# THE ROLE OF THE SYMPATHETIC NERVOUS SYSTEM IN THE DEVELOPMENT OF DECOMPENSATION DURING HEMORRHAGIC SHOCK

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This thesis is dedicated to my parents, for the motivation and the opportunity they provided; to my teachers for their patience and guidance; and to Donna and Elana, who made the effort meaningful.

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## ABSTRACT

The initial cardiovascular response to hemorrhagic hypotension may be termed compensatory in that it leads to an increase in blood pressure, or if the pressure is held constant, to a shift of blood from the animal to the bleeding reservoir. This period of compensation is associated with a movement into the vascular space of fluid with a low protein content, and thus hemodilution. The compensatory period is prolonged by adrenergic blockade with phenoxybenzamine (Dibenzyline), and shortened by the infusion of small amounts of noradrenaline.

After a period of hypotension the compensation changes quite abruptly to a phase of decompensation during which blood must be returned to the animal to maintain the established level of arterial pressure. The development of decompensation is characterized by progressive hemocontration, indicated by parallel increases in plasma protein concentration and hematocrit. The onset of decompensation is coincident with the onset of hemoconcentration, and factors promoting hemoconcentration appear to be important determinants of the subsequent course; the more pronounced the hemoconcentration, the less likely is survival. Adrenergic blockade delays and reduces the hemoconcentration and the decompensation, and even small doses of noradrenaline accelerate both.

Serial determinations of plasma volume (RISA space), plasma protein concentration and hematocrit show that the hemoconcentration is due to ultrafiltration of fluid from the vascular space, which quantitat-

ively accounts for the volume of blood required to maintain a constant arterial pressure during the early part of decompensation. This period is followed by a second decompensatory phase during which measured plasma volume continues to diminish without further hemoconcentration. The reduction in circulating blood volume during this phase appears to be due to the sequestration of blood within the vascular system. The second phase of decompensation is prevented by adrenergic blockade and accelerated by noradrenaline infusion.

Ultrafiltration from the vascular space during decompensation appears to be due to an increase in net capillary hydrostatic pressure. This occurs despite a constant arterial blood pressure and little change in central venous pressure, and must represent a change in the microvasculature. Either a decreased precapillary resistance, an increased postcapillary resistance or both could raise the capillary pressure. Precapillary resistances in the gastrointestinal tract, skeletal muscle, skin and kidney do not decrease during decompensation. However, postcapillary resistance increases progressively during hemorrhagic hypotension in both the intestine and skeletal muscle vascular beds, and appears to be responsible for both ultrafiltration of fluid from the vascular space and sequestration of blood in the tissues. The increased postcapillary resistance appears to be due to the activity of the sympathetic nervous system, since noradrenaline and phenoxybenzamine exert profound and opposite effects on the process.

Blood flows to the kidney, gastrointestinal tract, skeletal muscle and skin show important differences in their responses to hemorrhagic hypotension. Changes in vascular resistance and the effects of phenoxybenzamine pretreatment reveal a characteristic, highly organized sequence of vascular changes induced by the sympathetic nervous system in response

to hemorrhage. The magnitude of sympathetic vasoconstriction appears to be initially mesenteric > hindquarters > renal. The mesenteric and hindquarters vascular beds achieve a "maximal" constriction early in hemorrhage and this remains relatively constant until the immediate pre-terminal period. However, sympathetic constriction of the renal vasculature develops much later in the course of hemorrhage, and progresses with prolongation of the hemorrhagic hypotension.

After 2.5 hours or more of hypotension the decreased renal blood flow persists despite reinfusion of the shed blood and elevation of the arterial pressure, a response qualitatively different from that of the blood flow to intestine, skeletal muscle and skin. The depressed renal blood flow is associated with persistent oliguria, despite arterial pressures generally considered adequate for glomerular filtration. The degree of maintained renal vasoconstriction is related to the duration of hypotension. Pretreatment with phenoxybenzamine diminishes the maintained vasoconstriction and prevents the oliguria. After the vasoconstriction and oliguria develop they can be abolished by either denervation of the renal pedicle or intra-arterial administration of guanethidine and phenoxybenzamine. An infusion of noradrenaline producing renal vasoconstriction of quantitatively similar duration and degree does not result in maintained vasoconstriction following its termination. These findings suggest that the oliguria and persistently depressed renal blood flow following prolonged hemorrhagic hypotension are due to central nervous system activity mediated via efferent sympathetic nerves.

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## INTRODUCTION

A. The Problem of the Definition of Shock

Few processes have been subjected to more penetrating or diversified investigation than has "shock", the frequently lethal state that follows severe trauma. Despite this effort, there are few questions to which investigators who have worked in this area respond with any degree of unanimity. They all appear to agree, however, that no adequate definition of shock is available. Clearly, a universally acceptable definition must await a clearer insight into the pathophysiological processes involved. Cannon in 1923 wrote, "It seems to me that in such a complex as shock, definition is not a prime requisite. The important matter is ..... a careful description of the observed facts." Since an acceptable definition has remained elusive, a clinical description is necessary to facilitate communication. Cannon cites two descriptions of patients with the picture typical of shock. One patient had suffered a compound, comminuted fracture of a limb, the other a severe abdominal injury. They were cold and perspiring, pale, somewhat cyanosed, listless and apathetic; their pulse rates were rapid and blood pressures low. Injury in these individuals had led to a state of severe physical and mental prostration associated with a marked circulatory disturbance which followed a subacute course to death. Postmortem examination revealed nothing to explain the prostration, except the initial injuries.

There has been interest in a wide range of responses of the organism to shock inducing stress, but attention has been focussed primarily on the severe circulatory disturbance, since it

appears to be the decay of hemodynamic function which ultimately leads to death. The individual components of the cardiovascular apparatus, heart, arterioles, capillaries, and veins have been repeatedly investigated over the past seventy years, without the conclusive demonstration of any functional derangement to account for the progressive failure of cardiovascular function. (See Wiggers, 1950; Green, 1961.) Alterations in the function of some of these components have been observed, but only very late in the course of the shock process, well after the fate of the organism is sealed.

There appears to be uniform agreement that shock involves the organism as a whole, and can be initiated by any form of trauma or stress that results in generalized impairment of cardiovascular function. The cardinal feature of the syndrome appears to be an overall inadequacy of tissue perfusion, which in turn leads to impaired tissue function. This impairment is characteristically correctable early, but ultimately becomes "irreversible", i.e., refractory to current therapeutic measures, especially the transfusion of blood. The nature of the irreversibility to transfusion which results in an inexorable course to death in hours or days is the central problem in the field of shock today.

## B. The Choice of an Experimental Shock Model

A major area of advancement in the laboratory study of the shock process has been the development, during the last three decades, of techniques which induce shock in laboratory animals in a reasonably reproducible manner. Many of the techniques employed resemble stresses that are known to produce shock in man. These

include skeletal muscle and intestinal trauma, removal of blood, burns and prolonged regional ischemia of limbs or intestine. (See Wiggers, 1950; Fine, 1962; Selkurt and Rothe 1961.) Shock induced by hemorrhage was selected as the model in the present study because it is simple, highly reproducible, follows a well known course, and allows control of arterial blood pressure, which facilitates the study of cardiovascular function.

Somewhere between an acute lethal bleeding volume which results in rapid death due to cardiorespiratory failure, and a sublethal hemorrhage from which the animal recovers, lies a critical bleeding volume which leads more slowly to death through the induction of hemorrhagic shock. Animals bled somewhat less than the acute lethal bleeding volume, and then held at the resultant arterial blood pressure by the removal or reinfusion of blood, go through two characteristic stages. There is an initial stage which has been described as "compensatory" (Green, 1961) because if hemorrhage is stopped at this time, the blood pressure rises, and if the shed blood is returned, the majority of animals survive. If the blood pressure is held constant during this stage, the compensatory process expresses itself as continued loss of blood. Compensation is followed, after a variable but relatively short interval, by the development of a decompensatory stage in which the continuous reinfusion of blood is required to prevent the animal's blood pressure from falling. If reinfusion is withheld during the decompensatory stage, the blood pressure falls rapidly and the animal soon dies. During this period a progressively increasing percentage will die

despite the reinfusion of all the shed blood. There is no adequate explanation today for either the necessity of reinfusing blood during the period of decompensation, or its fate.

It is apparent from this description that there is a striking resemblance between the results of maintained hemorrhagic hypotension in the experimental animal and the course all too frequently seen in man during shock. The clinical pictures are also very similar. The animals are hypotensive and have a rapid, weak pulse, decreased pulse pressure, weak heart sounds, cold skin, oliguria, and if unanesthetized, apathy, muscular weakness and depressed reflexes.

C. The Purpose and Scope of the Present Study

The major purpose of the present investigation was to delineate the mechanisms of the development of decompensation during hemorrhagic hypotension in the dog. As the program developed it became necessary to investigate the following: (1) The role of transcapillary fluid shifts and the intravascular sequestration of blood in the development of decompensation, (2) the relation of peripheral hemodynamic changes to the course of the hypotension and the fluid shifts that occur, and (3) the role of the sympathetic nervous system in the development of decompensation.

Ancillary studies were also carried out to: (4) Assess blood flow redistribution during hemorrhagic hypotension, especially as influenced by sympathetic nervous system activity, (5) explore special factors in the response of the renal vascular bed to hemorrhage, particularly in relation to the development of oliguria following hemorrhagic hypotension, and (6) determine the phenoxybenz-

amine dose-response curve with respect to cardiovascular responses to hemorrhage.

# THE NATURE OF CIRCULATORY DECOMPENSATION IN SHOCK:

# REVIEW OF THE LITERATURE

A. Decreased Peripheral Resistance

The inability of patients or animals in shock to maintain their blood pressure is one of the most dramatic features of this syndrome, and so has attracted much attention. In terms of hemodynamics, a falling blood pressure can only be accounted for on the basis of a decrease in cardiac output or in peripheral resistance, or in both. Although it has been frequently suggested that shock represents failure of the organism to maintain peripheral vascular resistance, accumulated evidence has made this view untenable, and it is now of historical interest only. Crile (1915) postulated that the progressive cardiovascular decompensation in shock was secondary to "exhaustion" of the vasomotor center, resulting in the loss of arteriolar constriction. However, Cannon (1923) pointed out that, even late in the shock process, vascular denervation results in an increased blood flow, that incisions made during shock bleed very little, and that blood flow at a constant perfusion pressure remains depressed throughout the course of shock. It has subsequently been demonstrated many times that blood flow to various vascular beds remains depressed during shock, generally in association with a considerable increase in vascular resistance. In fact, the pendulum has swung to the point where many investigators now feel that the pathogenesis of shock involves too much, rather than too little vasoconstriction. (See Lillehei et al., 1964, Nickerson, 1964.) Gregg (1962) has reported studies of vascular resistance in hemorrhagic shock in unanesthetized dogs previously prepared with implanted electromagnetic flowmeter probes. He concluded that "the pathogenesis

of shock cannot be attributed to peripheral vascular collapse at the arteriolar site. The hemodynamic disturbance is postarteriolar in location."

B. Decreased Cardiac Output

As was pointed out above, if the blood pressure fall is not due to a decrease in peripheral resistance, it can be accounted for only on the basis of a fall in cardiac output. This fall in cardiac output has been amply demonstrated, both in man and in experimental animals. (See Blalock, 1934; Richards, 1944; Wiggers, 1950.) The decreased cardiac output, in turn, can be accounted for only on the basis of either inadequacy of myocardial function or of failure of venous return to the heart.

I. Myocardial Inadequacy. There has been continuing debate on the role of the heart per se in the failure of the organism to maintain cardiac output during the development of shock. Since the heart normally plays such an important role in maintaining the constancy of the circulation, it has not seemed unreasonable to suggest that it plays a major part in the circulatory derangement seen in shock. Many investigators have presented evidence that appeared to support the concept that failure of myocardial function is a crucial factor in the pathogenesis of shock. Unfortunately, they have rarely distinguished between failure of myocardial function as a late, preterminal event, and myocardial failure as a primary cause of the circulatory derangement leading up to the terminal episode.

Several early workers in the field of shock suggested that cardiac failure was of major etiological importance. (See reviews by Cannon, 1923; Moon, 1942; Simeone, 1963.) For several decades following the first World War, this view fell into disrepute, but Wiggers (1950) reopened the question with the observation that late in the course of shock, there was frequently an increasing atrial pressure in association with a falling cardiac output. This observation led him to conclude that myocardial inadequacy frequently played a role in the circulatory collapse that follows hemorrhage. In support of this viewpoint, Wiggers cited abnormal ventricular volume and pressure pulses, and abnormal electrocardiograms. Kohlstaedt and Page (1944) employing a roentgenographic technique, observed dilatation of the chambers of the heart during experimental hemorrhagic shock. However, these were late changes, becoming apparent only when the animal was about to die.

Other data suggested that coronary circulation decreased during shock (Wiggers, 1950), in association with an abnormal pattern of myocardial metabolism (Hackel and Breitenecker, 1963). A relationship between the abnormal coronary blood flow and abnormal functional responses was demonstrated by Sarnoff et al. (1954), who showed that the late rise in left ventricular filling pressure which appeared after an interval of hypotension could be reversed by increasing left coronary artery blood flow with a pump. However, this procedure did not prevent further deterioration of the animal.

Crowell and Guyton (1961, 1962) studied the effects of hemorrhagic hypotension on right and left atrial pressures with

cardiac output held constant. They found that after some delay these pressures rose, both when the cardiac output was held at a low level and when it was maintained at a normal level by extensive transfusion. They were also able to demonstrate myocardial deterioration by examining the cardiac response to increasing venous filling pressures induced by transfusion during various phases of shock. As shock progressed, the cardiac output response to transfusion diminished, particularly at high filling pressures.

Gomez and Hamilton (1964) were able to duplicate the results of Crowell and Guyton, showing that late in shock the response of the heart to transfusion in terms of work output was diminished. However, these authors noted, "the experiments give no definite evidence of deteriorating (myocardial) function at resting volume loads, the heart of the dog subjected to hypotension must be faced with a large load in order that it may be differentiated from a normal heart". Even late in the shock process, work output had to be increased more than ten times before an abnormality in the response became apparent. Moreover, these authors noted that as shock progressed, extremely large volumes of donor dog blood were required to maintain venous return and central venous pressure, suggesting the presence of a prepotent peripheral component.

There is considerable evidence to suggest that failure of the heart is not a major factor in the induction of shock irreversible to transfusion. It has been demonstrated that shock differing in no recognizable way from that induced by hemorrhagic hypotension can be produced in the dog in which thoracic aortic

blood pressure, and thus presumably coronary blood flow, is maintained during hypotension by a balloon in the thoracic aorta (Smith and Grace, 1957). This observation is relevant especially in the light of the observation of Sarnoff et al. (1954) that the myocardial decompensation induced by hemorrhage could be reversed by perfusion of the coronary arteries.

Weidner et al. (1961) studied the force of myocardial contraction by means of a Walton-Brodie strain gauge in dogs subjected to hemorrhagic hypotension. The force of contraction fell with hemorrhage, but promptly returned to normal with reinfusion of the shed blood, even late in the decompensatory stage of shock. Thereafter, normal donor dog blood transfused in volumes sufficient to maintain a mean arterial pressure of 80 mm Hg maintained cardiac performance. They concluded that the basic defect causing progressive deterioration of the circulation was a failure of venous return to the heart.

Rothe and Selkurt (1964) have also recently reported studies on cardiac performance in the dog during hemorrhagic shock which suggest that a decline in cardiac filling, rather than primary myocardial failure, is the major cause of the progressive, ultimately fatal decline in cardiac output. They found that although mild inadequacies were occasionally demonstrable early in the shock process, as indicated by the relationship of ventricular end-diastolic pressure to cardiac work, or cardiac circumference to cardiac work, cardiac failure generally could not account for the falling output. They were able to demonstrate that dogs in shock tolerate transfusions of as much as three times their normal blood volume with minimal evidence of cardiac failure.

The failure of digitalis preparations in adequate dosage to change the course of shock also suggests that myocardial inadequacy is not a major factor in the progressive circulatory deficiency (Blalock, 1927; Glasser and Page, 1948).

II. Inadequate Venous Return to the Heart. Inadequate venous return would explain much of the available hemodynamic data in shock. However, this explanation suffers from one major flaw; to date there has been no adequately documented mechanism to account for a failure of venous return. Failure of venous return has been variously ascribed to loss of tone in the venous segments of the vascular system, promoting pooling of blood in the large and small veins, or to a decrease of intravascular volume caused by a progressive loss of blood from the vascular space.

There is little direct information on venous tone in shock. It is a common clinical experience that it is extremely difficult to cannulate veins in the extremities in shock, suggesting that venous dilatation does not occur there. Cannon (1923) pointed out that no evidence of dilatation of large veins of the splanchnic area is found during surgery on cases of traumatic and hemorrhagic shock in man. Alexander (1955) carried out extremely careful studies on dogs subjected to hemorrhage, and found no loss of venous tone before the terminal stage. In fact, recent studies by Lewis and Mellander (1962) suggest that the veins are much more resistant to the local effects of diminished blood supply than are the arterioles.

The alternative to venous dilatation to explain decreased venous return to the heart is a progressive loss of blood from the

vascular space. Malcolm first suggested this possibility in 1909. Decreased blood volume during shock has subsequently been demonstrated many times, both in laboratory animals and in man (Keith, 1919; Gasser et al., 1918; Freeman, 1933; Gibson et al., 1947; Huggins et al., 1957; Dunn et al., 1958; Fozzard and Gilmore, 1959; Crenshaw et al., 1962; Longerbeam et al., 1962; Suzuki and Shoemaker, 1964). There is considerable evidence in man not only that a blood volume deficit exists in shock, but that the severity of the signs and symptoms of shock, and the probable outcome are related to the magnitude of the blood volume deficit. [See Reeve (1961) for references concerning blood volume deficits in man in shock.] However, except in those situations where local tissue destruction could account for continuing blood or fluid loss at some site, as in burns, trauma, or peritonitis (Fine and Seligman, 1943, 1944), it appears to have been tacitly assumed that the missing volume was lost during the initial insult. Although this might be true in some situations, such loss would not explain the progressive nature of the syndrome, as would continuing loss of volume from the circulating vascular compartment. Cannon (1923) recognized this, and postulated continuous loss of plasma from damaged capillaries as the progressive factor in shock. He believed that the capillary damage was secondary to the release of toxic substances from traumatized areas.

Several different lines of investigation have failed to provide evidence of generalized capillary damage in shock. Plasma protein tagged with radioisotopes (Fine and Seligman, 1943, 1944) or with T-1824 (Gregersen and Root, 1947) does not disappear from the

circulation faster than normal in irreversible shock, and the limited quantity of labelled protein found in the tissues also suggests that capillary permeability is not increased. There is an increased rate of appearance of tagged protein in lymph from traumatized but not from nontraumatized tissues in shock (Fine and Seligman, 1944; Cope and Moore, 1944), again suggesting that a generalized increase in capillary permeability to protein does not occur. Total lymph flow is reduced (Wessely, 1958), and the transcapillary flux of sodium (Gellhorn et al., 1944), thiocyanate (Holmes and Painter, 1947), and deuterium oxide (Fogelman et al., 1952) are reduced approximately 50% in shock, which is not consistant with the hypothesis of increased capillary permeability, and suggests rather a decreased capillary surface available for exchange. The recent observation of a markedly reduced radioactive sulfate space is also consistent with the latter possibility (Crenshaw et al., 1962). Indeed, the capillary membrane appears to be extremely resistant to the effects of hypoxia (Hendley and Schiller, 1954). Recent studies by Lewis and Mellander (1962) also suggest that the capillary wall is resistant to the effects of markedly reduced blood flow, net transcapillary exchange of fluid continuing to occur mainly on the basis of the balance between hydrostatic and oncotic pressures.

As the theory of diffuse capillary damage in shock became untenable, the possibility that continuous loss of vascular volume contributed to the progression of shock was apparently rejected. However, other data indicate that important fluid shifts can occur during the devlopment of shock. Hemoconcentration, as indicated

by an increased blood specific gravity, was first noted in rabbits that went into shock following laparotomy (Sherrington and Copeman, 1893); hemodilution after hemorrhage was also noted by these workers. Subsequently, hemoconcentration was so frequently found in association with shock that Cannon (1923) devoted an entire chapter in his monograph to this phenomenon. Later, Moon (1942) considered hemoconcentration to be such an integral part of the shock process that he included it in his attempt to define shock as "... a circulatory deficiency, not cardiac or vasomotor in origin, characterized by a decreased volume of blood and cardiac output, and by hemoconcentration." By hemoconcentration, Moon and his contemporaries meant an increased concentration of erythrocytes, determined from the erythrocyte count, the hemoglobin concentration, or the whole blood specific gravity. There appears to have been considerably less interest in changes in the concentration of plasma proteins, although the theory of circulating volume loss due to generalized capillary damage rested on the supposition that "hemoconcentration" represented disappearance of whole plasma from the vascular space. A loss of whole plasma would indicate a loss of integrity of the capillary wall, whereas loss of a low-protein ultrafiltrate of plasma would strongly suggest that the capillary wall was intact.

## C. Decompensation in the Hemorrhagic Shock Model

Moon (1942) argued that hemorrhage and shock are distinct entities despite certain obvious similarities, including death preceded by diminished blood pressure, pulse pressure and body temperature, and rapid pulse and respiration. He pointed out several apparent

major differences. In shock, there characteristically is evidence of hemoconcentration, whereas after hemorrhage, hemodilution occurs. After death from shock, the veins are collapsed and bloodless, whereas the capillaries are distended with blood and blood oozes from the parenchyma of various organs when they are cut. After hemorrhage, on the other hand, the tissues are characteristically anemic. Finally, the administration of adequate fluids after hemorrhage frequently results in survival, whereas shock is generally resistant to the therapeutic effects of transfusion. Blalock (1934) disagreed with this distinction, pointing out that if dogs are bled and the resultant hypotension maintained by small hemorrhages or infusions as required, hemoconcentration occurs, the animal becomes resistant to the therapeutic effects of transfusion, and develops the pathological changes characteristic of shock. Subsequently, shock induced by hemorrhagic hypotension became the most commonly used laboratory model, but apparently Moon's opinion concerning the absence of hemoconcentration in this preparation prevailed. In his monograph published in 1950, Wiggers stated that shock induced by hemorrhage was not associated with hemoconcentration, and there appears to have been little subsequent interest in hemoconcentration in shock, either with reference to the mechanism of its development or its significance in the overall shock process.

The induction of hemorrhagic shock in experimental animals by the method mentioned in the introduction offers a useful model for exploration of the cause of irreversibility to transfusion. After a variable period of hypotension at a fixed arterial blood pressure the animal begins to require the reinfusion of its shed blood to prevent

the blood pressure from falling. Since initially it is necessary to remove blood from the animal to prevent arterial pressure from rising, the change to a tendency for the pressure to fall unless blood is returned has been called "reversal". It has frequently been pointed out that after reversal, there is a change from a high probability of survival after reinfusion of all the shed blood, to a progressively increasing mortality in spite of transfusion (Frank et al., 1945; Wiggers and Ingraham, 1946; Glasser and Page, 1948; Lansing and Stevenson, 1957; Smith and Grace, 1957; Fine, 1962; Simeone, 1963). Despite the obvious importance of this phenomenon in relation to the ultimate fate of the animal subjected to hemorrhagic hypotension, there appears to have been little specific effort to account for reversal in hemodynamic terms. Lillehei et al. (1964) have suggested that "uptake of blood from the reservoir is a sign of failure of the dog to maintain vasoconstriction in the visceral and peripheral vascular beds. Consequently, the size of the vascular bed begins to expand, and blood must be taken up from the reservoir to fill this increasing space." Similarly, Bohr and Goulet (1962) suggested that the uptake of blood indicated an increased vascular capacity, and that this was due to failure of smooth muscle somewhere in the peripheral vascular bed to maintain constriction. Fine (1954), Lansing and Stevenson (1957) and Rushmer et al., (1962) have all suggested similar mechanisms to account for the uptake of blood from the reservoir during decompensation. However, as was pointed out earlier, no convincing evidence for a loss of tone at any level of the vascular bed exists, despite decades of research designed to demonstrate such a phenomenon.

An alternative explanation for uptake has been offered by Crowell and Guyton (1961, 1962) who suggested that uptake of blood from the reservoir is a manifestation of heart failure, the blood being required to increase venous filling pressure, and thus to drive the failing myocardium to sustain the arterial pressure. However, as was pointed out above, there is little evidence of important myocardial inadequacy at any stage of shock except immediately prior to death.

D. The Role of the Sympathetic Nervous System

Considerable evidence has accumulated to suggest that the vasoconstriction induced by activation of the sympathetic nervous system during shock is one of the major factors responsible for the development of irreversibility to transfusion. The evidence for this view has been extensively reviewed in several recent publications, and will not be examined in detail here. (See Nickerson, 1955, 1964; Nickerson and Gourzis, 1962.)

The administration of adrenaline or noradrenaline can result in both extreme vasoconstriction and the development of irreversible shock which is indistinguishable from that induced by trauma or hemorrhage (Erlanger and Gasser, 1919; Yard and Nickerson, 1956). Similarly, cerebral decortication produces extreme activity of the sympathetic nervous system, vasoconstriction and shock (Freeman, 1933). These procedures also produce hemoconcentration and diminish blood volume. Furthermore, the circulatory failure engendered by hemorrhage is potentiated by the administration of relatively small amounts of adrenaline, or by the increased sympathetic activity

induced by buffer nerve section (Remington et al., 1950). Similarly, in shock induced by limb trauma, section of the dorsal roots to reduce sensory input decreases both reflex vasoconstriction and lethality (Wang et al., 1947), and conversely, reflex vasoconstriction induced by sciatic nerve stimulation potentiates the shock process, despite a significant increase in arterial pressure (Overman and Wang 1947).

It has frequently been noted in both patients and experimental animals that hypotension associated with vasodilatation is remarkably well tolerated (Phemister et al., 1945; Page, 1961). Animals in which vasoconstriction mediated by the sympathetic nervous system has been reduced or abolished by sympathectomy (Freeman et al., 1938), ganglionic or adrenergic blockade (See Nickerson and Gourzis, 1962 for references.) or depletion of noradrenaline stores (Seifen et al., 1964) are considerably more resistant than normal controls to shock induced by many different procedures.

The exact mechanism by which increased sympathetic nervous system activity potentiates the shock process is not clear; at least in part, this appears to be due to decreased perfusion of tissues. One purpose of the following studies was to delineate more clearly the role of the sympathetic nervous system in producing cardiovascular decompensation in shock.

# GENERAL METHODS AND DEFINITION OF TERMS

Experiments were carried out on dogs of both sexes ranging in weight from 5 to 26 kg and free of obvious disease or malnutrition. Animals were rejected if they were pregnant, lactating, very young or had been used for any previous study. The animals were housed in a constant environment at a temperature of approximately 68° F for several days prior to use. Their diet consisted of standard Purine Chow pellets and water ad libitum. Food was withheld for approximately 18 hours before each experiment. Survival studies and all studies during the summer months when the environmental temperature rose above 75° F were carried out in the animal quarters at 68° F. Other experiments were performed in a room whose temperature varied between 70° and 75° F.

The dogs were anesthetized with pentobarbital sodium (33 mg/kg) administered intravenously by way of a foreleg vein, and a 10 to 15 minute equilibration period was then allowed before shaving or any surgery was performed. Additional pentobarbital was administered as required to maintain surgical anesthesia, as determined by diminished corneal reflexes, and absence of spontaneous limb movements and of responses to painful stimuli.

Arterial and venous cannulations were carried out after surgical exposure of the vessels, utilizing thin-walled hard polyethylene catheters of the maximum bore permitted by the vessel. The catheters were inserted well up into the vessel and fitted with three-way stopcocks to allow irrigation with heparinized 0.9% NaCl as required to maintain patency. Mean arterial blood pressure (BP) was measured by means of either a mercury manometer or a Statham

P 23A pressure transducer connected to a catheter introduced into the aorta via either the left brachial or left femoral artery. In all cases where the animal was subjected to hemorrhagic hypotension, the BP during hypotension was determined with a saline manometer. Central venous pressure was measured with either a saline manometer or a Statham P 23D pressure transducer connected to a catheter inserted into the thoracic venous system by way of the right external jugular vein. The zero reference point for all pressure measurements was one-third the distance from the sternum to the animal table with the dog lying on its back.

All surgical procedures were carried out using cautery to aid in hemostasis since the blood was rendered incoagulable with heparin in most experiments. At least 30 minutes was allowed after surgery before heparin was administered, and hemostasis was not a problem. In most experiments the brachial arteries were exposed bilaterally for the measurement of arterial BP and for connection to a blood reservoir. In the studies in which plasma volumes were determined, a femoral vein was exposed for the injection of the indicator, and sampling was carried out through a catheterized femoral artery. Blood samples for determination of plasma proteins and hematocrits were taken from an arterial catheter after removing all blood filling the dead space.

Exposure of the superior mesenteric artery and terminal aorta for blood flow measurements was through a midline abdominal incision. The origin of the superior mesenteric artery from the aorta was located, the overlying mesenteric sheath incised parallel

to the artery, and the artery carefully freed from its sheath by blunt dissection so as to damage the perivascular nerves as little as possible. Exposure of the left renal artery was through a flank incision and a retroperitoneal approach to the renal pedicle. This was found to allow easy access to the renal artery and ureter with minimal disturbance of the kidney and the renal vein, which was of importance since handling the kidney appeared to influence renal blood flow. The left ureter was cannulated one to 2 inches below the pedicle with a large bore polyethylene catheter which was passed up to but not into the renal pelvis. The tubing emptied into a graduated cylinder fixed below the level of the animal table for the collection and measurement of urine.

Plasma protein determinations were always done on the same day as the experiment, using a modification of the gravimetric method of Van Slyke et al. (1950). By arranging the copper sulfate solutions in gradations of 0.0005 specific gravity unit, a 1% dilution of the plasma proteins could be detected.

Hematocrits were determined in duplicate on well-mixed arterial blood using microhematocrit tubes centrifuged for 5 minutes at 11,000 RPM in an International microhematocrit centrifuge. Centrifugation for 10 minutes did not further decrease the hematocrit reading. All hematocrits reported are uncorrected for trapped plasma.

Plasma volume determinations were carried out by the indicator dilution method using $I^{131}$ tagged human serum albumin (RISA). An arterial blood sample was taken just before the injection of RISA and at 8, 16, 24 and 32 minutes after the injection. The activities

of these samples were plotted on semilogarithmic graph paper and a line of best fit, determined by the method of least squares, was extrapolated to zero time. The zero time indicator dilution was used to calculate the plasma volume. The volume injected was determined from the change in weight of the syringe, and the number of counts injected from suitable standards.

Blood flow measurements were carried out in the renal, superior mesenteric and terminal aorta (hindquarters) vascular beds using a sine-wave electromagnetic flowmeter (Medequipment Co.). A range of probe sizes was available so that a good approximation of the artery and probe was always possible. The flowmeter was calibrated *in vitro* with blood and saline and *in vivo* on the femoral artery. The flowmeter output varied less than 5% when the hematocrit was varied from 0 to 70%, so changes in hematocrit were ignored in calculating blood flows. In preliminary experiments, zero flow was checked frequently by both electrical and mechanical methods, but since these values had a constant relationship, only electrical zero was used during the course of each of the reported shock experiments. The determination of mechanical zero requires reopening the dog and stopping the blood flow for a time, and it was felt that this might alter the course of the shock process. However, electrical and mechanical zeros were compared at the beginning of each experiment and at the time of death; in every case, electrical and mechanical zero coincided. Mean flow was monitored, using electrical integration, and the flows and blood pressures (determined with a Statham P 23A transducer) were recorded on a Grass Model 5 Polygraph. In most

experiments, blood flows to 2 vascular beds, and in some, those to 3 vascular beds were measured. The flowmeter handled only one input at a time, but it was possible to switch the probe inputs mechanically so that each blood flow was sampled intermittently. The probes were coupled to a relay which made it possible to sample each of three flows up to twice a minute. Generally, somewhat longer intervals were used.

Hemorrhagic hypotension was produced and maintained with Beck's (1954) modification of the reservoir technique first described by Lamson and Deturk (1945). The dog's blood was rendered incoagulable with heparin, 5.0 mg/kg intravenously, and a further 1.0 mg/kg was placed in the glass blood reservoir. The dog was allowed to bleed from a femoral or brachial artery into the reservoir, which consisted of an inverted 1-liter cylinder with 10 ml graduations. The rate of hemorrhage was controlled with a screw clamp on the tubing between the dog and the reservoir. Oxygen was continuously bubbled through the blood in the reservoir at a carefully controlled pressure. This served not only to keep the blood in the reservoir well mixed and oxygenated, but also to control the animal's arterial BP during hypotension. If the dog's arterial BP tended to rise to a level greater than that in the reservoir, additional blood was forced into the reservoir bringing the arterial BP down. Conversely, if the arterial BP tended to fall below the pressure in the reservoir, blood was forced back into the dog. In this manner, the dog's arterial BP was held within one or 2 mm Hg of any desired level, and any tendency for it to change was recorded as a change in the volume of blood in the reservoir.

The dogs were bled over approximately 30 minutes to a predetermined BP, in most experiments either 35 or 50 mm Hg, and maintained at this level until death, or until all blood remaining in the reservior was returned to the animal, depending on the requirements of the experiment. In experiments involving blood flow measurements, the dogs were held at a BP of 50 mm Hg, since they were more sensitive to the deleterious effects of hypotension as a result of the extensive surgery required to implant the probes. Also, in the case of smaller dogs, blood flow to the kidney at a pressure of 35 mm Hg occasionally became too low to measure accurately (less than 10 ml/min).

The volume of blood in the reservoir at any time during an experiment is referred to as the "bleeding volume", expressed in ml/kg. The volume of blood in the reservoir when the BP first reached the predetermined hypotensive level is referred to as the "initial bleeding volume" (IBV), and the largest volume of blood in the reservoir at any time during the course of a procedure as the "maximum bleeding volume" (MBV). During the interval between the IBV and the MBV, blood was continuously removed from the dog to prevent the BP from rising. This is the "compensatory" stage of shock described above. After reaching MBV, the dog required continuous reinfusion of the shed blood to prevent its BP from falling. This change from removal to uptake of blood is referred to as "reversal", and the return of blood to the dog without a change in arterial pressure is termed "uptake of blood". The interval following reversal during which uptake of blood occurred is the "decompensatory" stage of shock.

In some experiments, all of the blood remaining in the reservoir at some specified time was returned to the animal intra-arterially under increased pressure, a procedure which is referred to as "reinfusion".

All means are reported with the standard error (S.E.) of the mean. Unless otherwise indicated, all P values reported were calculated from the Student "t" test for paired data. The designation "n.s." in the tables denotes differences which were not statistically significant at the 5% level.

## SPECIFIC EXPERIMENTS, RESULTS AND COMMENT

### A. The Mechanism of Circulatory Decompensation during Hemorrhagic Hypotension (Sections I to XI)

## Section I

## Relationship of Hemoconcentration to Decompensation during Hemorrhagic Hypotension

Hemodilution, resulting from a movement of fluid into the vascular space, is a well documented response to hemorrhage in both experimental animals and man. The converse, hemoconcentration, has often been described in association with the shock syndrome, but its presence has been denied in shock associated with hemorrhage (Moon, 1942; Wiggers, 1950). The following experiments were carried out to determine whether hemoconcentration is demonstrable in shock induced by hemorrhage, and whether any relationship exists between the development of decompensation and changes in intravascular volume.

### Methods

Eleven dogs were bled into a reservoir as described under general methods. The first four dogs were carried through a modified Wigger's technique that had been in use in this laboratory. The dogs were bled over 30 minutes to a BP of 35 mm Hg, and held at this level for 50 minutes. This was followed by 30 minutes at 60 mm Hg and then by a 2-hour interval during which the connection between the dog and the reservoir was clamped off. Because the BP changes during the various stages of this procedure made interpretation of the experiments difficult, another type of protocol was adopted for the next seven dogs. In these experiments the dogs were bled to a BP of 35, 45 or 55 mm Hg and maintained at this level until death. Arterial

blood samples were taken every 15 minutes for the determination of plasma protein concentration and hematocrit.

