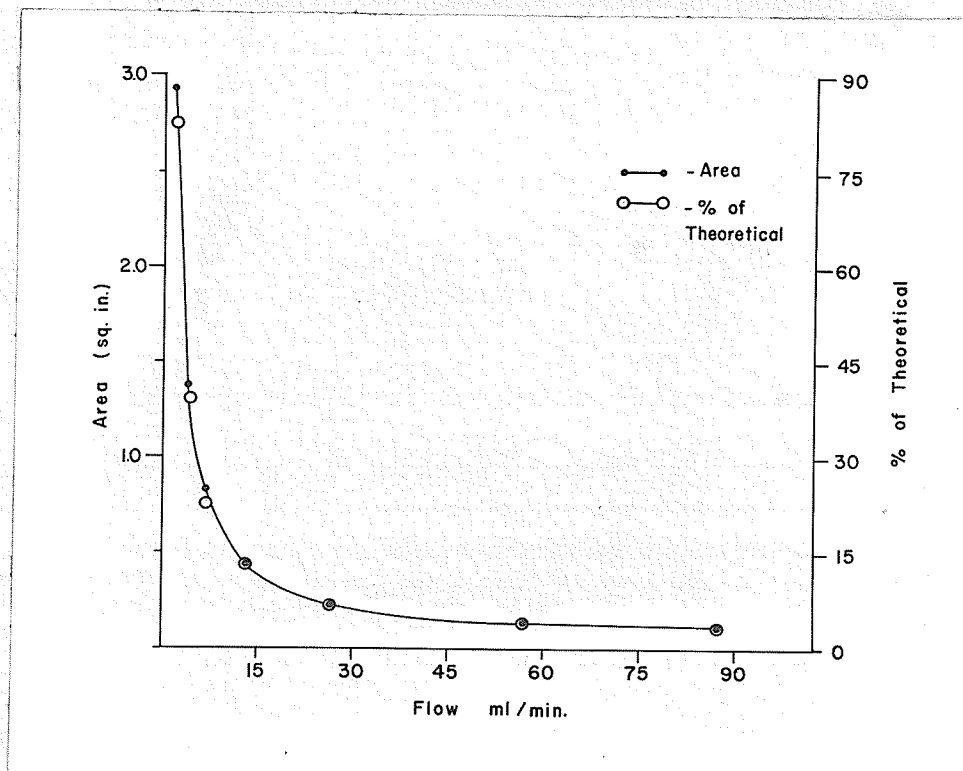


Figure 13

Relation of volume flow through coil in counter well to counting efficiency of scintillation crystal. The open circles represent per cent of theoretical counts obtained at different flow rates (cpm at zero flow/100%). See text for complete description.

at a flow rate of 19.7 ml/min, 10.5 per cent of the total counts in the venous blood were "seen" by the photomultiplier tube. If the area under the curve represented 8021 cps, then $\frac{8021}{.105} = 76,400$ cps were present in the outflow.

Flow-uptake experiments. Uptake of Ib^{86} by the vascular beds of the hind limb or ileum was measured during unimpeded blood flow and when flow was reduced progressively by partial occlusion of the regional artery or of the plastic tube carrying the venous return.

Experiments in regional vascular beds during cross-perfusion with oxygenated or hypoxic donor blood. Cross-perfusion was accomplished by connecting a large artery of a donor dog to the main arterial supply of the vascular bed under investigation in the recipient animal. Venous blood from this region was returned to the donor dog. Bilateral adrenalectomy was performed acutely on donor animals to prevent release ofpressoramines from the adrenal medulla during hypoxia. After cross-perfusion had been established, the flow-uptake pattern was determined during periods of perfusion when the donor animal was breathing room air, 15 per cent O_2 - 85 per cent N_2 , 10 per cent O_2 - 90 per cent N_2 , or 5 per cent O_2 - 95 per cent N_2 . Arterial and venous samples from the perfused vascular bed were analyzed for hemoglobin oxygen saturation according to the method of Johnson et al. (1949).

Ib^{86} uptake during hemorrhagic shock.

1) Hind limb: A single control determination of Ib^{86} uptake was made in 7 dogs prior to the induction of hemorrhage (Group I). In 3 addi-

tional dogs (Group II) a control flow-uptake pattern for ^{86}Rb was determined during progressive arterial occlusion. The animals were then subjected to the standard hemorrhagic shock procedure. ^{86}Rb uptake was determined at selected intervals during the hypovolemic period and after reinfusion of the shed blood.

3) Ileum: A single control determination of ^{86}Rb uptake was made in 10 dogs (Group I). In 6 additional dogs (Group II), a control flow-uptake curve was determined as in the hind limb experiments above. Hemorrhagic shock was then induced according to the standard procedure, with two modifications. The surgical trauma associated with preparation of the intestine increased the sensitivity of the animals to the shock procedure, and consequently, it was necessary to reduce the period during which the blood pressure was maintained at 33 mm Hg to 30 minutes. Secondly, the clamp-off period was maintained only until the animals' blood pressure first fell below 40 mm Hg if this occurred prior to the end of 3 hours. It has been our experience that untreated dogs whose pressure falls below 40 mm Hg during the clamp-off period almost invariably die. By reinfusing the shed blood at this point rather than maintaining the animals for the entire 3-hour period with several small blood transfusions, we provided an irreversibly shocked preparation which we could observe during the normovolemic shock phase without the development of rapid cardiovascular collapse.

^{86}Rb uptake during shock produced by prolonged infusions of noradrenaline.

Three experiments were performed on the hind limb and 6 on the ileum. Control flow-uptake curves were determined in all animals prior to the start of noradrenaline infusion. Noradrenaline was infused into a femoral vein in a dose of 2 $\mu\text{g}/\text{kg}/\text{min}$ (free base equivalent of the bitartrate)

for 3 hours. This procedure has been reported to produce irreversible shock in most dogs (Yard and Nicholson, 1956). Hb^{56} uptake was determined during the infusion.

Results

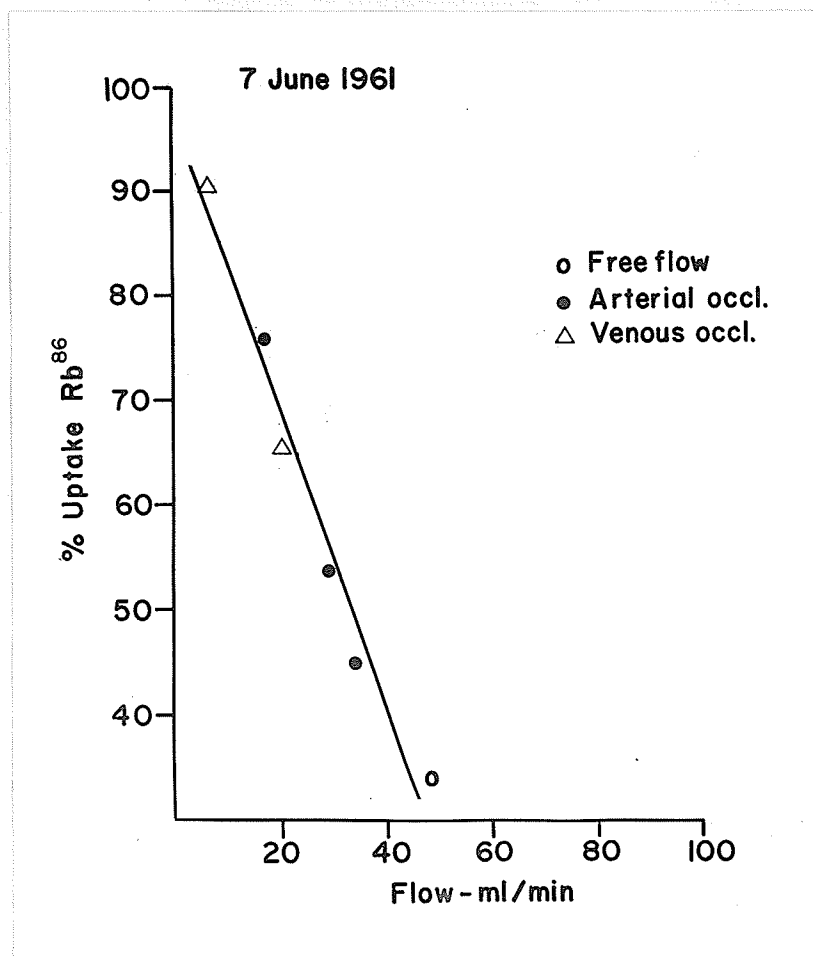
Flow-uptake experiments. Three hind limb and 4 ileal loop experiments were performed. The relation between Hb^{56} uptake and blood flow in these tissues is illustrated in Figures 14 and 15. In both vascular beds, as blood flow was reduced from the free-flow level by either arterial or by venous occlusion, uptake increased progressively. Within the range of blood flows covered by these experiments, Hb^{56} uptake was inversely and linearly related to blood flow in both areas. Assuming a constant distribution of the total flow, slower flow would allow a longer period for exchange to occur between blood and tissues. In every case, extrapolation to zero flow intercepts the ordinate very close to 100 per cent uptake.

As is shown in these figures, the pattern is characteristic for all experiments despite quantitative differences between the extraction ratios of the hind limb and ileum. During free flow uptake was greater in ileum than in hind limb. In addition, the slope of the lines relating flow to uptake in these experiments was considerably greater in the hind limb than in the ileum (mean values 1.90 and 0.79, respectively). If these data are combined with similar control measurements made on 8 additional animals each on the hind limb and ileum (*vide infra*), the difference becomes highly significant (means $\pm$ std. errors 1.60 ± 0.14 and 0.83 ± 0.06) ($P < 0.001$).

This relation was consistently reproducible and henceforth will be referred to as the flow-uptake relation for Hb^{56} .

Figure 14

Flow-uptake relations for Rb^{86} in the vascular beds of the hind limb in dogs. Note uptake during unimpeded flow and during reduced flow due to arterial or venous occlusion. Compare with Figure 15.



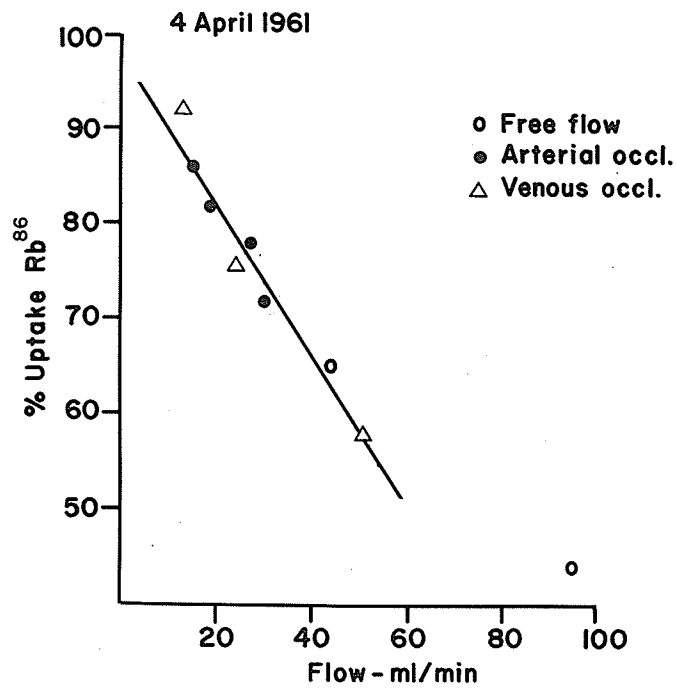
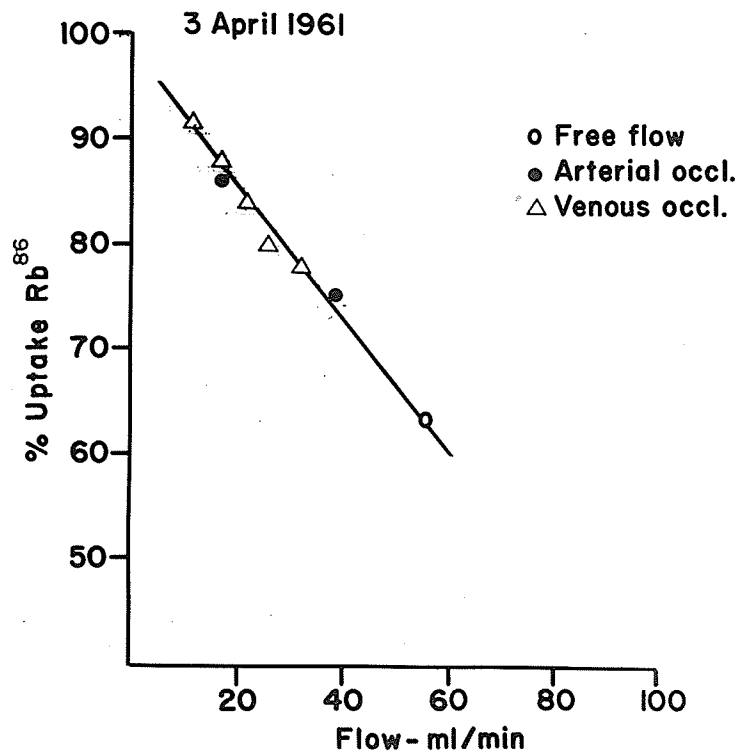


Figure 14

Figure 13

Flow-uptake relations for Ib^{86} in the vascular beds of the ileum in 4 days.
Note uptake during unimpeded flow and during reduced flow due to arterial
or venous occlusion. Compare with patterns in Figure 14.

