# MECHANISM OF THE RESPONSE OF THE SPLANCHNIC RESISTANCE VESSELS TO HEMORRHAGE: ROLES OF VASOPRESSIN AND ANGIOTENSIN

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ABSTRACT

The present study was devised to investigate the response of the splanchnic resistance vessels to hemorrhage and to elucidate the mechanisms involved in this response. It is well known that in response to hemorrhage, the intestinal and splenic resistance vessels constrict; the hepatic arterial resistance vessels dilate. However, the mechanisms of these responses are completely unknown. It has usually been assumed that the sympathetic nerves were the most important factor in the intestinal and splenic vasoconstriction. However, other vasoconstrictor factors might also play a role. Vasopressin is released and angiotensin is formed in response to hemorrhage and both of these substances are potent vasoconstrictor agents. The roles of these extrinsic factors in the mechanism of the intestinal and splenic vasoconstriction were investigated by removal of the major sources of these agents and by local denervation.

The investigation was carried out in cats anesthetized with sodium pentobarbital. Superior mesenteric, splenic, and hepatic arterial flows were recorded with a non-cannulating electromagnetic flowmeter and arterial pressures were recorded with pressure transducers. Splenic weight was recorded with a force-displacement transducer.

After rapid or slow hemorrhage, a marked and sustained intestinal vasoconstriction occurred. Following intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy, the vasoconstriction was greatly reduced and only a small vasoconstriction remained. If either the kidneys or the pituitary gland were left intact, the vasoconstriction was not significantly different from that which occurred when all the organs were intact. In the absence of both the kidneys and pituitary, the sympathetic nerves

and adrenal glands did not contribute to the small residual response. The intestinal vasoconstriction after hemorrhage was not altered by phenoxybenzamine. The data suggested that the intestinal vasoconstriction following hemorrhage was mediated by vasopressin secretion from the pituitary gland and angiotensin formation subsequent to renin release from the kidneys. The intestinal innervation and adrenal medullary secretions played no significant part.

Following hemorrhage, splenic resistance vessels constricted and splenic weight decreased. After denervation, adrenalectomy, nephrectomy, and hypophysectomy, the splenic vasoconstriction and the decrease in splenic weight were abolished. In the absence of the kidneys and pituitary, the sympathetic nerves and adrenal glands caused splenic vasoconstriction but the vasoconstriction was significantly reduced from that which occurred when all the organs were intact; the decrease in splenic weight was not altered. The data from these experiments and those reported in the literature suggested that the splenic vasoconstriction following hemorrhage was mediated by vasopressin, angiotensin, and the sympathetic nervous system, and that splenic contraction was mediated by the sympathetic nervous system.

If vasopressin and angiotensin play a role in the splanchnic response to hemorrhage, then intravenous infusions of these agents in amounts likely to be found in the blood after hemorrhage should cause a similar response. Therefore, the responses of the intestinal, splenic, and hepatic arterial resistance vessels were compared during intravenous infusions of vasopressin and angiotensin. Vasopressin and angiotensin caused marked vasoconstriction of the intestinal and splenic vascular

beds. Angiotensin also constricted the hepatic arterial bed but the response was small, whereas vasopressin caused vasodilatation of this bed. The data were consistent with the postulated roles for vasopressin and angiotensin in the splanchnic response to hemorrhage.

# PREFACE

I would like to express my appreciation to Dr. Clive V. Greenway for his valuable criticism and guidance throughout the course of this investigation and during the preparation of this manuscript. I am most grateful to him.

Because some of the technical procedures could not be carried out by one person, part of the work during this investigation was done in collaboration with others. Some experiments on the mechanisms of the intestinal and splenic vasoconstriction following hemorrhage were done in collaboration with Dr. Ronald D. Stark. The work on the responses of the splanchnic resistance vessels to intravenous infusions of vasopressin and angiotensin was done in cooperation with Marsha M. Cohen and Daniel S. Sitar while they were engaged in "rotational research" under the supervision of Dr. C.V. Greenway. The contribution of these people is gratefully acknowledged.

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# INTRODUCTION

The systemic circulation consists of numerous different circuits arranged in parallel. This permits wide variations in regional blood flow with or without changes in total systemic flow. With a few exceptions, each parallel-coupled circuit is tailored to provide a sufficient flow to meet the maximum metabolic requirements of the tissue or organ it supplies (Burton, 1965). Each individual parallel-coupled circuit in the systemic circulation contains a number of series-coupled sections of different design and function (Folkow, Heymans, and Neil, 1965; Mellander and Johansson, 1968). The "windkessel vessels" convert pulsatile outflow from the heart into fairly smooth blood flow to the tissues. These vessels correspond anatomically to the large arteries which possess large amounts of elastic tissue in their walls. The "resistance vessels" include a precapillary segment which corresponds to the small arteries and arterioles and a postcapillary segment which corresponds to the venules and veins. The small arteries and arterioles contain an abundance of smooth muscle and it is in these vessels that the largest drop in pressure energy occurs. The sum of the precapillary and postcapillary resistances determines the total resistance and, therefore, regional blood flow while the ratio of the two resistances determines capillary hydrostatic pressure and, therefore, fluid exchange. The capillaries comprise the "exchange vessels". They provide a large surface area with a high permeability to small molecules for diffusion and fluid exchange. Changes in activity of the "precapillary sphincter vessels" determine the number of capillaries open at any time and, therefore, determine the capillary surface area available for exchange. Beyond the capillaries are the venules and veins. Since most of the

regional blood volume is contained within these vessels, they are appropriately called "capacitance vessels". Changes in their tone have a profound effect on the distribution of the blood volume and, therefore, influence venous return and cardiac output. Finally, there are "shunt vessels". These are arteriovenous channels which permit a portion of the blood flow to bypass the exchange vessels. They have been demonstrated most clearly in the skin, where they are involved in thermoregulation.

The fundamental disturbance in hemorrhage and hemorrhagic shock is a decrease in blood flow through peripheral vascular beds. If the hemorrhage is severe enough, the decrease in blood flow may be so great as to cause tissue cell damage. Many investigators have suggested that irreparable damage may occur to splanchnic and renal tissue after severe hemorrhage (Lillehei, Longerbeam, and Rosenberg, 1962: Lillehei, Dietzman, and Movsas, 1967; Nickerson and Gourzis, 1962). The current study was devised to investigate the response of the splanchnic resistance vessels to hemorrhage and the mechanisms involved in this response.

Many investigations of the resistance function of the splanchnic vascular bed have involved arterial long-circuits and pump devices. However, the mere passage of normal blood through pump devices used in perfusion experiments may result in decreased basal tone and vascular reactivity (Folkow, 1953). Failure to detect autoregulation of blood flow in vascular beds has been attributed to some alteration in the bed as a result of changes in the blood due to contact with artificial structures or traumatization of the blood by the perfusion pumps. This effect was demonstrated clearly in the cerebral vascular bed where insertion of a perfusion pump altered the pressure-flow relationship

(Machowicz, Sabo, Lin, Rapela, and Green, 1961; Green et al., 1963). When autoperfused by the heart, cerebral vascular flow remained fairly constant as perfusion pressure varied from 40 to 100 mm Hg. With the perfusion pump in operation, flow did not remain constant but increased regularly over the same range of pressures. Similar observations on the use of arterial long-circuits and pump devices have been reported in the intestinal and hepatic arterial vascular beds (Dresel and Wallentin, 1966; Johnson, 1960; Greenway and Stark, 1971). Because of the inconsistent results obtained in studies in which arterial long-circuits and pump devices were used, little emphasis will be placed on such studies in the following sections. Since the experimental work for this thesis was carried out on cats, data on the cat will be emphasized. Indication will be made when the data was obtained from other species.

## The Splanchnic Vascular Bed

The splanchnic vascular bed receives a large proportion of the cardiac output. In cats anesthetized with pentobarbital, total hepatic flow is approximately 50 ml/min/kg of body weight and this accounts for approximately 35% of the cardiac output (Greenway and Lawson, 1966b). Of the total hepatic flow, one-third is supplied by the hepatic artery and the remaining two-thirds by the portal vein (Greenway and Lawson, 1966a).