Results

The results shown in Fig. 1 are typical of those obtained using the second protocol described above. The results for the group are similar in all respects to those shown in Tables I to VI, but the averages are not presented because several different levels of hypotension were used in these preliminary experiments.

In every dog, the plasma protein concentration began to fall within minutes after the onset of hemorrhage, and continued to decrease after IBV was reached. At MBV, the plasma protein concentration stopped falling, and coincident with the onset of uptake of blood (reversal) the plasma protein concentration began to increase, and generally exceeded the prehemorrhage level within 20 to 60 minutes after reversal. After an increase of variable magnitude, every dog showed a change in the slope of the plasma protein curve from the early rapid rise to a much more gradual rate of increase. This biphasic increase of plasma protein concentration after reversal occurred in spite of a relatively constant rate of uptake of blood from the reservoir. In the experiment for which data are shown in Fig. 1, the initial protein concentration was 5.87 g/100 ml and fell to 5.38 g/100 ml at MBV. The slope change occurred between 6.95 and 7.04 g/100 ml, at an uptake of 13.2 ml/kg (27.3% of MBV). Usually the slope change occurred somewhat earlier in the course of the experiment, at uptakes between 10 and 25% of MBV.

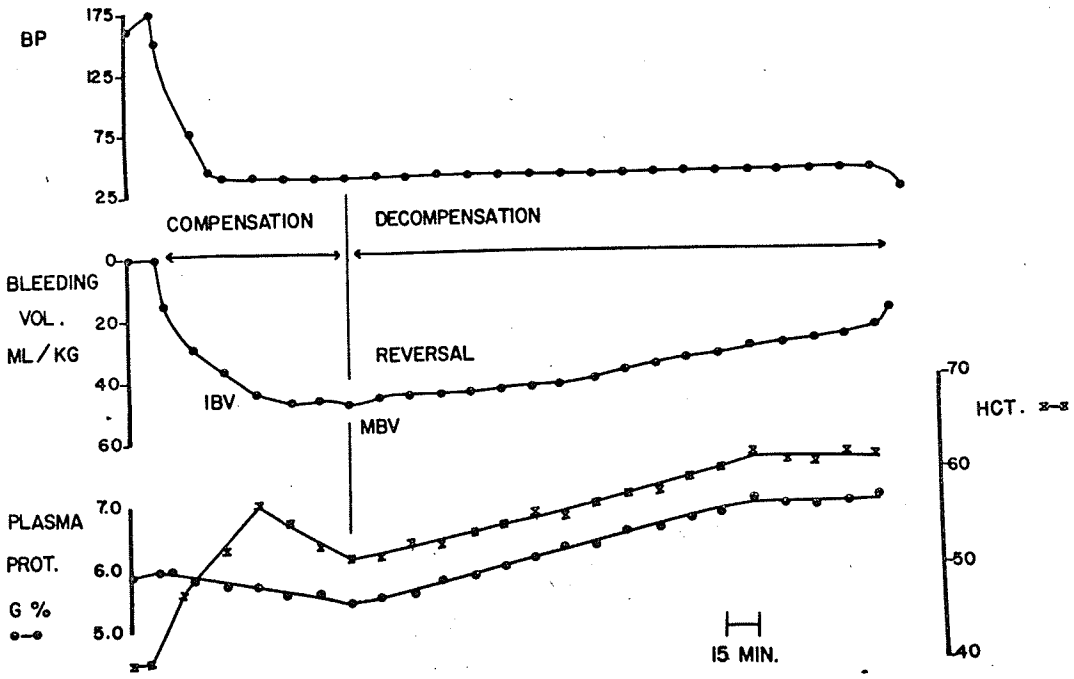


Figure 1. Association of Decompensation, Uptake of Blood and Hemoconcentration During Hemorrhagic Hypotension. Dog was maintained at an arterial pressure of 35 mm Hg until death, and showed typical changes in bleeding volume, plasma protein concentration and hematocrit (Hct.). "IBV" is the initial bleeding volume, the volume of blood removed to reduce the arterial pressure to the predetermined hypotensive level. "MBV" is the maximum bleeding volume, the maximum volume of blood removed from the animal at any time during the procedure. "Reversal" (vertical line) is the point at which uptake of blood from the reservoir became necessary to prevent the arterial pressure from falling, and corresponds to the onset of decompensation.

Changes in hematocrit following hemorrhage were more complicated than the changes in plasma protein concentration. All the dogs showed an initial increase in hematocrit of variable duration beginning shortly after hemorrhage started. In Fig. 1, it can be seen that the hematocrit had risen from 40 to 47% within 15 minutes of the onset of hemorrhage, and had reached an early peak of 56% within 45 minutes. At MBV the hematocrit had fallen to 52% in this dog. In all experiments, the hematocrit began to increase again after reversal and, when the slope of the plasma protein curve changed, the hematocrit increase showed a parallel tendency. The parallelism of the hematocrit and plasma protein concentration changes after reversal is evident in Fig. 1.

Comment

A very consistent relationship between the dogs' cardiovascular status after hemorrhage and changes in plasma protein concentration was observed. The period of compensation was always associated with hemodilution, as indicated by a decreasing plasma protein concentration. Hemodilution after hemorrhage has been described many times, and has been demonstrated to be due to an influx of a low-protein fluid into the vascular space. The observation that hemodilution always ended precisely with the compensatory stage was a new finding which suggests that the compensation is due, at least in part, to the intravascular movement of fluid tending to make up for the loss of blood. The increase in hematocrit during this early stage in the dog has been shown to be due to splenic contraction, and appears to be another compensatory mechanism (Wiggers 1950). The hematocrit increase was sufficient to mask the presence

of a considerable intravascular shift of fluid in every case.

An observation not previously reported was the association between reversal and increasing plasma protein concentration. The latter could have been due to movement of protein-poor fluid out of the vascular space, to movement of protein into the vascular compartment, or most simply, to the return of blood from the reservoir which had a protein concentration greater than that in the vascular space. While the latter possibility could not explain the protein concentration rising to values greater than initial, it could conceivably account for the immediate temporal association of protein concentration increase and uptake of blood. However, even during uptake of the first 10% of the shed blood following reversal, the amount of "excess" plasma protein received by the animal would be entirely inadequate to account for the increase observed. Using values for initial plasma volume and for movement of protein into the vascular space determined on a similar group of animals (Section V), it can be shown that the differential in protein content between the plasma in the reservoir and that in the dog would have to be at least 10 times as great as the maximum observed difference (initial concentration - concentration at MBV) for this explanation to be satisfactory.

## Section II

## Early Hemoconcentration in the Absence of Uptake of Blood

The quantitative considerations mentioned above made it unlikely that hemoconcentration was initiated by the uptake of

relatively protein-rich blood from the reservoir. However, because of the possible important theoretical implications of the precise temporal correlation between the onsets of hemoconcentration and of decompensation, the following experiments were undertaken to provide a final check on the possibility that the early protein concentration increase seen after reversal might in some way be related to the uptake of blood from the reservoir.

Methods

Seven anesthetized dogs were bled to an arterial pressure of 45 or 55 mm Hg, as previously described, and maintained at this pressure until reversal was detected by the uptake of 2 to 3% of the shed blood. The tubing connecting the blood reservoir to the dog was then clamped off, and the dog's arterial blood pressure allowed to change spontaneously. Arterial blood samples were taken every 15 minutes before clamp off, and at 5 to 10-minute intervals thereafter for the determination of hematocrit and plasma protein concentration.

Results

The results for this group are presented in Table I, and those from a typical experiment are shown in Fig. 2. After "clamp off", the BP fell progressively in all dogs and death ensued in 20 to 50 minutes. In every case, the plasma protein concentration and hematocrit increased steadily after clamp off, despite the falling arterial BP. The increases in plasma protein concentration and hematocrit are evident in Fig. 2, where the period during which the animal was disconnected from the reservoir is indicated by the

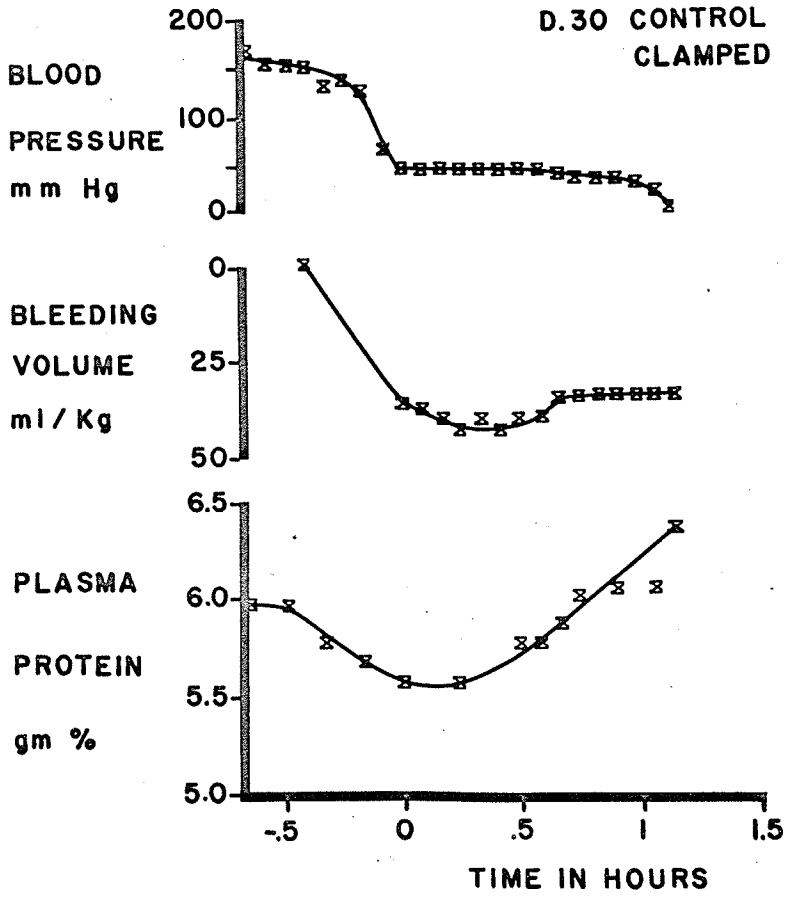


Figure 2. Effect of Preventing Uptake of Blood on the Development of Hemoconcentration. Note the progressive increase in plasma protein concentration in the absence of any change in bleeding volume and despite a falling arterial pressure.

Table I

Changes in Plasma Protein Concentration and Hematocrit during Hemorrhagic Hypotension without Reinfusion

| Dog Number | Initial | | At MBV | | Just before Death | |
|---|---|---|---|---|---|---|
| | Plasma Protein Conc. (g/100 ml) | Hematocrit (%) | Plasma Protein Conc. (g/100 ml) | Hematocrit (%) | Plasma Protein Conc. (g/100 ml) | Hematocrit (%) |
| 1 | 5.97 | 43.0 | 5.19 | 48.0 | 5.47 | 48.0 |
| 2 | 6.58 | -- | 4.73 | -- | 5.75 | -- |
| 3 | 6.48 | 38.5 | 5.93 | 40.5 | 6.39 | 43.5 |
| 4 | 6.30 | 45.5 | 5.28 | 46.0 | 5.65 | 48.0 |
| 5 | 6.71 | 40.5 | 6.49 | 40.5 | 6.67 | 44.0 |
| 6 | 6.39 | 38.0 | 5.84 | 43.5 | 5.93 | 43.0 |
| 7 | 6.02 | 43.0 | 5.65 | 43.0 | 6.39 | 52.5 |
| Mean ± S.E. | 6.35 ± 0.19 | 41.5 ± 1.6 | 5.58 ± 0.11 | 43.5 ± 1.8 | 6.04 ± 0.14 | 46.5 ± 1.8 |

| Interval | Percent Change | |
|---|---|---|
| | Plasma Protein Conc. | Hematocrit |
| Initial to MBV | - 12.1 | + 4.8 |
| MBV to Death | + 8.2 | + 8.0 |

constant bleeding volume. The initial plasma protein concentration was 6.02 g/100 ml; this fell to 5.65 g/100 ml at MBV, and rose to 6.39 g/100 ml before death. The latter change occurred during the period when there was no exchange of blood between the bleeding reservoir and the dog.

Comment

It is evident from these experiments that the rise in plasma protein concentration and hematocrit after reversal seen in the animals described in Section I, was not due to reinfusion of protein and erythrocyte rich blood. Whatever the cause of the progressive hemoconcentration after reversal, it overrides the effect of the falling arterial pressure, which, other things being equal, would tend to lower capillary pressure and thus produce intravasation of fluid (hemodilution). It is also apparent that after reversal, the dogs are in a state of serious cardiovascular decompensation, since prevention of uptake of blood at this time led in every case to a falling arterial BP and death in less than one hour, generally within 30 minutes. In contrast, experiments to be reported below (Section IX) will demonstrate that clamp off before reversal is associated with an increasing BP and the possibility of survival without the reinfusion of any of the shed blood.

The relative changes in hematocrit and in plasma protein concentration noted in these experiments are of some interest. Hematocrit tended to increase until MBV (ave. 4.8%), whereas the plasma protein concentration fell (ave. 12%). However, the increases after MBV were almost identical, 8.2% for plasma protein and 8.0%

for hematocrit. These findings confirm the parallel changes in plasma protein concentration and hematocrit after MBV noted in the experiments of Section I.

## Section III

## Effects of Adrenergic Blockade on Responses to Hemorrhagic Hypotension

Situations which increase sympathetic nervous system activity generally potentiate the lethality of shock-inducing procedures. (See Nickerson and Gourzis, 1962.) Conversely, adrenergic blockade decreases the lethality of various shock-inducing procedures including hemorrhage (Gourzis et al., 1963). (See the Review of the Literature for a more complete discussion and references.) Also, the sympathetic nervous system control of the precapillary and postcapillary vascular resistances can influence fluid shifts across the capillary wall by controlling the number of capillaries perfused and net capillary pressure (Mellander, 1960). The following experiments were undertaken to explore the effects of adrenergic blockade with phenoxybenzamine on the responses of dogs to hemorrhagic hypotension.

### Methods

Eight pairs of dogs were bled over 30 minutes to a BP of 35 mm Hg, and held at this level until death or for 8 hours. One of each pair was pretreated with phenoxybenzamine (POB), 5 mg/kg intravenously, given approximately 18 hours earlier. The animal

of each pair to be pretreated was selected by a toss of a coin. Experiments were carried out by two individuals who alternated in the preparation and management of the pretreated and control dogs. Arterial blood samples were taken every 15 minutes for the determination of hematocrit and plasma protein concentration, and BP and bleeding volume were recorded at 5-minute intervals.

Results

The results for this group are presented in Table II and those from a typical experiment are shown in Fig. 3. The responses of the control dogs were the same as noted in Section I, including hemodilution up to reversal, which always occurred within 60 minutes after reaching IBV. After reversal, the relatively rapid uptake of blood from the reservoir was associated with the rapid development of marked hemoconcentration. Also, as described in Section I, there was a clearly evident change in the slope of the plasma protein increase after reversal in every case. The data on hemoconcentration from 26 appropriate control animals from the experiments reported in Sections I to III are summarized in Table III, which also shows survival during the period of maintained hypotension. The first deaths occurred between 5 and 10 ml/kg uptake and the mortality was 73% by the time an uptake of 30 ml/kg was reached.

The differences between a POB-pretreated dog and its control shown in Fig. 3 were seen in all pairs. (See Table II.) The initial compensatory phase was considerably prolonged in the pretreated animals. The control dogs reached MBV in 42.2 ± 9.2 min after the IBV was reached, whereas the POB-pretreated dogs compen-

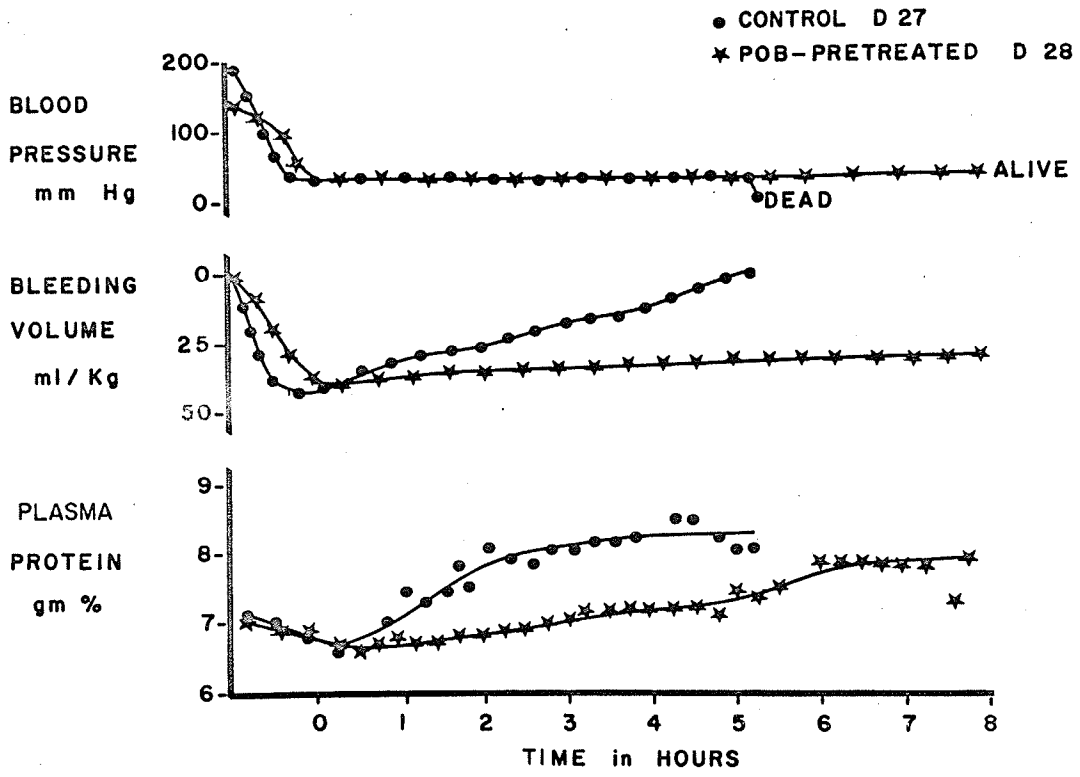


Figure 3. Effects of POB-Pretreatment on the Course of Hemorrhagic Hypotension. Paired control and POB-pretreated dogs bled to an arterial pressure of 35 mm Hg and held at this level until death or for 8 hours. Note the longer survival, slower uptake of blood and slower hemoconcentration of the pretreated animal.

TABLE II

Effects of POB Pretreatment on the Course of Hemorrhagic Hypotension

(Dogs Held at an Arterial Pressure of 35 mm Hg until Death or 8 Hours of Hypotension)

| Group | Initial BP (mm Hg) | Bleeding Volumes (ml/kg) | | | Rate of Blood Uptake (ml/kg/hr) |
|---|---|---|---|---|---|
| | | To Reduce BP 20 mm Hg | IBV | MBV | |
| Control (N = 9) | 158.5 ± 5.0 | 19.0 ± 2.2 | 45.1 ± 1.9 | 52.1 ± 1.7 | 15.8 ± 2.6 |
| POB-Pre-treated (N = 8) | 136.9 ± 4.2 | 4.1 ± 2.2 | 35.1 ± 1.5 | 51.2 ± 3.6 | 1.1 ± 0.1 |
| P | < 0.05 | < 0.01 | < 0.05 | n.s. | < 0.001 |

| Group | Time (Minutes) | | | Plasma Protein Conc. (g/100 ml) | | | |
|---|---|---|---|---|---|---|---|
| | To IBV | IBV to MBV | IBV to Death or 8 hrs | Initial | IBV | MBV | At Death or 8 hrs |
| Control (N = 9) | 30 | 42.2 ± 9.2 | 178.9 ±30.5 | 6.45 ± 0.18 | 6.04 ± 0.14 | 5.71 ± 0.18 | 6.95 ± 0.20 |
| POB-Pre-treated (N = 8) | 30 | 111.4 ±18.7 | 375.0 ±27.8 | 6.27 ± 0.17 | 5.82 ± 0.17 | 5.31 ± 0.25 | 6.18 ± 0.24 |
| P | | < 0.05 | < 0.01 | n.s. | n.s. | n.s. | < 0.05 |

TABLE III

Changes in Plasma Protein Concentration and Hematocrit, and Cumulative Lethality during Decompensation at an Arterial Pressure of 35 mm Hg

| Uptake of Blood (ml/kg) | Dogs Still Alive | | Plasma Protein Conc. (g/100 ml) | Hematocrit (%) | % Increase | |
|---|---|---|---|---|---|---|
| | No. | % | | | Plasma Protein Conc. | Hematocrit |
| 0 | 26 | 100 | 5.21 ± 0.11 | 40.8 ± 1.6 | 0 | 0 |
| 5 | 26 | 100 | 5.91 ± 0.20 | 46.0 ± 2.0 | 13.4 | 12.4 |
| 10 | 22 | 85 | 6.60 ± 0.22 | 50.5 ± 2.2 | 26.5 | 23.2 |
| 15 | 16 | 61 | 6.60 ± 0.21 | 51.2 ± 2.1 | 26.5 | 24.9 |
| 20 | 13 | 50 | 6.68 ± 0.23 | 52.1 ± 2.2 | 28.2 | 27.1 |
| 25 | 10 | 38 | 6.72 ± 0.40 | 51.0 ± 2.3 | | |
| 30 | 7 | 27 | 6.65 ± 0.24 | 48.5 ± 2.4 | | |

sated for 111.4 ± 18.7 min. In addition, the POB-pretreated dogs stayed at MBV for some time before the onset of uptake of blood from the reservoir, whereas control dogs always began to take up blood within 15 minutes after reaching MBV. When the POB-pretreated dogs began to take up blood, they did so at a much slower rate than did the controls. This was associated with a much longer survival, 375 ± 27.6 min, as compared to 178.9 ± 30.5 min for control animals. Actually, survival time of the POB-pretreated dogs was underestimated, 4 of the 8 being alive when the experiments were terminated after 8 hours of hypotension. These 4 dogs were included as 8-hour survivals in calculating survival time.

The prehemorrhage plasma protein concentrations, and the plasma protein concentrations at MBV were not significantly different in the two groups (Table II). However, after reaching MBV, a considerable difference became apparent. The plasma protein concentration did not increase during the interval between reaching MBV and the onset of uptake in the POB-pretreated dogs, and in parallel with their very slow uptake of blood, the plasma protein concentration increased only very slowly. Also, a clear change of slope in the plasma protein concentration increase was never apparent in the POB-pretreated animals.

The relationship between the volume of blood in the reservoir and the change in plasma protein concentration is shown in Fig. 4 for the same representative experiment illustrated in Fig. 3. This plot facilitates comparison of the POB-pretreated and control dog, since time is eliminated as a parameter. It is apparent that protein

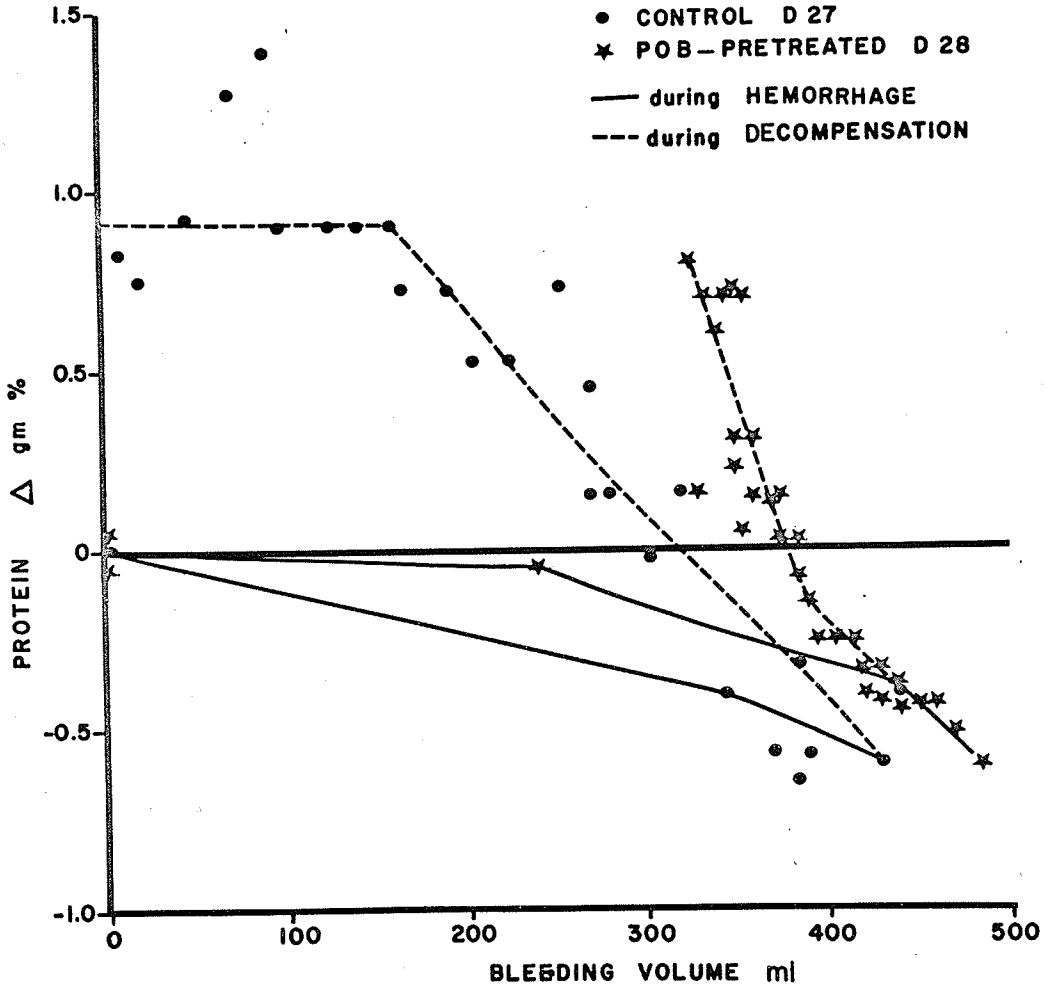


Figure 4. Relationship of Bleeding Volume to Plasma Protein Concentration. Data from the same pair of dogs shown in Fig. 3; much more time was required for the recorded changes in the POB-pretreated than in the control animal. Solid lines represent determinations during hemorrhage and dashed lines during uptake of blood from the reservoir (decompensation). POB did not change the relationship except that it eliminated the late phase of uptake of blood without concomitant hemoconcentration seen in the control animal.

concentration began to increase in association with the onset of uptake of blood from the reservoir in both dogs, rising to levels exceeding those before hemorrhage when 20% or less of the blood had been taken up. However, it is clear that the change in the slope of increasing protein concentration that is evident in the response of the control dog, did not occur in the POB-pretreated animal.

The increase in hematocrit always seen shortly after hemorrhage in control dogs did not occur in POB-pretreated animals. Their hematocrits fell with hemorrhage, and in general, paralleled the changes in plasma protein concentration.

The maximum bleeding volumes were not different in the two groups, 52.1 ± 1.7 ml/kg in the control and 51.2 ± 3.6 ml/kg in the POB-pretreated group. However, an increased sensitivity to hemorrhage in the latter was seen in the fact that they required only a 4.1 ± 2.2 ml/kg hemorrhage to produce the initial 20 mm Hg fall in BP, whereas the controls required 19.0 ± 2.2 ml/kg. Also, the initial bleeding volumes were smaller in the POB-pretreated group (35.1 ± 1.5 ml/kg) than in the controls (45.1 ± 1.9 ml/kg). The difference between the IBV and the MBV (the "secondary bleeding volume") was therefore 16.1 ml/kg in the POB-pretreated and 7.0 ml/kg in the control group.

Comment

Pretreatment with phenoxybenzamine considerably altered the changes associated with hemorrhagic hypotension in the dog. Pretreated dogs compensated for a longer time, decompensated much

more slowly and lived longer, 4 of 8 being alive after 8 hours of hypotension. Hemodilution was prolonged in parallel with the prolonged compensation. The relationship between uptake of blood and hemoconcentration seen in the controls was also evident in the POB-pretreated dogs, but its time course was very different. During the long period after MBV, before uptake began in the POB-pretreated dogs, no hemoconcentration occurred. Once uptake did begin, it progressed very slowly, as did the hemoconcentration. Finally, the slope change in the plasma protein concentration increase characteristic of the response of controls, was never evident in POB-pretreated dogs. While a slope change might have been difficult to see when the changes were plotted against time, because they occurred so slowly in POB-pretreated dogs, its occurrence should have been apparent in the plot of uptake versus plasma protein change, where time was not a factor (Fig 4).

The failure of the hematocrit to rise initially with hemorrhage in POB-pretreated dogs lends support to previous suggestions that the hematocrit increase associated with hemorrhage in the dog is due to splenic contraction (Wiggers, 1950). This has been shown to be mediated by sympathetic nerves and to be blocked by phenoxybenzamine (Green et al., 1960). It seems very likely that this early hematocrit increase obscured the relationship between uptake of blood from the reservoir and hemoconcentration in many previous studies.

## Section IV

## Effects of Noradrenaline Infusion on the Course of Hemorrhagic Hypotension

Although phenoxybenzamine is a relatively specific adrenergic blocking agent, it is also capable of blocking responses to other monoamines which might be released in response to hemorrhage, notably histamine and serotonin (5-hydroxytryptamine). The following experiments were carried out to determine whether a noradrenaline infusion during hemorrhagic hypotension would induce changes opposite to those due to phenoxybenzamine. If changes in the opposite direction did occur, they would lend support to the hypothesis that the modification of hemorrhagic hypotension by POB was due to adrenergic blockade, and thus implicate the sympathetic nervous system in the development of the phenomena under investigation.

### Methods

One of each of 7 pairs of dogs was treated with an infusion of noradrenaline. The drug was dissolved in saline (200 μg of base/ml) and infused intravenously at a rate of approximately 1.0 μg/kg/min from the time the dog reached its IBV until death. The control dog was simultaneously given an intravenous infusion of saline in a volume equal to that received by the noradrenaline-treated dog. The volumes infused were only approximately 0.005 ml/kg/min, e.g., 3 ml/hr to a 10 kg dog. Arterial blood samples were taken every 15 minutes for plasma protein determinations as in the experiments described above.

Results

The results for this group are presented in Table IV, and those from a typical experiment are shown in Fig. 5. The control dogs responded to hemorrhage essentially as did those described in Sections I and III. However, the noradrenaline infusion increased the rate at which the dogs passed through the various stages of hemorrhagic hypotension without changing either the bleeding volumes or the characteristic plasma protein changes. The period of compensation tended to be shorter in the noradrenaline-treated dogs, 6 of the 7 reaching reversal before their paired controls; the interval between IBV and MBV was 38.6 ± 7.7 min in the noradrenaline-treated dogs and 50.7 ± 4.2 min in the controls. During decompensation, the treated dogs took blood back from the reservoir at a much greater rate (15.6 ± 2.5 ml/kg/hr) than the control dogs (8.8 ± 1.6 ml/kg/hr). Uptake was associated with equal degrees of hemoconcentration, but it occurred much more rapidly in the treated animals. The noradrenaline-treated dogs also died after a much shorter period of maintained hypotension (127.9 ± 15.5 min) than did the controls (199.3 ± 16.2 min).

Comment

Noradrenaline infusion produced changes in the course of hemorrhagic hypotension in the dog opposite to those induced by pretreatment with phenoxybenzamine. The latter considerably prolonged the period of compensation, whereas noradrenaline infusion tended to shorten it. Phenoxybenzamine-pretreated dogs stayed at MBV for some time before uptake began, and then took back blood

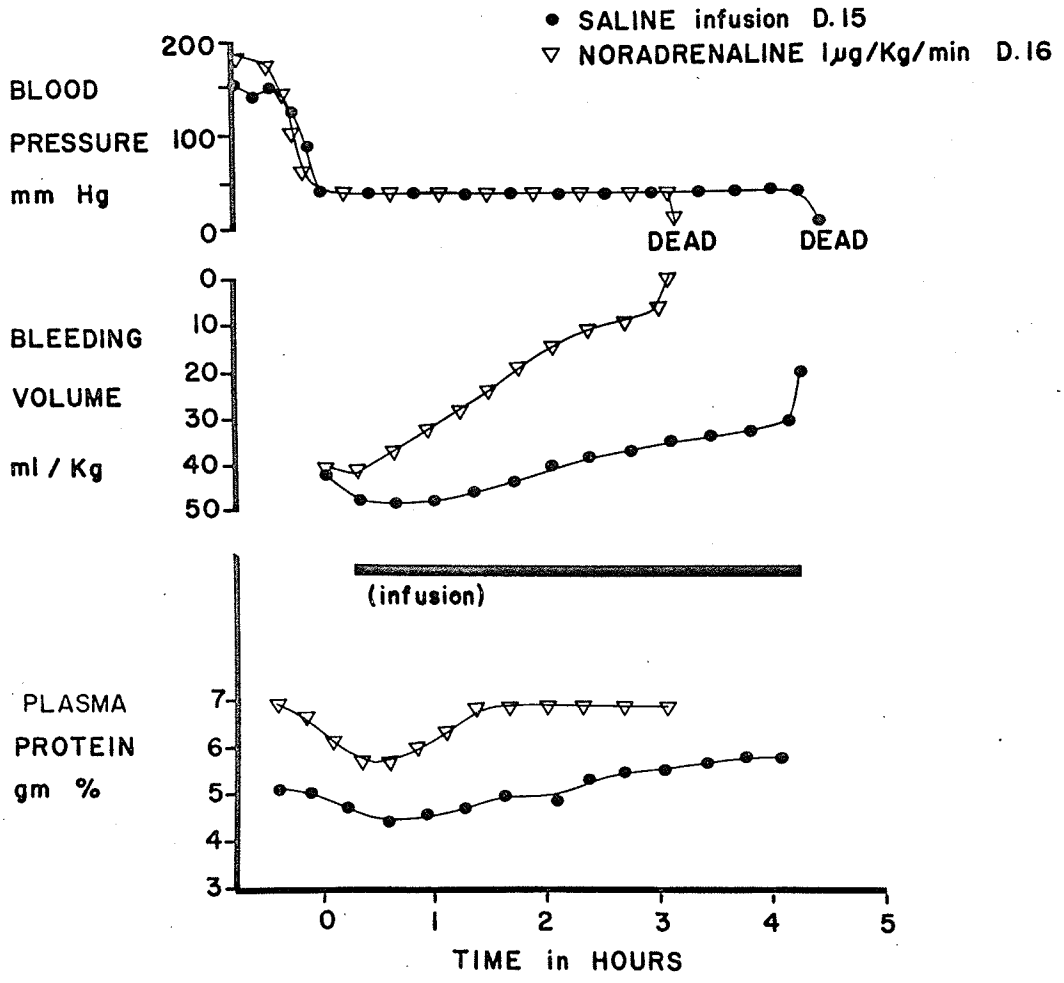


Figure 5. Effects of Infusion of Noradrenaline on the Course of Hemorrhagic Hypotension. Paired dogs infused with 0.9% NaCl solution with and without noradrenaline, beginning at IBV. Note that changes in all parameters are accelerated by the noradrenaline.