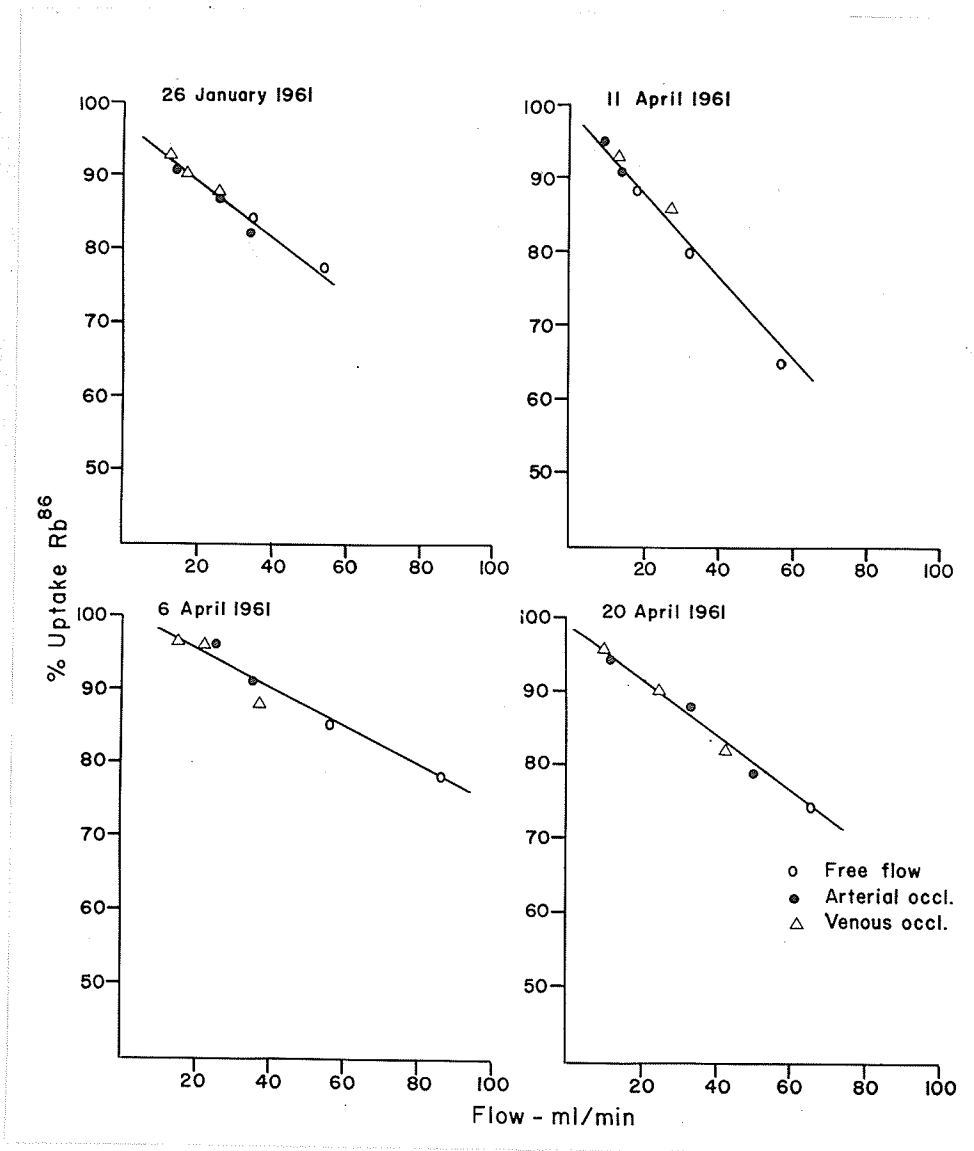


Figure 13

No differences could be detected between the flow-uptake relation obtained during arterial occlusion and that resulting from venous occlusion (Figures 14 and 15).

^{86}Rb uptake during cross-perfusion with hypoxic blood. Two experiments were performed on the hind limb and 2 on the intestine. In all cases, the flow-uptake relation obtained during free-flow perfusion with hypoxic donor blood was the same as that observed with fully oxygenated blood. Similarly, the relation determined with arterial occlusion during perfusion with hypoxic blood did not differ from that obtained with arterial occlusion during perfusion with fully oxygenated blood. Figure 16 illustrates the relations from each experiment. At similar rates of blood flow, A-V oxygen differences obtained during unimpeded blood flow or during arterial occlusion were approximately the same irrespective of the oxygen saturation of the arterial blood (Table 15).

Furthermore, even when venous oxygen saturation during arterial occlusion of the vascular bed perfused with fully oxygenated blood was the same as that during free-flow perfusion with hypoxic blood, ^{86}Rb uptakes at these points differed considerably (Figure 16) and in each case corresponded to that expected on the basis of flow rate.

Comparison of ^{86}Rb uptake by the hind limb and ileum of dogs subjected to hemorrhagic shock.

1) Group I animals. Uptake of ^{86}Rb by the hind limb was measured in 7 and that by the intestine in 5 animals during the development of hemorrhagic shock. Uptake by an intestinal segment was measured in 3 additional animals which had received phenoxybenzamine 1.0 mg/kg intravenously

Figure 16

Flow-uptake relations for Mn^{86} in the vascular beds of the canine hind limb and ileum during cross-perfusion with fully oxygenated or progressively hypoxic blood from a donor animal. Note experiment of 2/9/61. The arrow points to a closed circle (arterial occlusion, fully oxygenated blood). The oxygen saturations of venous blood at this point and at that represented by the open rectangle (free flow, hypoxic blood) are the same yet uptakes are significantly different.

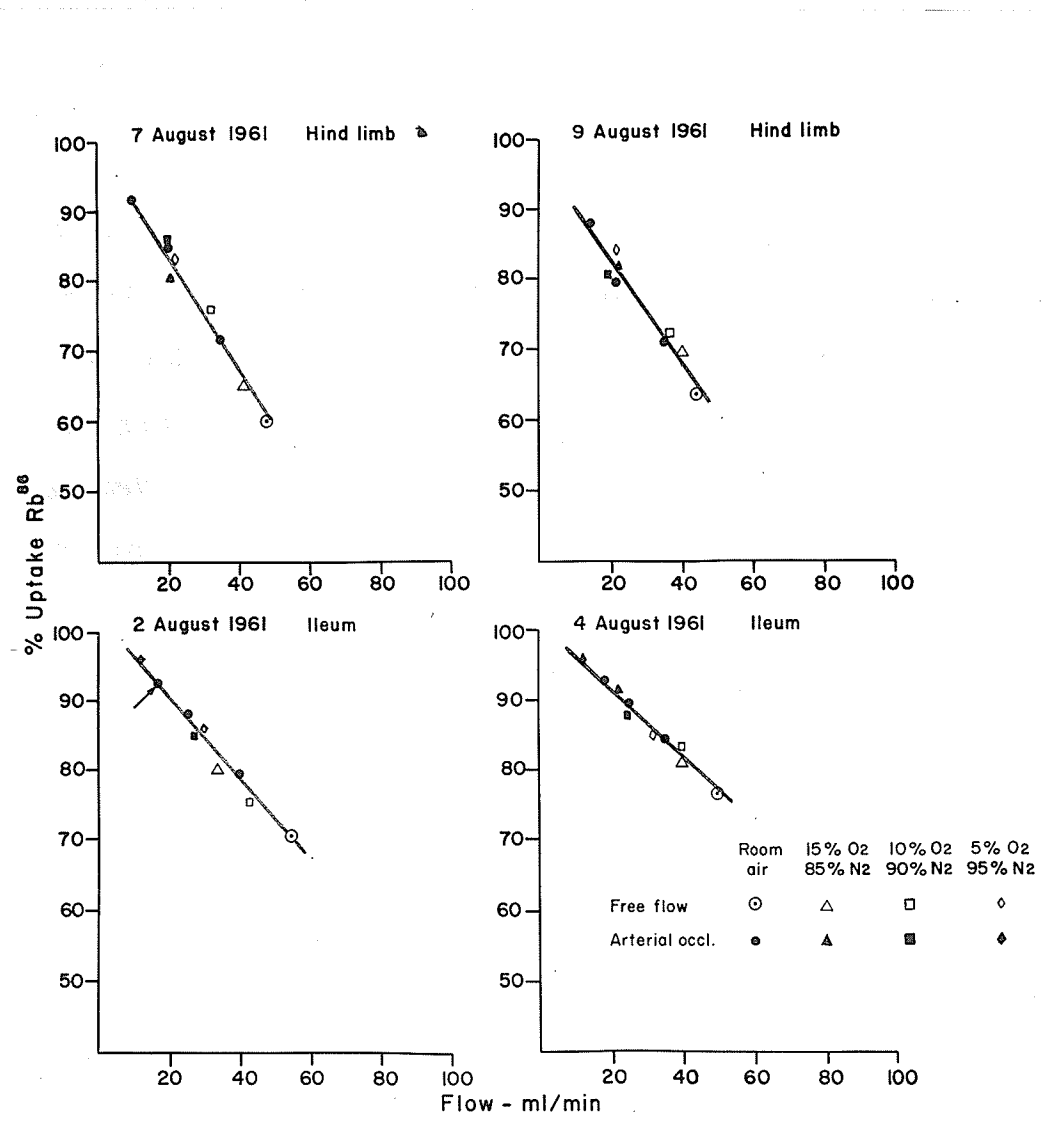


Figure 10

Table 13

Blood flows, arterial hemoglobin saturations and arteriovenous (A-V) oxygen differences in the vascular beds of the canine hind limb and ileum cross-perfused with fully oxygenated or progressively hypoxic donor blood.