Blood draining from the intestinal vascular bed comprises the major proportion of the portal vein flow. Attempts to measure "resting" blood flow in the intestine have resulted in values which vary greatly (Grim, 1963; Grayson and Mendel, 1965; Mellander and Johansson, 1968). This is not surprising since the values will vary considerably depending

upon the circumstances. It is difficult to define a resting state for the intestine since it shows varying degrees of activity in terms of motility and mucosal absorption, secretion, enzyme production, etc. The resting flow is sometimes defined as a state of reduced secretion and motility as after fasting and atropinization (Mellander and Johansson, 1968). Factors such as the anesthetic agent and depth of anesthesia will also have considerable influence. Other experimental variables such as accidental hypoxia, hypercapnia, and acidosis may also alter intestinal blood flow. Folkow, Lundgren, and Wallentin (1963) measured blood flow in short segments of jejunum and reported resting flows of 40-60 ml/min/100 g tissue. During maximal dilatation, flow increased up to 250 to 275 ml/min/100 g tissue (Folkow et al., 1963). These values are approximately 5 times greater than the corresponding values in skeletal muscle (Mellander and Johansson, 1968).

The intestine is composed of three main tissue layers, the mucosa, the submucosa, and the muscularis. The mucosa has relatively large metabolic requirements; as well, it transports absorbed material and provides material for glandular secretion. These properties place a large demand on the vascular bed of the mucosa. In contrast, the smooth muscle tissue has only moderate nutritional requirements. To satisfy the individual requirements of the different layers, the vascular beds of the three main tissue layers are connected in parallel permitting individualized adjustments of blood flow in each layer.

The distribution of the total blood flow to the different component tissue layers of the small intestine has been estimated by Lundgren (1967), and by Kampp and Lundgren (1968). They analyzed the

wash-out curve registered by a scintillation detector after intra-arterial injection of krypton$^{85}$. The elimination curve could be resolved into four exponential functions, and the corresponding tissue compartments were identified by several independent methods. Analysis was performed at "resting" blood flow and during various levels of vasodilatation. At rest, the mucosa received 40 to 60 ml/min/100 g tissue, the muscularis 10 to 15 ml and a small fraction located in the submucosa 400 to 600 ml. During maximal dilatation the flows increased from 2 to 3 times. The high flow rates in the submucosa may indicate some kind of shunting (Mellander and Johansson, 1968).

The splenic blood flow and vascular resistance have been measured using a variety of techniques. In cats anesthetized with pentobarbital, Ross (1967b) studied splenic blood flow using a non-cannulating electromagnetic flowmeter probe and found a mean splenic flow of 14 ml/min/cat. Greenway, Lawson, and Stark (1968) studied blood flow and volume simultaneously in the spleen with an uncannulated arterial supply and they reported a mean splenic flow of 10 ml/min/kg of body weight. They found no significant difference between innervated and denervated spleens. In a later study, they reported values of 18 ml/min/kg (Greenway and Stark, 1969).

The measurement of hepatic arterial blood flow and the limitations of the methods available have been reviewed recently by Greenway and Stark (1971). They pointed out the deleterious effects of arterial long-circuits on vascular reactivity and emphasized the importance of obtaining data in experiments where the arterial supply is intact. In cats anesthetized with pentobarbital, hepatic arterial flow measured

with a non-cannulating electromagnetic flowmeter was 16-18 ml/min/kg of body weight (Greenway, Lawson, and Mellander, 1967; Greenway, Lawson, and Stark, 1967). However, the spleen had been removed in these studies and hepatic arterial flow in cats with an intact spleen is probably less (Greenway and Stark, 1971).

Hemorrhage

Following hemorrhage there is generally a larger percentage decrease in cardiac output than in arterial pressure. An increase in total peripheral resistance was found in a wide variety of studies in the dog and these studies, together with others concerned with the changes in resistance to flow in the various peripheral vascular beds, have been reviewed recently by Chien (1967) and by Haddy, Overbeck, and Daugherty (1968). The resistance to blood flow increased in cutaneous, skeletal muscle, and renal vascular beds, while coronary vascular resistance and cerebral vascular resistance decreased. In the anesthetized cat, Greenway and Lawson (1966b) used a venous long-circuit technique to measure blood flows in the superior vena cava and in the hepatic, renal, and iliac segments of the inferior vena cava. Following hemorrhage, the flows in all the venae cavae segments decreased. However, the proportion of the reduced venous return draining from the superior vena cava increased, while the proportion draining from the renal and iliac segments decreased. Thus, the blood flow through the head was partially maintained at the expense of the kidneys and hindlimbs.

The response of the splanchnic vascular bed to hemorrhage is complex. The decrease in total hepatic flow following hemorrhage has been

associated with an increase in the calculated splanchnic resistance (Bounous, Hampson, and Gurd, 1963, dog; Frank, Frank, Jacob, and Fine, 1962, dog; Frank, Frank, Jacob, Weizel, Korman, and Fine, 1956, dog; Bearn, Billing, Edholm, and Sherlock, 1951, human; Sapirstein, Sapirstein, and Bredemeyer, 1960, rat). Other workers have reported that total splanchnic resistance did not change significantly (Heinemann, Smythe, and Marks, 1953, dog; Reynell, Marks, Chidsey, and Bradley, 1955, dog; Lacroix and Leusen, 1967, dog). In the cat, Greenway and Lawson (1966b) reported an increase in resistance to flow through the liver but it was much less than the corresponding increases in renal and hindlimb resistances.

There is considerable evidence to indicate that the reduction of blood flow through the splanchnic vascular bed after hemorrhage is not as great as through other beds. Werner, MacCanon, and Horvath (1952, dog) reported that the splanchnic fraction of the cardiac output was unchanged after mild hemorrhage. When arterial pressure was reduced to 50 mm Hg, Reynell, Marks, Chidsey, and Bradley (1955, dog) found that the splanchnic fraction of the C.O. increased from 24% to 31% and that the fraction was unchanged from control when arterial pressure was reduced to 40 mm Hg. Sapirstein, Sapirstein, and Bredemeyer (1960, rat) found that following a mild hemorrhage (10 ml/kg), the splanchnic fraction of the cardiac output increased by 6% while a more severe hemorrhage (25 ml/kg) resulted in a decrease of 2%. Lacroix and Leusen (1967, dog) found that splanchnic blood flow decreased to a slightly lesser extent than cardiac output. In the cat, Greenway and Lawson (1966b) reported that the proportion of the venous return draining from the hepatic segment of the

inferior vena cava was changed little in some experiments and rose slightly in others. Thus, there is clear evidence in a variety of studies that blood flow through the liver is not disproportionately reduced following hemorrhage. Similar to the cerebral and coronary vascular beds, blood flow to the liver is maintained at the expense of the cutaneous, skeletal muscle, and renal vascular beds.

Total hepatic flow consists of portal vein flow and hepatic arterial flow. Following hemorrhage, portal vein flow decreased markedly (Muller and Smith, 1963, dog; Selkurt and Brecher, 1956, dog; Bounous et al., 1963, dog; Kelso and Townsend, 1967, dog). In the cat, Greenway, Lawson, and Stark (1967) reported that a marked decrease in portal vein flow occurred after hemorrhage. The decrease was much greater than could be accounted for by the drop in arterial pressure. Part of the decrease in portal flow was due to marked vasoconstriction of the splenic resistance vessels which occurred after hemorrhage (Greenway and Stark, 1969). There is considerable evidence that, in the dog, the intestinal vascular bed also vasoconstricts in response to hemorrhage (Bounous et al., 1963, dog; Corday and Williams, 1960, dog; Cull, Scibetta, and Selkurt, 1956, dog; Selkurt, 1958, dog; Selkurt, Alexander, and Patterson, 1947, dog; Lillehei et al., 1962, dog; Lintermans, Appel, Bloom, Mullins, and Guntheroth, 1967, dog; Mundschau, Zimmerman, Gildersleeve, and Murphy, 1966, dog). In the cat, the response of the intestinal resistance vessels to hemorrhage is not clear. Oberg (1964) reported a transient intestinal vasoconstriction. Baker and Mendel (1967) observed very little change in intestinal vascular resistance; however, their studies involved cannulation of the superior mesenteric artery. Greenway and Lawson (1966b) concluded

from their experiments that marked intestinal vasoconstriction did occur. The results presented in this thesis show that in the cat, as in the dog, vasoconstriction of the intestinal resistance vessels occurs in response to hemorrhage. This conclusion will be assumed in the following discussion.