TABLE IV

Effects of Noradrenaline Infusion on the
Course of Hemorrhagic Hypotension

(Dogs Held at an Arterial Pressure of 35 mm Hg until Death)

| Group | Initial BP mm Hg | Bleeding Volumes (ml/kg) IBV | MBV | At Death | Rate of Blood Uptake (ml/kg/hr) |
|---|---|---|---|---|---|
| Control (N = 7) | 153.7 ± 4.8 | 36.5 ± 3.3 | 45.0 ± 3.9 | 22.4 ± 5.5 | 8.8 ± 1.6 |
| Noradrenaline (N = 7) | 158.0 ± 3.2 | 37.6 ± 4.0 | 47.9 ± 4.1 | 27.7 ± 6.3 | 15.6 ± 2.5 |
| P | n.s. | n.s. | n.s. | n.s. | < 0.025 |

| Group | Time (Minutes) To IBV | IBV to MBV | IBV to Death | Plasma Protein Conc. (g/100 ml) Initial | IBV | MBV | At Death |
|---|---|---|---|---|---|---|---|
| Control (N = 7) | 30 | 50.7 ± 4.2 | 199.3 ±16.2 | 6.35 ± 0.91 | 5.88 ± 0.25 | 5.54 ± 0.20 | 6.52 ± 0.17 |
| Noradrenaline (N = 7) | 30 | 38.6 ± 7.7 | 127.8 ±15.5 | 6.11 ± 0.14 | 5.52 ± 0.14 | 5.20 ± 0.14 | 6.46 ± 0.26 |
| P | | < 0.01 | < 0.01 | n.s. | n.s. | n.s. | n.s. |

very slowly. Conversely, noradrenaline-treated dogs reversed abruptly, and took back blood at an accelerated rate. In parallel with the above changes, POB-pretreated dogs tended to hemoconcentrate very slowly after reaching MBV, whereas noradrenaline-treated dogs hemoconcentrated at a much more rapid rate than did the controls. Finally, POB pretreatment prolonged the survival of the dogs during hemorrhagic hypotension, whereas noradrenaline infusion led to much earlier death. The results with noradrenaline indicate that the changes in the course of hemorrhagic hypotension in the dog induced by POB pretreatment were in fact due to its activity as an adrenergic blocking agent. This, in turn, suggests that the activity of the sympathetic nervous system is involved in the development and progression of the decompensatory stage of hemorrhagic hypotension. It should be noted that the dose of noradrenaline infused (1.0 μg/kg/min) is relatively small. Maximal adrenal catecholamine output appears to be approximately 5.0 μg/kg/min (Cellander, 1954).

The parallelism of uptake of blood from the reservoir and hemoconcentration noted in untreated dogs was also seen in dogs in which the onset and rate of uptake were changed by the administration of either phenoxybenzamine or noradrenaline, further suggesting that hemoconcentration and uptake are related.

## Section V

## Plasma Volume Changes during Hemorrhagic Hypotension

As was suggested above, the progressive rise of plasma protein concentration after reversal could represent loss of a protein-

poor fluid from the vascular space, entrance of protein at a greater rate than water into the vascular space, or the infusion of protein-rich blood from the reservoir. The last possibility was ruled out by calculation and by the experiments presented in Section II. The parallel rise of hematocrit and of plasma protein concentration following reversal suggests that the increase reflects the loss of an ultrafiltrate of plasma from the vascular compartment. Plasma volume determinations using radioiodinated serum albumin (RISA) were carried out to assess this possibility.

Methods

Eleven pairs of dogs were carried through a period of hemorrhagic hypotension at 35 mm Hg, as described previously. As in the experiments described in Section III, one of each pair had been pretreated with phenoxybenzamine, 5.0 mg/kg intravenously, approximately 18 hours before study. All blood remaining in each reservoir was reinfused when the control dog of a pair had taken up 35 to 45% of its MBV to facilitate study of the later stages of shock. Four determinations of plasma volume were carried out with RISA on each dog, using the technique described under General Methods. The first determination was done before hemorrhage, the second, 30 minutes after reaching IBV, the third, when the control dog had taken up 25% of its MBV, and the fourth, 30 minutes after reinfusion of all shed blood. The plasma volumes reported for the second and third determinations include the volume of plasma in the reservoir at that time, calculated from the volume and hematocrit of the blood in the reservoir. Serial plasma protein concentration and hematocrit deter-

minations were carried out as in previous series.

Results

The results for this group are presented in Tables V and VI and those from a representative experiment are shown in Fig. 6. The course of hemorrhagic hypotension in these animals resembled that seen in previous groups. POB-pretreated dogs compensated for a longer time, stayed at MBV for some time before reversal, and then decompensated much more slowly than did the controls. A constant relationship between the onset of decompensation and of hemoconcentration was also evident in this group.

Measured plasma volume increased in 10 of the 11 control dogs following hemorrhage (Table V), rising from 49.2 ± 2.7 ml/kg to 55.9 ± 2.6 ml/kg 30 minutes after IBV. This was associated with a fall in plasma protein concentration in every case. By the time 25% of the MBV had been taken up, the plasma volume had returned to 49.0 ± 3.3 ml/kg. This decline progressed and at the time of the final determination, 30 minutes after reinfusion of all shed blood, plasma volume of the control dogs had fallen to 36.6 ± 3.3 ml/kg.

POB-pretreated dogs had an initial plasma volume of 55.8 ± 3.1 ml/kg, signficantly greater than that of the control group. The increase in plasma volume 30 minutes after reaching IBV was similar to that in the control group, to 63.5 ± 3.5 ml/kg. However, at the time the controls had reached 25% uptake, and had returned to their initial plasma volumes, the POB-pretreated dogs still had a plasma volume of 61.2 ± 4.5 ml/kg, which was not significantly less than the volume 30 minutes after IBV. Thirty minutes after

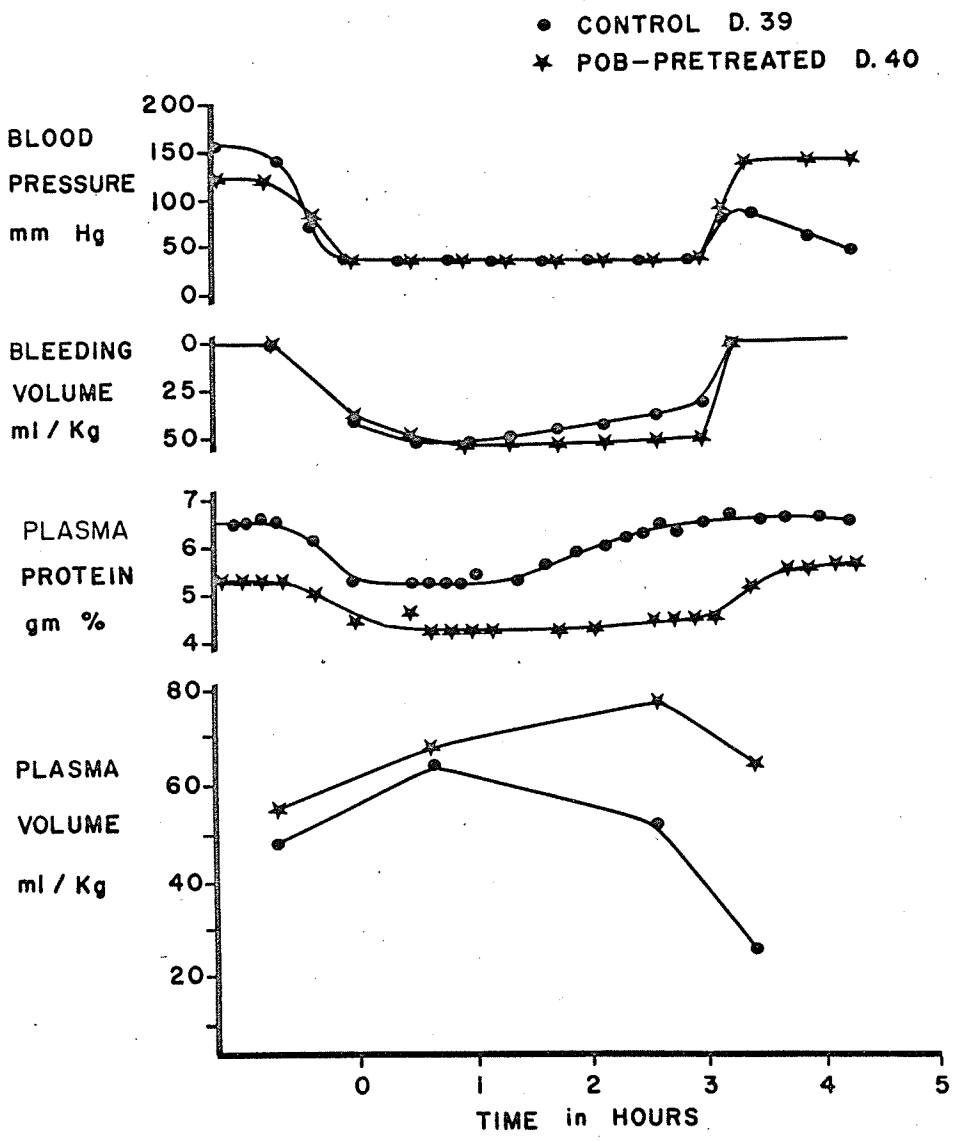


Figure 6. Plasma Volume Changes during Hemorrhagic Hypotension in Control and POB-Pretreated Dogs. Paired dogs, both of which were reinfused with all remaining shed blood when the control animal had spontaneously taken up 40% of MBV. Note that the measured plasma volume of the control animal fell markedly during the last interval without progression of the hemoconcentration. The POB-pretreated animal maintained an elevated plasma volume throughout the period of hypovolemia and returned toward the prehemorrhage value only after reinfusion.

Table V

Changes in Plasma Volume and Arterial Pressure during and after Hemorrhagic Hypotension in POB-Pretreated and Control Dogs

| Group | Plasma Volume (ml/kg) | | | |
|---|---|---|---|---|
| | Initial | 30 Min after IBV* | At 25% Uptake* | 30 Min after Reinfusion |
| Control | 49.2 ± 2.7 (11)** | 55.9 ± 2.6 (11) | 49.0 ± 3.3 (9) | 36.6 ± 3.3 (7) |
| POB-Pre-treated | 55.8 ± 3.1 (11) | 63.5 ± 3.5 (11) | 61.2 ± 4.5 (11) | 54.4 ± 3.9 (11) |
| P | 0.05 | 0.05 | < 0.05 | <0.05 |

* Includes plasma in blood reservoir
** Number of animals in each group

| Group | Arterial Pressure | | | | |
|---|---|---|---|---|---|
| | Initial | Following Reinfusion | | One Hour After Reinfusion | |
| | (mm Hg) | mm Hg | % of initial | mm Hg | % of initial |
| Control | 153.4 ± 6.5 (11)** | 100.1 ± 2.8 (7) | 65.8 ± 9.6 | 78.3 ± 8.6 (7) | 51.0 ± 4.2 |
| POB-Pre-treated | 126.8 ± 5.2 (11) | 105.8 ± 4.5 (11) | 84.0 ± 5.1 | 101.1 ± 7.0 (11) | 80.0 ± 5.3 |
| P | < 0.05 | n.s. | n.s. | n.s. | < 0.05 |

** Number of animals in each group

reinfusion, the plasma volume of POB-pretreated dogs had returned to the prehemorrhage volume (54.4 ± 3.9 ml/kg).

The difference in the courses of the control and pretreated dogs was also evident in the number of dogs in each group alive at each stage of the procedure. By the time 25% uptake had occurred, 2 of the 11 control dogs were dead, and 4 were dead 30 minutes after reinfusion of the shed blood. In contrast, all of the 11 POB-pretreated dogs lived to the end of the experiment.

As described in Section III, the degree of hemodilution was similar in the two groups (Table VI), but in the control dogs, hemoconcentration began earlier, progressed more rapidly and reached a level significantly higher than the initial concentration (7.10 ± 0.21 g/100 ml vs. 6.42 ± 0.12 g/100 ml). In the POB-pretreated dogs, the final plasma protein concentration (6.76 ± 0.23 g/100 ml) was not significantly greater than that preceding hemorrhage (6.52 ± 0.21 g/100 ml).

Reinfusion of the shed blood raised the BP of the control dogs to a maximum of 100.1 ± 2.8 mm Hg, or 65.8% of the level before hemorrhage (Table V). One hour after reinfusion, the BP of the animals still alive had fallen to 78.3 ± 8.6 mm Hg, 51% of their initial value. The maximum BP reached by the POB-pretreated dogs was 105.8 ± 4.5 mm Hg, 84% of their initial BP, and an hour later, it was 101.1 ± 7.0 mm Hg.

The plasma volume following reinfusion of all the shed blood measured in each dog is plotted in Fig. 7 against the BP at that time, expressed as a percentage of the prehemorrhage value.

TABLE VI

Bleeding Volumes and Changes in Plasma Protein Concentration during Hemorrhagic Hypotension in POB-Pretreated and Control Dogs

| Group | Bleeding Volume (ml/kg) | | Uptake of Blood | |
|---|---|---|---|---|
| | IBV | MBV | ml/kg | % of MBV |
| Control | 39.2 ± 2.7 (9)* | 49.5 ± 2.6 (9) | 18.6 ± 2.7 (7) | 38.2 |
| POB-Pretreated | 31.1 ± 1.9 (11) | 43.3 ± 2.6 (11) | 6.5 ± 3.2 (11) | 14.9 |
| P | 0.05 | n.s. | 0.01 | |

| Group | Plasma Protein Conc. (g/100 ml) | | | | |
|---|---|---|---|---|---|
| | Initial | At IBV | At MBV | At Plasma Vol. #3 | At Plasma Vol. #4 |
| Control | 6.42 ± 0.12 (11)* | 5.65 ± 0.13 (11) | 5.36 ± 0.03 (11) | 6.30 ± 0.11 (9) | 7.10 ± 0.21 (7) |
| POB-Pretreated | 6.52 ± 0.21 (11) | 5.63 ± 0.20 (11) | 5.42 ± 0.21 (11) | 5.83 ± 0.22 (11) | 6.76 ± 0.23 (11) |
| P | n.s. | n.s. | n.s. | n.s. | n.s. |

* Number of Animals in each group

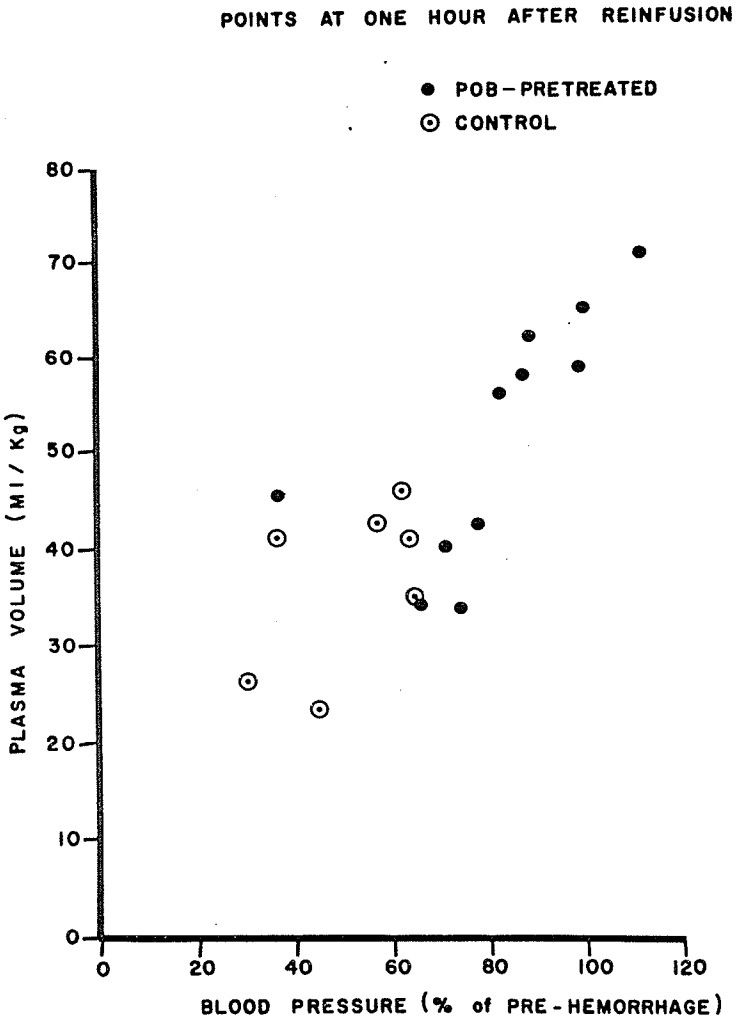


Figure 7. Relationship of Plasma Volume to Arterial Pressure after Reinfusion of all Shed Blood. Control and POB-pretreated animals show the same relationship, but the latter tend to have higher absolute values.

The relationship between plasma volume after reinfusion, and recovery of the BP is apparent in this figure, as is the difference between the POB-pretreated and the control animals.

The dilution of RISA following hemorrhage was compared to the dilution of plasma protein at the time of the second plasma volume determination (Table VII). The counts/min in the blank sample for the second plasma volume determination were plotted on the RISA decay slope from the first determination in each dog. In every case, the counts 30 minutes following hemorrhage fell below the expected value on the decay line, showing hemodilution, which was also indicated by the plasma protein determinations. The dilution of the RISA 30 minutes following hemorrhage was 22.2 ± 3.2%, whereas the total plasma protein dilution was only 14.7 ± 1.3%, a highly significant difference ($P < 0.005$).

Comment

The courses of the control and POB-pretreated dogs in this series were similar to those seen in the previous experiments. In this group, however, it was possible to compare the responses to reinfusion of the shed blood after identical periods of hypotension. The POB-pretreated animals reached 85% of their initial BP after reinfusion, and were still at 81% one hour later. The seven control dogs still alive at the time of reinfusion reached a BP only 66% of that before hemorrhage, and this had fallen to 51% one hour later. There was a clear positive correlation between the recovery of BP and the plasma volume after reinfusion.

TABLE VII

Comparison of the Dilution of Total Plasma Protein and of Plasma RISA 30 Minutes after Hemorrhage

| Dilution from Prehemorrhage Level (%) | | |
|---|---|---|
| Dog No. | Plasma Protein | RISA |
| 1 | 13.3 | 21.0 |
| 2 | 13.5 | 35.3 |
| 3 | 16.7 | 21.2 |
| 4 | 15.8 | 18.2 |
| 5 | 16.5 | 12.3 |
| 6 | 9.7 | 17.8 |
| 7 | 12.3 | 11.7 |
| 8 | 12.5 | 26.0 |
| 9 | 16.2 | 26.5 |
| 10 | 21.0 | 16.0 |
| 11 | 12.5 | 27.4 |
| 12 | 9.6 | 12.8 |
| 13 | 19.8 | 38.7 |
| 14 | 19.0 | 18.3 |
| 15 | 11.7 | 22.7 |
| 16 | 20.3 | 20.0 |
| 17 | 16.5 | 14.5 |
| 18 | 14.5 | 16.0 |
| 19 | 9.7 | 27.3 |
| Mean | 14.7 | 22.2 |
| S.E. | $\pm$ 1.3 | $\pm$ 3.2 |

The increase in plasma volume occurring shortly after hemorrhage indicates that the hemodilution represents a movement of fluid into the vascular compartment which is low in protein relative to plasma, in agreement with the findings of many other workers. (See Wiggers, 1950; Green, 1961.) Since there was a relatively short interval between the onset of hemorrhage and the second plasma volume determination, the plasma volume replacement was incomplete, and thus the total was underestimated. From the dilution of plasma proteins, it can be estimated that the increase in plasma volume due to influx of low-protein fluid was at least 16.8%. Lewis et al. (1950) found a 17.1% dilution of plasma proteins after a similar hemorrhage in the dog. However, other investigators have demonstrated that appreciable protein enters the vascular space after hemorrhage in the dog, the fluid entering the vascular compartment having a protein concentration approximately 50% that of the plasma (Dunn et al., 1958; Deavers et al., 1963). This finding is in agreement with the present observation that after hemorrhage, the RISA was diluted 50.2% more than the total plasma protein. By combining the values for hemodilution and protein gain, it is possible to estimate the total plasma volume replacement after hemorrhage, 25.2% of 49.2 ml/kg, of 12.4 ml/kg (61.6 ml/kg total at MBV) for the control animals under the conditions of the present experiments. This hemodilution is very similar to that reported by Deavers et al., (1963), who found a 29% gain in plasma volume after a hemorrhage of similar magnitude in the dog, associated with an increase in total circulating plasma protein of 14%. It should be noted that the initial plasma volume found by this group (Huggins

et al., 1963) in 372 dogs (49.8 ml/kg) was very close to that measured in our animals.

The plasma volume fell after reversal, in association with the onset of hemoconcentration. By the time 25% uptake of blood had occurred, the plasma volume had fallen from 61.6 to 49.0 ml/kg. Since the uptake had been 25% of MBV (49.5 ml/kg), 12.4 ml/kg of blood had been returned to the dogs at that time. Therefore the plasma volume loss (61.6 - 49.0 = 12.6 ml/kg) was almost identical to the volume of blood taken up from the reservoir. The relationship between uptake of blood and plasma volume decrease is explored in greater detail in the next section, where data on many more dogs are available. An extremely high correlation exists between these two parameters.

At 25% uptake the plasma protein concentration of the control dogs had increased from 5.36 ± 0.03 to 6.30 ± 0.11 g/100 ml, a 17.6% increase. From the plasma volume at MBV and the change in plasma protein concentration, it can be calculated that the dogs had lost a minimum of 10.8 ml/kg of protein-poor fluid from the vascular space. This indicates that at 25% uptake, at least 89% of the measured plasma volume deficit could be accounted for by a loss of protein-free fluid. The early, rapid hemoconcentration that occurs after reversal represents, therefore, chiefly the loss of a low-protein ultrafiltrate from the vascular compartment.

During the interval between the third and fourth plasma volume determinations in the control dogs, the plasma protein concentration continued to increase, but at a much slower rate, (6.30 ± 0.11 to 7.10 ± 0.20 g/100 ml), so that only 12.6% further hemoconcentration had occurred. This would represent a loss of 6.0 ml/kg of

protein-free ultrafiltrate during the interval. However, the plasma volume decreased a further 12.4 ml/kg (49.0 ± 3.3 ml/kg to 36.6 ± 3.3 ml/kg) and thus, only a part of the measured plasma volume loss during this interval could be accounted for by ultrafiltration. The loss of plasma volume during the period after 25% uptake therefore appears to represent, in part, a loss of whole plasma. Furthermore, since the changes in hematocrit after reversal paralleled those in plasma protein concentration very closely, the decrease after 25% uptake appears to involve the loss of whole blood. The period before 25% uptake included mainly the interval before the change in slope of the plasma protein and hematocrit increases occurred. It therefore appears that the decrease in intravascular volume that occurred before the slope change, represented the loss of a low-protein ultrafiltrate of plasma, and that after the slope change, involved disappearance of whole blood.

The initial plasma volume in the dogs pretreated with phenoxybenzamine 18 hours before the experiment was 55.8 ± 3.1 ml/kg, significantly greater than the plasma volume of the control group, which was 49.2 ± 2.7 ml/kg. POB has been previously demonstrated to increase the plasma volume in both the chick and the dog (Williams and Rodbard, 1960; Gourzis, 1962). However, in their experiments, only the early effects of POB were studied, and the increase in plasma volume was associated with hemodilution, the gain of plasma volume being quantitatively accounted for by an influx of low-protein fluid into the vascular space. However, in the present experiments, it was found that 18 hours after the administration of POB, the plasma volume

remained greater than that of the control animals, but the plasma protein concentrations in the two groups were the same. This suggests that in the intervening time, the POB-pretreated dogs had added protein to the protein-poor fluid gained earlier, converting it to normal plasma.

POB pretreatment did not affect the maximum gain of plasma volume after hemorrhage, but slowed the rate of increase, although the arterial pressures reached 35 mm Hg at the same time in the two groups. This suggests that some factor other than the fall in BP was involved in the plasma volume gain. Mellander (1960) has shown that in the normal animal, sympathetic nerve stimulation results in a change in the ratio of precapillary to postcapillary resistance such that capillary pressure falls and fluid moves into the vascular space. It is possible that POB pretreatment interfered with this response, and thus slowed this component of compensation. Noradrenaline infusion appeared to produce the opposite effect. (See Section IV.)

POB pretreatment also prevented the late loss of intravascular volume despite identical periods of hypotension. The possible mechanism of this effect will be discussed in detail in Sections X and XI.

## Section VI

## Relationship of Loss of Fluid from the Vascular Space to Uptake of Blood from the Reservoir

Quantitative aspects of the relationship between uptake of blood from the reservoir and change in plasma volume provide one possible basis for evaluating mechanisms responsible for the former. These will be examined more fully in this section, in which the relevant data from a large number of dogs has been combined.

### Methods

Thirty-three anesthetized dogs were bled to a mean arterial pressure of 35 mm Hg and carried through a period of hemorrhagic hypotension as previously described. To produce a wide range of plasma volume changes and of volumes of blood taken up from the reservoir, the periods of hypotension were varied from 45 to 150 minutes. The plasma volume of each dog was determined by the RISA dilution technique prior to hemorrhage and 30 minutes after reinfusion of all blood remaining in the reservoir. Plasma protein concentration was determined at 15-minute intervals as in previous experiments. At the end of each experiment, the vessels which had been cannulated were tied off, the incisions closed with sutures, and the dogs returned to their cages to determine survival.

### Results

In Fig. 8 (lower section) the changes in plasma volume from prehemorrhage to the determination following reinfusion of

all dogs for which the relevant data were available (33 dogs in this group and 18 of those described in Section V) have been plotted on the ordinate and the uptake of blood on the abscissa, both in ml/kg. These two parameters are highly correlated, analysis of variance indicating a P value of $< 0.005$. A curve relating increase in plasma protein concentration after reversal to uptake of blood in 26 dogs from the experiments of Sections I to III is shown at the top of Fig. 8 for comparison. (See also Table III.)

The dogs in which plasma volume was measured by RISA dilution (lower part of Fig. 8) were divided into two groups on the basis of uptakes greater than or less than 10 ml/kg. This point of division was selected because the slope of the plasma protein increase changed at approximately this uptake. In the 42 dogs with uptakes less than 10 ml/kg, the mean plasma volume loss was 3.6 ± 0.8 ml/kg, very similar to the mean uptake of blood from the reservoir, 3.9 ± 0.6 ml/kg. In the 9 dogs with uptakes exceeding 10 ml/kg, the average measured plasma volume loss was 12.2 ± 1.4 ml/kg, and the average uptake 19.0 ± 0.7 ml/kg, which is significantly greater than the plasma loss ($P < 0.005$).

Comment

These results show a high correlation between uptake of blood from the reservoir and disappearance of plasma from the vascular compartment. It appears that uptake can be quantitatively accounted for by the disappearance of plasma during the early phase of decompensation, to an uptake of approximately 10 ml/kg. After that point, the measured plasma volume loss is less than the uptake of blood. This change occurs at approximately the point at which

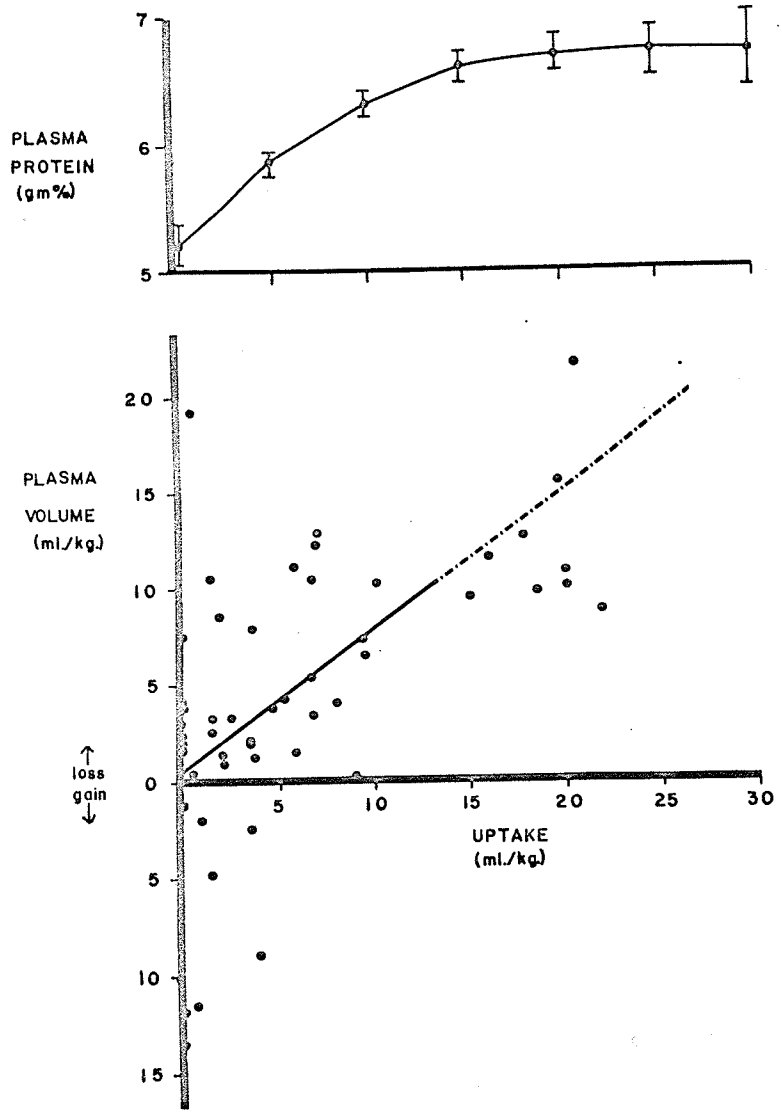


Figure 8. Relationship of Uptake of Blood to Plasma Volume and Plasma Protein Concentration. The line in the lower part of the figure is fitted to the points below 10 ml/kg uptake; above this, uptake is significantly greater than plasma volume loss. Note that this division is at approximately the point where hemoconcentration stops progressing, although uptake continues (upper part of figure). Vertical bars on plasma protein curve indicate S.E. of each point.

the increase in plasma protein concentration and hematocrit changes to a much slower rate. If uptake of blood represents replacement of fluid lost from the vascular compartment, as was suggested in the previous section, the measured decrease in plasma volume would be expected to account quantitatively for the uptake as long as the loss was of plasma only. However, if whole blood disappears from the vascular space after the slope change, as is suggested by the parallel changes in hematocrit and plasma protein concentration, the plasma volume decrease would be less than the uptake of blood, since red cell mass lost to the circulation also must be replaced.

The observations described above are all consistent with the hypothesis that uptake of blood from the reservoir during hemorrhagic hypotension represents replacement of volume lost from the intravascular compartment. This loss appears to be initially due to the disappearance of a low-protein ultrafiltrate of plasma, followed by the disappearance of whole blood. The mechanisms responsible for these two phenomena will be discussed more fully in connection with the observations to be presented in Sections X and XI.

## Section VII

## Effects of Hemorrhage and of Withholding Reinfusion on Central Venous Pressure

In the experiments presented in Section II, it was demonstrated that if uptake of blood was prevented after reversal, the BP fell rapidly and death quickly ensued. The decrease in BP was associated with a rapid increase in hematocrit and plasma protein concentration. Subse-

quent experiments (Sections V and VI) demonstrated that the increase in plasma concentration after reversal was due to the loss of protein-poor fluid from the vascular compartment. Since it is well known that a net deficit of intravascular volume reduces the central venous pressure (CVP) (Wiggers, 1950), CVP was used in this series of experiments as an index of the contribution of the volume loss to the hemodynamic deterioration characteristic of hemorrhagic shock. CVP should decrease during the fall in arterial BP when uptake of blood is prevented if the latter is due to loss of fluid from the vascular space, and thus to decreased venous return. Conversely, if the fall of BP were due to cardiac failure, the CVP should increase.

Methods

Arterial BP, plasma protein concentration, and bleeding volume were measured in dogs subjected to hemorrhage as in previous groups. In addition, central venous pressure was measured by passing a catheter through an external jugular vein into the thoracic venous system, as described under General Methods. This catheter was connected to a Statham P 23BB pressure transducer which was calibrated so that a pressure change of one centimeter of saline produced a pen excursion of 4 mm on the Polygraph. A paper speed, fast enough to allow measurement of the presystolic CVP during the expiratory pause, was used in most experiments. In some, an electronically integrated mean was recorded.

Each dog was initially bled to a BP of 30 mm Hg, and then brought up to 60 mm Hg with a small reinfusion of blood and held at this pressure until a predetermined uptake of blood had occurred, from 5 to 30 ml/kg (10% to 71% of MBV). After the preselected volume of blood

had been taken up, the line connecting the dog's arterial system to the reservoir was clamped, and the BP allowed to vary spontaneously. During the subsequent fall in BP, which occurred in all of the dogs, arterial blood samples were taken every 5 minutes for plasma protein determination. In three dogs the initial hemorrhage was continued to death in order to study the relationship between arterial pressures below 30 mm Hg and CVP during acute hemorrhage.

Results

The results for this group are presented in Table VIII, and examples of the two types of response observed are shown in Fig. 9. CVP fell during hemorrhage in all of 15 dogs, and in 14 of these, the fall continued through the BP decrease from 60 to 30 mm Hg.

The CVP did not increase in any dog during the interval when arterial pressure was maintained at 60 mm Hg, despite the uptake of very large volumes of blood by some animals. In fact, the CVP immediately before clamp off (-5.3 ± 0.6 cm of saline) was lower than that when the animals first reached an arterial pressure of 60 mm Hg (-4.0 ± 0.5 cm of saline). After clamp off, CVP continued to fall with the decrease in arterial BP in every dog. In all of 5 dogs that had taken up less than 25% of their MBV prior to clamp off, the fall in CVP paralleled that seen during hemorrhage, so that at an arterial pressure of 30 mm Hg, the CVP was equal to or less than that at the same arterial pressure during the initial hemorrhage. At a BP between 15 and 25 mm Hg, these dogs stopped breathing, arterial pressure fell abruptly and CVP rose rapidly, generally to the range of 0 to -2 cm of saline. The heart stopped beating very soon after CVP began to rise. In 6 of the 10

TABLE VIII

Changes in Central Venous Pressure and Plasma Protein Concentration during Arterial Pressure Reduction from 60 to 30 mm Hg by the Initial Hemorrhage and following Reversal when Reinfusion Was Prevented

| Uptake at "Clamp Off" (% MBV) | Prehemorrhage | | During Hemorrhage | | | | After Reversal | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CVP (cm saline) | Plasma Protein Conc. (g/ml) | CVP (cm saline) | | Plasma Protein Conc. (g/100 ml) | | CVP (cm saline) | | Plasma Protein Conc. (g/100 ml) | |
| | | | 60 mm Hg | 30 mm Hg | 60 mm Hg | 30 mm Hg | 60 mm Hg | 30 mm Hg | 60 mm Hg | 30 mm Hg |
| 5 | +1.3 | 5.93 | -1.7 | -3.7 | 5.75 | 5.28 | -4.2 | -5.2 | 5.47 | 5.93 |
| 5 | +1.0 | 5.64 | -1.2 | -1.3 | 4.82 | 4.82 | -1.0 | -1.2 | 5.01 | 5.01 |
| 10 | +1.0 | 5.75 | -5.5 | -6.8 | 5.45 | 5.10 | -6.3 | -7.5 | 4.91 | 5.33 |
| 10 | +0.5 | 5.28 | -2.3 | -3.5 | 4.99 | 4.79 | -4.5 | -5.3 | 4.73 | 4.82 |
| 15 | -3.5 | 5.56 | -5.0 | -5.5 | 5.28 | 5.21 | -4.5 | -5.5 | 5.20 | 5.48 |
| 29 | -1.3 | 6.39 | -3.0 | -5.3 | 5.80 | 5.75 | -4.3 | -5.8 | 5.93 | 6.27 |
| 30 | -1.0 | 6.49 | -3.5 | -4.3 | 6.39 | 6.21 | -7.3 | -6.8 | 6.58 | 6.86 |
| 33 | -2.5 | 7.69 | -6.8 | -7.8 | 6.67 | 6.49 | -7.0 | -5.8 | 7.60 | 7.68 |
| 37 | -1.8 | 5.81 | -4.5 | -6.5 | 5.38 | 4.73 | -7.0 | -8.3 | 5.10 | 5.19 |
| 49 | -4.0 | 6.12 | -5.4 | -8.3 | 5.93 | 5.38 | -7.3 | -5.0 | 5.65 | 6.02 |
| 52 | -3.0 | 6.02 | -7.0 | -7.8 | 5.84 | 5.56 | -6.0 | -1.0 | 6.12 | 6.39 |
| 60 | +0.5 | 5.84 | -4.0 | -4.8 | 5.47 | 4.82 | -4.5 | -5.0 | 6.02 | 6.49 |
| 66 | -0.5 | 6.49 | -1.5 | -1.6 | 6.99 | 6.30 | -1.4 | -1.5 | 7.13 | 7.32 |
| 67 | -1.3 | 5.28 | -1.7 | -0.8 | 5.47 | 5.28 | -2.0 | -2.7 | 6.39 | 6.67 |
| 71 | -5.5 | 6.12 | -6.5 | -7.5 | 5.84 | 5.75 | -6.8 | -7.0 | 5.56 | 5.84 |

dogs that took up more than 25% of their MBV, the fall in CVP following clamp off was similar to that in the dogs which had taken up less blood. In the remaining 4 dogs, CVP began to decrease following clamp off, but at an arterial pressure between 39 and 50 mm Hg, it began to increase again, and these dogs died very rapidly. One response of each type is shown in Fig. 9.