Table 15

| | | Blood Flow
ml/min | | Per cent Art.
Oxygen Saturation | | A-V O ₂ Diff.
Vol. Per cent | |
|--|------------|----------------------|------|------------------------------------|------|---|------|
| | Kind 1/2 # | 1 | 2 | 1 | 2 | 1 | 2 |
| Room
Air | Free flow | 48.0 | 44.2 | 94.1 | 96.4 | 22.2 | 22.9 |
| | Art. occl. | 35.1 | 34.2 | 96.0 | 98.0 | 31.0 | 32.8 |
| | Art. occl. | 20.0 | 21.4 | 97.5 | 95.2 | 35.5 | 36.7 |
| | Art. occl. | 10.2 | 14.6 | 97.2 | 95.4 | 48.0 | 43.7 |
| 15% O ₂
85% N ₂ | Free flow | 41.1 | 39.8 | 86.2 | 90.1 | 25.9 | 28.5 |
| | Art. occl. | 20.4 | 22.2 | 86.1 | 89.3 | 34.0 | 36.3 |
| 10% O ₂
90% N ₂ | Free flow | 32.0 | 36.3 | 82.0 | 79.4 | 25.6 | 29.4 |
| | Art. occl. | 20.4 | 19.7 | 82.6 | 84.2 | 37.0 | 37.9 |
| 5% O ₂
95% N ₂ | Free flow | 22.1 | 21.4 | 70.0 | 73.6 | 35.3 | 36.5 |
| Room
Air | Flow # | 1 | 2 | 1 | 2 | 1 | 2 |
| | Free flow | 34.2 | 30.0 | 96.1 | 96.3 | 29.0 | 31.5 |
| | Art. occl. | 40.0 | 33.1 | 97.2 | 95.0 | 32.0 | 32.5 |
| | Art. occl. | 25.1 | 25.2 | 93.1 | 95.4 | 41.0 | 38.6 |
| | Art. occl. | 16.7 | 18.2 | 95.0 | 95.1 | 45.5 | 43.0 |
| 15% O ₂
85% N ₂ | Free flow | 39.3 | 40.1 | 84.0 | 86.1 | 34.0 | 33.6 |
| | Art. occl. | 23.2 | 22.2 | 87.5 | 87.1 | 37.1 | 41.4 |
| 10% O ₂
90% N ₂ | Free flow | 42.5 | 40.2 | 84.0 | 80.2 | 34.0 | 36.5 |
| | Art. occl. | 27.0 | 24.1 | 80.2 | 78.1 | 40.3 | 40.1 |
| 5% O ₂
95% N ₂ | Free flow | 29.4 | 31.1 | 73.4 | 71.0 | 34.1 | 38.1 |
| | Art. occl. | 11.7 | 10.2 | 72.6 | 71.5 | 48.2 | 48.0 |

18 hours before hemorrhage. This treatment schedule previously was shown to protect dogs against irreversible shock without appreciably altering bleeding volumes (Part V). Blood-flow data from these experiments are recorded in Table 16. Induction of hemorrhage was accompanied by a rapid decline in blood flow in all three groups. However, the control flow in the intestine was higher in the phenoxybenzamine-treated animals and was maintained at a higher level than in the untreated dogs during all stages of the shock-inducing procedure. In the immediate post-reinfusion period blood flow in the hind limb of untreated animals was elevated considerably above control levels, and flow in the intestine was slightly higher than the control value. Intestinal blood flow in animals pretreated with phenoxybenzamine remained 33 per cent below the prehemorrhage value after reinfusion. However, this was only 10 per cent below the prehemorrhage flow rate of the untreated animals. Furthermore, although flows in the hind limb and intestine of the untreated dogs diminished sharply over the ensuing hour (62 and 64 per cent, respectively), intestinal blood flow was relatively well maintained in the treated dogs (29 per cent decrease) and was 160 per cent of the intestinal flow in the untreated series at this time.

The treated dogs maintained their blood pressure better during the clamp-off period. In none of these animals did the blood pressure fall below 40 mm Hg during the 2-hour period, whereas the pressure of untreated dogs fell below this level in an average of 33 minutes (hind-limb experiments) and 41 minutes (intestine experiments). This indicated a poor prognosis for the untreated animals, and probable protection from death in the treated group. However, because of the surgery involved, survival was not determined in these animals.

Table 16

Mean Flow in Regional Vascular Beds of Dogs Subjected to Standardized Hemorrhagic Shock

| Group | No. of Dogs | Blood Flow (ml/min) | | | | | | | |
|--|-------------|---------------------|----------|----------|-----------|------------------|---------|---------|---------|
| | | Control | 35 mm Hg | 60 mm Hg | Clamp-off | After Reinfusion | | | |
| | | | | | | Immed. | 20 min. | 40 min. | 60 min. |
| Head Limb
Untreated | 7 | 24 ± 3 | 6 ± 2 | 8 ± 1 | 6 ± 1 | 37 ± 8 | 21 ± 4 | 18 ± 2 | 14 ± 2 |
| Ileum
Untreated | 5 | 38 ± 2 | 7 ± 2 | 17 ± 2 | 8 ± 1 | 42 ± 6 | 27 ± 3 | 21 ± 2 | 19 ± 3 |
| Ileum
Pretreated with
Phenylbutazone | 5 | 32 ± 6 | 13 ± 3 | 26 ± 3 | 17 ± 3 | 34 ± 4 | 29 ± 5 | 26 ± 4 | 24 ± 4 |

All figures are mean ± S.E.

Control values for Rb^{86} uptake are recorded in Table 17. Uptake by the hind limb averaged 67 ± 1 per cent in 3 and 35 per cent in 2 of the 7 dogs. No explanation for this apparently sharp division into 2 groups has yet been found. Uptake by the intestine was considerably higher in untreated than in pretreated dogs (90 ± 1 and 70 ± 3 per cent, respectively). Reference to the previous flow-uptake curves from unshocked animals (Figures 14 and 15) indicates that this difference may be explained by the higher flow rates in the treated dogs. Measurements of Rb^{86} uptake were made at selected intervals during the shock procedure. Scanty and erratic flow in the hind limbs precluded measurements in 2 animals during hypovolemia. Inasmuch as determinations were not made on the remaining 3 animals at corresponding times, the individual figures for each experiment are recorded in Table 18. Uptake of Rb^{86} increased during the period of severe hypovolemia (35 mm Hg). This was expected on the basis of the flow-uptake relation described above. The extent of this increase was a function of the control uptake. When the blood pressure was raised from 35 to 60 mm Hg, flow increased and uptake fell.

Uptake was measured again as soon as the blood flow had become reasonably stable after reinfusion. During this immediate post-reinfusion period, uptake by the hind limb fell in all animals. In 3 of 7 animals, the decreases in uptake were greater than could be expected from the increases in blood flow (Table 19). This observation is compatible with a preferential decrease in flow through "nutrient" capillaries. After this initial period, blood flow in the hind limb fell progressively, accompanied in all cases by a sharply increasing uptake of Rb^{86} . This general pattern is shown in Figure 17 which illustrates the flow-uptake relation during the development of shock and after reinfusion in a representative ani-

Table 17

Uptake of Ba^{86} by Regional Vascular Beds

Prior to Hemorrhage

| Group | No. of
Days | Per cent Uptake
($\pm$ S.E.) |
|--------------------------------------|----------------|----------------------------------|
| Blind Limb | 5 | 67 $\pm$ 1 |
| Untreated | 2 | 35 |
| Ileum | 5 | 90 $\pm$ 1 |
| Untreated | | |
| Ileum | 5 | 70 $\pm$ 3 |
| Pre-treated with
Phenoxybenzamine | | |

Table 18
Uptake of Ru^{86} by Regional Vascular Beds
During Hypovolemic Shock in Dogs

| Type of Experiment | Per cent Uptake | | | |
|--|-----------------|----------|----------|-----------|
| | Control | 35 mm Hg | 60 mm Hg | Clamp-off |
| Hind Limb | 68 | 93 | 55 | ----- |
| | 63 | ----- | 98 | 94 |
| | 69 | 90 | ----- | 83 |
| | 65 | 91 | 88 | ----- |
| | 65 | ----- | 74 | ----- |
| Ileum | 92 | 99 | 97 | 96 |
| | 87 | 94 | 92 | 91 |
| | 95 | 99 | ----- | 97 |
| | 83 | 91 | 90 | 89 |
| Ileum
Pretreated with
Phenoxybenzamine | 68 | 92 | 79 | 83 |
| | 74 | 94 | 96 | 94 |
| | 87 | 97 | 95 | 95 |
| | 75 | 96 | 93 | 94 |
| | 65 | 91 | 91 | 92 |

Table 19

Uptake of Rb^{86} During the Immediate Postreinfusion Period by the Vascular
Beds of Hind Limbs in Dogs Subjected to Hemorrhagic Shock

| Expt. No. | Rb^{86} Uptake | | Blood Flow
(Per Cent of Control) |
|-----------|-------------------------|---------------------|-------------------------------------|
| | Per cent | Per cent of Control | |
| 1 | 24 | 77 | 76 |
| 2 | 32 | 80 | 76 |
| 3 | 48 | 71 | 132 |
| 4 | 51 | 79 | 100 |
| 5 | 69 | 99 | 177 |
| 6 | 60 | 92 | 107 |
| 7 | 20 | 31 | 110 |

nal of this series.

Ib^{86} uptake by the intestine immediately after reinfusion was below or equal to the control values in all 5 animals (Table 20), tending to be less than would have been expected on the basis of the normal flow-uptake relation. In addition, the Ib^{86} uptake consistently remained at or below the control value during the ensuing period of declining blood flow (Table 21). This failure of uptake to increase as flow decreased, the reverse of the relation in the hind limb or in normal intestine, suggests that a considerable portion of the blood flowing through the intestinal segment does not traverse "nutrient" channels. More complete data from a representative experiment in this series are shown in Figure 18. Ib^{86} uptake fell below the control value after reinfusion and remained below despite the subsequent drastic reduction in blood flow.

The animals pretreated with phenylephrine presented a very different picture in the post-reinfusion period. A slight fall in uptake occurred immediately after reinfusion, but it always remained above the control value (Table 22). In addition, uptake remained considerably elevated during the ensuing period of moderately decreasing blood flow (Table 23). A typical experiment is shown in Figure 19. It appears that phenylephrine altered the blood flow distribution in the intestine of the animals subjected to a hemorrhagic shock procedure to permit more effective exchange between blood and tissue cells.

Changes in the tracer outflow curves recorded from the hind limb during the shock procedure also are of interest. Figure 20 shows representative curves of tracer concentration in the venous outflow before and during hypovolemic shock. In the control, tracer reached the counting chamber

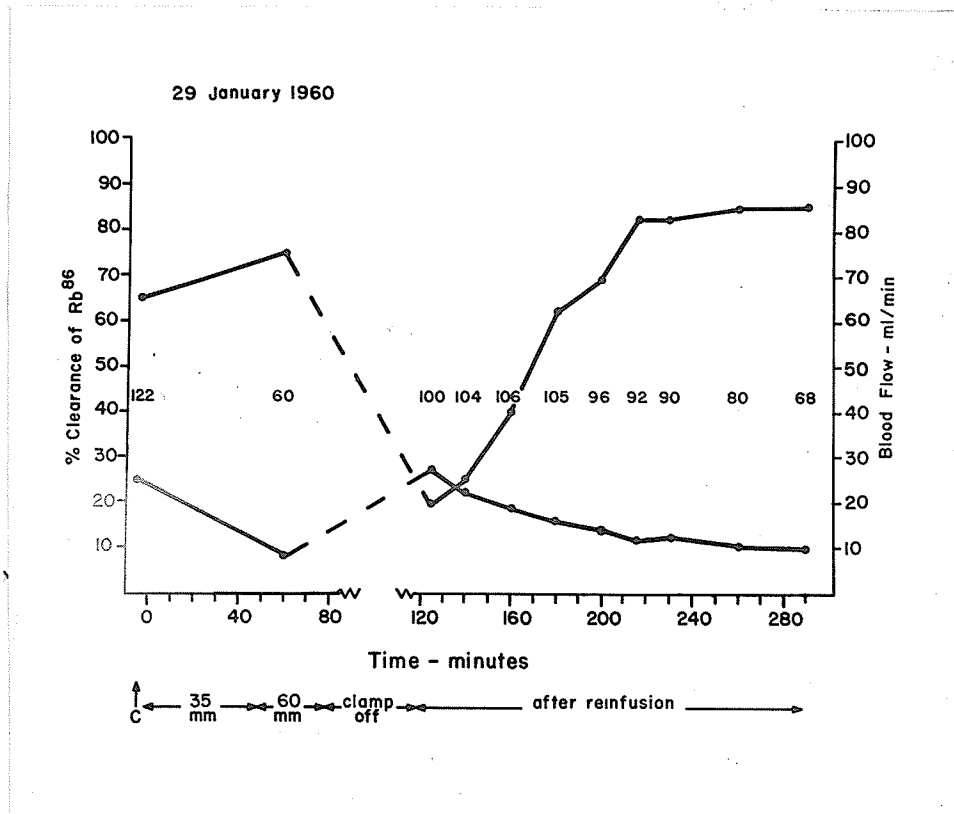


Figure 17

Flow-uptake relation: in the vascular bed of the hind limb of a dog subjected to hemorrhagic shock. The upper line represents uptake of Rb^{86} and the lower line blood flow. Blood pressures during this period are shown between these lines. Note the decline in tracer uptake after reinfusion, although flow is only 10% above the control, and the greater increase in uptake during the ensuing period of decreasing blood flow.