Following hemorrhage the portal venous flow was reduced to a much greater extent than the hepatic arterial flow, so that the relative contribution of the hepatic arterial flow increased (Corday and Williams, 1960, dog; Muller and Smith, 1963, dog; Mundschau et al., 1966, dog; Smith, Reeves, and Hinshaw, 1965, dog; Kelso and Townsend, 1967, dog). Most investigators have reported either little change or a decrease in hepatic arterial resistance (Kelso and Townsend, 1967, dog; Mundschau et al., 1966, dog; Muller and Smith, 1963, dog; Smith, Reeves, and Hinshaw, 1965, dog). In the cat, Greenway, Lawson, and Stark (1967) reported that, although portal venous flow decreased markedly during hemorrhage, hepatic arterial flow did not decrease unless arterial pressure fell below 80 mm Hg. Thus, hepatic arterial resistance decreased. The mechanism of the hepatic arterial vasodilatation in response to hemorrhage is not clear. However, it is known that hepatic arterial resistance decreases in response to reductions in portal pressure or flow, hepatic venous pressure, and hepatic arterial pressure, and that this response is probably myogenic in nature (see page 15).

In summary, the intestinal resistance vessels and splenic resistance vessels vasoconstrict in response to hemorrhage; the hepatic arterial resistance vessels vasodilate. Since the hepatic arterial flow forms a greater proportion of the total liver flow, the oxygen supply to the liver tends to be maintained.

In view of the importance of the splanchnic vascular bed, it is surprising how little is known about the mechanisms involved in the response of the splanchnic resistance vessels to hemorrhage.

Mechanism of Splanchnic Response to Hemorrhage

The resistance of a peripheral vascular bed is the ratio of pressure difference across the bed to the flow through it. Cardiovascular regulatory mechanisms tend to maintain a fairly constant perfusion pressure and the length of the vessels and the blood viscosity do not vary appreciably. Thus, changes in regional blood flow are primarily the result of changes in the luminal diameter of the resistance vessels (Folkow, Heymans, and Neil, 1965; Green, Rapela, and Conrad, 1963).

Changes in the luminal diameter of blood vessels may be active or passive (Grayson and Mendel, 1965; Haddy, Overbeck, and Daugherty, 1968). An active change refers to any change which results from an alteration in the contractile state of the vascular smooth muscle. The contractile state is influenced by such factors as autonomic nerves, blood-borne vasoactive substances, locally produced chemicals (metabolites), and physical factors such as transmural pressure. A passive change refers to any change other than an alteration in the contractile state of vascular smooth muscle, for example, distension or collapse of the vessels due to changes in transmural pressure.

The resistance function of a vascular bed is controlled partly by local or intrinsic factors. Contractile activity of vascular smooth muscle decreases when the perfusion pressure decreases or metabolism increases. Conversely, contractile activity increases when perfusion

pressure increases or metabolism decreases. This altered contractile activity results from changes in transmural pressure which evoke a myogenic or Bayliss response (Folkow, 1962) and from changes in the concentrations of vasoactive chemicals such as oxygen, carbon dioxide, hydrogen ions, potassium, adenine nucleotides, etc. (Haddy and Scott, 1968). Examples of local resistance control are seen in skeletal muscle following exercise (exercise hyperemia), following release of arterial occlusion (reactive hyperemia), and following changes in perfusion pressure (autoregulation).

Extrinsic factors also play an important role in the control of resistance vessels. This remote control results from active changes in the contractile activity of vascular smooth muscle due to changes in nerve activity or changes in the concentrations of circulating vasoactive chemicals.

The resistance to flow at any given moment is the net effect of local factors and extrinsic factors exerting their influence on the vascular smooth muscle. Thus, a decrease in arterial pressure activates sympathetic nerves which, in turn, cause an increase in resistance. In autoregulating beds, the decrease in transmural pressure would diminish the contractile activity of the vascular smooth muscle and this, together with an increase in vasodilator metabolites resulting from the decrease in flow, would tend to cause a decrease in resistance to flow.

To analyze the mechanisms of the intestinal and splenic vasoconstriction in response to hemorrhage, the quantitative importance of both intrinsic and extrinsic factors must be determined.

Intrinsic Factors

Green and coworkers (1963) have emphasized the importance of pressure-flow curves in studying the resistance function of vascular beds. The simplest relationship between pressure and flow occurs in "passive" vascular beds. The curves are curvilinear and convex to the pressure axis due to the passive distension of the vessels as pressure increases. With an increase in vasomotor tone the curves are shifted toward the pressure axis so that at any given pressure the flow is less.

In many vascular beds the pressure-flow relationship in passive beds is modified by the occurrence of autoregulation of blood flow. Broadly defined, autoregulation is the ability of an organ to adjust its blood flow in accordance with its needs. The term is more frequently applied to the intrinsic tendency of an organ to maintain a constant blood flow despite changes in the perfusion pressure. The initial response to an abrupt decrease in perfusion pressure is a decrease in flow; however, in autoregulating vascular beds, a secondary adjustment in vascular smooth muscle tone takes place and flow returns towards its control level. An increase in perfusion pressure results in an initial increase in flow and then a secondary decrease towards the flow prior to the increase in pressure. Thus, in contrast to passive vascular beds, resistances decrease as perfusion pressures decrease and the pressure-flow curves are concave to the pressure axis. Autoregulation of blood flow is characteristic of many vascular beds and it has been demonstrated in kidney (Shipley and Study, 1951, dog), brain (Carlyle and Grayson, 1956, sheep, cat, rabbit; Green et al., 1963, dog), skeletal muscle (Folkow and Oberg, 1961, cat), liver (Greenway, Lawson, and Mellander, 1967), and intestine (Johnson, 1960, dog).

Metabolic and myogenic hypotheses have been suggested to explain autoregulatory responses. The metabolic hypothesis accounts for autoregulation on the basis of accumulation and wash-out of locally produced vasodilator substances. It is a flow-dependent mechanism. The myogenic hypothesis suggests that an increase in transmural pressure across the arteriolar wall causes an increase in the rhythmic contractile activity of the vascular smooth muscle. Attempts to distinguish between the two mechanisms have involved elevation of venous pressure. According to the metabolic hypothesis, the resulting decrease in blood flow would result in the accumulation of vasodilator metabolites and thus lead to vasodilatation. However, according to the myogenic hypothesis, the increased intravascular pressure which follows venous pressure elevation should cause vascular constriction. The problem is not entirely resolved. It is probable that both mechanisms are operative and that the role played by each varies considerably from one vascular bed to the next.

Autoregulation of blood flow has been clearly demonstrated in the intestine (Johnson, 1960, dog). Resistance to blood flow generally decreased in the intestine as arterial pressure was reduced from 100 mm Hg to approximately 30 mm Hg (Johnson, 1960, dog; Johnson, 1964, dog; Johnson, 1967, dog). The mechanism of autoregulation was not a local reflex, a change in intestinal volume, or a change in tone of intestinal muscle (Johnson, 1960, dog; Johnson, 1964, dog). Changes in the concentration of metabolites and oxygen tension of tissues were also eliminated. Intestinal autoregulation did not appear to be a flow-dependent phenomenon since arterial constriction was produced by either arterial or venous pressure elevation (Selkurt and Johnson, 1958, dog; Johnson, 1959, dog;

Johnson, 1967, dog). It was concluded that autoregulation of intestinal blood flow was the result of a myogenic response to changes in transmural pressure (Johnson, 1960, dog; Johnson, 1964, dog; Johnson, 1967, dog). The degree of intestinal autoregulation varied considerably in different preparations (Johnson, 1960). Autoregulation diminished as the preparation deteriorated, and the inclusion of an artificial pump system decreased vascular reactivity (Dresel and Wallentin, 1966; Selkurt and Johnson, 1958).

In contrast to the intestinal vascular bed, there is little evidence of "autoregulation of splenic blood flow" to changes in perfusion pressure. Studies in the perfused spleen of the dog showed an approximately linear relationship between flow and the perfusion gradient (Frohlich and Gillenwater, 1963, dog). Similarly, an approximately linear relationship between splenic flow and arterial pressure was found in the cat in which the splenic artery was not cannulated (Greenway and Stark, 1969).