In the 3 dogs bled to death acutely, the CVP fell with decreasing arterial pressure to 15, 17 and 20 mm Hg, respectively, at which time it abruptly began to increase, respiration ceased, and the heart stopped. Attempts to resuscitate the dogs by reinfusion of the shed blood intra-arterially, one to 2 minutes after the heart stopped, were unsuccessful.

The plasma protein concentration rose after clamp off in all of the dogs, despite the decrease in both arterial BP and CVP. This response is similar to that of the dogs described in Section II.

Comment

The fall in CVP that occurs during hemorrhage is generally ascribed to a decreased venous return to the heart (Wiggers, 1950). In the present experiments, a considerable decrease in CVP occurred and progressed throughout the period of hemorrhage. The experimental design allowed comparison of the changes in CVP associated with the falls in arterial pressure from 60 to 30 mm Hg during initial hemorrhage, and following clamp off. Experiments described in previous sections suggested that uptake of blood from the reservoir indicated a decrease in intravascular volume. If loss of fluid from the vascular space were the cause of the fall in arterial pressure during circulatory deterioration, it should be associated with a decreasing CVP, similar to the response to hemorrhage.

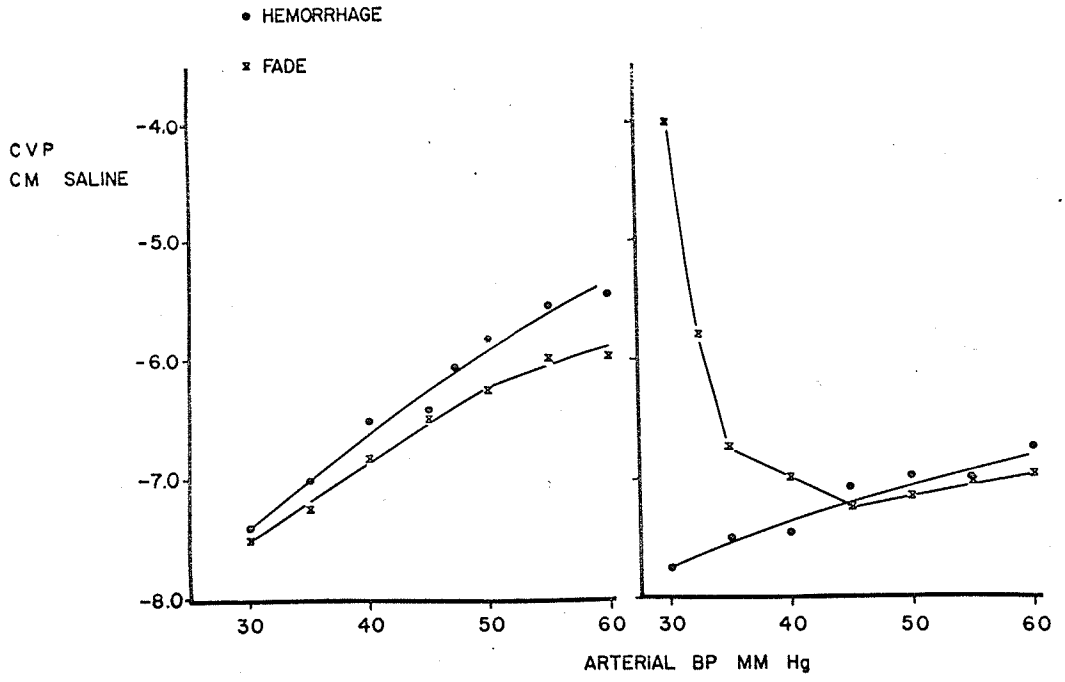


Figure 9. Relationships of Central Venous Pressure to Arterial Pressure during Hemorrhagic Hypotension. Solid circles show the relationship as arterial pressure was reduced by the initial hemorrhage, the crosses, that recorded when the pressure fell because uptake of blood was prevented during decompensation (fade). The usual relationship is shown on the left. Only 4 of 15 dogs followed the pattern shown on the right, with a preterminal rise in central venous pressure.

This did occur, and in all but 4 of 15 animals the CVP continued to fall until the arterial pressure reached 30 mm Hg or below. At still lower arterial pressures, there was a terminal increase in CVP in all dogs dying from either acute hemorrhage or prolonged hemorrhagic hypotension. In 4 of the dogs, the CVP began to fall after clamp off, but rose again shortly before death, although the arterial pressure had not yet fallen to 30 mm Hg, probably as a result of some terminal myocardial inadequacy.

The finding that CVP did not tend to increase following reversal is of some interest, since Crowell and Guyton (1961, 1962) have reported that CVP does increase following reversal, and have considered this to be evidence that myocardial inadequacy is a major contributor to the genesis of irreversibility to transfusion. In the present experiments, evidence of myocardial inadequacy appeared only after the spontaneous uptake of relatively large volumes of blood, i.e., well beyond the stage where transfusion can result in survival. Even at this terminal stage of decompensation, the index employed failed to show evidence of heart failure in most of the dogs studied.

The relationship between the parameters studied here and survival will be discussed in association with the results of the experiments reported in Sections VIII and IX.

## Section VIII

## Relationship of Hemoconcentration to Survival after Maintained Hemorrhagic Hypotension

The results of experiments reported in previous sections have demonstrated a close relationship between hemoconcentration and the uptake

of blood from the reservoir to prevent the arterial pressure from falling after reversal, and that hemodynamic failure and death are accelerated if uptake is prevented. These observations suggest that the hemoconcentration is closely, perhaps etiologically, related to the cardiovascular deterioration. However, it is well known that events during hemorrhagic hypotension can lead to death many hours later, after a period of relatively adequate cardiovascular function, and these experiments did not provide conclusive evidence that the observed hemoconcentration affects ultimate survival. The experiments described here and in Section IX were undertaken to clarify the relationship between hemoconcentration and survival.

Methods

The procedures for the induction of hemorrhagic shock and for the determination of plasma protein concentration, hematocrit and CVP were the same as in the groups reported above. Thirty-three of the dogs included in this group are the same as those described in Section VI, and had had their plasma volumes determined by RISA dilution before and after hemorrhage. An additional 31 dogs were studied with determinations of plasma protein concentration and CVP at various stages of the hemorrhagic procedure, but without direct plasma volume determinations. Animals alive 48 hours after hemorrhage were considered to have survived the procedure.

Results

The results for this group are presented in Table IX and Fig. 10. There was a very high negative correlation between the rise in plasma

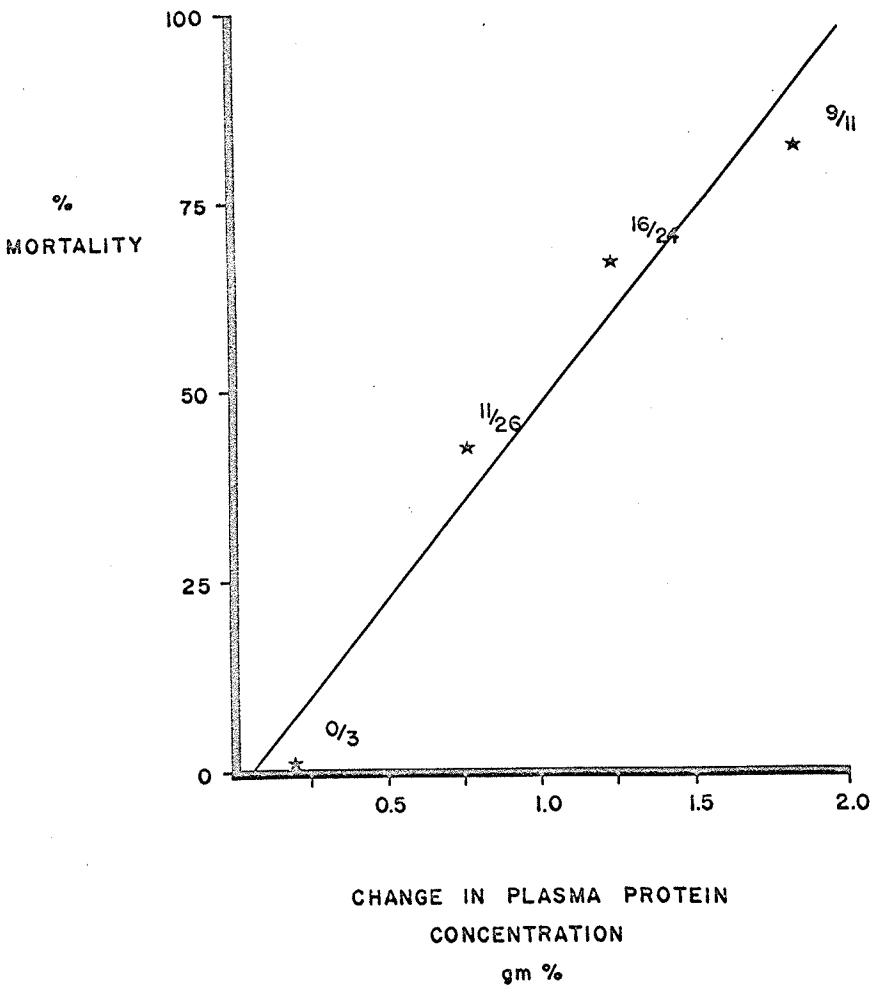


Figure 10. Relationship of Hemoconcentration to Mortality after Hemorrhagic Hypotension. Increases in plasma protein concentration during the stage of decompensation, from reversal to the reinfusion of all shed blood, are plotted on the abscissa. The figures beside each point indicate the number of fatalities over the total number of dogs in each group.

protein concentration after reversal, and the probability of survival (Fig. 10). Only 2 of 11 dogs with a plasma protein increase of 1.5 g/100 ml or more survived for over 48 hours (18%). At the other extreme, all of 3 dogs with a plasma protein increase of less than 0.5 g/100 ml survived.

The animals are classified as survivors or fatalities in Table IX. The initial plasma protein concentrations and plasma volumes of the two groups were the same, and the maximum bleeding volumes were also not significantly different. The only significant difference was in the plasma protein concentration 30 minutes after reinfusion, the fatalities having a plasma protein concentration of 6.98 ± 0.20 g/100 ml, and the survivors 6.53 ± 0.18 g/100 ml, significant at the 5% level. The plasma volumes following reinfusion were not significantly different. Although the uptake of blood from the reservoir was 38% higher among the fatalities, this difference was not significant at the 5% level because of the wide range of uptake in both groups.

Comment

The correlation between plasma protein increase and survival suggests that the factors leading to hemoconcentration have a considerable influence on the hemodynamic deterioration after hemorrhagic hypotension. The greater the hemoconcentration, the more likely the dog was to die of hemorrhagic shock. Since the plasma protein concentrations of nonsurvivors were higher, and this hemoconcentration was demonstrated to be due to loss of a low protein ultrafiltrate of plasma, they would have been expected to have a significantly smaller plasma volume after reinfusion. However, it appears that these animals were only in the early stages of decompensation at the time of reinfusion. Their uptake had been only

Table IX

Comparison of Responses of Dogs that Did and Did Not Survive Hemorrhagic Hypotension

| Group | No. | MBV (ml/kg) | Time to Reversal (min) | Uptake of Blood | | Plasma Protein Conc. (g/100 ml) | | | Plasma Vol. (ml/kg) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ml/kg | ml/kg/min | Initial | At MBV | Final | Initial | Final |
| Survivors | 11 | 45.3 ± 2.1 | 48.1 ± 6.7 | 1.6 ± 0.6 | 0.105 ± 0.013 | 6.41 ± 0.13 | 5.52 ± 0.20 | 6.53 ± 0.18 | 44.5 ± 1.6 | 42.6 ± 2.4 |
| Fatalities | 22 | 46.2 ± 1.9 | 49.0 ± 4.2 | 3.8 ± 1.0 | 0.145 ± 0.019 | 6.61 ± 0.17 | 5.68 ± 0.16 | 6.98 ± 0.20 | 47.1 ± 1.4 | 43.8 ± 1.9 |
| P | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | 0.05 | n.s. | n.s. |

3.8 ± 1.0 ml/kg, (8.2% of MBV). After reinfusion of the shed blood, most of them reached arterial pressures very close to their control levels, and most did not die for at least 18 hours after reinfusion. If reinfusion is delayed until after a greater uptake has occurred, the BP recovers only partially, a plasma volume deficit is readily demonstrable, and the animals die much more rapidly (Section V). It therefore appears that the fatalities in the present group were still in the earlier stages of decompensation. The 5.6% increase in plasma protein concentration was statistically significant because the variance with this determination is very small, whereas the 7.2% decrease in plasma volume was not significant because of the considerably larger variance.

## Section IX

## Relationship of Hemoconcentration to Survival after a Single Large Hemorrhage

The relationship between hemoconcentration and survival after hemorrhage was investigated in this group of dogs utilizing a different method for the induction of hemorrhagic shock. If dogs are subjected to a single, large, relatively rapid hemorrhage, 50% survival occurs with removal of 35 to 50% of the blood volume (Allen et al., 1959). This protocol was adopted to see if parameters which appeared to be critically related to survival under conditions of maintained hypotension were still important when the BP and CVP were allowed to vary spontaneously, and when no blood was returned to the dog from the reservoir.

### Methods

Dogs were anesthetized with pentobarbital and prepared for

hemorrhage as in previous studies. Two experimental protocols were followed. In one group of 16 dogs, the animals were bled over 15 minutes to an arterial pressure of 50 mm Hg; the connection to the reservoir was then clamped and the dog's arterial pressure allowed to vary spontaneously. In the second group of 9 dogs, the animals were bled 25 or 35 ml/kg; the connection to the reservoir was clamped and the BP allowed to vary spontaneously. In both groups, arterial pressure and CVP were monitored for 5 hours following hemorrhage and arterial blood samples were taken at 30-minute intervals for the determination of plasma protein concentration. The incisions were then closed and the animals returned to their cages for the determination of survival. All dogs alive 48 hours following the procedure were considered to be survivors.

Results

The results for this group of experiments are shown in Table X. All of the dogs initially responded to hemorrhage with a fall in arterial pressure and hemodilution, as indicated by a falling plasma protein concentration. After the cessation of hemorrhage, all the dogs showed a tendency for the arterial pressure to return toward normal, associated with a continued fall in plasma protein concentration. After one to 2 hours, the plasma protein concentration began to increase in all of the dogs. However, the increase was clearly different among the survivors and non-survivors. During the 5 hours following hemorrhage, the plasma protein concentration of 17 of 19 survivors remained below the control value, and it reached, but did not exceed, the control value in the remaining two. In contrast, the plasma protein concentration exceeded the control

Table X

Changes in Plasma Protein Concentration and Central Venous Pressure following a Single Large Hemorrhage without Reinfusion

| Dog No. | Bleeding Volume (ml/kg) | Resultant BP (mm Hg) | Plasma Protein Conc. (g/100 ml) | | | CVP (cm saline) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Initial | Lowest | At 5 hours | Initial | Lowest | At 5 hours |
| | | | Survivors | | | | | |
| 1 | 25 | 98 | 6.02 | 5.38 | 5.93 | -3.0 | -5.5 | -4.7 |
| 2 | 35 | 60 | 5.33 | 4.28 | 5.28 | -1.8 | -5.4 | -5.3 |
| 3 | 35 | 110 | 6.93 | 5.93 | 6.02 | 0 | -1.5 | 0 |
| 4 | 35 | 90 | 5.10 | 4.27 | 5.10 | 0 | -2.5 | -2.0 |
| 5 | 35 | 75 | 6.02 | 5.65 | 5.75 | 0 | -4.5 | -4.0 |
| 6 | 35 | 60 | 5.47 | 4.91 | 5.19 | +1.5 | -2.5 | -2.0 |
| 7 | 35 | 75 | 6.30 | 5.93 | 6.25 | -2.5 | -6.0 | -6.0 |
| 8 | 35 | 50 | 5.01 | 4.73 | 4.82 | -1.0 | -4.8 | -3.5 |
| 9 | 35 | 50 | 6.39 | 5.93 | 6.12 | +5.0 | +3.0 | +2.5 |
| 10 | 35 | 50 | 6.39 | 6.12 | 6.30 | 0 | -4.5 | -3.0 |
| 11 | 35 | 50 | 6.12 | 5.10 | 5.10 | -1.1 | -5.5 | -3.0 |
| 12 | 35 | 50 | 6.12 | 5.47 | 5.56 | +1.3 | -2.2 | -0.8 |
| 13 | 23 | 50 | 5.19 | 5.01 | 5.10 | +1.2 | -5.0 | -2.0 |
| 14 | 33 | 50 | 6.30 | 5.84 | 6.12 | 0 | -3.0 | -2.5 |
| 15 | 27 | 50 | 5.10 | 4.64 | 5.10 | 0 | -4.5 | -3.0 |
| 16 | 21 | 50 | 5.93 | 5.19 | 5.28 | -1.1 | -2.2 | -1.3 |
| 17 | 32 | 50 | 6.30 | 5.93 | 6.02 | +2.3 | -1.0 | -1.0 |
| 18 | 17 | 50 | 5.47 | 5.19 | 5.28 | 0 | -2.5 | -1.3 |
| 19 | 31 | 50 | 5.84 | 5.38 | 5.56 | -1.1 | -5.0 | -5.0 |
| | | | Fatalities | | | | | |
| 20 | 33 | 50 | 5.10 | 4.28 | 4.42 | -2.0 | -6.0 | -4.5 |
| 21 | 25 | 50 | 6.25 | 5.93 | 6.30 | -1.0 | -6.4 | -2.8 |
| 22 | 17 | 50 | 6.02 | 5.84 | 6.12 | -1.2 | -4.5 | -2.0 |
| 23 | 19 | 50 | 5.28 | 5.10 | 6.02 | -3.3 | -6.6 | -6.2 |
| 24 | 25 | 70 | 6.49 | 6.02 | 6.58 | -4.7 | -8.0 | -4.2 |
| 25 | 35 | 58 | 6.34 | 6.21 | 6.49 | -4.8 | -7.2 | -6.5 |
| Survivors | 31.26 ± 1.3 | 61.47 ± 4.3 | 6.03 ± 0.14 | 5.31 ± 0.10 | 5.57 ±0.60 | -0.65 ± 0.34 | -3.74 ± 0.94 | -2.81 ± 0.94 |
| Fatalities | 25.7 ± 2.5 | 54.7 ± 2.6 | 5.91 ± 0.22 | 5.50 ± 0.31 | 5.98 ±0.24 | -2.78 ± 0.68 | -6.53 ± 2.01 | -4.32 ± 0.65 |

value within 5 hours in 5 of the 6 fatalities. The plasma protein concentrations of survivors and nonsurvivors 5 hours after hemorrhage were significantly different at the $< 5\%$ level by Chi-squared analysis. Central venous pressure fell with hemorrhage in all dogs and remained low. It did not differ significantly in survivors and nonsurvivors, although it tended to be lower in the latter throughout.

Comment

The results of this group of experiments corroborate the observations reported in Section VIII, which indicated that the progression of hemoconcentration following hemorrhage was associated with a decreasing probability of survival. The present protocols, however, eliminated any possibility that either the blood returned to the dog from the reservoir, or the prevention of a compensatory rise in arterial pressure contributed to the observed correlation.

The protocols used in the experiments reported in this section also made it possible to examine the efficacy of compensatory processes. It is apparent that even without reinfusion of the shed blood, a considerable proportion of dogs will survive a hemorrhage that rapidly becomes lethal if the effects of compensatory processes are balanced by a protocol of maintained hypotension.

It is not possible to provide a definitive explanation of the plasma protein concentration increase in these experiments without information regarding changes in the total intravascular plasma protein. The increasing plasma protein concentration following initial hemodilution may in part, reflect a movement of protein into the vascular space to replace that which was lost. However, it appears likely that the marked

increase in the nonsurvivors involves a loss of protein-poor fluid from the vascular space, as was shown to occur in previous experiments. The finding that central venous pressure did not differ signicantly in the survivors and nonsurvivors, and indeed, tended to be lower in the latter, indicates that myocardial inadequacy did not play a major role in determining survival, at least during the first 5 hours after hemorrhage.

## Section X

### Role of Precapillary Resistance Vessels in the Development of Hemoconcentration during Hemorrhagic Hypotension

The preceding experiments demonstrated that the development of decompensation in the dog subjected to hemorrhagic hypotension is characterized by progressive hemoconcentration, and that the factors resulting in hemoconcentration appear to be important determinants of the dogs ultimate fate. The hemoconcentration has been shown to result from the ultrafiltration of fluid with a low protein concentration from the vascular space. This occurred despite constant arterial and central venous pressures, and was accelerated by the administration of noradrenaline, and delayed and reduced in magnitude by adrenergic blockade with phenoxybenzamine.

According to Starling's hypothesis, an increase in net ultrafiltration could result from a decreased interstitial hydrostatic pressure or an increased interstitial oncotic pressure, but these factors are generally considered to be quantitatively relatively unimportant, and are not known to be influenced by noradrenaline or phenoxybenzamine.

These agents do, however, mimic and block, respectively, effects of sympathetic nervous system activity. The sympathetics have well characterized, quantitatively important effects on the vascular smooth muscle which regulates the pre- and postcapillary resistances, and thus a major effect on net capillary pressure, and consequently, on fluid shifts across the capillary wall (Mellander, 1960).

The most likely explanation for the increased ultrafiltration of fluid from the vascular space during decompensation appears to be an increase in capillary hydrostatic pressure. Since both arterial and central venous pressures were constant through reversal, an increased capillary pressure must have been due to a change in the ratio of precapillary to postcapillary resistance (Pappenheimer and Soto-Rivera, 1948). Either a decrease in precapillary resistance, an increase in postcapillary resistance or a combination of these could increase capillary hydrostatic pressure despite constant arterial and venous pressures.

At a constant perfusion pressure, the major determinant of blood flow through a vascular bed is the precapillary resistance. This is predominantly in the arterioles, but it also includes components attributable to the metarterioles and precapillary sphincters (Zweifach, 1961.) In most of the experiments reported above, the arterial pressure was held constant after IBV, and consequently, any failure of the precapillary resistance vessels to maintain their tone following reversal would increase blood flow. Using blood flow at constant pressure as an index of precapillary resistance, the following experiments were carried out to explore the possibility that decreased precapillary vessel tone was involved in the loss of intravascular fluid after reversal. Since

these experiments measured the blood flow to several major vascular beds during hemorrhagic hypotension, they also provided information regarding the distribution of blood flow and the effects of POB pretreatment on this distribution.

Methods

A total of 32 dogs was studied in 2 groups, 22 in the first and 10 in the second. The experimental protocols differed only in the duration of hypotension; animals in the first group were subjected to 2.5 hours of hypotension at 50 mm Hg, and those in the second group to 4.5 hours or longer.

Half of the dogs were pretreated with phenoxybenzamine, 5 mg/kg intravenously, 18 hours before the experiment. Each dog was prepared with electromagnetic flowmeter probes around the appropriate arteries, as described in General Methods. Flow was measured in at least 2 of the 3 vessels studied (superior mesenteric, renal and terminal aorta) in all animals, and all three were measured in 3 control and 3 POB-pretreated dogs. Urine output from the left kidney was measured in 9 control and 10 POB-pretreated dogs by catheterizing the ureter approximately 2 inches distal to the renal pelvis, and draining the urine into a graduated cylinder by gravity.

After the preparation was completed the incisions were closed with clips and the dog allowed to stabilize for at least 30 minutes. The dogs were then bled over a 30-minute period, as in the previous experiments, and were held at an arterial pressure of 50 mm Hg for 2.5 hours. At the end of this interval, the blood remaining in the reservoir was reinfused intra-arterially and the measurements

continued for 2 to 3 hours, during which time most of the control dogs died.

In all 32 dogs, plasma protein determinations were carried out every 15 minutes, and the volume of blood in the reservoir was recorded every 5 minutes.

Results

The results obtained in this group of experiments which are relevant to the interpretation of fluid shifts during hemorrhagic hypotension will be presented in this section. Other data from these experiments will be presented and discussed in Section XII.

Data on the group of animals maintained hypotensive for 2.5 hours are given in Table XI. The courses of the control and POB-pretreated dogs were similar to those seen in previous groups. The maximum bleeding volumes were not different, but the time to MBV was considerably longer in the POB-pretreated dogs, 92.3 ± 16.6 min vs. 48.6 ± 9.1 min in the controls. Reversal occurred in all animals during the 150 minutes of hypotension. However, the uptake of blood by the end of the period of hypotension was considerably different, the control dogs having taken up 38.7% of their MBV, and the POB-pretreated dogs only 9.3%. The initial plasma protein concentrations and the hemodilution at MBV were not significantly different in the control and pretreated animals. However, by the end of 150 minutes at 50 mm Hg, the plasma protein concentration of the control dogs had risen to 5.89 ± 0.19 g/100 ml, while that of the POB-pretreated animals was only 5.15 ± 0.19 g/100 ml. This difference was also evident after reinfusion. In the last sample before death, or 2 hours after rein-

TABLE XI

Effects of POB Pretreatment on Responses to Hemorrhagic Hypotension of Dogs in which Regional Blood Flows Were Measured

| Group | No. | Weight (kg) | Bleeding Volume (ml/kg) IBV | MBV | At 2.5 hours | Uptake % |
|---|---|---|---|---|---|---|
| Control | 11 | 14.1±1.0 | 25.6±3.1 | 36.9±4.6 | 22.6±4.4 | 38.7 |
| POB-Pretreated | 11 | 14.0±1.1 | 26.3±1.5 | 38.8±2.6 | 35.2±2.7 | 9.3 |
| P | | n.s. | n.s. | n.s. | < 0.01 | |

| Group | No. | Plasma Protein Conc. (g/100 ml) Initial | At IBV | At MBV | At Reinfusion | At Death or 2 Hrs After Reinf. |
|---|---|---|---|---|---|---|
| Control | 11 | 5.83±0.14 | 5.59±0.19 | 5.41±0.19 | 5.89±0.19 | 6.30±0.26 |
| POB-Pretreated | 11 | 5.71±0.10 | 5.45±0.14 | 5.06±0.19 | 5.15±0.19 | 5.82±0.14 |
| P | | n.s. | n.s. | n.s. | 0.05 | < 0.05 |

| Group | No. | Vessel | Blood Flow at 50 mm Hg (ml/min) IBV | IBV + 75 min | IBV + 150 min |
|---|---|---|---|---|---|
| Control | 11 | Mesenteric | 43.1± 8.3 | 37.1± 8.2 | 38.0± 8.1 |
| POB-Pretreated | 11 | Artery | 98.9±19.9 | 89.4±18.6 | 92.3±19.2 |
| Control | 11 | Terminal | 52.9±12.0 | 51.0±16.0 | 48.4±11.5 |
| POB-Pretreated | 11 | Aorta | 63.4±12.7 | 65.4±12.8 | 66.8±12.6 |
| Control | 11 | Renal | 52.3±17.1 | 23.9± 7.7 | 23.3± 7.7 |
| POB-Pretreated | 11 | Artery | 85.1± 9.4 | 51.9± 2.9 | 48.6± 4.2 |

fusion in the 2 animals that had not died by that time, the plasma protein concentration of the control dogs was 6.30 ± 0.26 g/100 ml. Ten of the 11 POB-pretreated dogs were still alive 2 hours after reinfusion and their plasma protein concentration was only 5.82 ± 0.17 g/100 ml, not significantly greater than their plasma protein concentration before hemorrhage.

Differences in the progression of hemorrhagic shock in the control and POB-pretreated dogs are also evident in Table XII, where the arterial blood pressures of the individual dogs at the time of reinfusion and at 30-minute intervals thereafter are presented. After reinfusion, the POB-pretreated dogs had a BP 101.7 ± 4.15% of their initial level, whereas the control dogs reached only 73.7 ± 5.3%, a difference significant at the 0.001 level. Six of the control dogs died within one hour after reinfusion, and within 2 hours, 9 of the 11 were dead. Only one of the 11 POB-pretreated dogs died within 2 hours of reinfusion. This difference in survival 2 hours after reinfusion is significant at the 0.0015 level by Chi-squared analysis.

The individual effects of hemorrhage and of POB pretreatment on blood flow distribution will be presented in a later section. The data relevant to the interpretation of fluid shifts are the changes in flow after the animals reached IBV (Table XI). Since the dogs were held at 50 mm Hg from this time until reinfusion, any tendency of the precapillary resistance vessels to lose their tone would be evident as an increase in flow.

When the control dogs first reached a BP of 50 mm Hg, the superior mesenteric blood flow had fallen to 43.1 ± 8.3 ml/min. Sev-

Table XII

Effects of POB-Pretreatment on Blood Pressure Response to Reinfusion after 2.5 Hours of Hemorrhagic Hypotension

| | Arterial Blood Pressure (mm Hg) | | | | | |
|---|---|---|---|---|---|---|
| Group | Initial | After Reinfusion | + 30 min | + 60 min | + 90 min | + 120 min |
| Control | 115 | -- | -- | -- | -- | -- |
| | 112 | 65 | -- | -- | -- | -- |
| | 105 | 75 | -- | -- | -- | -- |
| | 125 | 75 | -- | -- | -- | -- |
| | 160 | 130 | 70 | -- | -- | -- |
| | 145 | 95 | 60 | -- | -- | -- |
| | 105 | 100 | 105 | 100 | -- | -- |
| | 135 | 125 | 120 | 115 | 115 | -- |
| | 95 | 75 | 70 | 65 | 50 | -- |
| | 140 | 110 | 100 | 95 | 90 | 90 |
| | 145 | 125 | 125 | 120 | 120 | 105 |
| Survivors | 11 | 10 | 7 | 5 | 4 | 2 |
| POB-Pre-treated | 135 | 120 | 95 | 70 | 65 | -- |
| | 90 | 80 | 80 | 75 | 75 | 65 |
| | 105 | 130 | 120 | 120 | 95 | 80 |
| | 120 | 115 | 85 | 85 | 85 | 85 |
| | 100 | 105 | 105 | 100 | 95 | 90 |
| | 130 | 110 | 110 | 100 | 100 | 100 |
| | 90 | 105 | 100 | 100 | 100 | 100 |
| | 115 | 105 | 130 | 130 | 110 | 110 |
| | 120 | 135 | 110 | 110 | 105 | 110 |
| | 125 | 145 | 140 | 115 | 115 | 110 |
| | 140 | 135 | 125 | 120 | 110 | 110 |
| Survivors | 11 | 11 | 11 | 11 | 11 | 10 |

enty-five minutes later, after reversal in all dogs, it was 37.1 ± 8.2 ml/min, and after 2.5 hours of hypotension, when the dogs showed marked cardiovascular decompensation, having taken up a mean of 39% of their MBV from the reservoir, the flow to the mesenteric vascular bed was 33.0 ± 8.1 ml/min. There was clearly no tendency for the resistance to blood flow in this vascular bed to "give out". In fact, there was a tendency for the flow to fall slightly with time, but this change was not statistically significant. The mesenteric blood flow in the POB-pretreated dogs also showed no tendency to increase at, or after, reversal. In Fig. 11, the superior mesenteric blood flow at IBV is plotted on the abscissa against the flows 75 and 150 minutes later on the ordinate for all experiments in this group. In this plot, any tendency of the precapillary resistance vessels to lose tone would result in the points falling above the line of equality, but no such trend was detected.

Blood flow in the renal artery had fallen to 52.3 ± 17.1 ml/min when the control dogs first reached a BP of 50 mm Hg. In contrast to the mesenteric flow, the renal blood flow continued to fall during the initial period of hypotension, reaching 23.9 ± 7.7 ml/min after 75 minutes, and 23.3 ± 7.7 ml/min after 2.5 hours of hypotension. This reduction was significant at the 5% level. The renal blood flow at IBV is plotted against the flows after 75 and 150 minutes at 50 mm Hg in Fig. 12, which illustrates the tendency for renal blood flow to fall with time.

Blood flow to the hindquarters (terminal aorta) was 52.9 ± 12.0 ml/min when the control dogs first reached a BP of 50 mm Hg. After 75 and 150 minutes of hypotension, the blood flows to this vascular bed

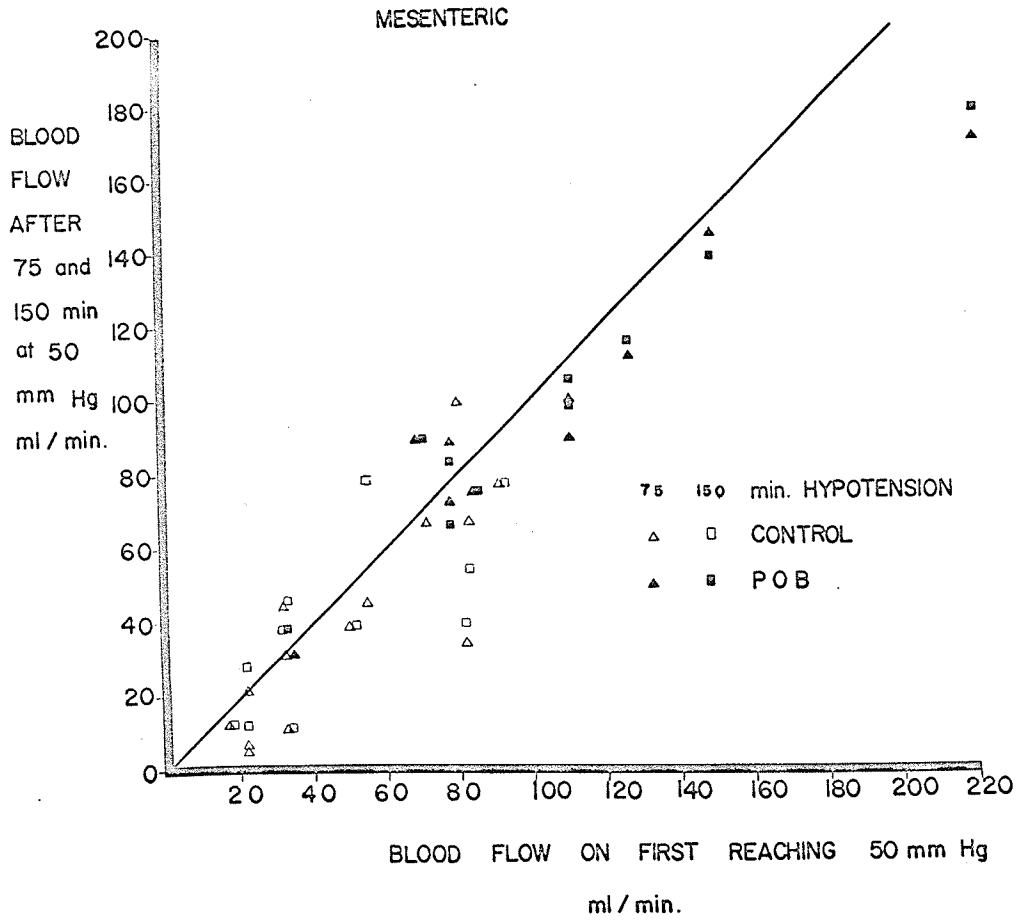


Figure 11. Relationship of Mesenteric Blood Flows at IBV to Those at the Same Pressure 75 and 150 Minutes Later. Arterial pressure was maintained at 50 mm Hg throughout the period of measurement. Points determined after 75 (early decompensation) and 150 minutes (late decompensation) of hypotension tend to fall below the line of equality, indicating that there was no decrease and may have been a slight increase in the resistance of the mesenteric vascular bed during the period of hypotension.