Table 20

Uptake of Hb^{56} During the Immediate Postreinfusion Period by the
Vascular Bed of a Segment of Ileum in Dogs Subjected to Hemorrhagic Shock

| Exper. No. | Hb^{56} Uptake | | Blood Flow
(Per cent of Control) |
|------------|-------------------------|---------------------|-------------------------------------|
| | Per cent | Per cent of Control | |
| 1 | 67 | 73 | 100 |
| 2 | 90 | 98 | 103 |
| 3 | 87 | 97 | 85 |
| 4 | 87 | 92 | 90 |
| 5 | 83 | 100 | 50 |

Table 31

Uptake of Hb^{86} One Hour after Reinfusion by the
Vascular Beds of a Segment of Ileum in Dogs Subjected to Hemorrhagic Shock

| Expt. No. | Hb^{86} Uptake | | Blood Flow
(Per cent of Control) |
|-----------|-------------------------|---------------------|-------------------------------------|
| | Per cent | Per cent of Control | |
| 1 | 74 | 51 | 48 |
| 2 | 92 | 100 | 45 |
| 3 | 84 | 96 | 42 |
| 4 | 92 | 96 | 37 |
| 5 | 83 | 100 | 30 |

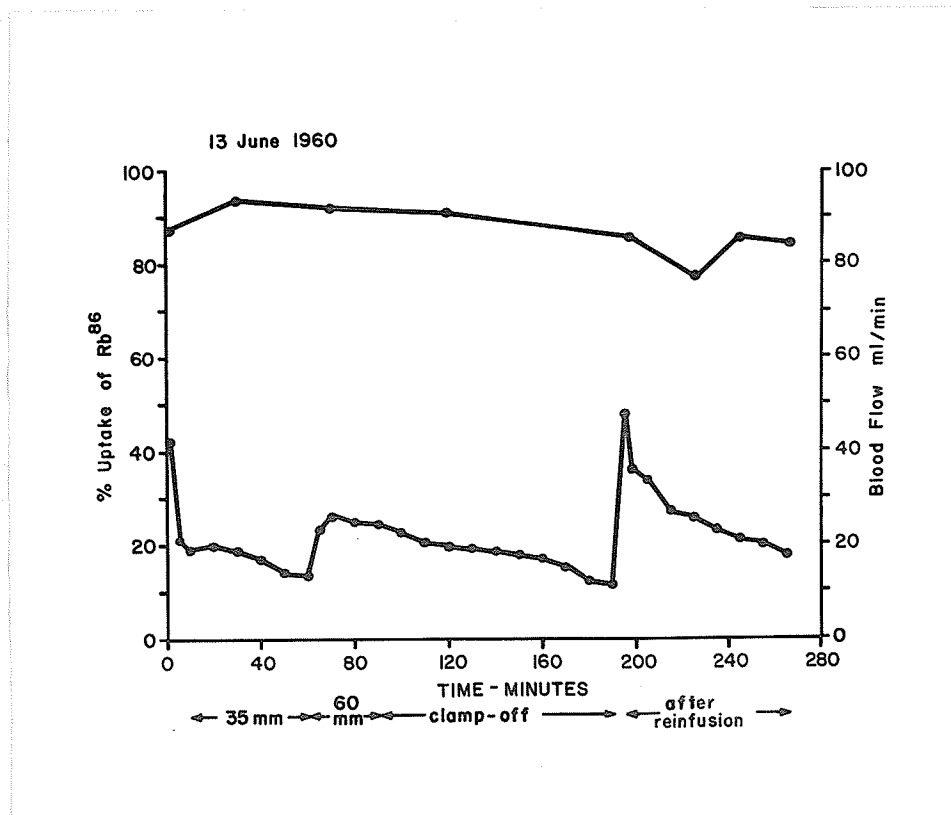


Figure 14

Flow-uptake relation in the vascular bed of a segment of ileum of a dog subjected to hemorrhagic shock. The upper line represents Rb^{86} uptake and the lower line blood flow. Uptake of tracer fell after reinfusion and remained below the control level thereafter despite reduced blood flow.

somewhat less than one minute after injection and its concentration rapidly reached a sharp peak. No significant amount of tracer was present in the venous outflow after 5 minutes. In contrast, the pattern obtained during hypovolemic shock showed an increased latency, a slow rise to a lower peak concentration and a prolonged persistence of the tracer in the venous blood. These changes probably are related to a decreased rate of "delivery" of the tracer to the tissue and to variable rates of passage through various vascular beds. They provide a partial explanation for our previous observations regarding the diminished rate of clearance of Rb^{86} from the blood of shocked animals. The tracer outflow curve after reinfusion presented an interesting pattern, which is illustrated in Figure 21. Rb^{86} injected 5 minutes after reinfusion appeared in the venous outflow earlier and its concentration rose more rapidly to a higher peak than that of the control. During the ensuing 140 minutes, when flow was declining, the delay in appearance of the tracer in the venous blood increased, the peak concentration decreased and a plateau appeared. The pattern eventually became similar to that observed during hypovolemic shock (cf. Figure 20). Thus, the outflow pattern also suggests the possibility of increased arterio-venous shunting in the hind limb during the immediate postreinfusion period.

2) Group II animals. As in Group I experiments, induction of hypovolemia decreased blood flow and increased Rb^{86} uptake in the hind limbs of 3 dogs. The increased uptake of tracer during hypovolemia corresponded to the control flow-uptake relation. After reinfusion, blood flow increased initially to above prehemorrhage levels and Rb^{86} uptake at this time was less than would have been predicted on the basis of flow rate (cf. Group I). However, during the ensuing period of declining pressure

Table 22

Uptake of Eb^{86} During the Immediate Postreinfusion Period by the
Vascular Beds of a Segment of Ileum in Dogs Pretreated with Phenoxybenzamine
and Subjected to Hemorrhagic Shock

| Expt. No. | Eb^{86} Uptake | | Blood Flow
(Per cent of Control) |
|-----------|-------------------------|---------------------|-------------------------------------|
| | Per cent | Per cent of Control | |
| 1 | 72 | 106 | 89 |
| 2 | 94 | 127 | 42 |
| 3 | 96 | 110 | 57 |
| 4 | 90 | 119 | 56 |
| 5 | 85 | 131 | 72 |

Table 23

Uptake of Rb^{86} One Hour After Reinfusion by the
Vascular Bed of a Segment of Ileum in Dogs Pretreated with Phenoxylbenzamine
and Subjected to Hemorrhagic Shock

| Exper. No. | Rb^{86} Uptake | | Blood Flow
(Per cent of Control) |
|------------|-------------------------|---------------------|-------------------------------------|
| | Per cent | Per cent of Control | |
| 1 | 72 | 108 | 60 |
| 2 | 93 | 128 | 32 |
| 3 | 93 | 109 | 37 |
| 4 | 89 | 118 | 40 |
| 5 | 85 | 131 | 57 |

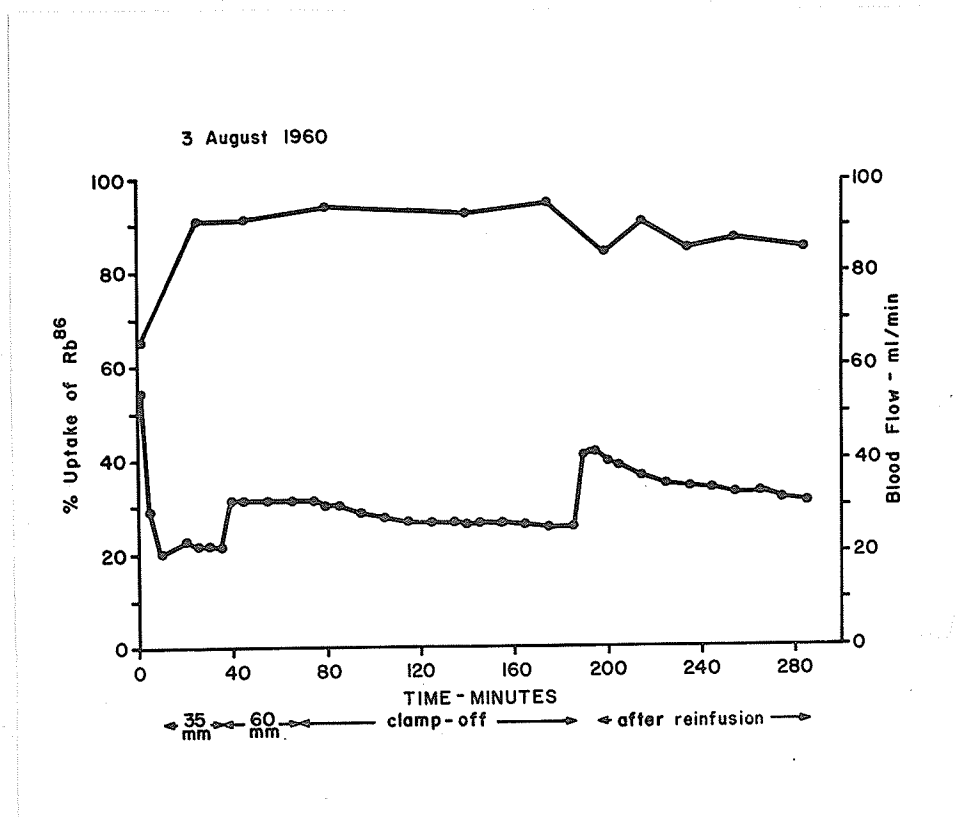


Figure 19

Flow-uptake relation in the vascular bed of a loop of ileum in a dog pretreated with phenoxybenzamine and subjected to the hemorrhagic shock procedure. The upper line indicates Rb⁸⁶ uptake and the lower blood flow. Uptake remained elevated throughout hypovolemia and, despite a slight decrease secondary to reinfusion, remained at a level considerably above that of the control.

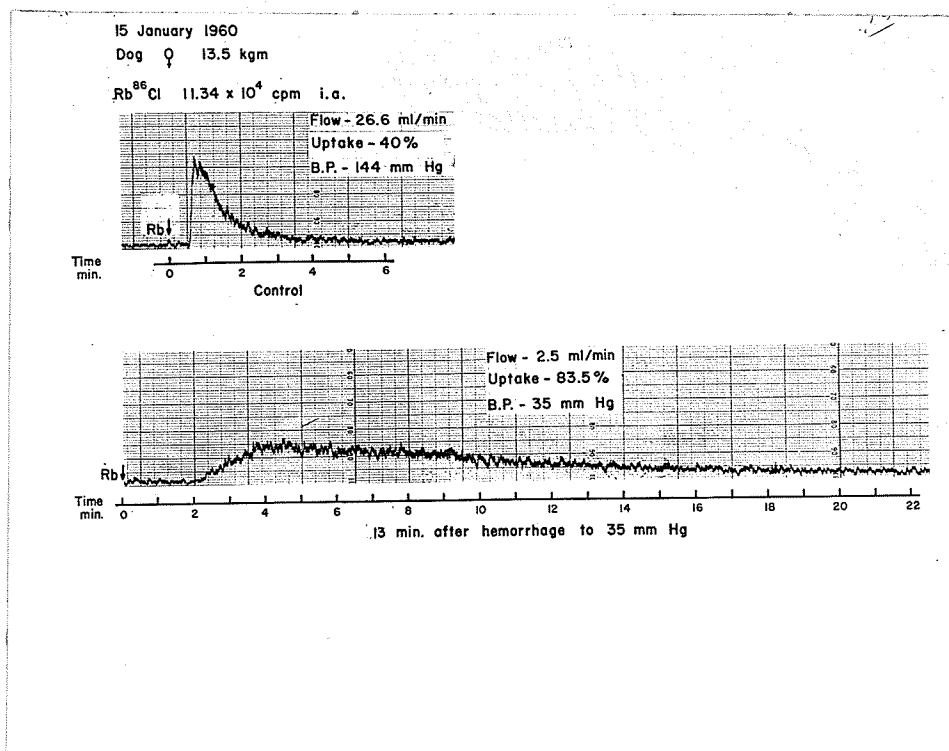


Figure 20

Oscillographic recording of radioactivity in the venous outflow of the hind limb of a dog before and during the hypovolemic period of hemorrhagic shock. Note the increased latency, slower rise and greater persistence of the tracer in the venous outflow during hypovolemia as compared to the control.