The hepatic arterial resistance vessels are influenced by changes in arterial pressure, portal pressure or flow, and hepatic venous pressure. The relationship of hepatic arterial flow to arterial pressure has been studied in experiments where the hepatic artery was long-circuited or the liver perfused <u>in vitro</u>. Some workers reported autoregulation of blood flow (Hanson, 1964, dog; Takeuchi, Kitagawa, Kubo, Murai, and Tone, 1966, dog; Condon, Chapman, Nyhus, and Harkins, 1962, calf; Torrance, 1961, dog); other workers did not observe autoregulation (Price, McFate, and Shaw, 1964, dog; Shoemaker, 1964, dog; Field and Andrews, 1968, dog). Greenway, Lawson, and Mellander (1967) studied the relationship of hepatic

arterial pressure in cats using a method which did not involve cannulation of the hepatic artery. At pressures above 80 mm Hg, consistent and marked autoregulation of blood flow occurred. The pressure-flow curve was concave to the pressure axis. Autoregulation occurred after denervation of the liver (Hanson, 1964, dog; Greenway, Lawson, and Mellander, 1967) and after the administration of atropine or hexamethonium (Torrance, 1961, dog). In anesthetized animals, a decrease in portal pressure or flow was associated with an increase in hepatic arterial flow (Ackroyd, Mito, and McDermott, 1966, dog; Cohn and Kountz, 1963, dog; Hanson and Johnson, 1966, dog; Price, Britton, Peterson, Reilly, Vorhees, 1965, dog; Ternberg and Butcher, 1965, dog). The results have been confirmed in the conscious dogs (Price et al., 1965). Temporary occlusion of the portal vein in human patients also caused a marked increase in hepatic arterial flow (Schenk, McDonald, McDonald, and Drapanas, 1962). The administration of reserpine (Ternberg et al., 1965, dog) or denervation of the liver (Cohn et al., 1963, dog; Sancetta, 1953, dog) had little effect on the response. An increase in hepatic venous pressure was followed by a marked increase in hepatic arterial resistance (Hinshaw, Reins, and Wittmers, 1965, dog; Lutz, Peiper, and Bauereisen, 1968; Hanson and Johnson, 1966, dog). From these considerations it is clear that the hepatic arterial resistance is increased by increases in hepatic arterial pressure, portal pressure or flow, and hepatic venous pressure. The most probable explanation of these results is that of a myogenic response of the arteriolar smooth muscle to changes in transmural pressure (Hanson and Johnson, 1966, dog; Lutz et al., 1968; Greenway and Stark, 1971). Hanson and Johnson (1966, dog) have shown that the changes in hepatic arterial resistance were influenced to a much greater

extent by changes in portal pressure and in hepatic venous pressure than by changes in hepatic arterial pressure. They pointed out that transmural pressure in the terminal portions of the hepatic arterioles was probably influenced to a greater extent by pressure in the sinusoids than by that in the large hepatic arteries.

Summary: Changes in arterial pressure produce local or intrinsic responses which appear to be largely myogenic. At present, there is little evidence for a role for metabolites in these responses. The responses are prominent in the intestinal and hepatic arterial vascular beds but are weak in the splenic vascular bed. Following hemorrhage, these responses would tend to cause a decrease in resistance to flow. Since the hepatic arterial bed vasodilates in response to hemorrhage, intrinsic factors can account for this response. However, since the intestinal and splenic vascular beds vasoconstrict in response to hemorrhage, the intrinsic control mechanisms must be overidden by extrinsic vasoconstrictor factors.

## Extrinsic Factors

Various extrinsic vasoconstrictor factors which may play important roles in the splanchnic response to hemorrhage include the sympathetic nerves, adrenal medullary secretions, vasopressin, and angiotensin.

## Sympathetic Nerves

The sympathetic nervous system plays a major role in compensatory mechanisms which tend to maintain homeostasis following hemorrhage. Control of the resistance function, capacitance function, and fluid exchange function of peripheral vascular beds by the sympathetic vasoconstrictor fibre system has been reviewed recently by Mellander and

Johansson (1968). Following hemorrhage, the sympathetic nervous system is activated and, in general, this leads to stimulation of the heart, venoconstriction, constriction of arterioles, and alteration of capillary hydrostatic pressure through changes in the pre- to postcapillary resistance ratio. The role of the sympathetic nervous system in the control of these vascular functions during hemorrhage has been reviewed by Chien (1967).

Following hemorrhage there is an increase in total peripheral resistance due to vasoconstriction of the resistance vessels in peripheral vascular beds (see earlier section). The magnitude of the vasoconstriction varies considerably in the different vascular beds. Some beds constrict more than others, while some dilate. Intense vasoconstriction has been observed in the vascular beds of skeletal muscle, skin, kidney, intestine, and spleen (Chien, 1967). The exact mechanism of the vasoconstriction is not clear but most investigators have assumed that the vasoconstriction is due to increased sympathetic nerve activity. However, an increase in resistance to flow does not necessarily imply sympathetic vasoconstriction. Other blood-borne vasoconstrictor factors might also be involved. There is little doubt that sympathetic vasoconstrictor fibres play some role in the resistance response to hemorrhage, but their quantitative importance may differ greatly in different vascular beds.

Vasoconstriction occurs in skeletal muscle after hemorrhage (Haddy, Overbeck, and Daugherty, 1968; Chien, 1967). Rothe, Schwendenmann, and Selkurt (1963) studied the effect of hemorrhage in dogs whose gracilis muscle was perfused at constant pressure with a pump, using blood from a donor dog. The only connection between the bled dog and its gracilis

muscle was through its innervation. There was a decrease in flow and an increase in vascular resistance in the muscle after hemorrhage. Cooling of the nerves eliminated the response. Thus, in these experiments, the nerves caused vasoconstriction. However, this type of experiment does not rule out the possibility that vasoactive substances circulating in the blood of the bled dog could have caused vasoconstriction had they been allowed access to the muscle. In the cat, there was an increase in resistance to flow in the calf muscle under conditions of natural flow (Lundgren, Lundwall, and Mellander, 1964). After the lumbar sympathetic trunks had been sectioned, there was no increase in vascular resistance after hemorrhage (Mellander and Lewis, 1963). Similarly, Oberg (1964) found an increase in vascular resistance in a hindquarter preparation after hemorrhage and the increase in resistance was eliminated by sympathetic denervation. These studies indicate that the vasoconstriction of skeletal muscle resistance vessels is mediated by the sympathetic nerves. Haddy, Scott, and Molnar (1965, dog) reported that active vasoconstriction of the forelimb resistance vessels in response to hemorrhage was due to a baroreceptor-induced sympathico-adrenal discharge and to some nonadrenal, nonrenal circulating vasoconstrictor substance. However, the quantitative importance of the substance in the response was not studied.

It is dangerous to extrapolate results from one vascular bed to another. There is little evidence to indicate that the intestinal and splenic vasoconstriction following hemorrhage is mediated by the sympathetic nerves. However, the responses of the intestinal, splenic, and hepatic arterial vascular beds in the normovolemic cat to postganglionic sympathetic stimulation have been studied in some detail and the possible

responses of these beds to increased sympathetic nervous activity during hemorrhage may be predicted from these studies.

The response of the intestinal resistance vessels to activation of the sympathetic vasoconstrictor fibres is complex. Venous outflow from a segment of small intestine was measured before and during supramaximal stimulation of the splanchnic nerves (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a). The resistance response consisted of two phases. There was an initial pronounced decrease in flow reaching a maximum within 30 to 40 seconds. During the next 1 to 3 minutes of continued stimulation, flow recovered towards the prestimulatory control level and reached a new steady-state level. The decline in resistance to a new steady-state value was called "autoregulatory escape" from the constrictor fibre influence. Both the initial decrease in flow (peak response) and the extent of the "autoregulatory escape" increased markedly with increasing rates of stimulation. At low frequencies of stimulation the steady-state phase was usually only 20 to 40% below the control level. At higher frequencies the steady-state flow values were close to the control values and sometimes were even above the control values. Cessation of stimulation was followed by a marked "reactive hyperemia" which occurred even when the steady-state flow had returned to or above the control level. The same pattern of response was obtained using a constant flow technique (Dresel and Wallentin, 1966). Both the peak response and autoregulatory escape diminished as the preparation deteriorated (Dresel and Wallentin, 1966). Reflex activation of the vasoconstrictor fibres to the intestine by decreased baroreceptor activity elicited a resistance response similar to that described above and the

steady-state resistance was usually only 10 to 25% above control (Oberg, 1964). Thus, the response of the intestinal resistance vessels to vasoconstrictor fibre stimulation is quite different from that in skeletal muscle where the initial large response is well maintained over prolonged periods of stimulation (Mellander, 1960).