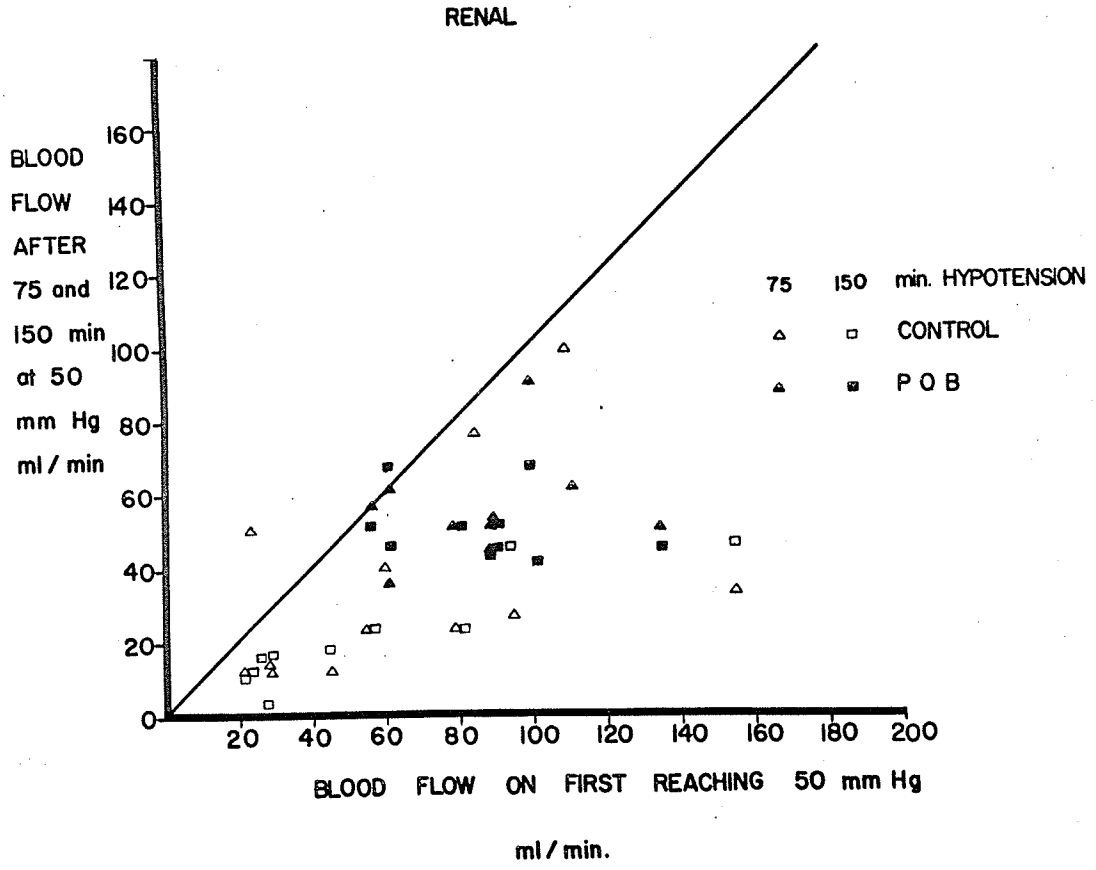


Figure 12. Relationship of Renal Blood Flows at IBV to Those at the Same Pressure 75 and 150 Minutes Later. Arterial pressure was maintained at 50 mm Hg throughout the period of measurement. Most of the points determined after 75 (early decompensation) and 150 minutes (late decompensation) of hypotension fall well below the line of equality, indicating a progressive increase in renal vascular resistance during the period of hypotension.

were 51.0 ± 16.0 and 48.4 ± 11.5 ml/min, respectively. As in the other vascular beds studied, no evidence for a loss of vascular resistance in either the control or the POB-pretreated dogs could be detected (Fig. 13).

In order to determine if a tendency for the precapillary resistance vessels to lose their tone would appear during even more prolonged hypotension, the second group of 10 dogs was held at a BP of 50 mm Hg for 4.5 to 5.5 hours. No tendency for blood flow to increase in any of the vascular beds studied was observed despite severe decompensation, indicated by uptakes of blood as great as 71% of the MBV.

Comments

The results of experiments on this group of 32 dogs demonstrated that there is no tendency for the "resistance vessels" to lose their tone during hemorrhagic hypotension, either at the time of reversal or at considerably later stages of decompensation. Since the major portion of the vascular resistance to blood flow lies in the precapillary vessels, i.e., arterioles, metarterioles and precapillary sphincters, these results indicate that loss of precapillary tone in the regions studied plays no part in the genesis of the fluid shifts noted in previous groups. Since the regions studied, i.e., gastrointestinal tract, kidney, skeletal muscle and skin, make up a very large part of the body and take a large percentage of the cardiac output, it is very unlikely that any major loss of precapillary vessel tone occurred. Therefore, if the loss of fluid from the vascular space is due to an increased capillary hydrostatic pressure, it is probably due to an

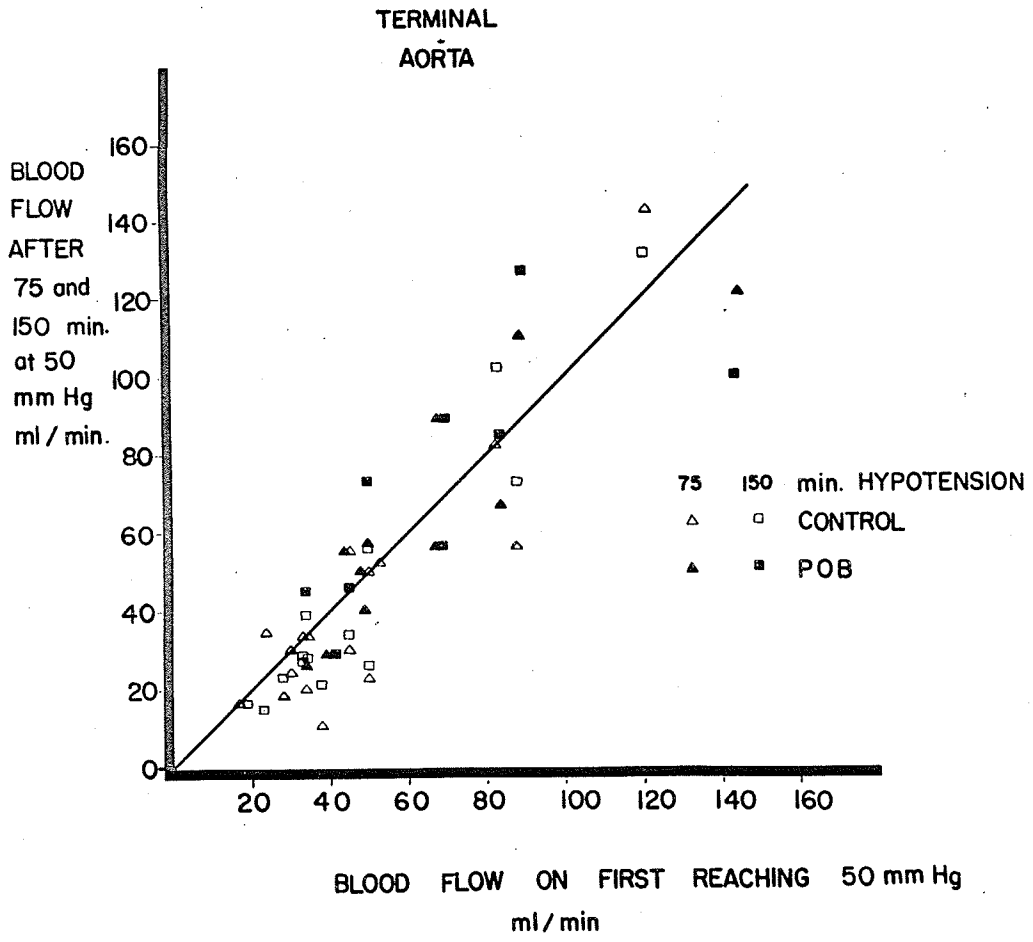


Figure 13. Relationship of Hindquarters Blood Flows at IBV to Those at the Same Pressure 75 and 150 Minutes Later. Arterial pressure was maintained at 50 mm Hg throughout the period of measurement. Points determined after 75 (early decompensation) and 150 minutes (late decompensation) of hypotension cluster about the line of equality, indicating that there was no change in the resistance of this vascular bed during the period of hypotension.

increase in the tone of the postcapillary vessels.

## Section XI

## Role of Postcapillary Resistance Vessels in the Development of Hemoconcentration during Hemorrhagic Hypotension

The postcapillary resistance, which is a small part of the total peripheral resistance, is commonly attributed to the venules and small veins, but there is no clear evidence regarding which vessels make the most important contribution. Most of the techniques in common use today for the study of venous tone monitor the weight or volume of a tissue segment, and thus responses of the larger capacity veins dominate the results. Responses of the postcapillary resistance vessels may be inferred from net movements of fluid into and out of the vascular space in conjunction with information on changes in precapillary resistance, as in the experiments described in Section X, but this provides only indirect evidence. The technique that most closely approximates a measurement of postcapillary resistance is that developed by Haddy et al. (1957). Segmental pressures are monitored in a vascular bed at the levels of a large artery, a small artery, a small vein and a large vein. The "small vein" pressure is measured through a small polyethylene catheter introduced into a vein in a retrograde direction and "wedged" as far peripherally as possible, presumably at the level of veins too small to allow the catheter to enter. This provides a measurement of the end pressure in vessels just proximal to the catheter. Generally, the vascular bed is perfused by a constant flow pump so that changes in arteriolar resistance are expressed as changes in perfusion pressure.

Methods

A modification of the Haddy technique was used in experiments on 8 dogs in which the vasculature of the hindquarters was studied, and on 6 dogs in which the mesenteric vascular bed was studied. No perfusion pump was used since there is evidence that pumps adversely affect vascular reactivity (Folkow, 1952), and since the blood flow to the hindquarters and gastrointestinal tract remained relatively constant during the critical interval when arterial pressure was held at 50 mm Hg (Section X).

Perfusion pressure to the hindquarters was measured by cannulating the artery to the tail and placing the catheter tip at the bifurcation of the aorta. Blood flow was measured with an electromagnetic flowmeter as in the experiments reported in Section X with the probe around the aorta just proximal to the bifurcation. Small vein wedge pressure was measured by passing a polyethylene catheter with an outside diameter of 0.039 inches (Clay Adams P.E. 50) as far as possible in a retrograde direction through one of the small veins draining the gracilis muscle. A sudden increase in pressure was seen when the catheter was wedged. Patency of the catheter was assessed periodically by disconnecting it from the pressure transducer, and checking backflow. Large vein pressure was measured through a catheter placed in a tributary so that its tip was flush with the wall of the femoral vein. Pressures from both peripheral veins were recorded with Statham P 23AC transducers. Central venous pressure was measured with a saline manometer, as in previous experiments. During the period of hypotension, arterial perfusion pressure was also measured with a saline manometer.

After a 30 to 60 minute control period the dogs were bled to an arterial blood pressure of 50 mm Hg and held at this pressure until at least 20% uptake of blood from the reservoir had occurred. After this, phenoxybenzamine was administered intra-arterially to the hindquarters of 5 of the dogs in graded doses of 0.5 to 1.0 mg/kg, generally at 5 to 10 minute intervals, to a total dose of 2.0 to 5.0 mg/kg. In 2 of these dogs, and in 2 others, atropine and mepyramine were administered intra-arterially, the former in serial doses of 0.5 to 1.0 mg/kg to a total dose of 2.0 mg/kg and the latter in one or 2 doses of 5.0 mg/kg.

In another group of 6 dogs, similar experiments were performed on the circulation of the gastrointestinal tract. Superior mesenteric artery blood flow and perfusion pressure in the abdominal aorta at that level were monitored. Small vein wedge pressure was obtained by catheterizing a 0.5 to 1.0 mm diameter vein in a mesenteric venous arcade in a retrograde direction until the tip of the catheter wedged in a small vein near the bowel wall. The same vein was also catheterized centrally and the catheter advanced to measure the pressure in a large mesenteric vein. The experimental protocol was essentially the same as that used in studies on the hindquarters. Plasma protein concentrations and bleeding volumes were followed in both groups, as in the experiments reported in previous sections.

Results

Results typical of experiments on the hindquarters vascular bed are shown in Fig. 14 and of those on the mesenteric vascular bed in Fig. 15. The bleeding volumes and changes in plasma protein concentration were not different from those reported above for other groups. The

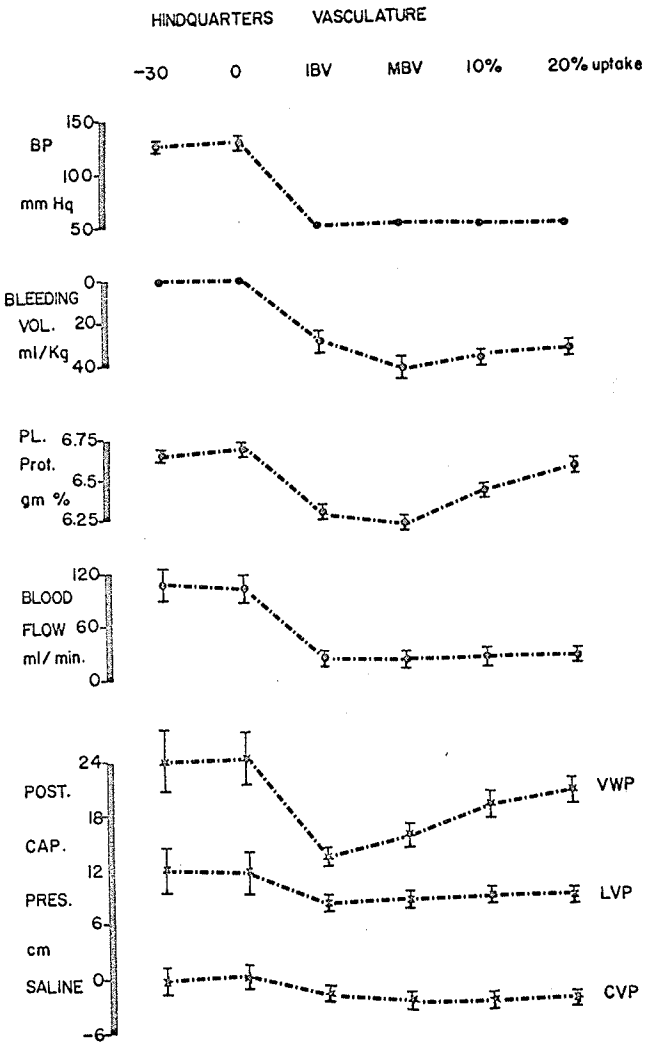


Figure 14. Relationship of Postcapillary Pressures to Other Parameters during Hemorrhagic Hypotension (Hindquarters Vascular Bed). VWP = Small vein wedge pressure, LVP = Large (femoral) vein pressure, CVP = Central venous pressure. Note the rising venous wedge pressure following IBV, despite constant arterial, femoral and central venous pressures, and a constant blood flow. Vertical bars indicate S.E. of each point.

mean femoral venous pressure was 12.1 ± 2.7 cm of saline before hemorrhage, and the venous wedge pressure was always higher, averaging 24.0 ± 3.6 cm of saline.

During the initial part of the hemorrhage, to a bleeding volume of approximately 15 ml/kg, there was generally little or no fall in arterial pressure, and only a moderate decrease in central and femoral venous pressures. During this interval, hindquarters' blood flow fell precipitously, suggesting intense constriction of the arterioles in that vascular bed. The venous wedge pressure fell in parallel with the decreased flow. After an arterial pressure of 50 mm Hg was reached, the hindquarters blood flow remained relatively constant, as did femoral and central venous pressures. The only parameter to show a consistent change was venous wedge pressure, which generally began to increase as soon as the arterial pressure had stabilized at 50 mm Hg. It was 13.3 ± 1.7 cm of saline at IBV, and had risen to 20.1 ± 1.0 cm saline at 20% uptake. In most experiments, the venous wedge pressure had stopped increasing by the time 20% uptake was reached, but termination of the increase in wedge pressure bore no consistent relationship to the slope change of the plasma protein concentration curve.

Events in the mesenteric vascular bed (Fig. 15) were similar to those in the hindquarters except that the venous wedge pressure tended to increase earlier and more rapidly. In some cases, the increase began during early hemorrhage, before an arterial pressure of 50 mm Hg had been reached. Small venous wedge pressure initially fell with the decrease in flow as in the hindquarters vascular bed. However, even during early hemorrhage, the wedge pressure tended to rise during intervals when the flow was not decreasing. After IBV was reached, the wedge pressure rose

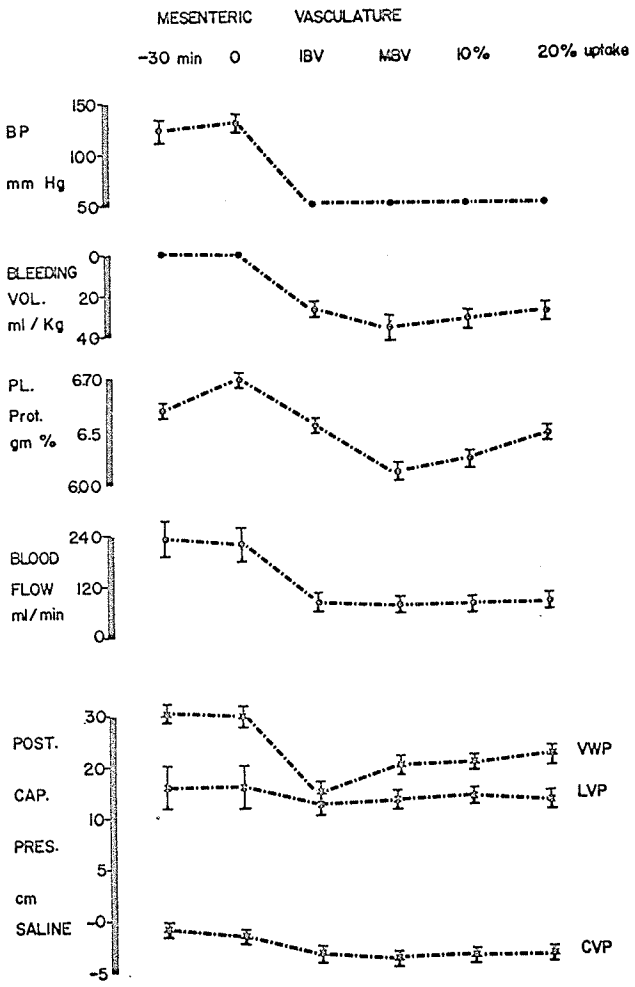


Figure 15. Relationship of Postcapillary Pressures to Other Parameters during Hemorrhagic Hypotension (Mesenteric Vascular Bed). VWP = Small vein wedge pressure, LVP = Large mesenteric vein pressure, CVP = Central venous pressure. Note the rising venous wedge pressure following IBV, despite constant arterial, large mesenteric and central venous pressures, and a constant blood flow. Vertical bars indicate S.E. of each point.

rapidly, from 15.6 ± 1.3 to 22.0 ± 1.2 cm of saline at 20% uptake.

The administration of atropine intra-arterially to the hindquarters vascular bed in doses larger than those required to block the cholinergic vasodilator system (Uvnäs, 1961) (Fig. 16) had no effect on either blood flow or segmental vascular pressures during hemorrhagic hypotension. Mepyramine administered intra-arterially induced a transient arteriolar dilatation, but flow returned to the preinjection level within one to 2 minutes after each injection. Phenoxybenzamine injected intra-arterially increased hindquarters blood flow in every experiment. The increase in flow was often associated with a transient increase in venous wedge pressure. However, this was always followed by a fall in the wedge pressure, generally to well below the level prior to phenoxybenzamine administration, although the blood flow remained elevated.

Comment

The pressure measured with a venous catheter wedged in a retrograde direction is that of the closest peripheral collateral vessels through which blood is flowing. This pressure is determined by the arterial pressure and its transmission across the intermediate resistance vessels, the pressure in the large veins draining the vascular bed, and the tone of the small veins at the level of measurement. After the hypotensive level of 50 mm Hg was reached, arterial pressure was held constant, and flow, reflecting precapillary resistance, did not change appreciably. Thus, an increased transmission of arterial pressure could not have been responsible for the increase in wedge pressure which was observed in every animal. Also, since large vein pressure was constant or falling, the increased venous wedge pressure could not have been due to "back pressure"

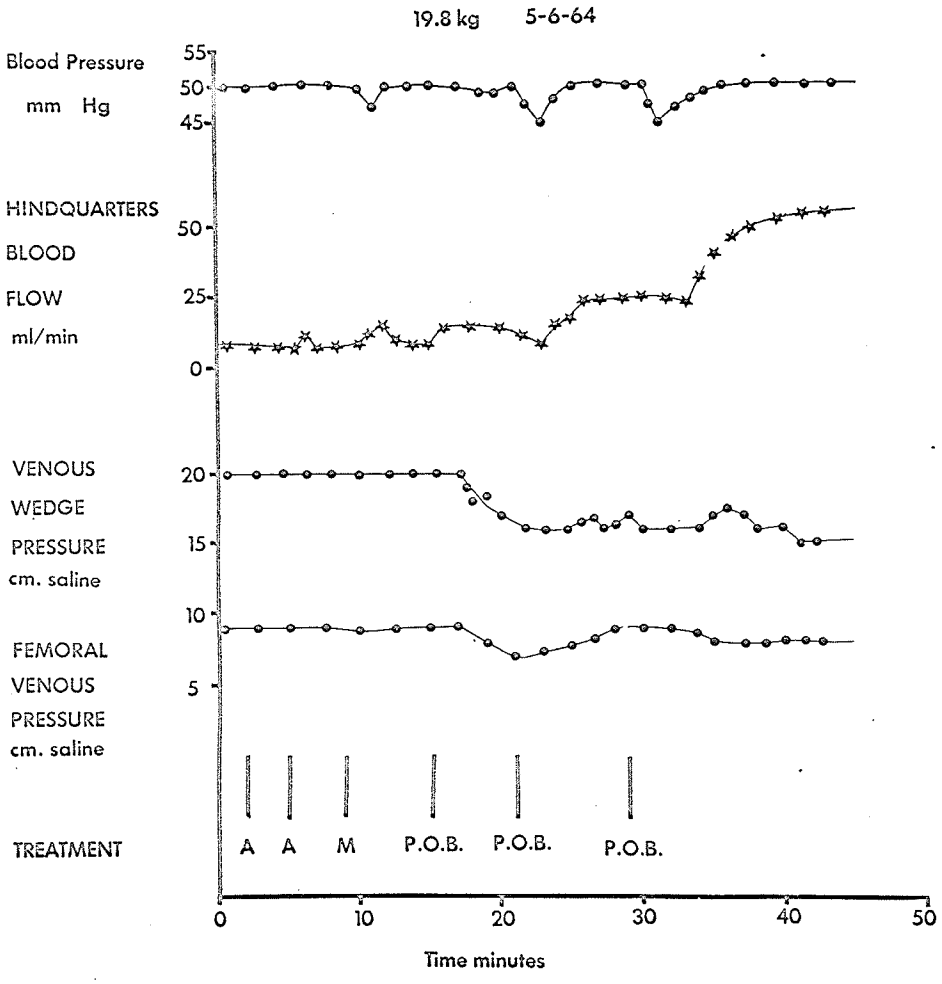


Figure 16. Effects of Atropine (A), Mepyramine (M) and Phenoxybenzamine (P.O.B.) on Increased Small Vein Pressure during Hemorrhagic Hypotension (Hindquarters Vascular Bed). All drugs administered intra-arterially. Note the sustained fall in venous wedge pressure induced by phenoxybenzamine despite increased blood flow.

from larger veins or the right atrium. This indicates that the increase in venous wedge pressure after IBV was due to constriction of small veins near the level of measurement. This interpretation is consistent with the hypothesis that ultrafiltration of fluid from the vascular space during decompensation is due to a change in the ratio of precapillary to postcapillary resistance, with precapillary resistance staying relatively constant and postcapillary resistance progressively increasing. The results of the experiments in which administration of phenoxybenzamine or noradrenaline, respectively, delayed or potentiated the loss of fluid from the vascular space suggest that the increased postcapillary resistance during hemorrhagic hypotension is due to activity of the sympathetic nervous system. The administration of phenoxybenzamine in experiments of the present group lowered the small vein wedge pressure despite an increase in blood flow, and thus an increased transmission of arterial pressure to the region in which wedge pressure was measured. The failure of atropine, an anticholinergic agent, and mepyramine, an antihistaminic, to reduce the wedge pressure indicates that the effect of phenoxybenzamine was not due to inhibition of effects of histamine or acetylcholine, but to block of the actions of catecholamines on the $\alpha$ receptors of postcapillary resistance vessel smooth muscle, and thus of vasoconstriction due to sympathetic nervous system activity.

# SPECIFIC EXPERIMENTS, RESULTS AND COMMENT

## B. Ancillary Studies on Sympathetic Nervous System Involvement in Hemorrhagic Shock

## (Sections XII to XVII)

## Section XII

## Effects of Hemorrhagic Hypotension and of Adrenergic Blockade on Blood Flows to the Kidney, Gastrointestinal Tract and Hindquarters of the Dog

It is frequently stated that shock is associated with considerable vasoconstriction, but there is a relative paucity of information on its distribution, and, in fact, some disagreement as to whether quantitatively important generalized arteriolar constriction does occur (Green, 1961). In addition, it is quite clear that adrenergic blocking agents can change the course of shock, and it is suggested that this is due to improved perfusion of vital structures, but there is little quantitative information on the effects of adrenergic blockade on blood flow to various vascular beds during the development of shock. Data from the experiments reported in Section X, which were designed to explore the possibility that the precapillary resistance vessels lose their tone during hemorrhagic hypotension, have been utilized also in an analysis of changes in the pattern of regional blood flows during hemorrhage and hypotension, and of the effects of adrenergic blockade. The weights of the control and POB-pretreated dogs were very similar, 14.1 ± 1.0 and 14.0 ± 1.1 kg, respectively, which made it possible to compare blood flows in the two groups quantitatively. The Methods employed in these experiments were described in Section X.

### Results

Blood flows to the mesenteric, renal and hindquarters vascular beds in control and POB-pretreated dogs at various stages in the hemorrhagic shock procedure are shown in Table XIII.

The mesenteric blood flow in the control dogs was 178.6 ± 25.3 ml/min 30 minutes before, and 164.0 ± 20.1 ml/min just before the onset of hemorrhage. By the time an arterial pressure of 50 mm Hg was reached (IBV), the mesenteric blood flow had fallen to 43.1 ± 8.3 ml/min. The characteristic relationship between changes in arterial pressure and in mesenteric blood flow during hemorrhage is shown in Fig. 17. In all cases, a precipitous fall in mesenteric blood flow occurred during the first 15 minutes of hemorrhage (15-20 ml/kg), while arterial pressure fell very little. Mesenteric flow had generally fallen to a third or less of the control value before arterial pressure began to decrease rapidly. In the dog shown in Fig. 17, mesenteric blood flow had fallen from 450 to 135 ml/min when the BP had decreased only 15 mm Hg. After this, the arterial pressure fell much more rapidly, and the mesenteric flow, much more slowly. As pointed out in Section X, flow changed very little during the period when arterial pressure was held at 50 mm Hg.

The increase in mesenteric flow during reinfusion was also characteristically related to the increase in arterial pressure, as can be seen in Fig. 17. The flow in this vascular bed was greater during reinfusion than during hemorrhage at all perfusion pressures in every dog studied. However, the highest mesenteric blood flow attained by the control group at reinfusion was 137.4 ± 39.1 ml/min, considerably below the prehemorrhage value, because the perfusion pressure did not reach the control level. (See Section X.) During the terminal spontaneous fall in arterial pressure after reinfusion, the mesenteric pressure-flow curve closely resembled that during the initial hemorrhage. This relationship is also evident in Fig. 17.

TABLE XIII

Regional Blood Flows before and during Hemorrhagic Hypotension in Control and POB-Pretreated Dogs

| | 30 min Before Hemorrhage | Immediately Before Hemorrhage | At IBV | 75 min After IBV | 150 min After IBV | Max. Flow After Reinfusion |
|---|---|---|---|---|---|---|
| | | | Mesenteric Blood Flow (ml/min) | | | |
| Control | 178.6 ± 25.3 | 164.0 ± 20.1 | 43.1 ± 8.3 | 37.1 ± 8.2 | 38.0 ± 8.1 | 137.4 ± 39.1 |
| POB-Pretreated | 318.6 ± 52.0 | 302.8 ± 47.5 | 98.9 ± 19.9 | 89.4 ± 18.6 | 92.3 ± 19.2 | 213.3 ± 42.2 |
| Difference (%) | 78.2 | 85.7 | 130.1 | 140.5 | 142.0 | 55.4 |
| P | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | n.s. |
| | | | Hindquarters Blood Flow (ml/min) | | | |
| Control | 157.6 ± 11.0 | 159.0 ± 11.1 | 52.9 ± 12.0 | 51.0 ± 16.0 | 48.4 ± 11.5 | 133.1 ± 26.8 |
| POB-Pretreated | 246.5 ± 36.6 | 254.4 ± 42.1 | 63.4 ± 12.7 | 65.4 ± 12.8 | 66.8 ± 12.6 | 222.8 ± 27.9 |
| Difference (%) | 56.3 | 59.7 | 20.7 | 27.4 | 39.5 | 67.6 |
| P | < 0.05 | < 0.05 | n.s. | n.s. | n.s. | n.s. |
| | | | Renal Blood Flow (ml/min) | | | |
| Control | 159.4 ± 25.4 | 150.4 ± 29.1 | 52.3 ± 7.1 | 23.9 ± 7.7 | 23.3 ± 7.7 | 94.4 ± 16.6 |
| POB-Pretreated | 184.5 ± 19.9 | 180.1 ± 20.8 | 85.1 ± 9.4 | 51.9 ± 2.9 | 48.6 ± 4.2 | 144.6 ± 7.9 |
| Difference (%) | 16.3 | 20.0 | 63.4 | 116.6 | 113.0 | 54.2 |
| P | n.s. | n.s. | < 0.05 | < 0.01 | < 0.01 | < 0.05 |

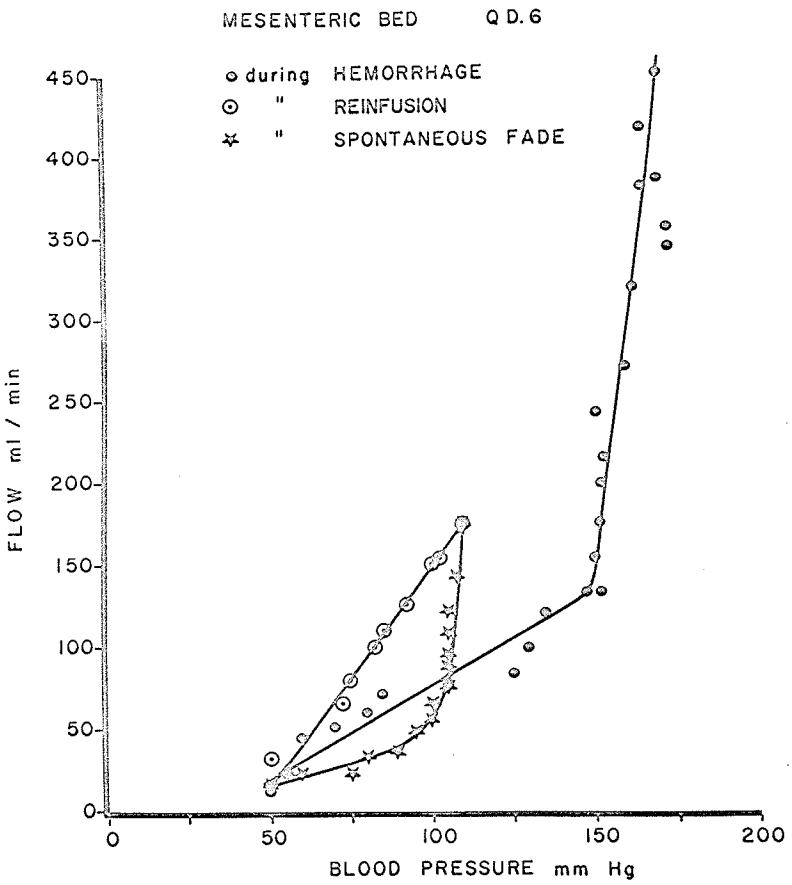


Figure 17. Relationship of Mesenteric Blood Flow to Arterial Pressure during the Development of Hemorrhagic Shock. Note the precipitous decrease in flow at the beginning of hemorrhage, before arterial pressure fell appreciably, the absence of change during 4.5 hours of hypotension at 50 mm Hg, the considerable vasodilatation associated with reinfusion of the shed blood, and the re-establishment of effective vasoconstriction during the subsequent terminal fall in arterial pressure (fade).

Phenoxybenzamine pretreatment induced major changes in mesenteric blood flow both in the control period and during hemorrhage. Thirty minutes before hemorrhage, the mesenteric blood flow in pretreated animals was 318.6 ± 52.0 ml/min, and it was 302.8 ± 47.5 ml/min just before hemorrhage, both significantly greater than those of the control dogs ($P < 0.01$). The relationship between the fall in arterial pressure and the mesenteric flow during hemorrhage was qualitatively similar to that of the control dogs, but the flows were higher at all perfusion pressures. The mesenteric blood flow of POB-pretreated dogs was significantly greater than that of the control animals at IBV and throughout the entire period of hypotension. There was no tendency for the flow to increase while the pressure was maintained at 50 mm Hg in any of the animals studied.

Mesenteric blood flow increased with the increase in perfusion pressure during reinfusion, in a manner qualitatively the same as in control dogs, but the curve was higher on the flow axis. Both mesenteric blood flow and perfusion pressure reached higher levels and were better maintained in the POB-pretreated group. The difference between the mesenteric blood flows of POB-pretreated and control dogs increased as the cardiovascular system was stressed by hemorrhage. Flow was about 80% greater in pretreated animals prior to hemorrhage, and this differential increased to 130% at IBV and 142% after 150 minutes of hypotension. It decreased to 55% after reinfusion.