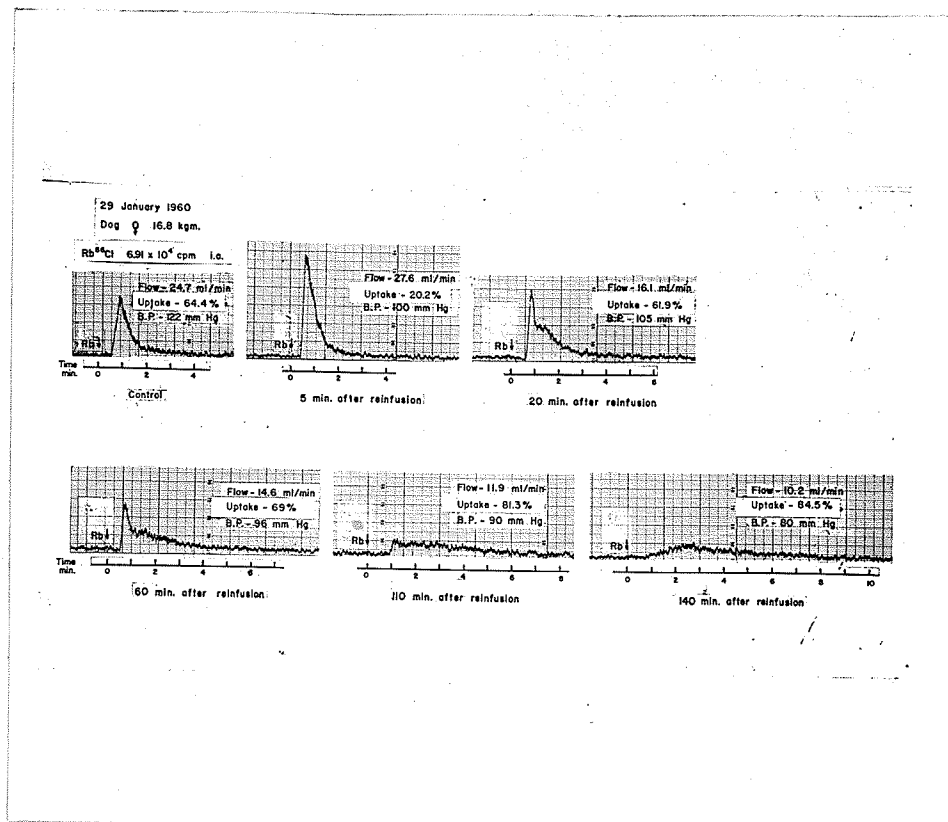


Figure 21

Oscillographic recording of radioactivity in the venous outflow of the hind limb of a dog before hemorrhage and in the period immediately after reinfusion of blood. The pattern during the early postreinfusion period shows decreased latency and a higher peak. Thereafter, latency increased and the peak decreased with the appearance of a plateau. Eventually, the pattern approximates that seen during hypovolemic shock. (See Figure 30).

and flow, uptake increased and the flow-uptake pattern was not different from that of the control period. Flow-uptake relations for these experiments are illustrated in Figure 22.

In 3 untreated dogs, induction of hypovolemia was accompanied by decreased blood flow and increased uptake of Ib^{86} by loops of ileum. During this period, the increased uptake of Ib^{86} corresponded to the control flow-uptake relation. After reinfusion, there was a gradual departure of the Ib^{86} uptake from this relation in the direction of decreased uptake. As in Group I animals, this occurred despite a progressive decrease in total blood flow. The results from these experiments are shown in Figure 23. In contrast, no deviation from the control flow-uptake relation was seen in loops of ileum of 3 dogs pretreated with phenoxybenzamine. The relations observed in these animals are illustrated in Figure 24.

Ib^{86} uptake during shock produced by prolonged infusions of noradrenaline.

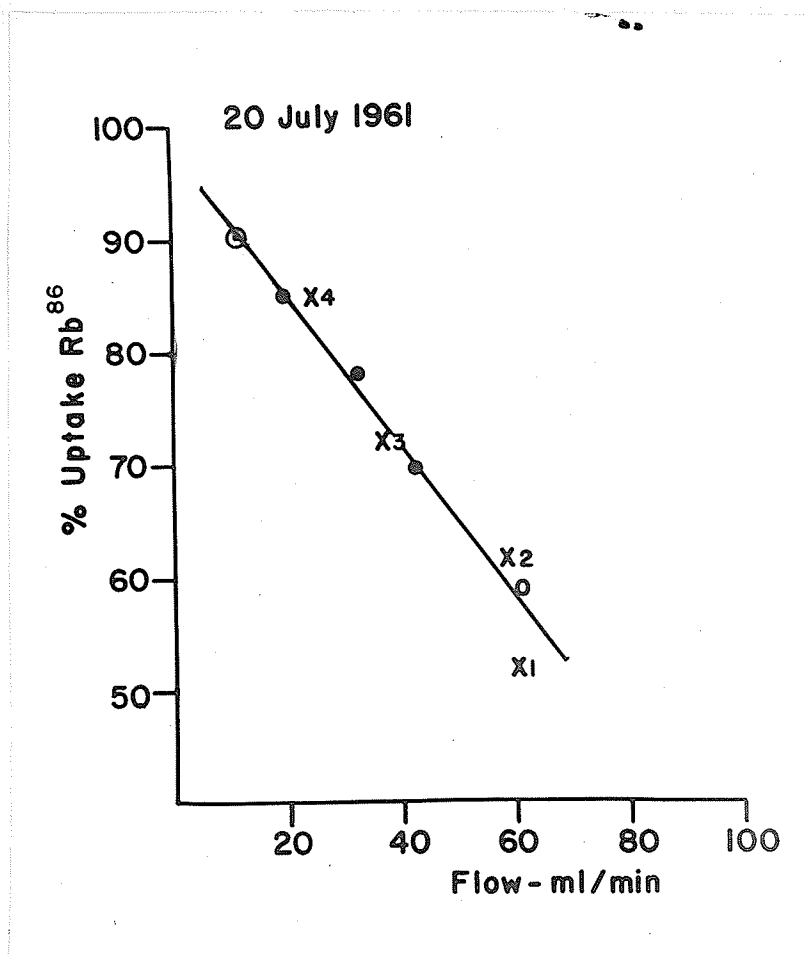
Hind limb and intestinal uptakes of Ib^{86} were measured during infusions of noradrenaline. Uptake by an intestinal segment was measured also in animals which had received phenoxybenzamine (1.0 mg/kg) intravenously 18 hours previously.

Infusion of noradrenaline did not alter the flow-uptake relation of the hind limb in 3 dogs even though the infusion was continued until shock developed. Blood flow changes during noradrenaline infusion were similar in all 3 animals. There was some increase in flow during the first 10 to 15 minutes of the infusion which was followed by a progressive decline during the course of infusion. The results of these experiments are illustrated in Figure 25.

Striking changes, however, were produced by noradrenaline in the

Figure 22

Flow-uptake relations for Rb^{86} in the vascular beds of canine hind limbs before and during shock induced by the standard hemorrhagic procedure. Control relations were determined during free flow (open circles) and during arterial occlusion (closed circles). Uptakes during the shocking procedure as follows: 60 min Rb period (closed triangles); clamp-off period (target symbols; 1, 20, 40 and 60 minutes after reinfusion (X1 to X4, respectively).



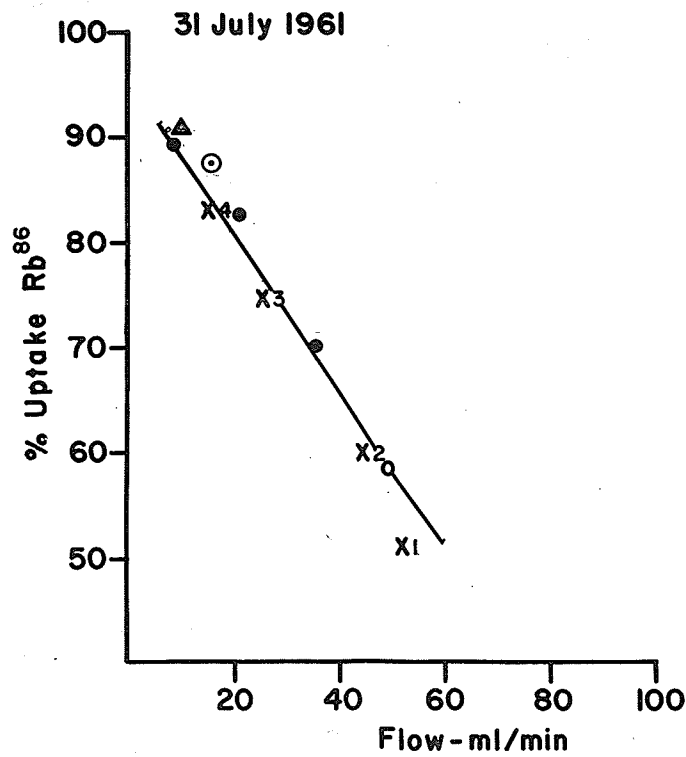
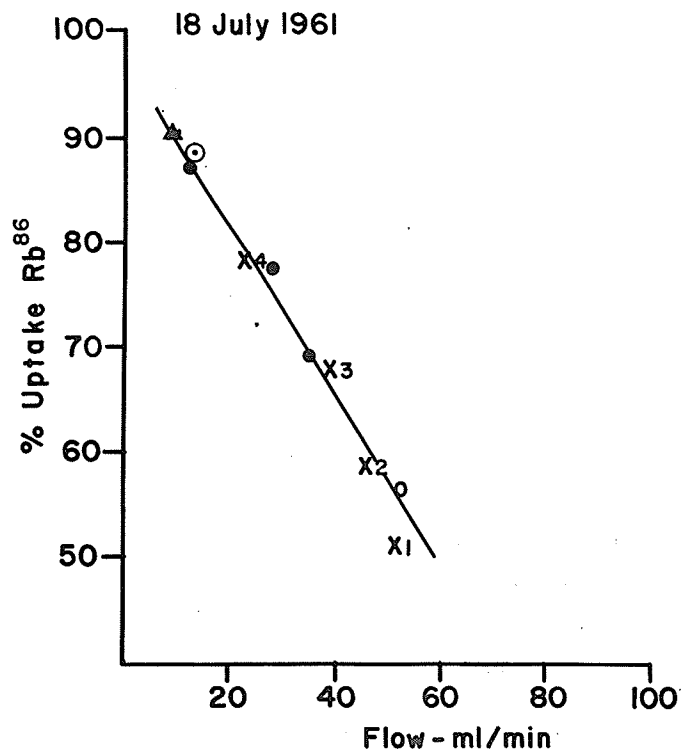
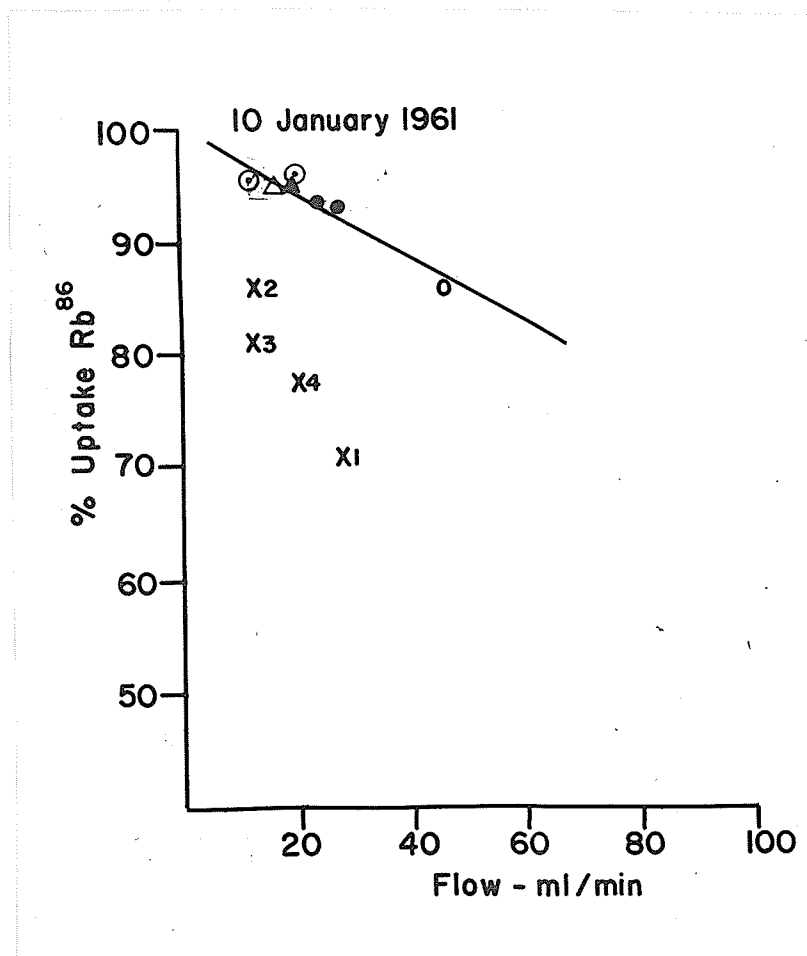


Figure 22

Figure 22

Flow-uptake relations for Rb^{86} in the vascular beds of the canine ileum before and during shock induced by the standard hemorrhagic procedure. Control relations were obtained during free flow (open circles) and during arterial occlusion (closed circles). Uptakes during the shocking procedure as follows: 35 and 60 mm Hg periods (open and closed triangles, respectively); clamp-off period (target symbols; 3, 20, 40, 60 and 90 minutes after reinfusion (X1 to X5, respectively)).