The mechanism underlying "autoregulatory escape" in the intestinal vascular bed is not clear. It is unlikely that it is due to failure of adrenergic impulse discharges or to transmitter release since the constrictor response of the small precapillary vessels and of the capacitance vessels is well maintained (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a and 1964b). The phenomenon is not dependent upon a decrease in flow since it also occurs under conditions of constant flow (Dresel and Wallentin, 1966). Intra-arterial infusions of noradrenaline resulted in the same pattern of response as that seen during nerve stimulation; whereas, infusions of vasopressin resulted in a well maintained decrease in flow with no autoregulatory escape (Dresel and Wallentin, 1966). Autoregulatory escape was not blocked by atropine (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a), nor by the β-blocking agent, propranolol, (Mellander and Johansson, 1968). The capillary filtration coefficient, or CFC, was estimated before and during stimulation of the splanchnic nerves (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964b). CFC is determined by volumetric or gravimetric recording of the rate of net fluid filtration produced by a known rise in mean hydrostatic pressure and it indicates the capillary surface area available for exchange (Mellander and Johansson, 1968). Folkow and coworkers (1964b) found that sympathetic activation caused a reduction in CFC which was well maintained

over prolonged periods of stimulation, even though blood flow due to autoregulatory escape had returned to a steady-state level near the control value. The results indicated a decrease in the capillary surface area available for exchange. Cessation of stimulation resulted in a large increase in CFC during the period of reactive hyperemia. The decrease in capillary surface area during stimulation without an increase in resistance to flow suggests a redistribution of the blood flow within the intestine. The distribution of intra-arterial injections of India ink was studied before and during activation of the splanchnic nerves (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964b) and the results suggested a redistribution of blood flow from mucosal to submucosal tissue during nerve stimulation. Unfortunately, these studies did not show that the distribution of the Ink was an accurate reflection of the flow distribution. Richardson and Johnson (1969, dog) found that the degree of autoregulatory escape was not dependent on the initial vascular resistance of the bed. On this basis they argued that a redistribution of blood flow to explain autoregulatory escape was unlikely and they suggested that escape occurs by a secondary relaxation of the same vascular elements affected by the constrictor agent. However, until further evidence is obtained, the mechanism of autoregulatory escape remains unsolved.

In summary, stimulation of the postganglionic nerves does not result in a maintained vasoconstriction of the intestinal resistance vessels and autoregulatory escape occurs. These results suggest that the intestinal vasoconstriction following hemorrhage cannot be mediated by the sympathetic nerves. This argument is supported by the observation that when the intestinal flow is reduced by partial occlusion of the

superior mesenteric artery, the response to sympathetic nerve stimulation is impaired (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a).

Until the recent studies of Greenway and Stark very little was known about the response of the spleen to activation of the sympathetic nerves. Barcroft and Stephens (1927) observed that splenic volume decreased during exercise or hemorrhage and the contraction was dependent on the splenic innervation. Green, Ottis, and Kitchen (1960, dog) found that splenic flow and splenic weight decreased simultaneously during activation of the sympathetic nerves. Other workers have confirmed the increase in splenic vascular resistance during sympathetic nerve stimulation (Boatman and Brody, 1964, dog; Haefely, Hürlimann, and Thoenen, 1965). However, all these studies involved cannulation of the splenic artery. Greenway and Stark were the first to study blood flow and volume simultaneously in the spleen with an uncannulated arterial supply (Greenway, Lawson, and Stark, 1968). They measured splenic flow with a non-cannulating electromagnetic flowmeter probe and recorded splenic weight. Supramaximal stimulation of the splenic nerves resulted in a marked decrease in splenic flow which then recovered towards the control level. After stimulation for 10 minutes, 37% recovery had occurred. The recovery of flow was much slower than that observed during autoregulatory escape in the intestine (Folkow et al., 1964a). Splenic weight decreased more slowly and the response was well maintained. Neither the flow response nor the weight response was impaired by prolonged reductions in splenic blood flow resulting from a lowered arterial pressure (Greenway, Lawson, and Stark, 1968). This was in contrast to the responses in skeletal muscle (Lewis and Mellander, 1962) and intestine (Folkow et al., 1964a).

Thus, the sympathetic nerves may be important in the splenic vasoconstriction following hemorrhage.

The response of the hepatic vascular bed to prolonged electrical stimulation of the hepatic plexus has been studied in cats in which the hepatic artery was not cannulated (Greenway, Lawson, and Mellander, 1967). Hepatic arterial flow decreased markedly during the first 30-40 seconds of stimulation and then gradually recovered towards the prestimulatory control level during the following 2-3 minutes of stimulation. Thus, similar to the intestine, "autoregulatory escape" occurred. Cessation of stimulation resulted in a small reactive hyperemia. The response was the same when a constant flow perfusion technique was used. Reflex activation of the constrictor nerves by occlusion of the carotid arteries also resulted in a similar flow response.

In summary, the results of these studies suggest that the sympathetic nerves may play a role in the splenic vasoconstriction following hemorrhage but they are unlikely to be important in the intestinal and hepatic arterial responses.

Adrenal Medullary Secretions

The roles of adrenaline and noradrenaline released from the adrenal medulla in response to hemorrhage have not been analyzed. However, the extensive study in the cat by Celander (1954) indicates that the quantitative importance of adrenal medullary hormones released after a variety of stimuli is insignificant when compared to the effects of activation of the regional sympathetic nerves.

The responses of the intestinal, splenic, and hepatic arterial

beds to the administration of adrenaline and noradrenaline have been studied in the cat and the possible responses of these vascular beds to these agents released during hemorrhage may be predicted from these studies. Injections of these drugs are of little value since the hormones are not released in a single bolus but are continually secreted. Infusions of the drug provide a better insight into their physiological role.

The intestinal response to intra-arterial infusions of noradrenaline is similar to that following nerve stimulation. There is a peak constrictor response followed by "autoregulatory escape" and a return of resistance towards the control levels (Dresel and Wallentin, 1966; Baker and Mendel, 1967). Infusions of adrenaline, on the other hand, result in increased flow through the superior mesenteric artery and propranolol blocks the response (Greenway and Lawson, 1966a; Ross, 1967a; Greenway and Lawson, 1968).

The splenic flow responses and splenic weight responses to intravenous infusions of catecholamines have been investigated using methods which did not involve cannulation of the splenic artery (Greenway and Stark, 1970). Intravenous infusions of noradrenaline caused a marked decrease in splenic arterial flow and splenic weight and the decreases were well maintained during the 5 minute infusion period. Thus, there was no evidence of autoregulatory escape. The responses were unchanged after administration of propranolol. Intravenous infusions of adrenaline caused a marked decrease in splenic weight but the flow response varied. There was either an increase, a decrease, or no change in flow. After propranolol, adrenaline caused only vasoconstriction which was abolished by phenoxybenzamine. After phenoxybenzamine, adrenaline caused only

vasodilatation which was abolished by propranolol. Thus, the response of the splenic resistance vessels to adrenaline is a balance between the α- and β-receptor actions of the drug while, in the case of noradrenaline, the α-receptor action predominates. Both noradrenaline and adrenaline cause splenic contraction which is mediated by α-adrenergic receptors. Adrenaline was more potent in this respect than noradrenaline (Ahlquist, Taylor, Rawson, and Sydow, 1954, dog; Greenway and Stark, 1970).