The renal blood flow of the control dogs was 159.4 ± 25.4 ml/min 30 minutes before hemorrhage, and 150.4 ± 29.1 ml/min just before bleeding. During hemorrhage, the relationship of flow to perfusion pressure was distinctly different from that of the mesenteric bed, as can be seen in

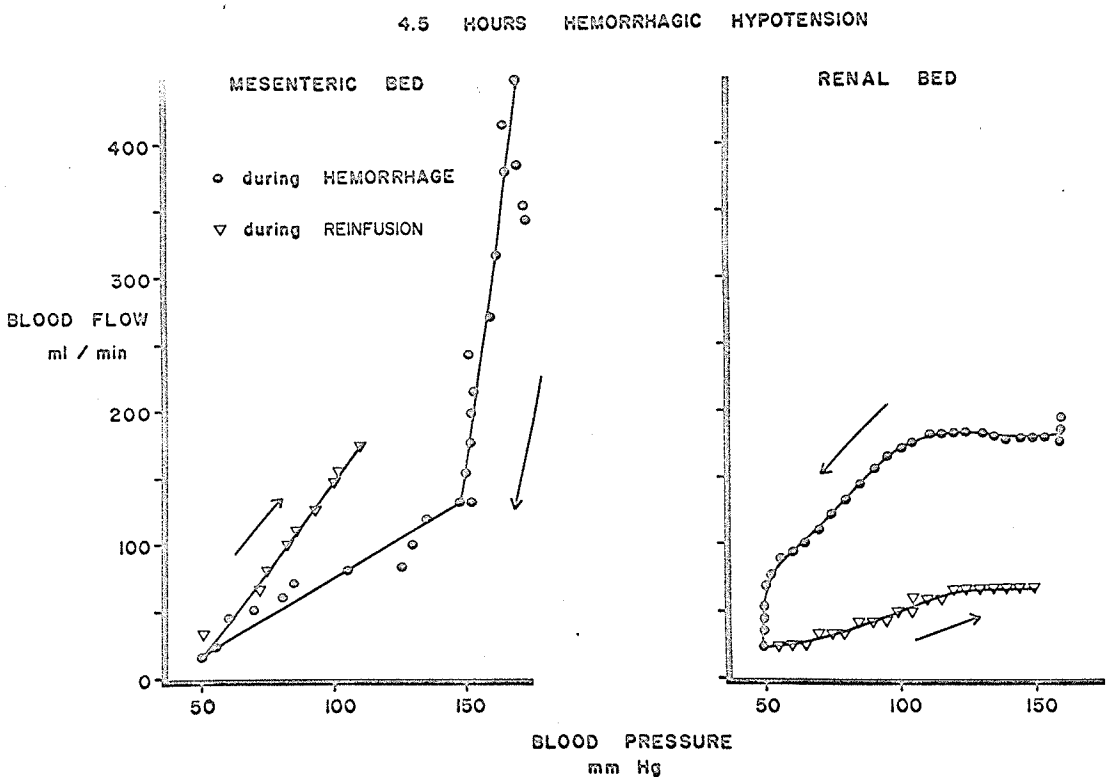


Figure 18. Comparison of Mesenteric and Renal Blood Flows during Hemorrhage, Hypotension and Reinfusion. Arterial pressure reduced to 50 mm Hg over 30 minutes. Note particularly the early rapid decrease in mesenteric flow, the initial period of "autoregulation" in the renal vascular bed, the progressive reduction in renal flow during sustained hypotension (4.5 hours), and that during reinfusion mesenteric flows are all above and renal blood flows all below those at comparable pressures during the initial hemorrhage.

Fig. 18. Instead of an initial steep fall in flow, with relatively little decrease in perfusion pressure, the renal blood flow remained relatively constant through a considerable fall in perfusion pressure. Flow began to decrease at arterial pressures between 80 and 100 mm Hg, but never as rapidly as it did in the mesenteric vascular bed. Renal blood flow decreased somewhat more rapidly when the pressure fell below 70 mm Hg. Mesenteric blood flow remained relatively constant while the arterial pressure was held at 50 mm Hg, but the renal blood flow fell progressively during this period, and the value just before reinfusion was only 44% of that at IBV. The two vascular beds also responded quite differently to reinfusion. Blood flow in the mesenteric bed was higher at each perfusion pressure during reinfusion than it was during hemorrhage, but after periods of hemorrhagic hypotension of 2.5 or 4.5 hours, blood flow to the kidney was always much lower (Fig. 18). During terminal hemodynamic deterioration, the renal blood flow tended to fall along the same pressure-flow curve it followed during reinfusion. The elevated renal vascular resistance which persisted in spite of reinfusion and increased arterial pressure, and factors in its genesis are explored further in Sections XIII to XVI.

In contrast to that of the intestine, the renal blood flow was not significantly greater in POB-pretreated than in control dogs prior to hemorrhage. During hemorrhage, the relationship between perfusion pressure and renal blood flow was similar in pretreated and control dogs except that flows tended to remain higher in the former. At IBV, the renal blood flow in the POB-pretreated dogs was significantly greater (63.4%) than that of the controls, and remained significantly greater

throughout the period of hypotension, being more than double the control value (116% greater) just prior to reinfusion. Renal blood flow also showed less tendency to remain depressed after reinfusion in the POB-pretreated animals.

Differences in the renal vascular responses to hemorrhagic hypotension in POB-pretreated and control dogs were paralleled by differences in urine output following reinfusion (Fig. 19). The marked reduction in renal blood flow was associated with anuria in 8 of the 9 control dogs despite arterial blood pressures exceeding 100 mm Hg following reinfusion. All 10 POB-pretreated dogs diuresed at this time, and in 5 the rate of urine production was higher after reinfusion than before hemorrhage.

Responses of the vasculature of the hindquarters (skin and muscle) to hemorrhagic hypotension were similar in several respects to those of the mesenteric vascular bed. Hindquarters blood flow fell precipitously during the early period of hemorrhage, although perfusion pressure was well maintained. The flow had fallen from a control value of 159.0 ± 11.1 to 52.9 ± 12.0 ml/min at IBV, and remained close to this level for the entire period of hypotension. There was no tendency for flow to increase. As in the mesenteric bed, blood flow to the hindquarters was higher at every perfusion pressure during reinfusion than it had been during hemorrhage, and during terminal cardiovascular decompensation, it fell along the same pressure-flow curve as during the initial hemorrhage.

Hindquarters blood flows of POB-pretreated dogs before hemorrhage were significantly greater ($P = 0.05$) than those of the controls. During hemorrhage, the flow fell from 254.4 ± 42.1 to 63.4 ± 12.7 ml/min at IBV, with a pressure-flow relationship qualitatively similar to that

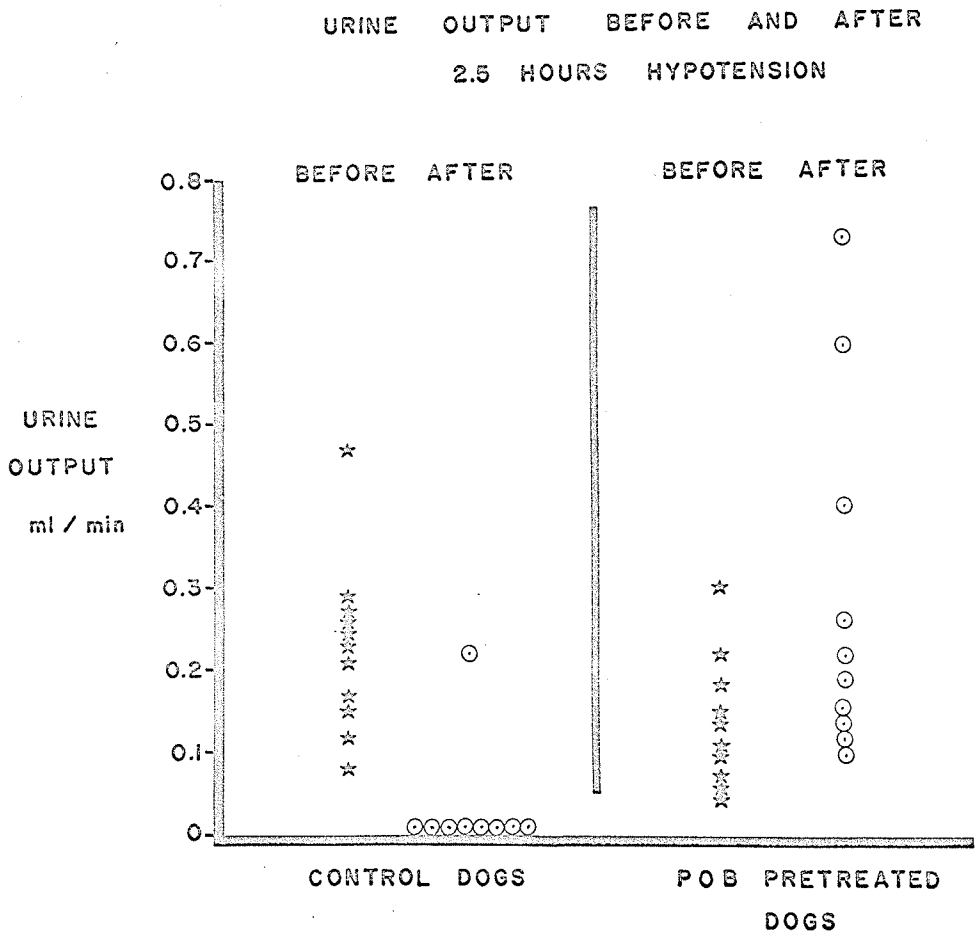


Figure 19. Urine Output of Control and POB-Pretreated Dogs before and after Hemorrhagic Hypotension. Arterial pressure reached at least 100 mm Hg in all dogs after reinfusion. Note anuria in 8 of 9 control dogs after reinfusion and diuresis in POB-pretreated animals.

of the controls. At no time during the period of maintained hypotension was the blood flow to the hindquarters in POB-pretreated dogs significantly greater than that of the control animals. As in the other vascular beds studied, there was no tendency for hindquarters blood flow to increase while the arterial pressure was maintained at 50 mm Hg. The response of blood flow to reinfusion was the same in POB-pretreated and control dogs.

The overall effect of POB pretreatment on the response to hemorrhage was entirely different in the hindquarters as compared to the mesenteric and renal vascular beds. Hindquarters blood flow was significantly greater (55 to 60%) in the pretreated group before hemorrhage, but this differential decreased during hemorrhage, to as little as 20.7%. In contrast, hemorrhage caused a progressive increase in the difference between pretreated and control dogs in the mesenteric and renal blood flows. This reached 142% and 116% greater flows in the mesenteric and renal beds, respectively, of pretreated animals late in the period of hemorrhagic hypotension.

Comment

The results reported in this section indicate important differences both in the responses of different vascular beds to hemorrhagic hypotension, and in the effects of POB pretreatment on these responses. The mesenteric vessels constricted severely early in hemorrhage, and the vasculature of the hindquarters responded only slightly less. In contrast, the renal vessels tended to "autoregulate" through the early period of hemorrhage, and renal blood flow fell less rapidly than did arterial pressure. During the period when arterial pressure was held constant, the

mesenteric and hindquarters blood flows were stable, whereas the renal flow fell progressively during the first hour or more of maintained hypotension. Another important difference was apparent during reinfusion, when the mesenteric and hindquarters blood flows were higher at every perfusion pressure than those recorded during hemorrhage, whereas renal blood flow was well below what it had been at comparable perfusion pressures during hemorrhage.

Phenoxybenzamine pretreatment increased blood flow in all of the vascular beds studied at some phase of the experimental procedure. However, the patterns and magnitudes of the increased flow varied considerably. During the control (prehemorrhage) period, POB pretreatment increased blood flow most in the mesenteric vascular bed, somewhat less in the vascular bed of the hindquarters, and to only a slight extent (not statistically significant) in the renal vascular bed. The difference between the blood flows of pretreated and control dogs increased in the mesenteric and renal vascular beds during the induction and maintenance of hypotension, but decreased in the hindquarters. The differences between control and POB-pretreated dogs in mesenteric and hindquarters blood flows did not change during the period of maintained hypotension, but the difference in renal blood flow increased progressively.

If one accepts changes in vascular resistance and the effects of POB blockade as indices of sympathetic nervous system activity, the above findings suggest that there is a characteristic, highly organized sequence of regional vasoconstriction induced by the sympathetic nervous system in response to hemorrhage. During the control period, the dogs were under moderate stress from the anesthesthetic and the surgery necessary for the direct measurement of blood flow. At this time, phenoxybenz-

amine increased blood flow to the vascular beds studied, in the order mesenteric > hindquarters > kidney, suggesting this same distribution of sympathetic vasoconstriction. Addition of the severe stress of hemorrhage increased the vasoconstriction in the three vascular beds in quantitatively the same order, as indicated by the pressure-flow relationships. Differences between the POB-pretreated and control animals increased in parallel, indicating that the vasoconstriction was due to increased sympathetic nervous system activity. After IBV was reached, mesenteric and hindquarters vascular resistance remained constant, perhaps at a physiological maximum, but renal resistance and the difference in renal blood flow between POB-pretreated and control dogs continued to increase during the period of maintained hypotension. These observations suggest that the renal vascular bed, which showed little evidence of sympathetic vasoconstrictor tone during the control period and the initial portion of the hemorrhage, was "called upon" to constrict only after other vascular beds had approached their maximal response. However, once constriction of the renal vessels began, it was progressive and very marked.

The present findings indicate that the sympathetic nervous system does not induce vasoconstriction in response to stress in an "all-or-none" manner, but displays a high degree of selectivity. This concept has been clearly suggested recently (Folkow et al., 1961), but has not yet achieved wide acceptance.

A finding of particular interest was the maintenance of the increased renal vascular resistance after reinfusion. This phenomenon is apparent in the data of some earlier investigators, e.g., in Fig. 5 of Phillips et al. (1946), and was pointed out in the clinical study of Lauson et al. (1944), but the mechanisms involved have not been investi-

gated. The maintained increase in vascular resistance appears to occur only in the kidney, being absent from the other areas included in the present study and in several additional vascular beds such as those of the brain and myocardium where there is little vasoconstrictor response to cardiovascular stress (Green, 1961; Gregg, 1962). Furthermore, POB pretreatment partially inhibited this renal vascular response and completely prevented the accompanying anuria. These observations are of particular significance because the kidney is known to be very susceptible to damage from cardiovascular stress, acute renal failure being a relatively common sequel of shock (Franklin and Merril, 1960). The production of a persistent increase in renal vascular resistance and the mechanisms involved were investigated further in the experiments reported in Sections XIII to XVI.

## Section XIII

## Relationship of Renal Vascular Response to Rate of Hemorrhage

It was noted in the preceding experiments that the renal vascular bed "autoregulated" in response to hemorrhage, i.e., maintained a blood flow greater than expected on the basis of the decreased perfusion pressure. Similar observations have been made in other quite recent studies (Sapirstein et al., 1960; Abel and Murphy, 1962). However, earlier investigations indicated that the fall in renal blood flow associated with hemorrhage was greater than the fall in perfusion pressure, i.e., that active renal vasoconstriction occurred (Selkurt, 1945; Phillips et al., 1946). These two groups of studies differed in that clearance

techniques were used to measure renal blood flow in the early experiments. Because this became impossible when urine output ceased, bleeding tended to be done slowly to prolong the period preceding anuria. In the more recent studies, methods for measuring renal blood flow which are independent of urine output have been used and, probably for this reason, the hemorrhage employed has tended to be much more rapid, e.g., to reduce the arterial pressure to 50 mm Hg in 30 minutes in the present investigation. The following experiments were undertaken to assess the effect of rate of hemorrhage on the renal vascular response as a possible explanation for the above discrepancy.

## Methods

Dogs were prepared for hemorrhage and for the measurement of renal blood flow, urine output, arterial pressure and bleeding volume as in previous experiments. However, the rate of bleeding was varied. The arterial pressure was reduced to 50 mm Hg over 90 minutes in 4 dogs, and 5 were bled even more slowly, to reduce the arterial pressure to between 70 and 100 mm Hg over a period of more than 3 hours. In the latter group, phenoxybenzamine (5 mg/kg) was infused intravenously at the end of the hemorrhage, blood being reinfused slowly as required to prevent a precipitous fall in arterial pressure.

## Results

The results of a typical experiment involving hemorrhage over a 90-minute period are shown in Fig. 20. In all of 4 dogs subjected to this procedure, the autoregulation characteristically seen during a 30-minute hemorrhage was either much less marked, or absent, renal blood flow falling progressively as the perfusion pressure decreased. Blood

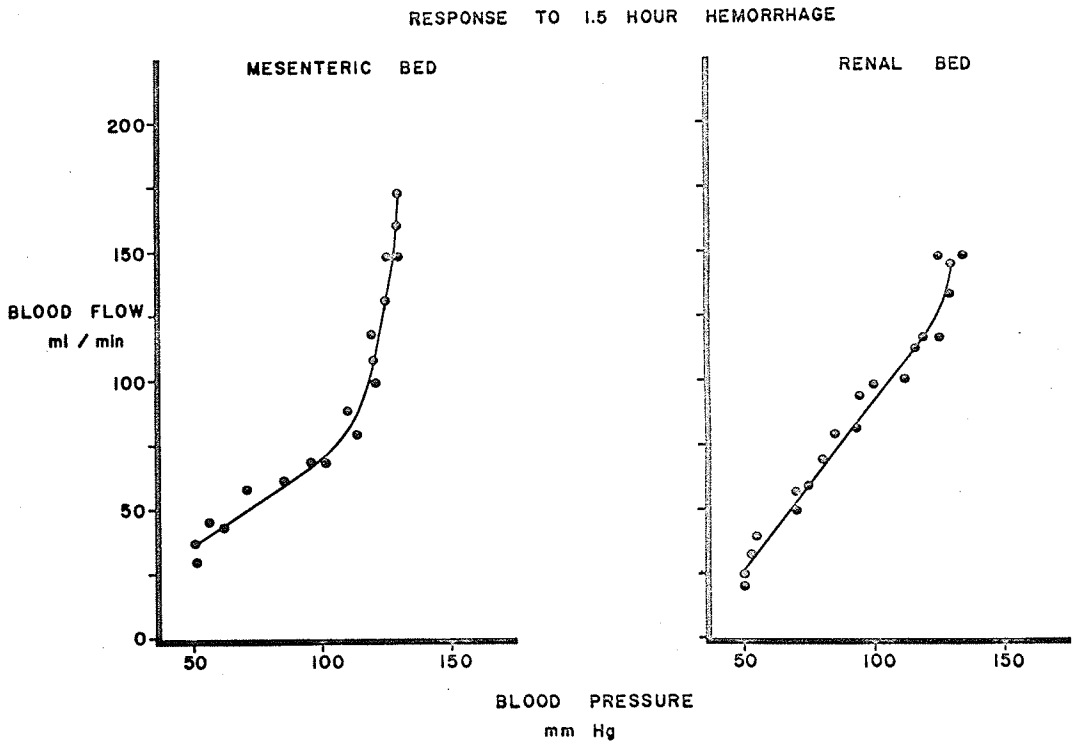


Figure 20. Responses of Mesenteric and Renal Blood Flows to Moderately Slow Hemorrhage. Arterial pressure reduced to 50 mm Hg over 90 minutes. Most of the "autoregulation" of the renal vascular bed in response to a 30-minute hemorrhage (Fig. 18) was eliminated by the slower bleeding, but the response of the mesenteric bed was changed very little.

flow in the mesenteric and hindquarters vascular beds fell precipitously prior to a major decrease in perfusion pressure, as in experiments involving more rapid hemorrhage.

In all of the 5 dogs bled even more slowly over a 3-hour period, renal blood flow decreased markedly and continuously despite relatively well maintained perfusion pressure (Fig. 21), much like the responses of the mesenteric and hindquarters vasculatures to more rapid hemorrhage. In association with the reduced blood flow, urine output fell progressively, and in most experiments ceased before the perfusion pressure fell below 90 mm Hg. After anuria had developed, the administration of phenoxybenzamine increased renal blood flow and caused resumption of urine production despite a further decrease in perfusion pressure during the drug administration in many of the animals. Rate of hemorrhage was plotted against change in renal vascular resistance for intervals of hemorrhage during which a volume of blood greater than 8 ml/kg was removed (Fig. 22). The correlation between decreased rate of hemorrhage and increased resistance change is obvious. It is apparent from these results that one of the determinants of renal blood flow during hemorrhage was the rate of bleeding. The effects of phenoxybenzamine suggest that not only was the increased resistance due to vasoconstriction mediated by the sympathetic nervous system, but that it was sufficient to induce anuria or oliguria despite perfusion pressures generally considered adequate to maintain glomerular filtration.

The demonstrated correlation between rate of hemorrhage and renal vasoconstriction provides an explanation for the discrepancies in previous reports on the relationship of perfusion pressure to renal blood flow during early hemorrhage. No adequate explanation for the effect of rate of hemor-

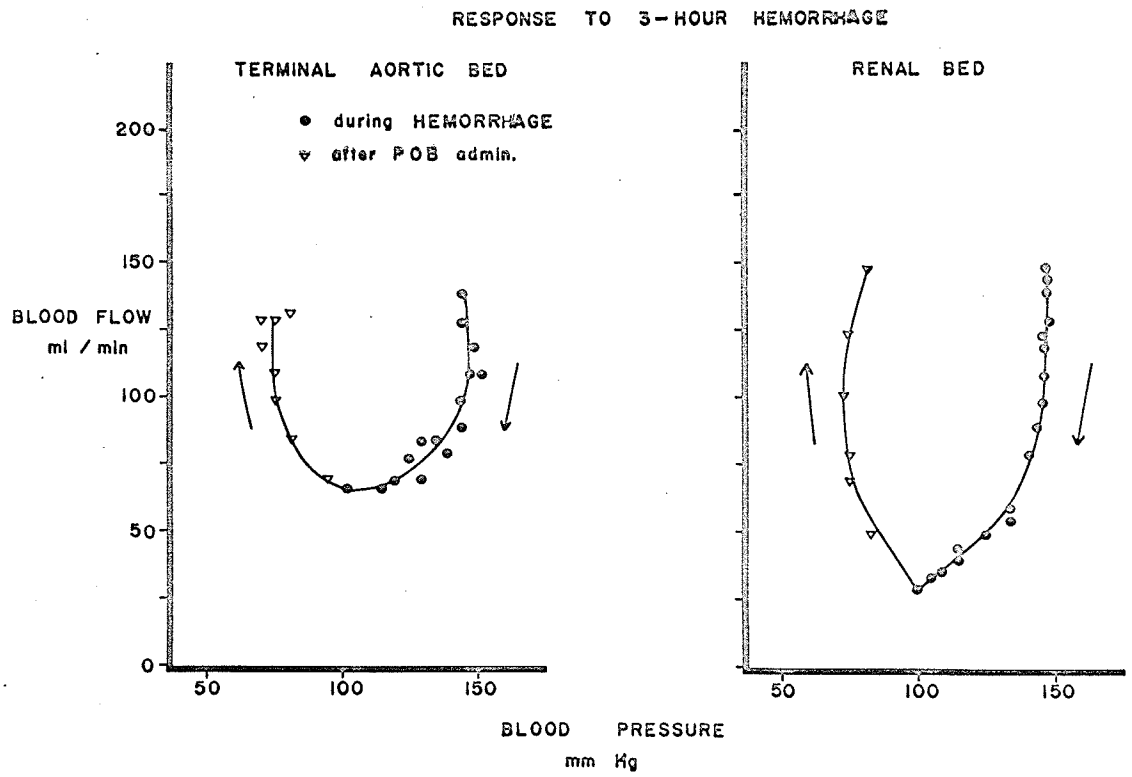


Figure 21. Responses of Terminal Aortic and Renal Blood Flows to Very Slow Hemorrhage. Arterial pressure reduced only to 100 mm Hg over 3 hours. Response of renal vascular bed is similar to that shown by mesenteric and aortic beds at all rates of hemorrhage. Administration of phenoxybenzamine caused a marked increase in flow in both beds and a diuresis despite a considerable further reduction in pressure. Reinfusion of blood was started 20 minutes after phenoxybenzamine (POB) to prevent arterial pressure from falling below 75 mm Hg.

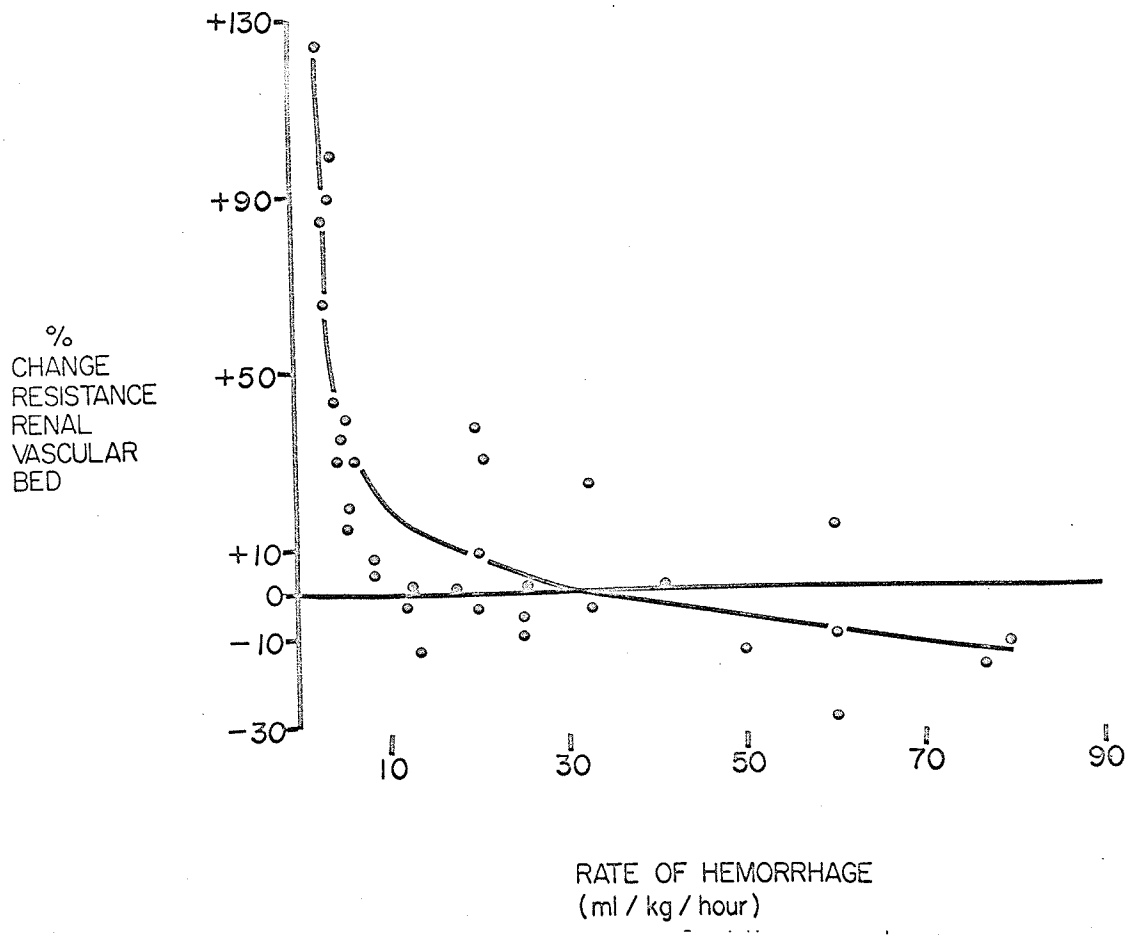


Figure 22. Relationship of Rate of Hemorrhage to Change in Renal Vascular Resistance. Note the marked increase in vascular resistance induced by very slow hemorrhage.

rhage is available, but it is apparent that if the arterial pressure falls slowly enough, sympathetic nervous system activity is capable of overcoming the renal autoregulation.

## Section XIV

## Relationship of Duration of Hypotension to the Maintained Increase in Renal Vascular Resistance

Renal blood flow increased very little when the arterial pressure was increased by reinfusion of the shed blood after a prolonged period of hemorrhagic hypotension, although reinfusion effectively dilatated the vascular beds of the hindquarters and intestine. Since the persistent decrease in renal blood flow was associated with anuria despite perfusion pressures adequate for glomerular filtration (Coelho and Bradley, 1964), this observation was of considerable interest. A similar oliguria or anuria, referred to as acute renal failure, is a relatively common sequel to a period of hemodynamic collapse in man (Lauson et al., 1944), which has frequently been attributed to decreased renal blood flow. The reason for a persistent decrease in flow, despite recovery of most cardiovascular functions is obscure. However, the incidence of acute renal failure following hemodynamic collapse in man appears to increase with the duration of the preceding interval of shock (Nickerson et al., unpublished observations). The following experiments were undertaken to determine the relationship of duration of hypotension to the resultant maintained increase in renal vascular resistance.

### Methods

Twenty-seven dogs were bled over 30 minutes to an arterial

pressure of 50 mm Hg, and all of the blood in the reservoir was reinfused after 1.0 minute or 1.0, 2.5, or 4.5 hours at this pressure; 14 of these dogs were pretreated with phenoxybenzamine (5 mg/kg) given intravenously 18 hours before the experiment. Renal blood flow and arterial blood pressure were measured continuously during the period of hypotension and for at least one hour after reinfusion. The dogs maintained at 50 mm Hg for 2.5 and 4.5 hours are the same as those described in Section X.

An index of maintained renal vasoconstriction was used to facilitate comparison of various animals and groups. This was the renal blood flow at an arterial pressure of 100 mm Hg during reinfusion, expressed as a percentage of the flow at the same perfusion pressure during the initial hemorrhage. An index of 100 indicates identical flows at the two points; an index of less than 100, that the flow was lower during reinfusion than during hemorrhage, etc.

Results

Data from this series of experiments are shown in Fig. 23. After 1.0 minute of hypotension, 5 of 6 control dogs had an index greater than 100 (mean 132), indicating that renal vascular resistance was lower during reinfusion than it had been during hemorrhage. After 1.0 hour of hypotension, 4 of 5 dogs had increased renal vascular resistances at reinfusion, and the mean index fell to 86. Further prolongation of the period of hypotension caused a further, progressive decrease in the index, to means of 62 and 42 after 2.5 and 4.5 hours, respectively.

Phenoxybenzamine did not change the renal vascular responses to the shorter periods of hypotension, but the indices of POB-pretreated dogs were significantly higher than those of the controls after prolonged

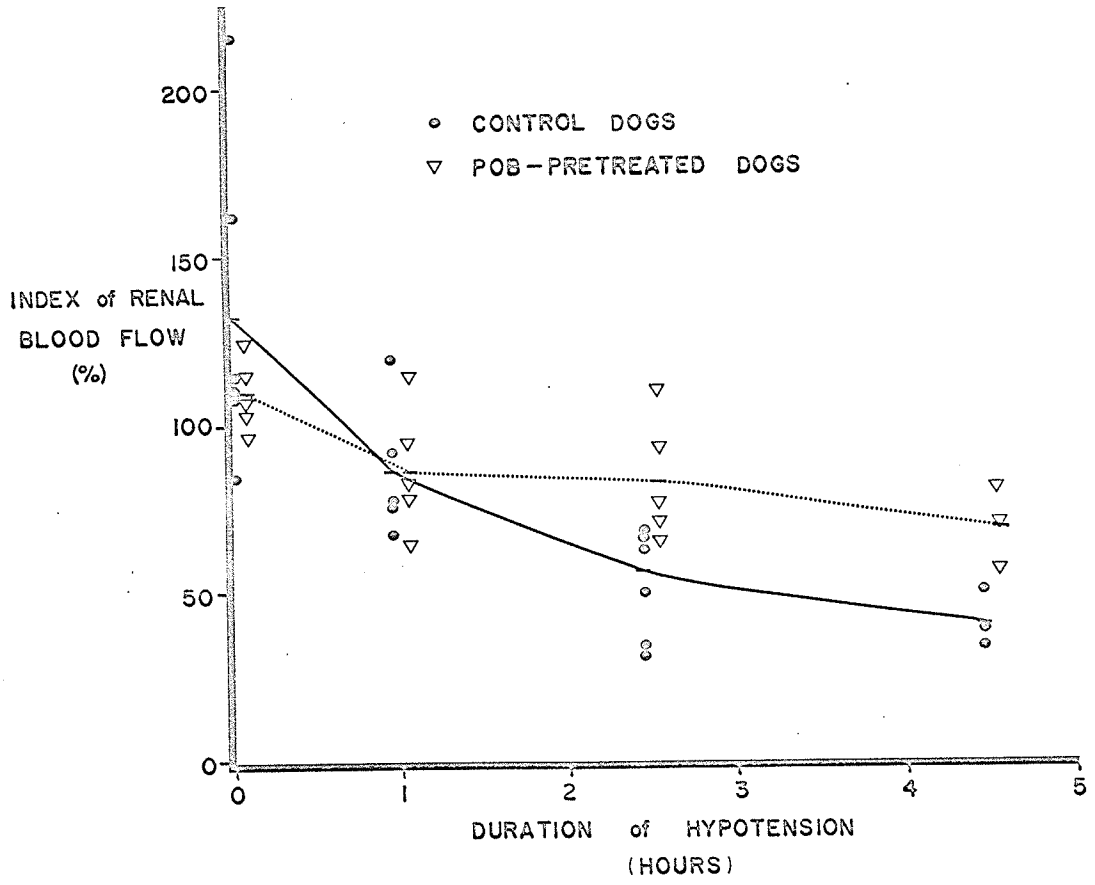


Figure 23. Relationship of Duration of Hemorrhagic Hypotension to Degree of Maintained Renal Vasoconstriction. The index of renal blood flow is the blood flow at an arterial pressure of 100 mm Hg during reinfusion divided by the blood flow at the same arterial pressure during the initial hemorrhage. Note the increase in renal vascular resistance and in the difference between POB-pretreated and control animals as the period of hypotension is prolonged.

hypotension ($P < 0.05$ by Chi-squared analysis).

Comment

It is apparent from the above experiments that the development of maintained renal vasoconstriction was dependent on relatively prolonged hypotension. After periods of 2.5 hours or more, the persistent vasoconstriction was associated with complete anuria. Pretreatment with phenoxybenzamine partially prevented the maintained renal vasoconstriction and completely prevented the anuria. This indicates that the sympathetic nervous system is involved in the genesis of both, either through local release of noradrenaline at efferent nerve endings, or via circulating catecholamines.

## Section XV

## Mechanism of Maintained Renal Vasoconstriction after Prolonged Hypotension

Current knowledge of sympathetic nervous system function provides no obvious basis for a persistence of vasoconstriction after reinfusion and the associated elevation of arterial pressure. However, a mechanism by which effects of sympathetic activity might persist after reinfusion is through an increase in renal tissue pressure. Since the kidney has an inelastic capsule, edema of the renal parenchyma could increase tissue pressure, and thus passively increase renal vascular resistance and reduce blood flow. This has been suggested as a possible mechanism in the induction of acute renal failure in man (Franklin and Merril, 1960). In this series of experiments, an attempt was made to determine the mechanism responsible for the maintained increase in renal

vascular resistance.

Methods

Ten dogs were bled to and maintained at an arterial pressure of 50 mm Hg. In addition to the measurement of renal blood flow and perfusion pressure, renal tissue pressure was continuously monitored by means of a 21-gauge needle inserted about 3/4 inch into the renal parenchyma and coupled to a Statham P 23BB pressure transducer. Zero pressure was taken at the level of the renal hilum. Patency of the system was checked at intervals by injecting 0.01 to 0.02 ml of 0.9% NaCl into the system and monitoring the resulting pressure transient.

The activity of the sympathetic nerves to the renal vasculature, after the period of hypotension and reinfusion, was assessed in 8 dogs. In 4, the kidney was denervated by sectioning the nerves in the renal pedicle between ligatures, and infiltrating 1.0% procaine solution into the area, care being taken to avoid intravascular injection. In another 4 dogs, guanethidine (10.0 mg/injection) was administered intra-arterially until additional doses had no further effect on renal blood flow. Phenoxybenzamine (5 mg) was then given intra-arterially. In the latter dogs, urine output, as well as renal blood flow, was measured.

In an additional series of 7 dogs, an attempt was made to determine the duration of the maintained vasoconstriction by denervating the kidney at various intervals after reinfusion. However, these dogs were in severe hemodynamic decompensation and tolerated the manipulations poorly. Their arterial pressures fell rapidly, and most of them died too soon to allow assessment of the effects of denervation. Only one dog had an arterial pressure high enough to allow renal denervation to

be carried out one hour after reinfusion, and its effects assessed.

Results

The relationship between renal tissue pressure and renal vascular resistance is shown in Table XIV and Fig. 24. Tissue pressure fell during hemorrhage, and rose again at reinfusion, but there was no tendency for the renal tissue pressure to increase to above the control level during the period of maintained renal vasoconstriction. As shown in Fig. 24, renal vascular resistance tended to remain high despite tissue pressures below the control values, and lower than those recorded at comparable arterial pressures during hemorrhage. Tissue pressure was greater during reinfusion than at the same arterial pressures during hemorrhage in only one of the 10 dogs, and this increase was only 0.5 mm Hg.