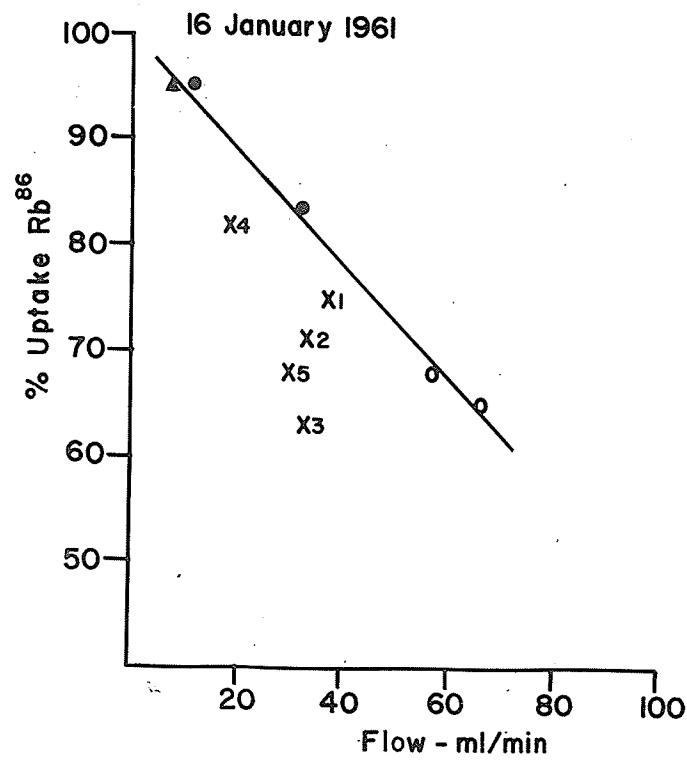
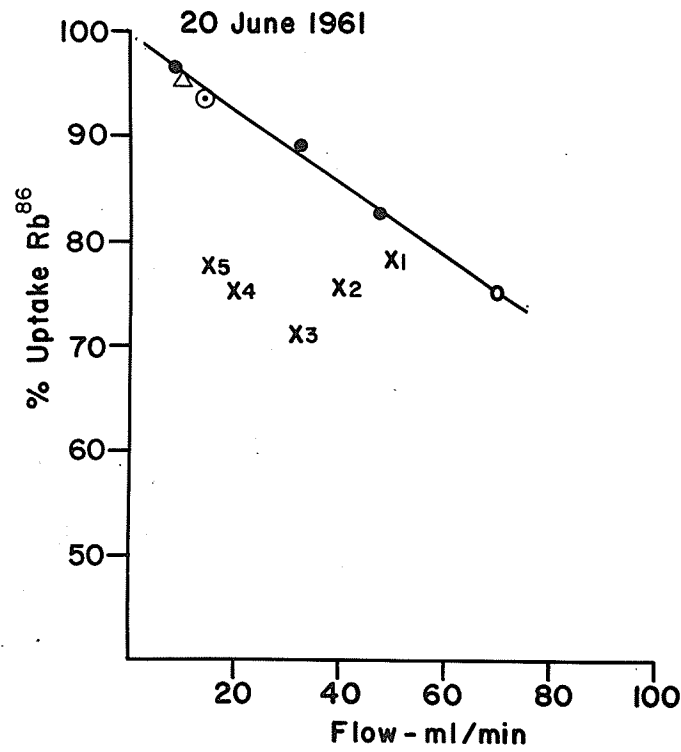
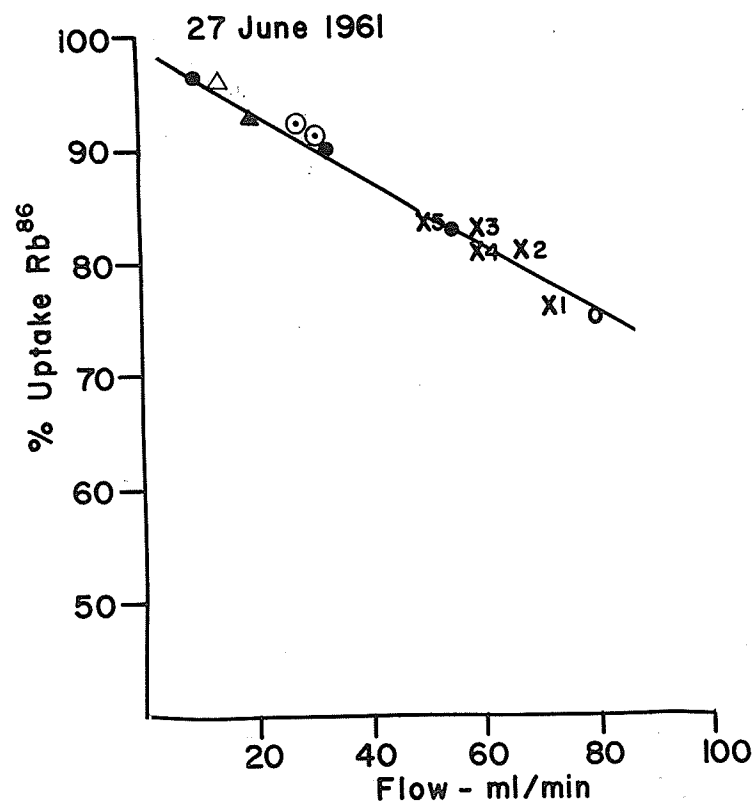
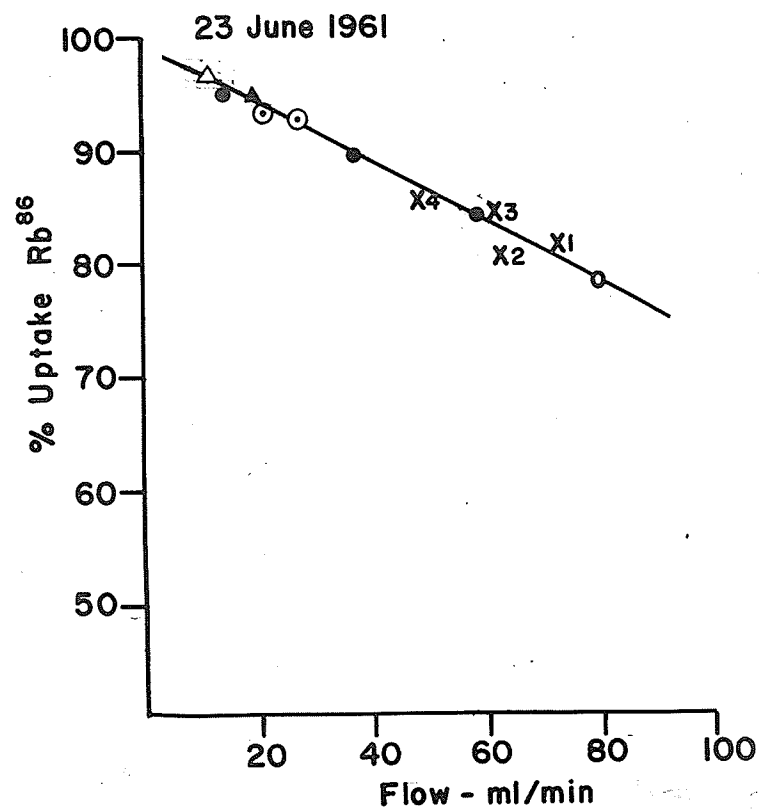
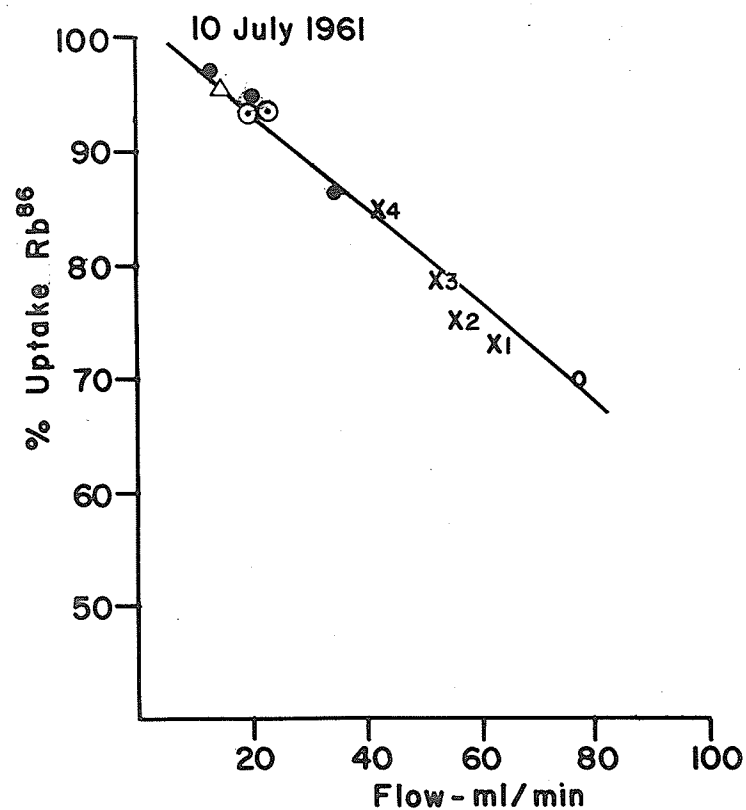


Figure 22

Figure 24

Flow-uptake relations for Rb^{86} in the vascular beds of the canine ileum, before and during the standard hemorrhagic shock procedure. The animals had received phenylephrine (1.0 mg/kg) 18 hours prior to the experiment. Control relations are represented by the open (free flow) and by the closed circles (arterial occlusion). Uptakes during the shocking procedure as follows: 35 and 60 sec periods (open and closed triangles, respectively); clamp-off period (target symbols); 5, 20, 40, 60 and 80 minutes after reinfusion (X1 to X5, respectively).





flow-uptake relation in the intestine. As in the leg, flow increased initially in response to noradrenaline and then decreased progressively. However, in contrast to the leg, uptake of Rb^{86} did not follow the control flow-uptake relation in any of the 3 animals. Figure 26 illustrates the changes observed in this vascular bed. Points X_1 , determined 15 minutes after the beginning of infusion, were below the expected uptake value, and this discrepancy was increased for determinations made 30 to 60 minutes after the start of the noradrenaline infusion. With one exception (experiment of 7/7/61), points determined after 2 to 3 hours of noradrenaline infusion show no further departure from the flow-uptake relations obtained prior to infusion.

Pretreatment with phenoxybenzamine prevented the changes in the flow-uptake relation induced by noradrenaline in the intestine. The pressor response to noradrenaline did not exceed 13 mm Hg at any time, and no evidence of developing shock was observed. Results from these animals are shown in Figure 27. All determinations of Rb^{86} uptake during and after infusion of noradrenaline conformed to the control flow-uptake relations.