The responses of the hepatic vascular bed to infusions of catecholamines have been studied in cats using non-cannulating electromagnetic flowmeters (Greenway, Lawson, and Mellander, 1967; Ross and Kurrasch, 1969). Intra-arterial infusions of noradrenaline caused changes similar to those following nerve stimulation. Hepatic arterial flow initially decreased and then autoregulatory escape occurred. Intra-arterial infusions of adrenaline in small doses produced variable effects. Following the administration of propranolol, vasoconstriction was produced while vasodilatation occurred after phenoxybenzamine (Greenway and Lawson, 1969; Ross and Kurrasch, 1969). Following intravenous infusion of adrenaline, total hepatic flow increased with little change in mean arterial pressure and an increased proportion of the cardiac output was distributed to the liver (Greenway and Lawson, 1966a). There was little change in hepatic arterial flow (Craig and Honig, 1963; Greenway and Lawson, 1966a; Scholtholt, Lochner, Renn, and Shiraishi, 1967, dog). The increased total hepatic flow was due to an increased portal inflow resulting from intestinal vasodilatation (Greenway and Lawson, 1966a; Greenway and Lawson, 1968; Ross, 1967a; Scholtholt *et al.*, 1967, dog).

These results suggest that, similar to nerve stimulation,

noradrenaline may cause a maintained splenic vasoconstriction following hemorrhage but it is unlikely to cause a maintained intestinal or hepatic arterial response. Adrenaline, on the other hand, dilates the intestinal vascular bed and has little effect on the splenic and hepatic arterial resistance vessels.

Vasopressin and Angiotensin

It is well known that large amounts of vasopressin are released in response to hemorrhage in the dog (Weinstein, Berne, and Sachs, 1960), cat (Beleslin, Bisset, Haldar, and Polak, 1967), and rat (Ginsburg and Brown, 1956), although the precise mechanism of release is not clear. Gauer and Henry (1963) postulated that stretch receptors were present in the left atrium and that activation of these receptors inhibited the release of vasopressin from the posterior pituitary. This idea was based on a number of earlier observations which showed that manoeuvers which increased the volume of the left atrium resulted in diuresis, whereas manoeuvers which decreased the volume of the left atrium resulted in antidiuresis (Henry, Gauer, and Reeves, 1956, dog; Murdaugh, Sieker, and Manfredi, 1959, human; Ginsburg, 1954, rat; Weinstein, Berne, and Sachs, 1960, dog). Share and Levy (1962, dog) reported that occlusion of both common carotid arteries in the dog resulted in a marked increase in the blood levels of vasopressin. The response was blocked by the simultaneous inflation of a balloon in the left atrium (Share, 1965, dog). Inflation of the balloon did not block the response if the vagi had been cut (Share, 1965, dog). In both conscious and anesthetized dogs which were subjected to nonhypotensive hemorrhage, there was a significant increase in vaso-

pressin levels (Henry, Gupta, Meehan, Sinclair, and Share, 1968; Share, 1967). However, if the vagi were cut and the carotid sinuses perfused at constant pressure, there were no significant changes in the vasopressin level even when as much as 40% of the blood volume was removed. In dogs anesthetized with chloralose and urethane, a hemorrhage of 8 ml/kg resulted in doubling of the plasma vasopressin level while a hemorrhage of 40 ml/kg resulted in plasma levels of 350 μU/ml, an elevenfold increase above control (Share, 1968, dog).

In the cat, Beleslin et al. (1967) reported that following hemorrhage, the concentration of vasopressin in the blood rose from 16 μU/ml to approximately 750 μU/ml. Oxytocin levels did not increase. Similarly, Clark and Rocha e Silva (1967) found independent release of vasopressin without oxytocin during hemorrhage in the cat. They also found that the afferent limb of the reflex arc for release of vasopressin involved fibres in the sinus nerves and vagi. The levels of vasopressin in this study rose from a control value of 47 μU/ml to 1020 μU/ml 5 minutes after hemorrhage, and remained high at 763 μU/ml 20 minutes after hemorrhage.

There is only limited data indicating possible vascular effects of endogenously released vasopressin. In cross perfusion studies, Christoforo and Brody (1968, dog) found vasoconstriction of the isolated dog gracilis muscle following halothane anesthesia of the donor dog. The vasoconstriction was not due to the direct effect of halothane and was not blocked by phentolamine. However, acute removal of the pituitary abolished the response, suggesting the vasoconstriction was due to vasopressin. A recent study by Rocha e Silva and Rosenberg (1969) published

during the course of the work reported in this thesis also provided some interesting results. They studied the release of vasopressin in response to hemorrhage and the effects of vasopressin infusions on blood pressure in dogs anesthetized with sodium pentobarbital. Hemorrhages which produced a decrease in diastolic pressure of 40 mm Hg resulted in blood levels of vasopressin of 150-350 μU/ml. In hypophysectomized animals whose blood pressure regulating mechanisms were suppressed by division of the vagi and sinus nerves, infusions of vasopressin which produced blood levels similar to those found after hemorrhage increased diastolic pressure by 30-40 mm Hg. They also showed that the high vasomotor tone which followed section of the vagi and sinus nerves was partly due to the presence of the pituitary gland; removal of the gland was followed by a decrease in blood pressure, the time course of which was similar to that which follows cessation of an infusion of vasopressin.

Following hemorrhage, renin is also released and the concentration of angiotensin in the blood is increased. Scornik and Paladini (1964, dog) reported blood levels of 2.4 ng/ml. Regoli and Vane (1966, dog) found an increase in the formation of angiotensin which was similar to infusions of angiotensin at the rate of 0.6 μg/min. Hodge, Lowe, and Vane (1966, dog) reported that mild hemorrhage of 14-26 ml blood/kg caused an increase of 0.25-1.5 μg/min in the rate of generation and an increase of 0.1-0.33 ng/ml in the blood concentration of angiotensin. The changes in the generation rate were not due to changes of renal arterial or venous pressure. They were eliminated by blocking the renal nerves with lignocaine. They showed a consistent inverse correlation with central venous pressure, but not with systemic arterial pressure. These workers concluded

that changes in blood volume bring about changes in the rate of generation of angiotensin by a reflex mechanism the efferent limb of which involved the renal nerves. The mechanism of the afferent limb was not clear, but it might involve vagal afferent impulses arising from atrial receptors (Hodge, Lowe, Ng, and Vane, 1969, dog).

The responses of the intestinal, splenic, and hepatic arterial vascular beds to infusions of vasopressin and angiotensin have not been studied in any detail. In contrast to noradrenaline, intra-arterial infusions of vasopressin cause a maintained vasoconstriction of the intestinal resistance vessels (Dresel and Wallentin, 1966). Other workers (Texter, Chou, Merrill, Laureta, and Frohlich, 1964, dog) reported that both vasopressin and angiotensin cause marked arterial vasoconstriction of the intestinal bed. In this latter study, however, the superior mesenteric artery was cannulated and a pump device was included in the arterial long-circuit. This may account for the high doses of the drugs required in this study to produce a response.

Intravenous infusions of vasopressin caused marked vasoconstriction of the splenic resistance vessels but there was very little effect on the splenic weight response (Greenway and Stark, 1970). Angiotensin constricted the splenic resistance vessels (Benelli, Della Bella, and Gandini, 1964; Boatman and Brody, 1964, dog; Davies, Gamble, and Withrington, 1968b, dog; Greenway and Stark, 1970). However, the effect of angiotensin on splenic volume was not clear. Boatman et al. (1964, dog) and Davies et al. (1968b, dog) found no significant splenic contraction. Greenway and Stark (1970) found no significant contraction when angiotensin was infused intravenously at infusion rates of less than

0.5 μg/min. At higher rates, splenic contraction did occur. Adrenalectomy did not alter the response.