The effects of denervation of the kidney were qualitatively similar in all experiments. The denervation was carried out after 2.0 to 2.5 hours of hypotension, while the dogs were still at an arterial pressure of 50 mm Hg, and resulted in an immediate large increase in renal blood flow. The flow increased further during reinfusion, reaching approximately 90% of the control level in 3 of the 4 dogs, and actually exceeding the control value in the experiment shown in Fig. 25.

The response of renal blood flow to guanethidine, given after reinfusion and demonstration that maintained vasoconstriction was present, had two characteristic components. In every case, the initial dose produced a transient decrease in blood flow, followed by a progressive increase. In one of 4 dogs, intra-arterial guanethidine, alone, raised renal blood flow to the control level (Fig. 26). In the other 3, guanethidine administration increased flow to 85% or more of the control value.

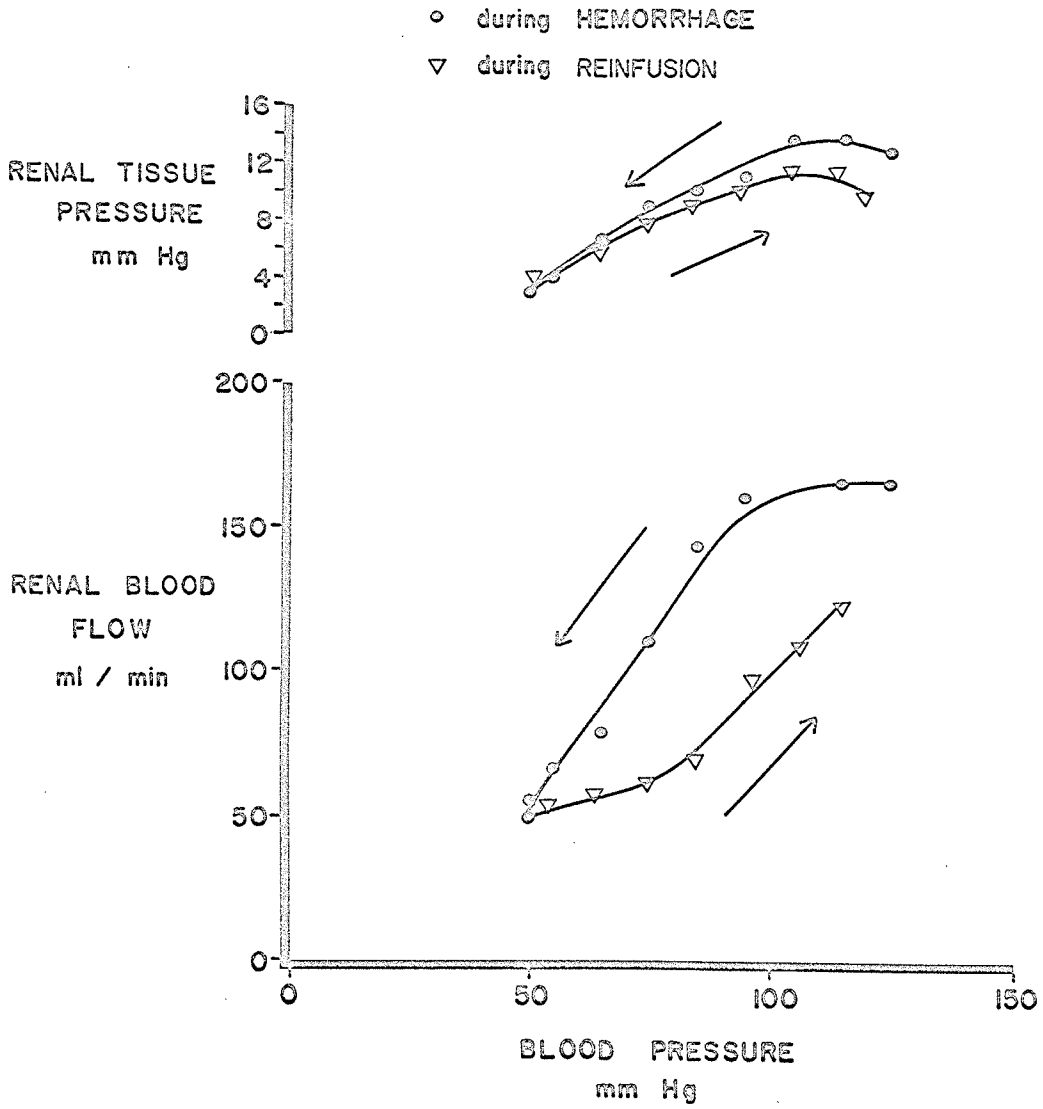


Figure 24. Relationship of Renal Tissue Pressure to Renal Blood Flow during Hemorrhage and Reinfusion. Renal tissue pressure was lower during reinfusion than during hemorrhage at all arterial pressures.

TABLE XIV

Changes in Renal Tissue Pressures and Blood Flows after Hemorrhagic Hypotension

| Treatment | Time at 50 mm Hg (hours) | Prehemorrhage | | | At 100 mm Hg Arterial Pressure | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | During Hemorrhage | | During Reinfusion | |
| | | Arterial Pressure (mm Hg) | Renal Blood Flow (ml/min) | Tissue Pressure (mm Hg) | Renal Blood Flow (ml/min) | Tissue Pressure (mm Hg) | Renal Blood Flow (ml/min) | Tissue Pressure (mm Hg) |
| Control | 2.5 | 115 | 95 | 6.5 | 125 | 5.0 | 70 | 4.0 |
| Control | 1.6 | 100 | 130 | 19.0 | 130 | 19.0 | 80 | 15.0 |
| Control | 1.5 | 135 | 150 | 27.0 | 190 | 18.0 | 105 | 5.0 |
| POB | 1.5 | 133 | 203 | 14.5 | 165 | 9.0 | 125 | 7.5 |
| POB | 1.0 | 132 | 160 | 24.0 | 150 | 16.0 | 115 | 15.0 |
| POB | 1.0 | 105 | 175 | 24.0 | 180 | 22.5 | 165 | 20.0 |
| POB | 1.0 | 150 | 180 | 21.0 | 160 | 14.0 | 160 | 14.0 |
| POB | 1.0 | 135 | 75 | 15.5 | 70 | 11.0 | 55 | 11.5 |
| Control | 1.0 | 125 | 180 | 12.0 | 175 | 8.5 | 165 | 6.5 |
| POB | 1.0 | 130 | 170 | 16.0 | 165 | 13.0 | 145 | 11.0 |

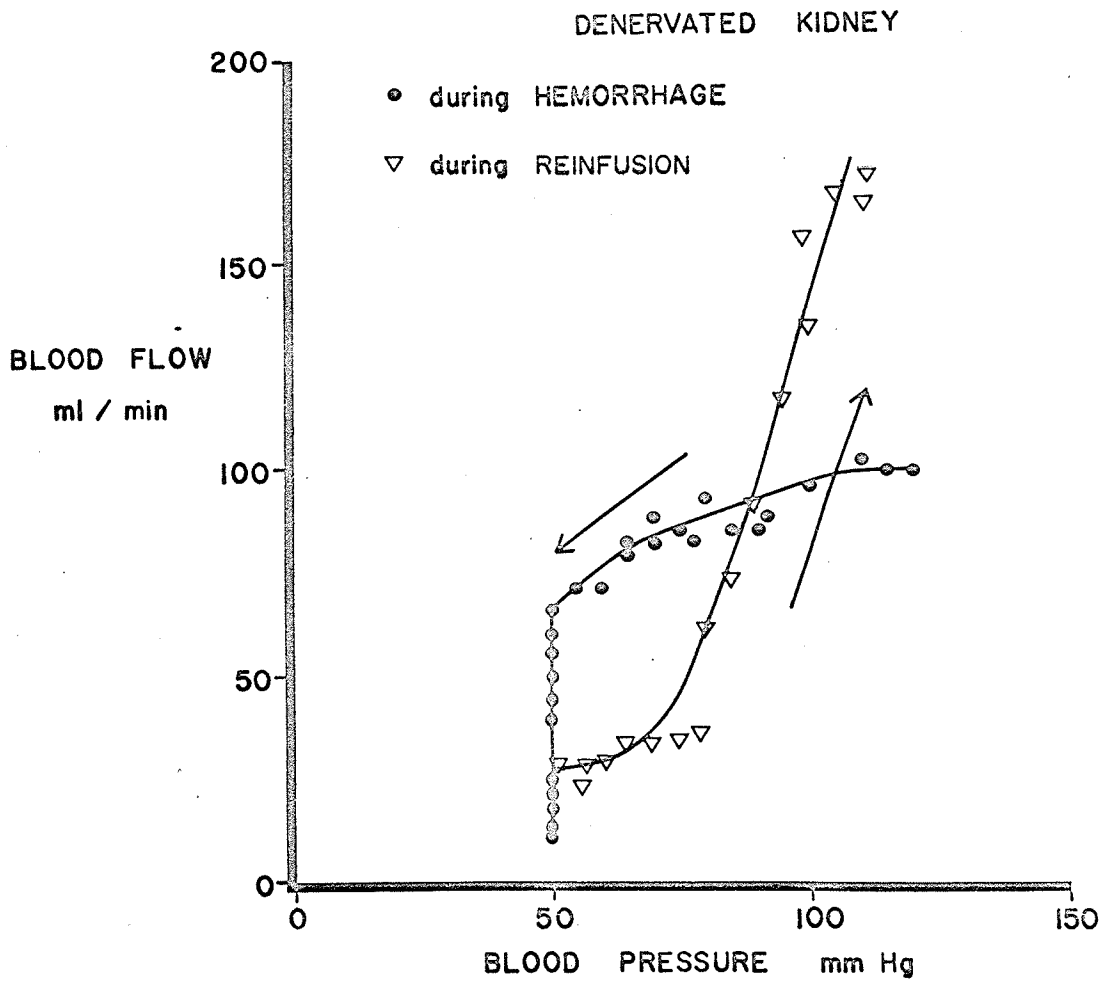


Figure 25. Effects of Renal Pedicle Denervation on Renal Blood Flow during Hemorrhagic Hypotension and Reinfusion. Denervation was done after 2.5 hours at an arterial pressure of 50 mm Hg. Blood flow increased immediately and continued to rise during reinfusion without evidence of the sustained vasoconstriction which would otherwise have been expected.

Subsequent intra-arterial administration of phenoxybenzamine resulted in blood flows equal to the control values, i.e., complete abolition of the maintained renal vasoconstriction. In the 3 dogs in which urine output was measured, abolition of the maintained vasoconstriction was associated with resumption of urine output (Fig. 27).

The response of the one dog, in which it was possible to denervate the renal pedicle one hour after reinfusion, was very similar to the responses to guanethidine and POB (Fig. 28).

Comment

The results of these experiments indicate that it is highly unlikely that renal interstitial edema plays any role in the genesis of the persistent decrease in renal blood flow seen immediately following prolonged hypotension. It appears probable that the decreased blood flow is largely due to vasoconstriction produced by sympathetic nervous system activity, which persists despite reinfusion and the associated increase in arterial pressure. Both denervation and the administration of guanethidine, an agent which interferes with the effects of sympathetic nerve activity, but not those of circulating catecholamines, resulted in complete or almost complete abolition of the maintained vasoconstriction.

## Section XVI

## Hemodynamic Effects of Prolonged Infusion of Noradrenaline in the Dog

Since prevention of maintained vasoconstriction by phenoxybenzamine pretreatment prevented oliguria and abolition of the maintained vaso-

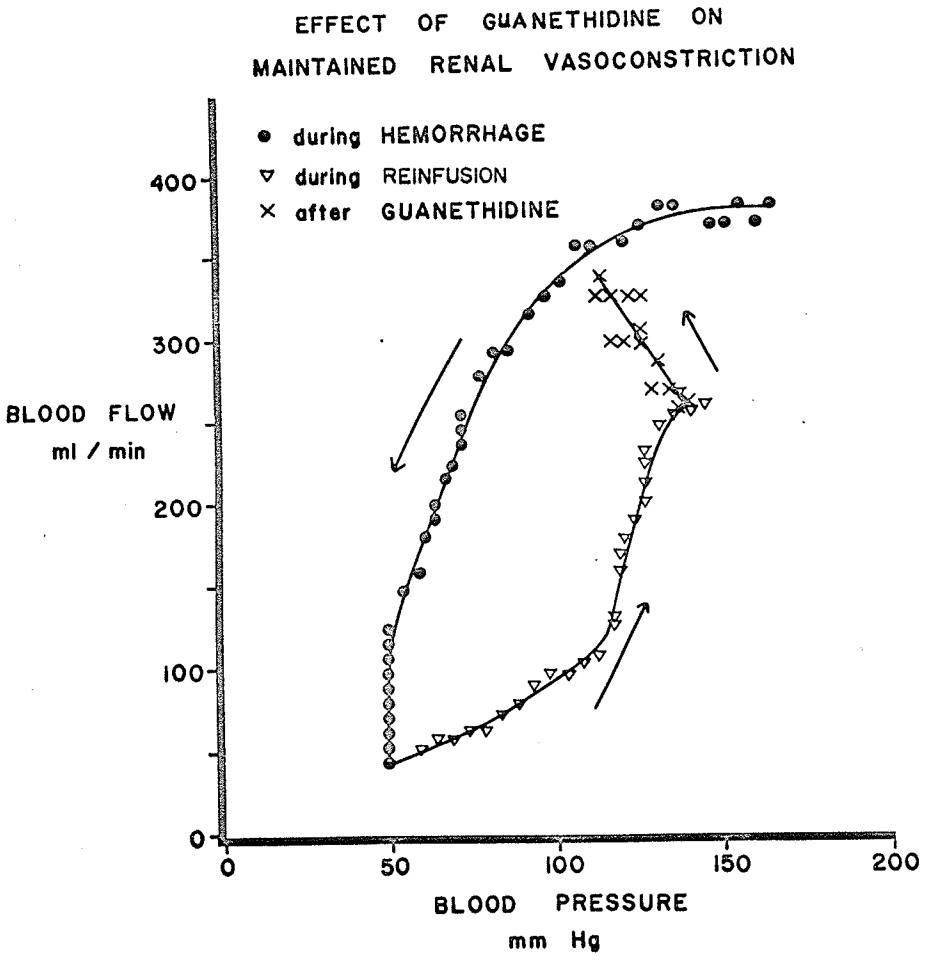


Figure 26. Effect of Guanethidine on Maintained Renal Vasoconstriction. The guanethidine was administered intra-arterially following the reinfusion of all shed blood after 2.5 hours of hemorrhagic hypotension. Abolition of the maintained renal vasoconstriction was associated with resumption of urine formation.

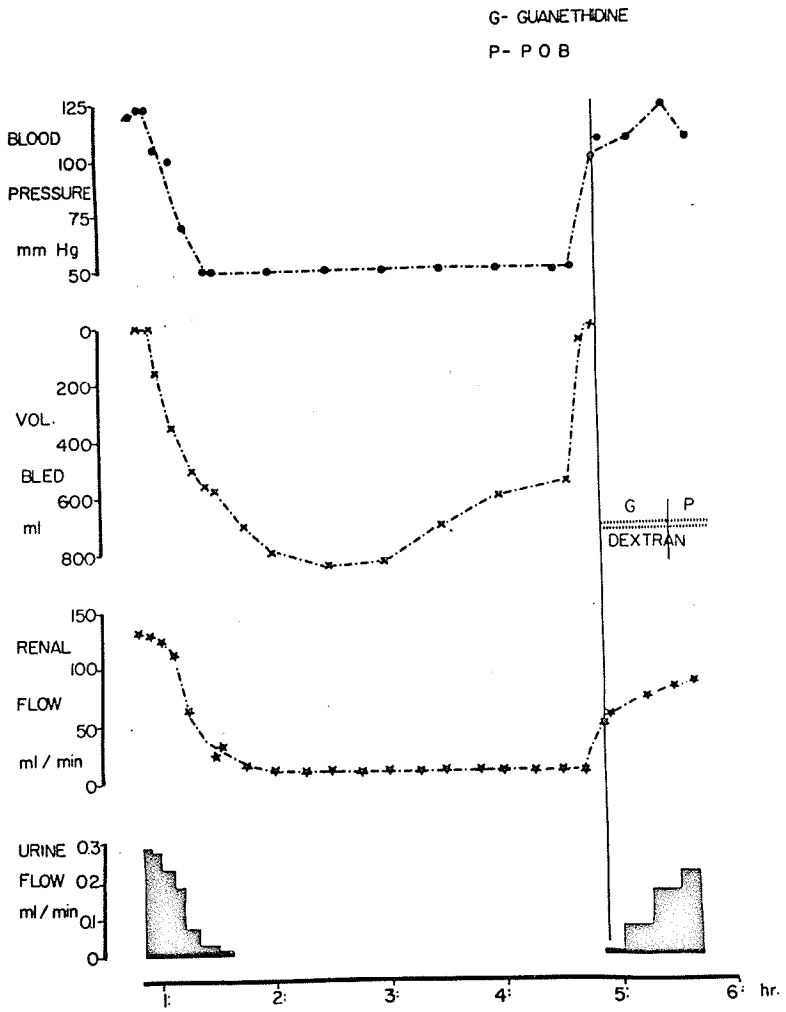


Figure 27. Effects of Guanethidine and Phenoxybenzamine on Renal Blood Flow and Urine Formation. The drugs were administered intra-arterially following the reinfusion of all shed blood after 4.5 hours of hemorrhagic hypotension. A small amount of 6% dextran in saline was given intravenously at the same time to prevent a marked fall in blood pressure.

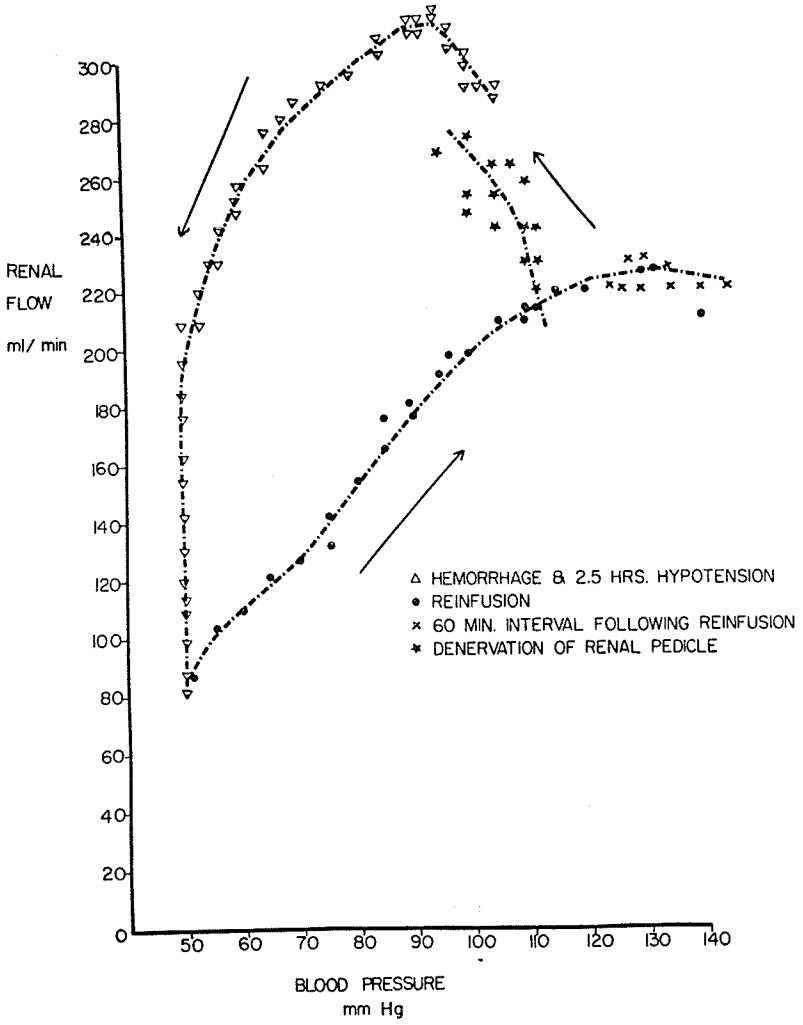


Figure 28. Effect of Renal Pedicle Denervation One Hour after Reinfusion on Renal Blood Flow. Note the prompt increase in flow despite a considerable fall in arterial pressure due to the manipulations incident to the denervation.

constriction after reinfusion restored urine output, it seemed likely that the anuria following prolonged hypotension in these dogs was due to the persistence of vasoconstriction induced by noradrenaline released at sympathetic nerve endings in renal vascular smooth muscle. However, these observations do not distinguish between an unusual sensitivity of the renal vessels and a persistent sympathetic discharge to explain the unique behavior of this vascular bed. The following series of experiments was performed to assess the contributions of these two factors to the maintained renal vasoconstriction.

Methods

Ten dogs were prepared for the measurement of arterial and central venous pressures, blood flows to the renal, mesenteric and hindquarters vascular beds and urine output. Arterial blood samples were taken serially for the determination of plasma protein concentration. After these parameters had been stable for at least 30 minutes, an infusion of noradrenaline (2.0 to 3.0 μg/kg/min, calculated as the base) was begun. The infusion was continued for 3 hours, and the dogs were studied for at least an hour after the end of the infusion. Three of the dogs then received an infusion of dextran sufficient to raise the arterial blood pressure to control levels, and renal blood flow and urine output were monitored for an additional period.

Results

The cardiovascular effects of a 3-hour infusion of noradrenaline are shown in Fig. 29. The arterial pressure rose initially in all of the dogs, reached a maximum within 5 minutes, and then began to fall progress-

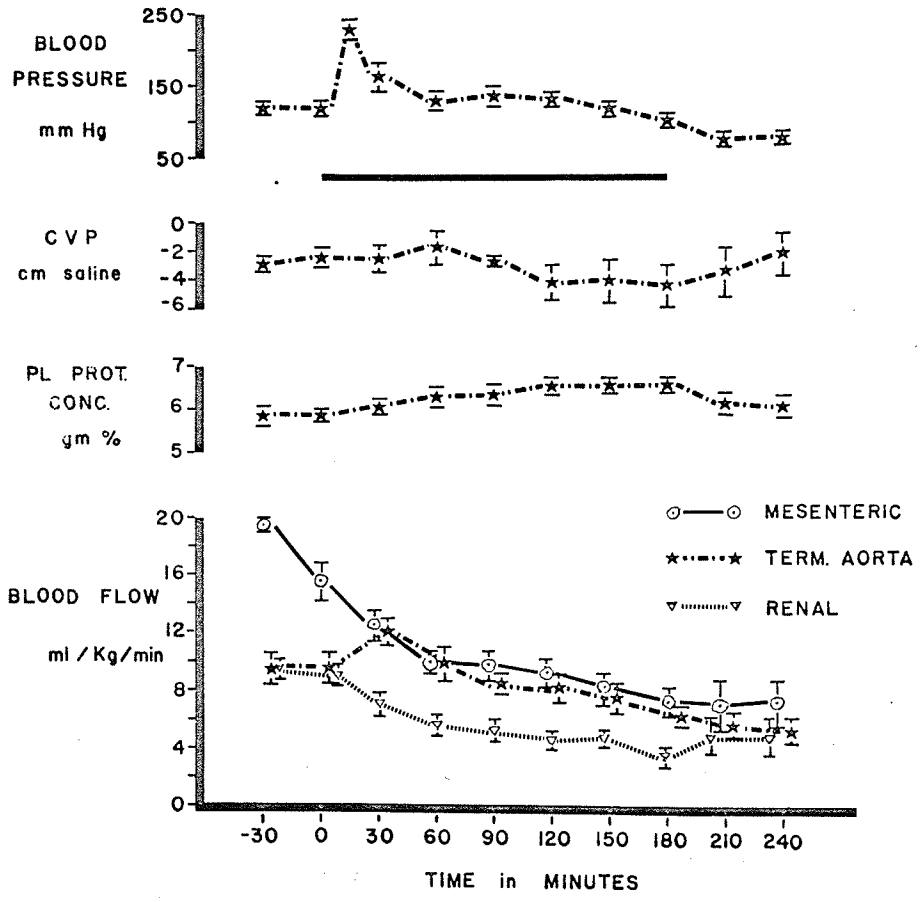


Figure 29. Cardiovascular Effects of Noradrenaline Infusion. Changes in arterial pressure, central venous pressure, plasma protein concentration and blood flow in the superior mesenteric and renal arteries and the terminal aorta during the infusion of noradrenaline (2.0 μg/kg/min) for 3 hours are shown. Period of infusion is indicated by the solid bar under the arterial pressure curve. Vertical lines indicate S.E. of each point.

ively. It generally reached the control level after 2 to 2.5 hours and was well below the control value by the end of the infusion. When the infusion was stopped, the arterial pressure fell precipitously, and did not rise significantly during the following hour. Central venous pressure tended to increase initially, but then fell progressively until the end of the infusion. Plasma protein concentration increased progressively, or rose rapidly to a maximum early in the course of the infusion and then stayed at this level. It often decreased again in association with a fall in arterial pressure, usually after the end of the infusion. After an initial increase in the hindquarters, blood flow fell progressively in all of the areas in which it was measured. Calculated vascular resistance increased in all 3 of the vascular beds studied (Fig. 30). Renal and mesenteric resistances initially increased very rapidly and continued to rise throughout the infusion. The resistance of the hindquarters vasculature did not show the early precipitous increase, but tended to rise progressively throughout the infusion. After cessation of the infusion, the resistances of the renal and hindquarters vascular beds fell rapidly to within 20% of the control values. The mesenteric vascular resistance also fell, but remained 60 to 70% above the control level.

In association with the increase in renal vascular resistance, urine output fell rapidly in every case, and ceased after a variable interval. It did not resume after the infusion of noradrenaline was terminated and the renal vascular resistance fell. However, arterial blood pressure at this time usually was below the level necessary for effective glomerular filtration, i.e., less than 70 mm Hg.

The relationship between arterial pressure and renal blood flow in a typical experiment is shown in Fig. 31, starting with the highest arterial

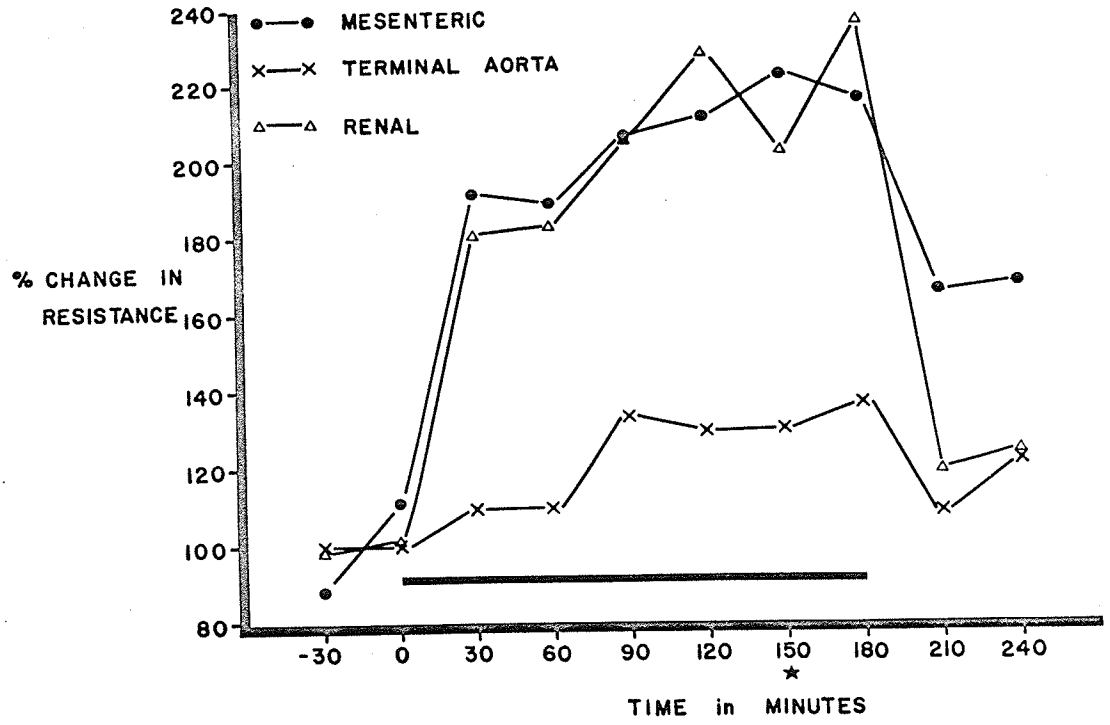


Figure 30. Changes in Vascular Resistance during Noradrenaline Infusion. Calculated resistances of the superior mesenteric, hindquarters (terminal aorta) and renal vascular beds are shown during 3-hour infusion at a rate of 3.0µg/kg/min. Arterial pressure had returned to the preinfusion level at 150 minutes. Note particularly the close correspondence of changes in the renal and mesenteric beds and the absence of maintained renal vasoconstriction after the infusion. This contrasts sharply with the characteristics of the renal vasoconstriction induced by hemorrhagic hypotension (Fig. 18).

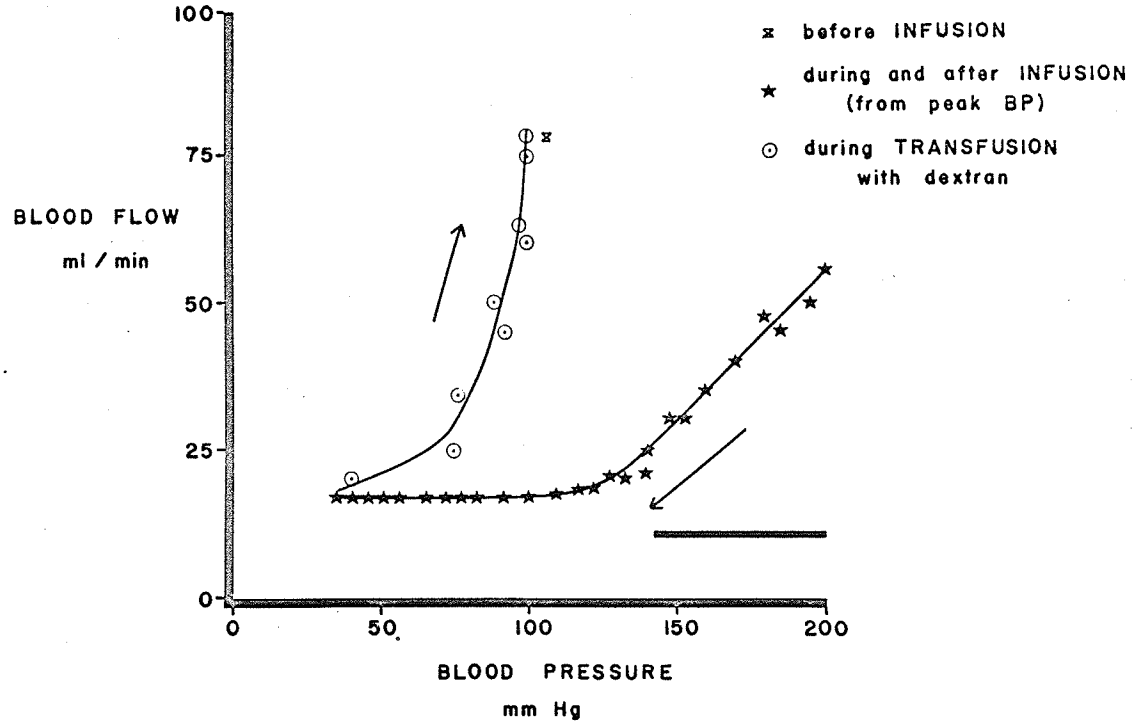


Figure 31. Relationship of Renal Blood Flow to Arterial Blood Pressure during and after Noradrenaline Infusion. Both parameters were low after termination of a 3-hour infusion (3.0 µg/kg/min), probably due to decreased intravascular volume. Infusion of 6% dextran in saline restored both pressure and renal blood flow to control levels, indicating the absence of maintained renal vasoconstriction.

pressure attained in this animal. The administration of dextran after completion of the noradrenaline infusion, increased arterial pressures and renal blood flows and caused resumption of urine output in all of 3 dogs to which this material was given.

Comment

After an initial increase, arterial pressure fell progressively during noradrenaline infusion despite increasing resistance in all of the 3 major vascular beds studied. This strongly suggests that the fall in arterial pressure was due to decreased cardiac output. Although a decreased resistance in other vascular beds could conceivably account for the falling arterial pressure, the areas studied represent about 70% of the total cardiac output, and are those in which the greatest change of peripheral resistance would be expected to occur. Central venous pressure increased initially, probably the result of a central shift of blood from peripheral venous reservoirs and the spleen, but then began to fall progressively in association with the development of hemoconcentration. It has previously been demonstrated that hemoconcentration during a noradrenaline infusion is associated with a progressive fall of plasma volume (Sutter, 1963).

In contrast to the response to hemorrhage, renal vascular resistance increased as rapidly and markedly as did mesenteric resistance during noradrenaline infusion. Also, resistance in all 3 vascular beds tended to increase progressively, whereas during hemorrhagic hypotension, the mesenteric and hindquarters beds reached an early maximum and then plateaued. After cessation of the noradrenaline infusion, the renal vascular resistance fell to near control levels, again in contrast to the

response to hemorrhage. The increased renal blood flow and the resumption of urine output induced by infusion of dextran further demonstrated that the persistent increase in renal vascular resistance seen following prolonged hemorrhagic hypotension, was not present after a prolonged noradrenaline infusion. These findings support the suggestion made above that the pattern of vasoconstriction seen during and following a period of hemorrhagic hypotension was not due to local peculiarities in the response to noradrenaline, but rather, to unequal release of the transmitter at different sites in response to different levels of activity of various segments of the sympathetic nervous system.

## Section XVII

### Effects of Graded Doses of Phenoxybenzamine on Bleeding Volume and Regional Blood Flows during Hypotension

There is relatively little published information on the dose of phenoxybenzamine required to achieve complete blockade of vascular responses to sympathetic nervous system activity. Pretreatment of dogs with phenoxybenzamine (5 mg/kg given 18 hours before each experiment) decreased the rate of progression of cardiovascular decompensation during hemorrhagic hypotension, and partially prevented the maintained renal vasoconstriction following reinfusion, but abolished neither. It was important to know whether these partial effects were associated with only partial adrenergic blockade. Consequently, the following studies were undertaken to determine the phenoxybenzamine dose-response curves for several cardiovascular parameters in the dog.

Methods

Ten dogs were bled at a constant rate to an arterial pressure of 50 mm Hg, and the shed blood reinfused immediately. This procedure was repeated serially. After the first hemorrhage and reinfusion in each dog, phenoxybenzamine in a dose of 1.0 mg/kg was given intravenously, and the bleeding repeated. Subsequent reinfusions were followed by cumulative doses of 5.0, 10.0, 15.0 and 20.0 mg/kg. A period of at least one hour was allowed between each dose of phenoxybenzamine and the following hemorrhage. No attempt was made to prevent a fall in arterial pressure during the administration of the blocking agent in these experiments, but in no case did the resting arterial pressure fall below 85 mm Hg. Bleeding volumes were noted at frequent intervals during each hemorrhage, and arterial pressure was recorded continuously.

In another series of 6 dogs, superior mesenteric, renal and hindquarters blood flows and arterial pressure were recorded, as previously. Serial hemorrhages and phenoxybenzamine administrations were carried out as described above, except that the total dose of phenoxybenzamine never exceeded 10.0 mg/kg. This limitation was necessary because of the extensive surgery required to implant the 3 flow probes. After higher doses of phenoxybenzamine, continuous oozing of blood from the operative sites made it difficult to maintain a satisfactory basal arterial pressure.

Results

The IBV before adrenergic blockade was 36.2 ± 1.9 ml/kg, a value similar to those recorded in previous groups of experiments.

The IBV did not change appreciably with successive hemorrhages in two dogs bled serially without the administration of phenoxybenzamine. In the group treated with serial doses of phenoxybenzamine, the IBV fell progressively with increasing doses to 10.0 mg/kg; higher doses did not further affect the bleeding volume. This dose-response curve is shown in Fig. 32.