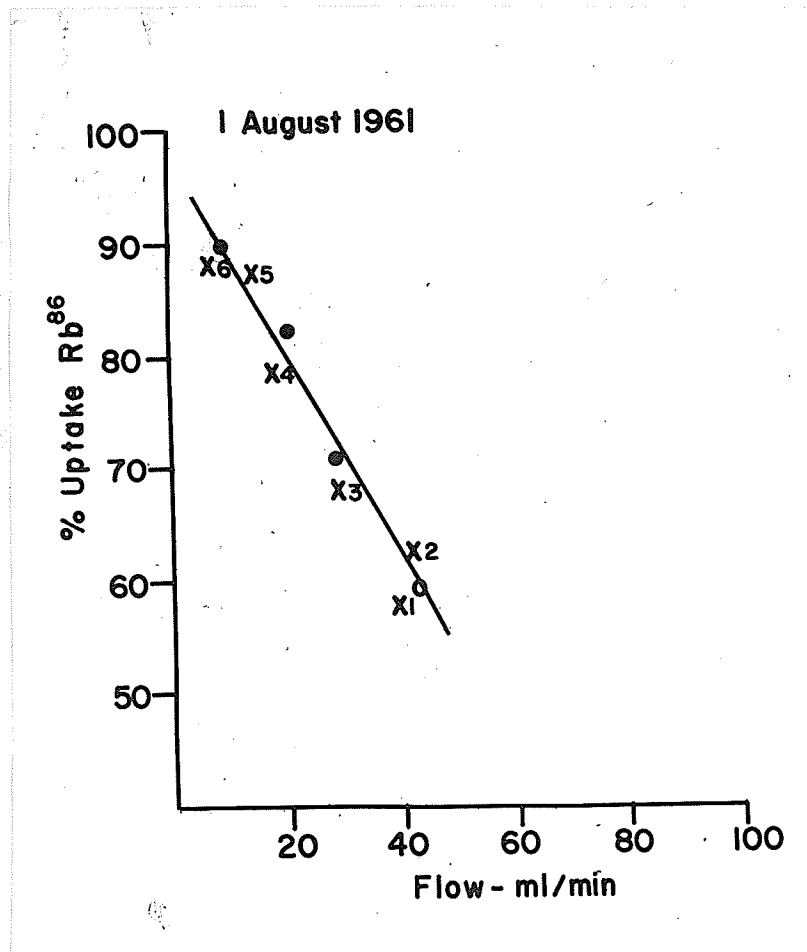
Discussion

In the range of blood flows covered by our experiments, Rb^{86} uptake by the hind limb and ileum of dogs was inversely and linearly related to blood flow. The flow-uptake relations of the hind limb and ileum were quantitatively different. The slope was considerably greater in the hind limb and uptake during free-flow perfusion was greater in the ileum.

Flow-uptake relations for Rb^{86} in the hind limb or loop of ileum obtained during alteration of flow by venous or by arterial occlusion were

Figure 25

Flow-uptake relations for Rb^{86} in the vascular beds of canine hind limbs before and during 3-hour infusion of noradrenaline. Control relations were obtained during free flow (open circles) and during arterial occlusion (closed circles). Uptakes after start of infusion as follows: experiment of 26/7/61, 15, 45, 90, 120 and 180 minutes (X1 to X5, respectively); experiment of 28/7/61, 15, 60, 120 and 180 minutes (X1 to X4, respectively); experiment of 1/8/61, 15, 30, 60, 90, 120 and 180 minutes (X1 to X6, respectively).



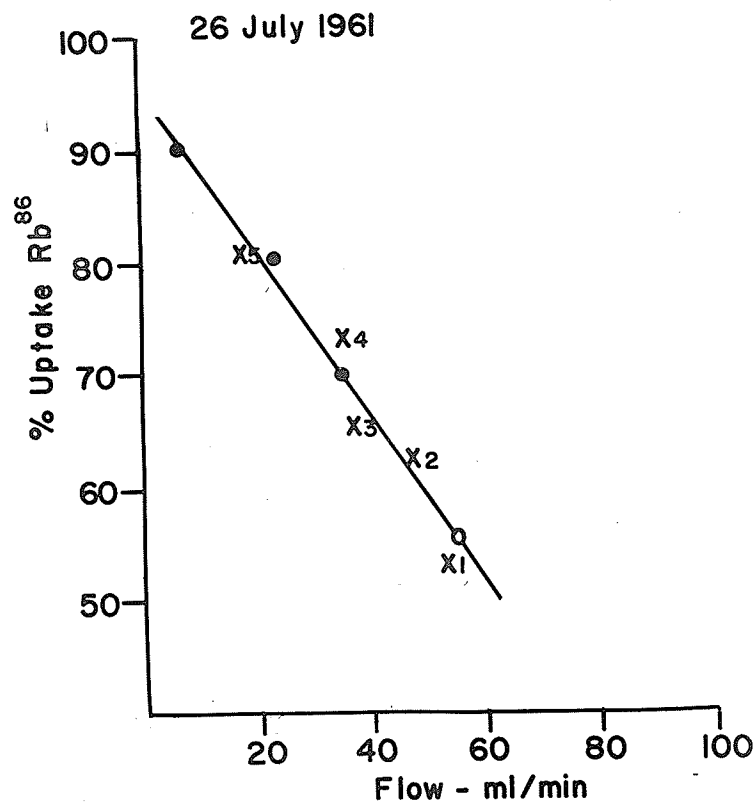
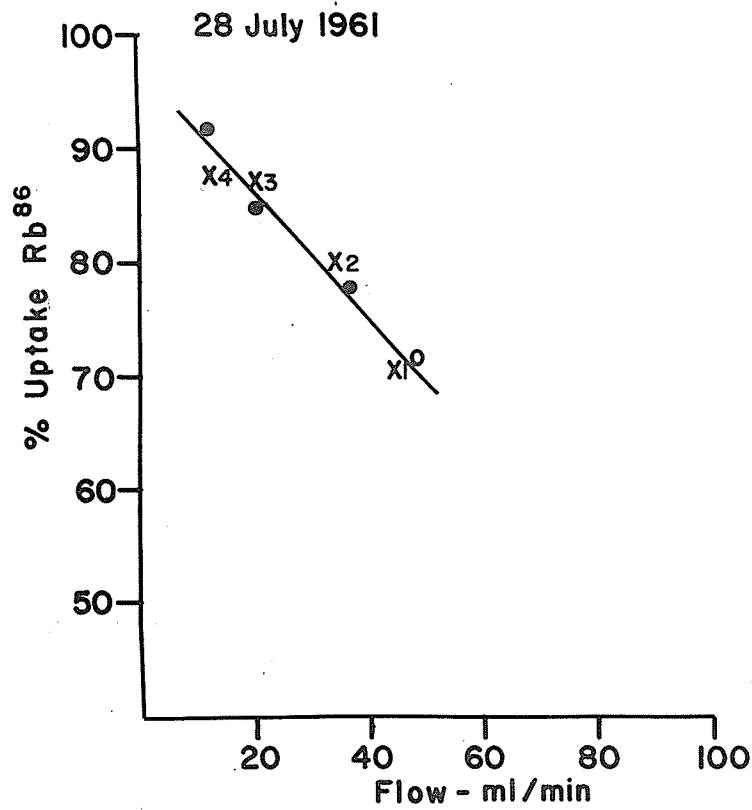
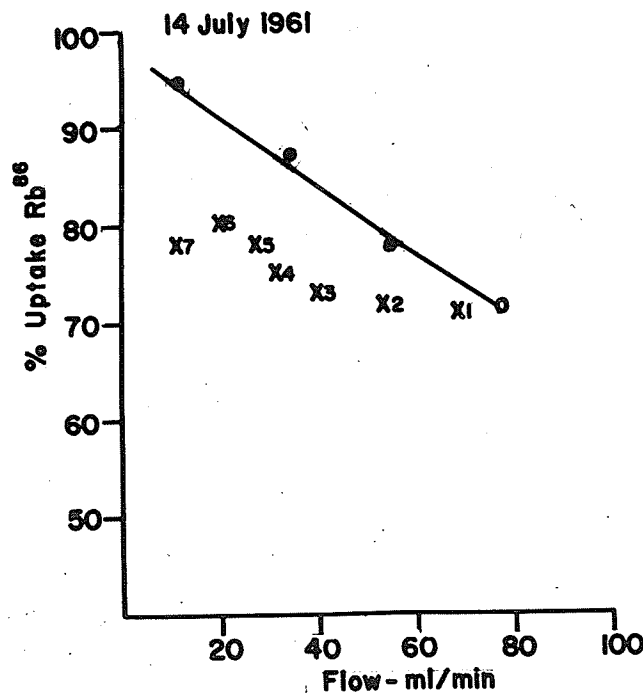


Figure 22

Figure 26

Flow-uptake relations for Rb^{86} in the vascular beds of the canine ileum, before and during 2 or 3-hour infusion of noradrenaline. Control relations were determined during free flow (open circles) and during arterial occlusion (closed circles). Uptakes after start of infusion as follows: 15, 30, 45, 60, 90, 120 and 180 minutes (X1 to X7, respectively).



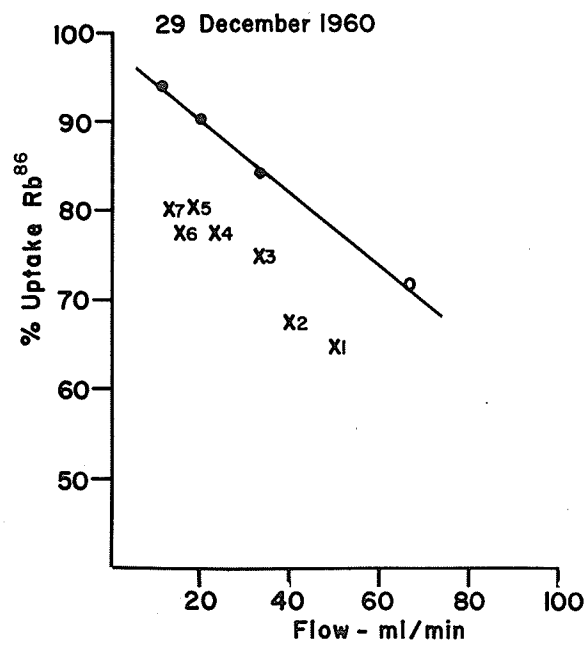
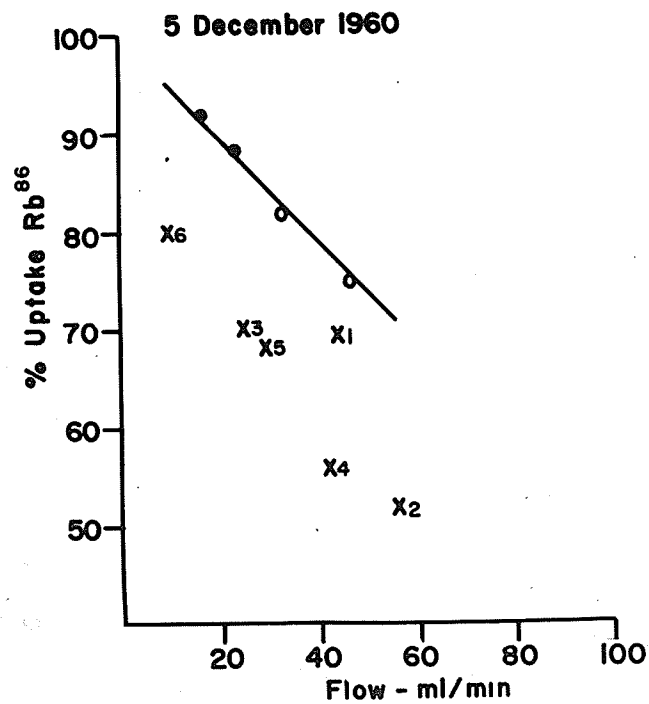
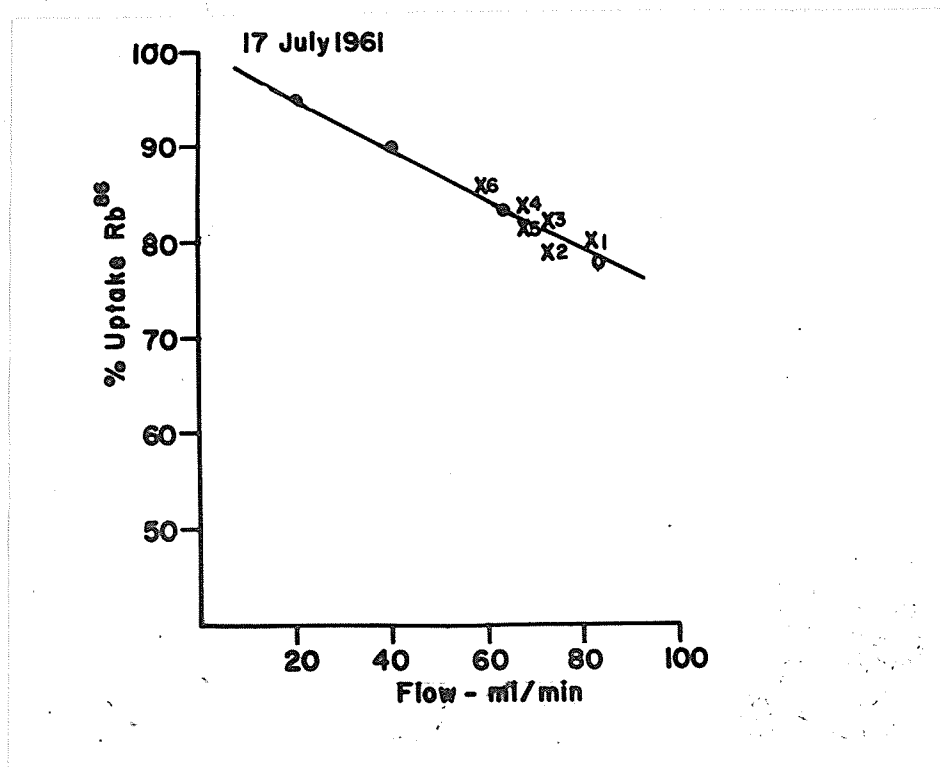


Figure 25

Figure 27

Flow-uptake relations for Rb^{86} in the vascular beds of the canine ileum, before and during 3-hour infusion of noradrenaline. The animals had received phenoxymethamine (1.0 mg/kg) 18 hours before the experiment. Control relations were determined during free flow (open circles) and during arterial occlusion (closed circles). Uptakes after start of infusion as follows: experiments of 17/7/61 and 21/7/61, 15, 30, 60, 90, 120 and 180 minutes (X1 to X6, respectively); experiment of 24/7/61, 15, 30, 60, 120 and 180 minutes (X1 to X5, respectively).