A variety of studies reviewed by Greenway and Stark (1971) have shown that vasopressin reduced portal pressure, portal flow, and total hepatic flow due to vasoconstriction of the splenic and intestinal vascular beds. The direct action of vasopressin on the hepatic arterial bed was vasoconstriction (Shoemaker, 1964, dog; Mahfouz and Aida, 1967, dog). After intravenous administration of vasopressin, hepatic arterial flow increased (Heimburger, Teramoto, and Shumacker, 1960, dog; Peskin, Miller, Johnson, MacVaugh, and Hardesty, 1961, dog) or decreased (Drapanas, Crowe, Shim, and Schenk, 1961, dog). Intravenous infusions of angiotensin have been reported to cause a decrease in total hepatic flow (Bashour, Taha, and Sellers, 1963, dog; Segel, Bayley, Paton, Dykes, and Bishop, 1963, human). This resulted partly from a decreased portal flow due to intestinal (Barer, 1961; Texter, Chou, Merrill, Laureta, and Frohlich, 1964, dog) and splenic (Boatman and Brody, 1964, dog; Greenway and Stark, 1970) vasoconstriction. The direct action of angiotensin on the hepatic arterial bed was vasoconstriction (Kelly and Nyhus, 1966, cow; Scholtholt and Shiraishi, 1968, dog). The effect of intravenous infusions of angiotensin on the hepatic arterial resistance vessels has not been established.

Summary: Large amounts of vasopressin and angiotensin are found in the circulation following hemorrhage. However, there is only limited data indicating possible vascular effects for these agents released endogenously. Furthermore, data on the responses of the intestinal, splenic, and hepatic arterial vascular beds to administration of exogenous vasopressin and angiotensin is not complete. Dose-response curves have not

been obtained and the sensitivities of the vascular beds to these agents have not been compared.

Conclusion and Experimental Design

The present study was devised to investigate the response of the splanchnic resistance vessels to hemorrhage and to investigate mechanisms involved in this response. Previous work has indicated that in response to hemorrhage the intestinal and splenic resistance vessels constrict; the hepatic arterial resistance vessels dilate. However, the mechanisms of these responses are completely unknown. It has usually been assumed that the sympathetic nerves were the most important factor in the intestinal and splenic vasoconstriction. However, other vasoconstrictor factors might also play a role. Vasopressin is released and angiotensin is formed in response to hemorrhage and both of these substances are potent vasoconstrictor agents. The roles of these extrinsic factors in the intestinal and splenic vasoconstriction following hemorrhage were investigated by removal of the major sources of these agents and by local denervation. Thus, the effect of hemorrhage was studied in cats with intact organ systems and in cats subjected to intestinal or splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy in various combinations.

Following hemorrhage, arterial pressure decreases. To quantitate the role of extrinsic factors in the intestinal and splenic vasoconstriction after hemorrhage, the local response of the vascular bed to changes in arterial pressure must be assessed. This may be done by studying the pressure-flow relationship of the vascular bed over a suitable range of

pressures and flows during the pre-hemorrhage control period. The role of extrinsic factors during hemorrhage can then be quantitated by comparing individual observations of pressure and flow during hemorrhage with the control pressure-flow values obtained prior to hemorrhage.

If vasopressin and angiotensin play a role in the splanchnic response to hemorrhage, then intravenous infusions of these agents in amounts likely to be found in the blood after hemorrhage should cause a similar response. Thus, experiments were devised to compare the relative sensitivities of the intestinal, splenic, and hepatic arterial vascular beds to intravenous infusions of vasopressin and angiotensin.

# METHODS

General

Cats of either sex and weighing between 2.2 and 4.0 kg were fasted for 24 hours. Anesthesia was induced by intraperitoneal injection of sodium pentobarbital (30 mg/kg, Nembutal, Abbott Laboratories, Ltd.). When reflex limb and ear movements returned, additional doses of 8 mg were given through a cannula which had been placed in a forelimb cutaneous vein. A heating element, positioned under the operating table, maintained the rectal temperature of the cats at 38°C. The cats respired spontaneously, but to ensure a free airway the trachea was cannulated. Systemic arterial pressure was recorded from a cannula placed in the left femoral artery. The abdomen was opened by a midline incision and the free edges of the peritoneum and skin were sewn together.

A) Response of Intestinal Resistance Vessels to Hemorrhage

1. Preparation: Figure 1 is a diagram of the methods used to measure superior mesenteric arterial flow and pressure. After exposing the superior mesenteric artery close to its origin at the aorta, a 1 cm length of artery was carefully dissected free from the surrounding nerves and tissue. A non-cannulating electromagnetic flowmeter probe was placed around the superior mesenteric artery (see appendix 1) and a micrometer-controlled screw clamp was placed just downstream from the flowmeter probe. The clamp served to steady the flowmeter probe and allowed controlled reductions in superior mesenteric arterial flow and pressure. The flowmeter zero was checked frequently by briefly occluding the artery. At the end of each experiment the flowmeter was calibrated

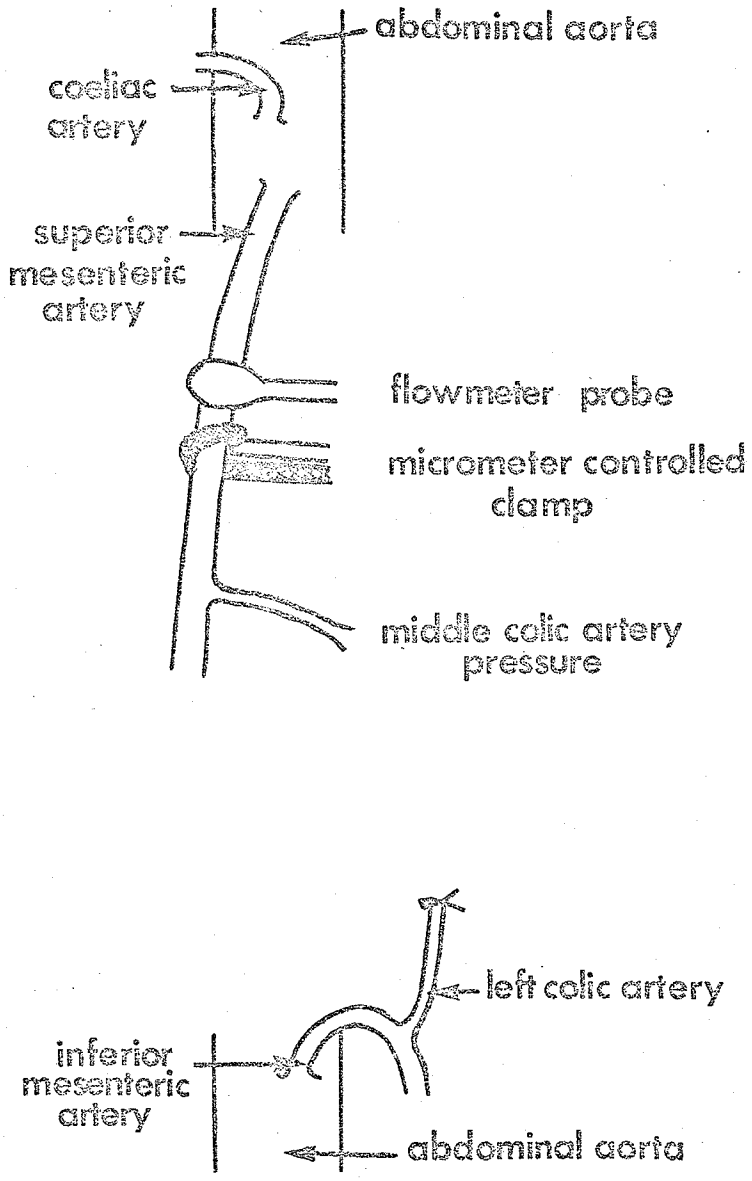


Figure 1. Diagram of the methods used to record superior mesenteric arterial flow and pressure.

(see appendix 1). The anastomsis between the inferior and superior mesenteric arteries, the left colic artery, was ligated. The middle colic artery was cannulated close to its junction with the superior mesenteric artery. The pressure in the superior mesenteric artery downstream from the flowmeter probe and clamp was recorded from this cannula. Anastomoses with the coeliac artery were judged to be of minor significance since occlusion of the superior mesenteric artery close to the aorta caused superior mesenteric arterial pressure to fall below 20 mm Hg. Furthermore, in an experiment where superior mesenteric venous flow was being measured, occlusion of the superior mesenteric artery caused the venous outflow to decrease from 84 ml/min to approximately 2 ml/min. In a few experiments the pressure in the portal vein was measured by inserting a cannula into the portal vein through a small vein draining the appendix. When the abdominal surgery was completed, the abdomen was covered with gauze soaked in warm saline. The animal was left undisturbed for one hour to recover from the surgery.