The relationship between the cumulative dose of phenoxybenzamine and blood flows in the 3 vascular beds at 50 mm Hg in a typical experiment is shown in Fig. 33. As in all of the dogs studied, the blood flows at 50 mm Hg in this animal increased progressively with increasing doses of phenoxybenzamine up to 10.0 mg/kg, the largest tested.

Comment

The volume of blood removed to reduce arterial pressure to 50 mm Hg is an index which probably combines many effects of the sympathetic nervous system, including constriction of arterioles and postcapillary capacitance vessels and intravascular fluid gain during the hemorrhage. The blood flow at 50 mm Hg offers an index of arteriolar resistance in the presence of the increased sympathetic nervous system activity induced reflexly by the reduced blood pressure. It is apparent from this series of experiments that phenoxybenzamine, in a dose of 5 mg/kg given 18 hours before an experiment, would not completely block the cardiovascular effects of the sympathetic nervous system activity induced by hemorrhage. These results suggest that failure of phenoxybenzamine to abolish completely the maintained renal vasoconstriction and the cardiovascular decompensation induced by hemorrhage could have been due to incomplete blockade of the sympathetic nervous system. Further studies would have to be carried out with higher doses of the blocking agent

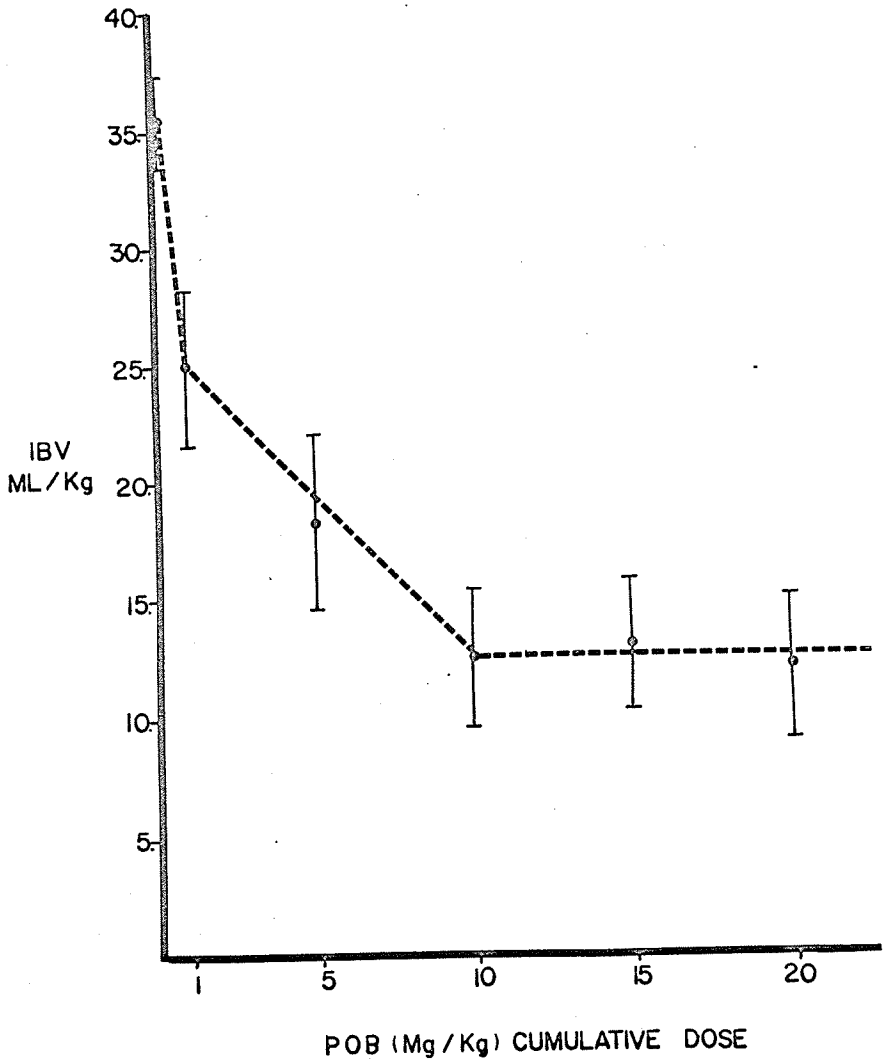


Figure 32. Dose-Response Curve for Effect of Phenoxybenzamine on IBV during Relatively Rapid Hemorrhage. Dogs were bled at a constant rate to a pressure of 50 mm Hg and the blood immediately reinfused. Vertical lines indicate S.E. of each point.

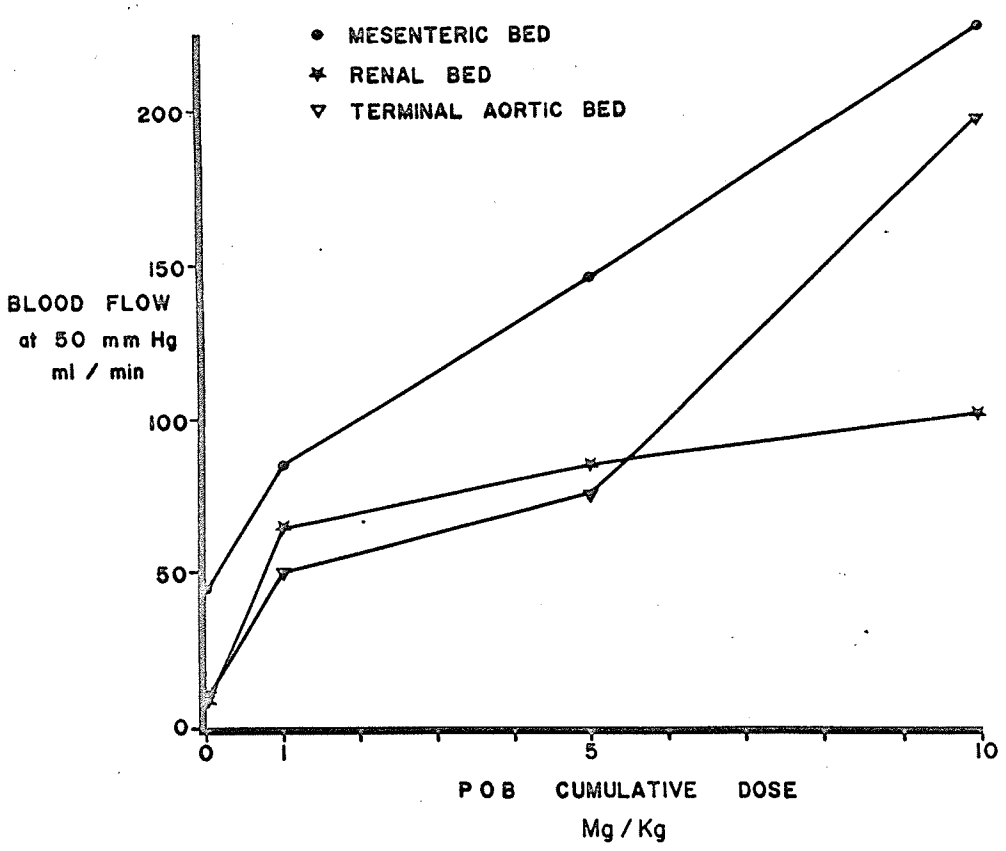


Figure 33. Dose-Response Curves for Effects of Phenoxybenzamine on Regional Blood Flows. Animals were hemorrhaged to an arterial pressure of 50 mm Hg to induce sympathetic vasoconstriction prior to each flow determination.

to determine the extent to which more complete blockade could further modify the responses.

# GENERAL DISCUSSION

Starling (1896) was the first to describe clearly the principal factors regulating the exchange of fluid across capillary walls. According to his formulation, the direction and rate of transcapillary fluid movement is determined by the relationship between the net hydrostatic and colloid osmotic pressures across the capillary wall and the functional properties of the capillary membrane. Net transcapillary hyrostatic pressure, capillary hydrostatic pressure less tissue pressure, tends to filter fluid from the blood. Tissue pressure is usually a negligible factor and may even be negative and thus act in the same direction as capillary hydrostatic pressure (Guyton, 1963). This outward movement of fluid is opposed by the colloid osmotic pressure gradient, which is maintained by the relative impermeability of the capillary wall to plasma proteins and by the lymphatic system, which returns the small amount of filtered plasma protein to the vascular system, keeping the interstitial protein concentration relatively low.

Landis (1934) determined capillary hydrostatic pressure and plasma colloid osmotic pressure directly, and his measurements provided experimental support for the Starling hypothesis. Further confirmation was provided by the studies of Pappenheimer and Soto-Rivera (1948). The latter investigators pointed out the importance of the ratio of precapillary to postcapillary resistance in determining effective capillary hydrostatic pressure. They also demonstrated that although precapillary resistance is the major determinant of blood flow through a vascular bed, a change in postcapillary resistance is 5 to 10 times more effective than one in precapillary resistance in changing capillary hydrostatic pressure.

Mellander (1960) showed that direct sympathetic nerve stimulation increased precapillary more than postcapillary resistance in the cat hind-

quarters, resulting in a fall in capillary pressure and a shift of fluid into the vascular space. Öberg (1964), working with the same experimental preparation, demonstrated that a similar movement of fluid occurred in response to hemorrhage, even when the arterial and venous pressures of the region studied were held constant. As he pointed out, this fluid shift would be potentiated by a decrease in arterial and venous pressures, as generally occurs during hemorrhage.

Hemodilution and plasma volume replacement following hemorrhage is a previously well documented phenomenon, as was pointed out in the Review of the Literature. It has generally been assumed that the predominant factor responsible for the movement of fluid into the vascular space following hemorrhage is the reduction in arterial and venous pressures. However, hemodilution has been observed to occur in response to moderate hemorrhage, which did not necessarily cause a fall in arterial and venous pressures (Chien, 1958, Baker and Remington, 1961). Furthermore, it has been demonstrated that the rate of hemodilution is significantly lower in totally sympathectomized than in intact dogs following hemorrhage, despite a considerably greater fall in arterial pressure (Chien, 1958). These findings are consistent with the conclusion of Öberg (1964) that increased sympathetic nervous system activity plays a part in the compensatory shift of fluid into the vascular space following hemorrhage.

In the experiments reported above, it was found that the degree of dilution of plasma protein induced by hemorrhage was identical in control dogs, and in animals infused with noradrenaline or pretreated with phenoxybenzamine. However, maximal dilution was achieved much more rapidly in noradrenaline-treated dogs, and conversely, phenoxybenzamine pretreat-

ment considerably delayed maximal dilution, despite an earlier fall in arterial pressure. These observations corroborate the earlier suggestions that increased sympathetic nervous system activity is an important factor promoting the shift of fluid into the vascular space following hemorrhage. However, it appears that the sympathetic nervous system acts only in determining the rate of fluid movement and does not affect the final equilibrium dilution reached.

The onset of decompensation during hemorrhagic hypotension is indicated by the animal's requirement for the return of shed blood to prevent the arterial pressure from falling below the predetermined level. (See Review of Literature for references.) The association between the onset of decompensation and of hemoconcentration observed in this study has not been reported previously. In retrospect, the association was apparent in protocols published over 25 years ago (Freeman et al.,1938). His figures show that in dogs subjected to hemorrhagic hypotension, the hemoglobin concentration rose when return of blood was required to prevent the arterial blood pressure from falling. However, the correlation was never mentioned, probably, either because there were too few experiments or because it was assumed that the hemoconcentration was due to the high hemoglobin content of the infused blood. It is also of interest, in the light of the present findings, that the protocol of a totally sympathectomized dog shows that the return of shed blood was not required to maintain the blood pressure. This was also true of a control dog which survived the hemorrhagic shock procedure. Blalock (1934) reported that dogs developed hemoconcentration after maintained hemorrhagic hypotension, but did not attempt to correlate that finding with either the return of blood or survival. These early relevant observations were apparently

soon forgotten, and Wiggers (1950) stated categorically in his monograph that hemoconcentration does not accompany the shock induced by hemorrhage.

Uptake of blood from the reservoir is highly correlated with lethality following hemorrhagic hypotension. (See Review of the Literature for references.) This has been known for at least 20 years, yet today there is no generally accepted hypothesis to explain the association. Crowell and Guyton (1961) suggested that the uptake of blood is a sign of heart failure. This appears unlikely. Much of the evidence suggesting that heart failure is not an important feature of shock except shortly before death was presented in the Review of the Literature, and the experiments reported here failed to show a significant degree of heart failure, either at reversal or at any stage of decompensation, except occasionally as a preterminal event.

An alternative concept that has been suggested by many workers (See Review of the Literature for references.) to account for uptake of blood and decompensation is "peripheral circulatory failure". This term was coined by Blalock, and has subsequently been widely used, presumably because it is euphonious, descriptive and directs attention away from the heart and to the peripheral circulation where most investigators in this field over the last 5 decades have believed the primary fault to lie. In recent reviews, a "giving out" of some component of the microcirculation has been cited as the most likely explanation for the uptake of blood, which was required to fill a progressively expanding space. The questions regarding what space expanded, and especially what caused it to expand remain unanswered, and many careful studies of the function of the arterioles, capillaries and veins have failed to demonstrate a loss of functional integrity of any of these components of the microcirculation

in shock, except as a terminal event.

The data presented here have led, by way of several different lines of investigation, to a third possible explanation to account for the necessity for uptake of blood to prevent the pressure from falling during sustained hemorrhagic hypotension: Loss of circulating intravascular volume. The hemoconcentration that occurred in the first phase of decompensation was shown to be due to the ultrafiltration of fluid from the vascular space, resulting in a progressive decrease in intravascular volume. There was a very high quantitative correlation between measured volume loss from the vascular space and the uptake required to prevent falls in both central venous pressure and arterial pressure. These findings indicate that uptake of blood from the reservoir represents replacement of volume lost from the vascular space. The observation that preventing uptake of blood during decompensation rapidly led to death with a falling central venous pressure, also supports this hypothesis.

The hemoconcentration reflected loss of an ultrafiltrate of plasma from the vascular space. The capillary wall is normally so highly permeable to water that a net shift of water across the capillary wall is much more likely to reflect a change in either the oncotic or hydrostatic pressure, the major factors normally regulating movement of water across the membrane, than primary changes in the capillary wall itself (Pappenheimer, 1953). This is particularly so in view of the finding that in hemorrhagic shock, total transcapillary flux of deuterium oxide is considerably lower than normal (Fogelman et al., 1952). Although ultrafiltration of fluid from the vascular space could theoretically have been due to decreased interstitial hydrostatic pressure, or increased interstitial oncotic pressure, these parameters tend to be quantitatively minor and

are likely to be only secondarily affected by noradrenaline and phenoxybenzamine, which profoundly influenced the course of the ultrafiltration. The hemoconcentration therefore probably reflected an increasing capillary hydrostatic pressure, which occurred despite a constant arterial pressure and a tendency toward a decreased central venous pressure. This suggests that some change in microcirculatory control of capillary hydrostatic pressure was responsible. Either a decreasing precapillary resistance, allowing greater transmission of arterial pressure to the capillary bed, or an increasing postcapillary resistance would result in an increasing capillary hydrostatic pressure. The former possibility was assessed by determining blood flow to 3 major vascular beds during sustained hypotension. Since the major determinant of blood flow to any vascular bed at a given arterial pressure is the precapillary resistance, and since in these experiments arterial pressure was held constant through the interval when reversal occurred, any reduction in the tone of precapillary resistance vessels would have resulted in an increased blood flow. This did not occur in any of the vascular beds studied, in agreement with the majority of previous investigations, which found no consistent loss of vascular resistance during the development of hemorrhagic shock (Green, 1961). However, most previous measurements were difficult to interpret because blood pressure was not held constant throughout the procedure. (See Wiggers, 1950.)

It was subsequently demonstrated (Section XI) that small vein wedge pressure in the vascular beds of both the hindquarters and the intestine increased progressively after the arterial pressure had been stabilized at the desired hypotensive level. This increase occurred despite relatively constant blood flows and large vein pressures. Since

arterial blood pressure and blood flow were constant, the transmission of pressure from the arterial system to the level of the wedged catheter must have been unchanged, and the constant large vein pressure indicated that the increase was not a "back pressure" from the large veins or right atrium. Consequently, it is necessary to postulate that the increase in postcapillary pressure was due to an event somewhere between the arterioles and the large veins. It is highly probable that the pressure increase was due to an increased resistance of the venous collaterals draining the area proximal to the small vein in which the catheter was wedged. The exact size of these vessels is not known, but they probably are of approximately the same size as the vein in which wedging occurred, 0.5 mm in diameter.

By the time 20% uptake of blood from the reservoir had occurred, the postcapillary wedge pressure in the hindquarters vascular bed had increased 6.8 cm of saline, or 5.0 mm Hg. The arterial pressure at this time was 50 mm Hg and, assuming the usual 60 to 70% drop in pressure at the arteriolar level, capillary pressure would have been in the neighbourhood of 15 to 20 mm Hg. Thus, the pressure change of 5.0 mm Hg would represent a 25 to 33% increase. The pressure drop across the arterioles was likely greater than assumed above, since total peripheral resistance was increased following hemorrhage. Thus, the 5.0 mm Hg probably represents an increase in capillary pressure of one-third or more.

Uptake occurred in the control animals at a rate of 15.8 ± 2.6 ml/kg/hour, or 0.026 ml/100 g/min. (Section III). The capillary filtration coefficient for the whole dog is not known, but Pappenheimer and Soto-Rivera reported it to be 0.014 ml/100 g/min/mm Hg for the dog hind limb.

Assuming that a similar figure applied to the dog as a whole, which is not unreasonable since skeletal muscle and skin form approximately 65% of a dog's tissue mass, the postcapillary pressure increase of 5.0 mm Hg was more than enough to account for the loss of intravascular volume that occurred. In fact, an increase in net filtration approximately three times greater than the observed loss of volume would have been expected. However, the effect of the increased pressure would have been partially offset by the increasing plasma colloid osmotic pressure and by the fact that total capillary surface available for exchange was probably reduced by the increased sympathetic vasoconstrictor activity. An increase of plasma protein concentration from 6.22 to 6.58 g/100 ml would result in an increase of plasma colloid osmotic pressure of 1 to 2 mm Hg (Landis and Pappenheimer, 1963).

The increase in postcapillary pressure during sustained hypotension was reversed by the administration of phenoxybenzamine, but not by the antihistamine, mepyramine, or the anticholinergic agent, atropine. Unfortunately, the effects of a specific serotonin antagonist on the postcapillary wedge pressure were not examined, but it has been shown that serotonin lowers small vessel resistance in the intact dog (Haddy et al., 1957). Phenoxybenzamine administration decreased the wedge pressure despite an increased blood flow and, therefore, an increased transmission of arterial pressure to the level of the wedged catheter. These findings, especially in the light of the previous demonstration of the effects of noradrenaline and phenoxybenzamine on the rate of intravascular fluid loss during hemorrhagic hypotension, suggest that the increasing postcapillary resistance measured in the hindquarters and intestinal vascular beds during

hemorrhagic hypotension are due to increased sympathetic nervous system activity.

Although the sympathetic nervous system affects both vascular segments, the postcapillary resistance in the mesenteric and hindquarters vascular beds increased progressively, whereas the precapillary resistance in these areas reached an early maximum which remained relatively constant throughout the period of hypotension. The reason for this difference in the responses of the pre- and postcapillary vessels is not completely clear. At least two explanations are tenable. One possibility is that a regionally specific pattern of efferent nervous activity emanates from the central nervous system. An alternative possibility is that the nervous activity to both components was similar, but that local factors prevented the arteriolar response from increasing beyond a certain point, whereas the postcapillary resistance vessels were capable of a progressively increasing response to increasing sympathetic activity. The observations of Lewis and Mellander (1962) lend support to the latter possibility. They found that if blood flow to the hindquarters of a cat was markedly restricted and the sympathetic nerve supply to the region stimulated intermittently, the response of the precapillary vessels to a given stimulus declined much more rapidly than that of the postcapillary vessels. This eventually resulted in a reversal of the net transcapillary fluid movement induced by sympathetic nerve stimulation from the initial shift of fluid into the vascular space to a loss from it. The decreased response of the precapillary resistance vessels was not due to loss of function of their sympathetic nerves, since the response to noradrenaline injected intra-arterially showed a similar decrease.

The results reported by Lewis and Mellander (1962) differ in one important qualitative respect from those reported here. In the dogs subjected to hemorrhagic hypotension, there was no evidence for a loss of responsiveness of precapillary resistance vessels. Their tone reached an early maximum which was usually maintained until the terminal stage. In contrast, the resistance of the postcapillary vessels increased progressively. This difference may be explained on the basis of another observation of Lewis and Mellander. They found that increasing the frequency of sympathetic nerve stimulation or the dose of noradrenaline restored the response of the precapillary resistance vessels. It seems likely that in the animals subjected to hemorrhage with the nervous system intact, the precapillary resistance response was maintained by a similar mechanism, i.e., by a progressively increasing sympathetic nerve activity. This also affected postcapillary vessels which responded with a progressively increasing resistance and, thus, the fluid shifts described above.

The factors which result in the fluid shifts and hemoconcentration appear to be important determinants of the fate of dogs subjected to prolonged hemorrhagic hypotension since there was a high correlation between the degree of hemoconcentration and the probability of a dog ultimately dying despite the reinfusion of all the shed blood.

Trapping of blood in the microvasculature (sequestration) in shock was first described on the basis of postmortem microscopic findings in both patients and experimental animals. (See Scudder (1940) and Moon (1942) for descriptions and many early references on this subject.) More recently, sequestration in late shock has been confirmed by microscopic examination in vivo (Zweifach, 1958; 1961), by measurements of circulating and tissue blood volumes with labelled erythrocytes and proteins (Gibson

et al., 1947; Dunn et al., 1958), and by analysis of the equilibration curves of injected labelled erythrocytes (Shoemaker and Iida, 1962; Suzuki et al., 1964, a and b). However, no generally acceptable mechanism has been suggested to account for the development of sequestration. Trapping has been most commonly ascribed to the "sluggish state" of the circulation, but more specific hemodynamic phenomena, which would allow blood to flow into but not out of a vascular bed, remain unclear.

Some observations from the present study suggest a plausible mechanism. The decompensatory stage of hemorrhagic hypotension has been divided into an early phase characterized by rapid, progressive hemoconcentration and a later phase during which hemoconcentration either progresses very slowly or not at all. During the first phase, both uptake of blood and loss of plasma volume can be quantitatively accounted for by ultrafiltration of fluid from the vascular space. During the second phase, uptake of blood continues at a similar rate and measured plasma volume continues to fall. However, hematocrit and plasma protein concentrations are relatively constant, and consequently, the loss of intravascular volume must represent the disappearance of whole blood from the measured pool, i.e., sequestration. It is of some interest that the second phase was never seen in phenoxybenzamine pretreated dogs.

The rate of uptake of blood from the reservoir reflects the rate of disappearance of fluid from the circulation. If the mechanism resulting in ultrafiltration of fluid from the vascular space were wholly unrelated to that resulting in sequestration, one might expect that the change from one process to the other would be reflected in some change in the rate of uptake. Such a change in rate was never seen in the above studies on over 200 dogs. It is therefore attractive

to suggest that sequestration of blood involves some mechanism which also contributes to ultrafiltration. An increased postcapillary resistance would increase capillary hydrostatic pressure and thus promote ultrafiltration from the vascular space, and a continuing increase could ultimately produce a resistance to outflow from the vascular bed too great to be overcome by the pressure transmitted from the arteries in the shock state, "critical closure". Such a mechanism is in line with the observations that phenoxybenzamine prevents the rise in postcapillary pressure (small venous wedge pressure), markedly slows ultrafiltration and prevents sequestration. However, further investigations will be required to fully substantiate this hypothesis.

The technique that offers the most promise for investigating sequestration is that developed by Shoemaker and coworkers (Shoemaker et al., 1962; Suzuki et al., 1964, a and b), employing $Cr^{51}$-labelled erythrocytes. This has been applied both in hemorrhagic shock in the dog and in shock of diverse etiology in man. In the normal animal and man the injection of labelled cells is followed by a rapid exponential fall of erythrocyte specific activity. Equilibrium is generally reached within 10 minutes and the specific activity then remains essentially constant for several hours. In shock, this early equilibrium was frequently not achieved. In the majority of cases there was a phase of gradually decreasing specific activity, followed after a variable period, generally 20 to 60 minutes, by an equilibrium plateau. In some cases equilibrium was not achieved during the period of study. Analysis of these curves allowed calculation of the sizes of "rapidly circulating" and "slowly circulating" red cell compartments. Subtracting these from the preshock

red cell mass also allowed determination of a "noncirculating" erythrocyte space. A recently reported study (Dagher and Moore, 1964) failed to substantiate these findings, but these workers did not mention the amount of uptake of blood at the time measurements were made, and their failure to find a fall in plasma volume suggests that they were done relatively early in the decompensatory period, probably before significant sequestration could be expected.

Shoemaker and coworkers suggested that the slowly circulating and noncirculating red cells were sequestered because of intravascular aggregation. Blood viscosity is increased in shock, and it has been suggested that at the lower rates of shear in capillaries and venules of animals in shock the increased blood viscosity predisposes to stagnation and red cell aggregation or "sludging" (Gelin, 1961; Bergentz et al., 1963). There is, as yet, no critical evidence on which to assess the role of increased blood viscosity and red cell aggregation either in producing sequestration or in determining survival in shock. It would be of some interest to compare the effects of adrenergic blockade and of low molecular weight dextran, which has been shown to decrease blood viscosity, on red cell mixing curves and on some of the hemodynamic parameters investigated in the present studies. The possibility exists that the microscopically evident red cell aggregates merely represent the appearance of functionally sequestered cells. A recent study has shown that the administration of noradrenaline to dogs subjected to hemorrhage increased both the lethality of the procedure and the microscopic evidence of trapping and sludging (Schumer and Durrani, 1963).

Cannon (1914) first suggested that the sympathoadrenal system was designed to discharge as a unit in an emergency, resulting in the

simultaneous activation of sympathetically innervated structures throughout the body in situations where the organism must prepare for "flight or fight". In Cannon's words, "The sympathetics are like the loud and soft pedals, modulating all the notes together". This view is still stated in relatively recent publications, e.g., Goodman and Gilman (1955). However, considerable evidence has recently accumulated to suggest that this is an oversimplification, and that there is a dynamic, readily adjustable control of the circulation mediated by the sympathetic nervous system, making possible both regionally differentiated and generalized, homogenous discharge patterns (Folkow et al., 1961; Löfving, 1961; Johansson, 1962; Killip, 1962). It is apparent from data obtained in the present study that even in response to a severe hemorrhage, a situation in which a simple massive sympathetic discharge might have been expected, a highly organized, differential activation of various effector units occurred.

During the control period before hemorrhage, phenoxybenzamine pretreatment resulted in a very large increase in blood flow to the gastrointestinal tract, a smaller but significant increase to skeletal muscle-skin areas, represented by the hindquarters, and no significant increase in renal blood flow. This suggests that under the moderate stress imposed by anesthesia and the surgery necessary to place the electromagnetic flowmeter probes, sympathetic vasoconstrictor activity was greatest in the mesenteric vascular bed, less marked in the hindquarters and minimal or absent in the renal bed. Vasoconstriction increased in response to the first part of a rapid hemorrhage, but this same differential was maintained. Mesenteric and hindquarters blood flows fell precipitously, the former more than the latter, before any major decrease in the arterial pressure

occurred. Renal blood flow fell very little at this time, and calculated renal vascular resistance frequently decreased. However, during the final part of the initial hemorrhage, as arterial pressure fell from approximately 85 to 50 mm Hg, renal flow tended to fall markedly, and concurrently the difference between the renal blood flows of control and phenoxybenzamine pretreated animals became significant. After the initial hemorrhage, when a stable arterial pressure of 50 mm Hg was maintained, mesenteric and hindquarters vascular resistances and the differences between the flows to these beds in control and phenoxybenzamine-pretreated dogs remained constant. However, renal blood flow decreased progressively during the interval at 50 mm Hg, and the difference between the renal flows of control and phenoxybenzamine-pretreated dogs became progressively greater. Evidence for differential activation of sympathetic vasoconstriction is also available from the studies on the effects of various rates of hemorrhage on the renal vascular resistance. A slow hemorrhage resulted in intense vasoconstriction, reversed by phenoxybenzamine, whereas there was no initial constriction when the hemorrhage was rapid. It is quite clear that these differences are due to differences in sympathetic nerve activity rather than in the sensitivity of the vascular smooth muscle to the mediator, noradrenaline, since the responses of the renal and mesenteric vascular beds to infused noradrenaline were nearly identical.

Another major difference in the responses of the vascular beds studied was apparent on reinfusion of the shed blood following a period of hemorrhagic hypotension; renal vascular resistance remained high whereas that of the intestinal and hindquarters vascular beds was less than at comparable arterial pressures during hemorrhage. The depressed renal

blood flow was associated with failure of the kidney to resume the elaboration of urine when the arterial pressure reached levels adequate for glomerular filtration. The failure of renal flow to increase when the arterial pressure was raised by reinfusion following a prolonged period of hemorrhagic hypotension was apparent in the protocols of some early studies, e.g., Phillips et al. (1946), but it did not attract the attention of the investigators. More recently Kramer and coworkers (See Kramer, 1962.) have recognized this phenomenon and its possible importance in the development of acute renal failure following shock. In his words, "...what causes persistent renal vasoconstriction ... is still obscure. The assumption that pressor agents, such as renin or other types of substance, are produced during circulatory shock has often been discussed. It seems necessary to reinvestigate the matter." The only relevant finding that Kramer could add was that there appeared to be a relationship between the severity of the stress applied and the degree of persistent vasoconstriction. In the present study it was found that the degree of maintained renal vasoconstriction was determined predominantly by the duration of hypotension. Pretreatment with phenoxybenzamine prevented both maintained renal vasoconstriction and oliguria. It was also possible to abolish the maintained renal vasoconstriction by denervation of the renal pedicle or by the intra-arterial administration of guanethidine and phenoxybenzamine. Abolition of the maintained renal vasoconstriction frequently resulted in a diuresis, suggesting that it was a major factor producing the oliguria. The available evidence suggests strongly that both the maintained renal vasoconstriction and the oliguria following prolonged hemorrhagic hypotension involve abnormal sympathetic nervous system activity. Failure of infused noradrenaline to produce comparable per-

sistent effects suggests that they are not due to a peculiarity in the response of the renal vascular bed to the transmitter released, but rather to the characteristics of the sympathetic efferent activity arising in the central nervous system following prolonged hemorrhagic hypotension.

The reason for the persistent sympathetic discharge to the renal vascular bed is not clear. In many situations involving sympathetic vasoconstriction, the renal vascular bed is rather less involved than are those of other organs (Folkow et al., 1961; Löfving, 1961; Johansson, 1962; Killip, 1962). The renal vascular bed is markedly involved in the "defence reaction" induced by hypothalamic stimulation (Feigl et al., 1964), which generally includes activation of the cholinergic vasodilator fibers to skeletal muscle (Uvnäs, 1961; Feigl et al., 1964). No evidence that this dilator system is activated during hemorrhagic hypotension in the dog was obtained in the present studies, and the fact that administration of atropine failed to influence skeletal muscle blood flow is strong evidence against its involvement. This suggests either that various components of the defence reaction can be activated selectively, or that the hypothalamic centers of this system are not involved in the response to hemorrhage in the anesthetized dog. Experiments now in progress in this laboratory have shown that spinal anesthesis abolishes the persistent renal vasoconstriction, but that spinal cord section at the level of $T_1$ does not, indicating that the sympathetic nervous activity is initiated at a spinal cord level. However, considerably more investigation will be required before a full explanation of this phenomenon can be given.

# SUMMARY AND CONCLUSIONS

It has been demonstrated in dogs subjected to hemorrhagic hypotension that decompensation, indicated by the need to reinfuse shed blood to prevent the arterial pressure from falling, is associated with a loss of intravascular volume. It has been possible to divide the stage of decompensation into two phases. In the first, an ultrafiltrate of plasma is lost, associated with progressive parallel increases in plasma protein concentration and hematocrit; in the second, the loss appears to represent sequestration of whole blood, and plasma protein concentration and hematocrit remain relatively constant. The ultrafiltration appears to be due to an increasing capillary hydrostatic pressure caused by a progressive increase in postcapillary vascular resistance, which probably arises predominantly in venules about 0.5 mm in diameter. It is postulated that the sequestration of blood during the second phase is caused by a further increase in this resistance. Since infusion of noradrenaline accelerates the development of decompensation, the rate of ultrafiltration and the onset of sequestration, and administration of phenoxybenzamine delays decompensation, slows ultrafiltration, prevents sequestration and reduces postcapillary resistance, it appears that sympathetic nervous system activity is an important factor increasing the postcapillary resistance. A very close correlation between the development of hemoconcentration and lethality was demonstrated, suggesting that these phenomena are major determinants of the ultimate fate of dogs subjected to hemorrhagic hypotension.

The sympathetic nervous system appears to act in a highly selective manner during the response of the dog to hemorrhage. Initially, the vascular beds of the gastrointestinal tract and of skeletal muscle and skin are constricted, followed by progressive constriction of the renal vessels after a stable level of near maximal constriction has been attained

in the other beds. The degree of constriction of the renal vascular bed during early hemorrhage has been shown to be a function of the rate of hemorrhage, the slower the hemorrhage the greater the early constriction. Following reinfusion of the shed blood after prolonged hemorrhagic hypotension, the renal vascular bed remains constricted, whereas the vascular beds of the intestine and hindquarters dilate. The maintained renal vasoconstriction can be prevented by pretreatment with phenoxybenzamine and is abolished by denervation of the renal pedicle or by the intra-arterial administration of guanethidine and phenoxybenzamine. Maintained renal vasoconstriction is associated with oliguria, which is also prevented by phenoxybenzamine and abolished by intra-arterial guanethidine, suggesting that both the renal vasoconstriction and the oliguria are due to increased sympathetic nervous system activity. Since it was not possible to produce maintained renal vasoconstriction by prolonged infusions of noradrenaline, it seems likely that it is not due to a unique local response of the renal vessels to noradrenaline, but rather to a selective discharge of sympathetic nerves innervating the renal vascular bed.

Phenoxybenzamine dose-response curves, using two different cardiovascular responses in the dog, indicate that the dose required to block completely responses to sympathetic nerve activity on peripheral vessels is approximately 10.0 mg/kg. Doses below this diminished but did not abolish the responses. Since a dose of 5.0 mg/kg was used in most of the present studies, it is possible that the residua of certain changes which were not completely eliminated by phenoxybenzamine were due to residual effects of sympathetic nervous system activity.

It is concluded that increased sympathetic nervous system act-

ivity can promote the development of shock irreversible to transfusion not only by inducing arteriolar constriction, and thus decreasing tissue perfusion, but also by causing an imbalance of the precapillary and postcapillary resistances, with a consequent net filtration of fluid from the vascular space. It also appears likely that the increased postcapillary resistance is responsible for the trapping of blood in the microvasculature (sequestration) in the later phase of circulatory decompensation. Both the increased ultrafiltration and the sequestration contribute to the progressive fall in effective circulating blood volume.

The sympathetic nervous system produces a highly organized redistribution of blood flow during and following a period of hemorrhagic hypotension. The skeletal muscle-skin and particularly the mesenteric vascular beds are predominantly involved during early hemorrhage, whereas the renal vascular bed is subjected to neurogenic constriction only after slow hemorrhage or a period of maintained hypotension. Neurogenic constriction in the renal bed can persist for long periods after the blood pressure has been elevated by reinfusion of the shed blood and this maintained renal vasoconstriction appears to be a major factor in the development of oliguria following hemorrhagic hypotension.

There is no convincing evidence for loss of the functional integrity of any component of the cardiovascular system during any except the immediately preterminal stage of hemorrhagic shock. Since all the phenomena described can be accounted for on the basis of increased tone or activity of various vascular elements, it is suggested that "peripheral circulatory failure" and analogous terms are misleading, and should be abandoned.

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