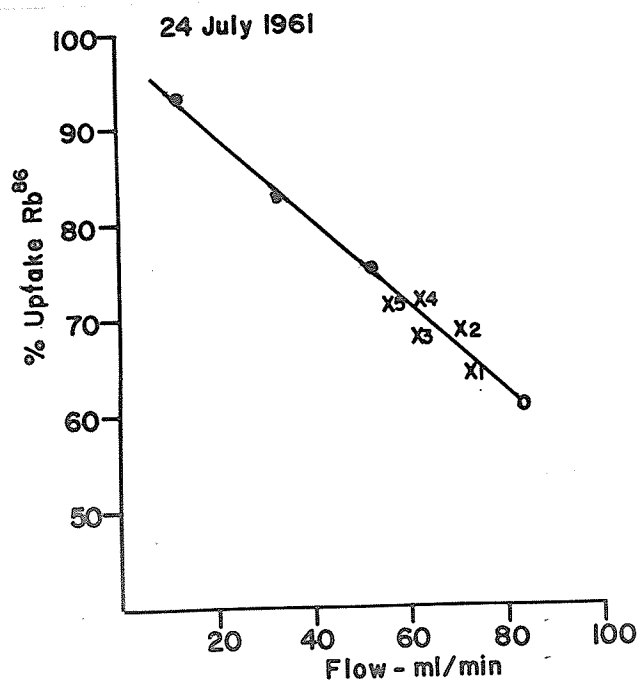
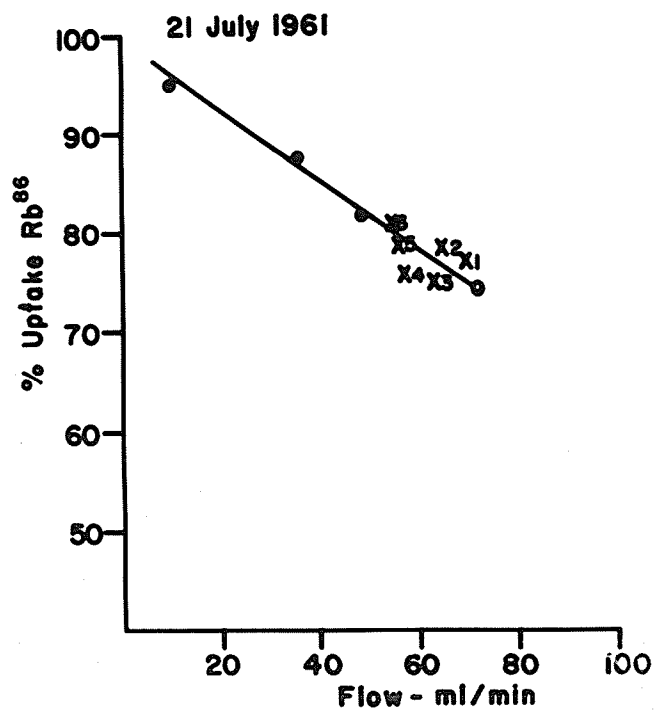


Figure XI

almost identical. Under conditions of constant arterial pressure, even small increments in venous pressure promote net efflux of fluid at the capillary level. Thus, reduction of flow by venous occlusion might be expected to result in a greater uptake of Rb^{86} than would occur during a comparable reduction in flow by arterial occlusion. However, the similarity of the changes induced by the two procedures indicates that Rb^{86} exchange occurs chiefly by diffusion and is not affected significantly by changes in the rate of mass movement of water across capillaries.

Exchange of Rb^{86} present in blood perfusing an organ and intracellular potassium will depend primarily on the nature of the vessel walls, as barriers to diffusion, and the time available for this exchange to occur. Because of the markedly increased barrier to diffusion in larger vessels, it may be assumed that most of the exchange occurs in true capillaries, as is the case with other metabolites. On this basis, Rb^{86} uptake following close intra-arterial injection appears to reflect accurately the distribution of total blood flow between "nutrient" vessels, i.e., those from which exchange with tissue cells occurs readily (true capillaries), and other parallel "non-nutrient" vessels.

The considerable difference in uptake values between the hind limb and ileum of control dogs under conditions of free flow raises another important point. Sapirostein's method for determining blood flow depends primarily on the assumption that interorgan extraction ratios are essentially the same. Although indirect evidence presented by Sapirostein (1956, 1958) and by Sheppard *et al.* (1953) supported this concept, the direct comparison afforded by our experiments appears to negate this premise. Consequently, the Sapirostein method for measuring total organ blood flow may be unreliable under basal conditions as well as during shock.

Hb^{86} uptake did not change from the control pattern when the vascular beds were perfused with progressively more hypoxic blood. This indicates that local hypoxia does not alter exchange of this tracer at the capillary level, at least within the range of oxygen saturations covered by these experiments (72 to 97 per cent). The relative uptakes of Hb^{86} obtained at comparable venous oxygen saturation but different flows are of particular importance. In every case, uptake appeared to depend on blood flow per se rather than on the oxygen content of venous blood. Assuming that venous blood oxygen saturation represents the maximum unsaturation of capillary blood, we can reasonably conclude that hypoxia is not an important determinant of Hb^{86} uptake.

The effects of shock induced by hemorrhage or by prolonged infusion of noradrenaline on the flow-uptake relation are most obvious in the vascular bed of the intestine. Uptake of Hb^{86} by the hind limb was minimally affected by either procedure even when the animals were irreversibly shocked. In contrast, Hb^{86} uptakes by the ileum were decreased considerably below those expected at the existing blood flows. Rayner *et al.* (1960), using H_2O uptake as a measure of true capillary flow, reported a similar picture in the intestine of dogs during the development of endotoxin shock. These observations strongly indicate that a considerable portion of the total blood flow perfusing the ileal segment may pass through channels which do not permit free exchange with tissue cells. The altered distribution of total blood flow in the intestine appears to be due to vasoconstriction per se. This conclusion is supported by uptake measurements made early during the period of noradrenaline infusion. In these animals, considerable deviation from the expected flow-uptake pattern occurred within 15 minutes after the onset of infusion, *i.e.*, well before the develop-

ment of shock. The resulting compromise of intestinal nutrition may very well explain the sensitivity of this organ in shock.

The ability of phenoxybenzamine to maintain the normal flow-uptake pattern in the intestines of dogs subjected to hemorrhage or nor-adrenaline infusion indicates that a major component of the protection provided by phenoxybenzamine may be promoting more efficient distribution of intra-organ blood flow.

Summary

The method of Sapirstein, which relies on rapid removal by the tissues of an exchangeable cation from the blood, is not directly applicable to the measurement of total organ blood flow in animals during the development of irreversible shock. This is most probably due to the fact that under these conditions plasma does not equilibrate with extracellular fluid as rapidly as it does in the normal animal. The reliability of the method in normal animals also must be questioned in view of demonstrated differences between interorgan extraction ratios.

The flow-uptake relations for Hb^{86} in the vascular beds of the hind limb and intestine were determined. The results show an inverse, linear relationship between Hb^{86} uptake and blood flow and indicate excellent reproducibility in a given dog and vascular bed. The similarity between the flow-uptake relation obtained during arterial occlusion and that resulting from venous occlusion indicates that Hb^{86} exchange occurs chiefly by diffusion and is not affected significantly by changes in the rate of mass movement of water across capillaries. Comparison of flow-uptake relations determined in the hind limb and intestine during perfusion

with fully oxygenated or hypoxic blood indicates that hypoxia is not an important factor in determining changes in the flow-uptake relation.

The flow-uptake relation for SB^{86} in the vascular beds of the hind limb was affected very little by shock-inducing procedures. However, in the ileum SB^{86} uptake was considerably depressed, even at low flows, throughout periods of noradrenaline infusion, even prior to the development of shock and during the normovolemic phase of hemorrhagic shock. These changes indicate that a decreased fraction of the total organ blood flow was traversing vessels from which exchange with tissue cells occurred readily. The normal flow-uptake relation was maintained throughout both shocking procedures in animals pretreated with phenoxybenzamine. A major component in the protection provided by phenoxybenzamine may be prevention of the deleterious redistribution of intraorgan blood flow characteristic of the development of shock, thus permitting more effective nutrition of compromised tissues.

GENERAL DISCUSSION

Review of the pertinent literature leaves little doubt as to the importance of vasoconstriction and of reduced effective regional blood flow in the development of shock. Three observations in particular give impressive support to this concept: 1) excessive vasoconstriction per se can induce shock and also can contribute to the development of irreversibility in shock due to other causes; 2) procedures and drugs which induce vasodilatation or limit vasoconstriction effectively prevent shock, and 3) shock-inducing procedures severely restrict blood flow in the viscera whereas maintaining an adequate blood flow through the splanchnic vascular bed prevents the development of shock. The present studies were concerned with possible mechanisms involved in the deleterious effects of excessive vasoconstriction in shock and in the protection provided by vasodilating agents, and particularly, with the relation between vasoconstriction and the local distribution of blood flow.

The foregoing results have shown that appropriate pretreatment with phenoxybenzamine protects against the development of irreversible hemorrhagic shock, reduces the mortality due to bacterial endotoxin and prevents the changes in the flow-uptake pattern for Mb^{86} in the vascular bed of the intestine induced by hemorrhage or by infusions of noradrenaline.

These effects of phenoxybenzamine appear to be related to its prolonged and highly specific adrenergic blocking activity. Both a reactive β -halogen and an appropriate aromatic group (phenoxyisopropyl, benzyl, etc.) are necessary structural components for this activity. Compounds related to

phenoxybenzamine but possessing only one of these moieties necessary for effective adrenergic blockade did not protect against shock. Furthermore, protection by phenoxybenzamine against the lethality of bacterial endotoxin, which may be released during shock and which may be an important determinant of shock, is related to the adrenergic blockade produced rather than to any direct inactivation of endotoxin.

The mechanisms involved in the protection provided by adrenergic blockade are of paramount interest. That an increased blood flow through otherwise constricted vascular beds may per se provide additional nutrition and thereby prevent dysfunction is obvious. Phenoxybenzamine provides an increase in the total plasma volume, and thus may reverse vasoconstriction-induced loss of intravascular fluid during shock, and it appears to decrease plasma skimming in the microcirculation. The importance of these changes in the distribution of blood flow within the microcirculation during shock becomes more apparent in simultaneous measurements of total blood flow and of extraction of Rb^{86} in a loop of ileum. During the development of shock there is a considerable decrease in the percentage extraction at a given flow rate, an indication that less of the total blood flow effectively exchanges metabolites with tissue cells. This change is particularly marked during the normovolemic period of hemorrhagic shock and during infusion of even relatively small doses of noradrenaline. In both cases the decreased uptake is largely prevented by phenoxybenzamine. This deleterious shift in the local distribution of blood flow appears to be promoted by adrenergic vasoconstriction and to be inhibited by the adrenergic blocking agent.

The experimental results reported above have defined more clearly the role of vasoconstriction and of reduced effective regional blood flow in the development of shock and allow formulation of the following working

hypothesis: Excessive vasoconstriction during shock results in a loss of intravascular fluid and a severe restriction of blood flow in certain critical organs. The reduction is not only in total flow, but also in the fraction of the total blood flow which traverses nutrient capillaries i.e. effective blood flow. Ultimately, irreparable dysfunction develops in critical organs, notably the intestines, and the shock becomes irreversible to retransfusion.

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PART VIII

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