2. Pressure-flow curves: In each experiment the relationship between superior mesenteric arterial flow and pressure was studied. Prior to bleeding the animal, a pressure-flow curve was obtained by graded clamping of the superior mesenteric artery which reduced superior mesenteric arterial pressure by approximately 20 mm Hg steps at 1 to 2 minute intervals. One hour after reinfusion of the shed blood, a second pressure-flow curve was obtained.

3. Hemorrhage: In all experiments blood was withdrawn through a cannula placed in a femoral artery. The blood was collected in a glass syringe containing heparin (5 mg) and was stored at $38^{o}C$. Two

types of hemorrhages were used - rapid and slow.

Rapid hemorrhage was induced by removing blood at a mean rate of 19 ± 0.78 ml/min/kg body weight (mean ± S.E.) until the blood pressure reached 50 mm Hg. Additional small volumes of blood were withdrawn to maintain the blood pressure at 50 mm Hg for one minute. Thereafter, no more blood was removed and the blood pressure was allowed to recover. The shed blood was returned to the animal 60 minutes following the start of hemorrhage. In some animals a second hemorrhage was induced, after the second pressure-flow curve had been obtained.

Slow hemorrhage was induced by removing blood over 25 minutes at the rate of 0.6 ml/min/kg by a Harvard constant infusion/withdrawal apparatus. Thereafter, no more blood was removed. The shed blood was returned to the animal 60 minutes following the start of hemorrhage. In some animals a second hemorrhage was induced.

In a few experiments, animals were bled slowly and continuously until the arterial pressure reached 50 mm Hg. Thereafter, the arterial pressure was maintained near 50 mm Hg for several hours by either removing or returning small additional volumes of blood.

4. Additional procedures: The following procedures were carried out in certain of the cats as described in the results.

The intestinal vascular bed was denervated by ligation and section of the nerves and tissue surrounding the superior mesenteric artery. Cotton wool soaked in 1% procaine was placed around the artery. Ligatures were tied around the adrenal glands and the glands removed. In these experiments hydrocortisone (5 mg/kg, Solu-Cortef, Upjohn Co.)

was administered intramuscularly (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a; Greenway and Stark, 1969). The kidneys were removed by ligation and section of the renal vessels and ureters. The pituitary gland was removed in the following way. A mid-line incision was made through the soft palate and the pterygoid hamuli were located. A line between these crossed the saggital mid-line at the site of a small foramen. This was the surface marking of the pituitary. A trephine (6 mm diameter) was used to penetrate the sphenoid. Bleeding from the bone was controlled with bone wax. The pituitary was identified and the meninges covering it were divided. The gland was then removed by suction. If bleeding occurred, it was quickly stopped with Surgicel (Johnson and Johnson). Blood loss during and after the hypophysectomy was seldom more than 1-2 ml. The completeness of the hypophysectomy and the other procedures was verified by dissection at the end of each experiment.

5. Drugs: Phenoxybenzamine hydrochloride (Dibenzyline, Smith, Kline, and French) was dissolved in propylene glycol (250 mg in 25 ml) acidified with 4 drops of 10 N HCL. Prior to injection, the required dose was diluted with 5 volumes of 0.9% NaCl.

A stock solution containing noradrenaline tartrate (British Drug Houses) in distilled water (1 mg base per ml solution) was diluted with 0.9% NaCl containing 0.2 mg/ml of ascorbic acid. The final concentration of noradrenaline was 1 $\mu$g/ml.

6. Calculation of results: Comparison of the changes in intestinal vascular resistance before and after hemorrhage is not the best method to assess the role of extrinsic vasoconstrictor factors. Changes in

calculated resistance may be due to extrinsic influences acting on the vascular smooth muscle elements such as circulating vasoconstrictor agents but they may also be due to local adjustments in response to changes in arterial pressure. Following hemorrhage, arterial pressure decreased and the pressure-flow curve for the intestinal vascular bed was not linear. Therefore, the effects of extrinsic factors were quantitated by comparing the actual flows at any time to the flows expected from the changes in arterial pressure. These latter flows were determined from the pressure-flow curves and, throughout this thesis, they will be referred to as the passive flows. The actual flows were expressed as percentages of the passive flows. Thus, the extent of deviation from 100% represents the extent to which flows were altered by extrinsic factors. This method of expressing the results also allowed comparison of the responses in different animals in which the arterial pressure values differed.

B) Response of Splenic Resistance Vessels to Hemorrhage

1. Preparation: The response of the splenic vascular bed to hemorrhage was studied in a similar way to that described for the intestinal vascular bed. Figure 2 is a diagram of the preparation. Because of the small size of the splenic artery, the flowmeter probe was placed on the coeliac artery after tying all its branches with the exception of the splenic artery. This was done as follows. The vessels in the gastro-splenic ligament and the inferior part of the lieno-renal ligament were tied. The ligaments were then divided to allow mobilization of the spleen. The pancreatic branches of the splenic artery were

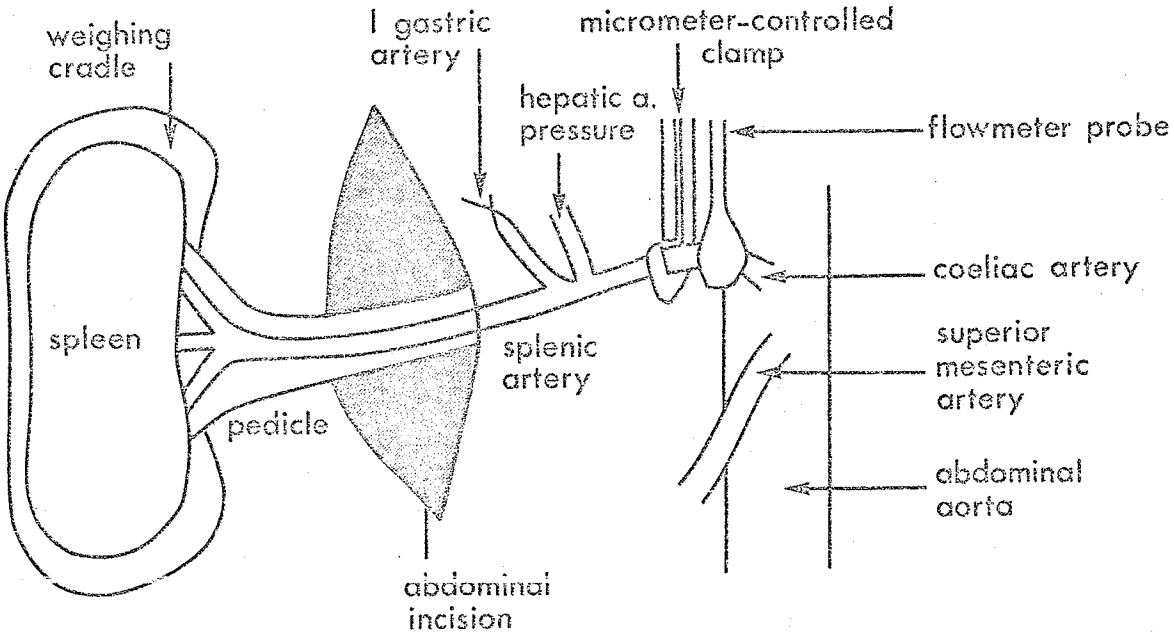


Figure 2. Diagram of the methods used to record splenic weight and splenic arterial flow and pressure.

tied. The left gastric arterial branch and the hepatic arterial branch of the coeliac artery were also tied. Therefore, all branches of the coeliac artery with the exception of the splenic artery were tied and flow through the coeliac artery was a measure of splenic flow. After exposing the coeliac artery close to its origin at the aorta, an approximately 1 cm length of artery was carefully dissected free from the surrounding tissue. This section of artery was just proximal to the point where the nerves from the coeliac ganglion joined the coeliac artery. A non-cannulating electromagnetic flowmeter probe was placed around the artery (see appendix 1). A micrometer-controlled screw clamp was placed immediately downstream from the probe. The clamp served to steady the flowmeter probe and allowed controlled reductions in splenic arterial flow and pressure. The flowmeter zero was frequently checked by briefly occluding the artery. A cannula was placed in the hepatic branch of the coeliac artery so that its tip lay close to the junction of the two arteries. Pressure recorded from this cannula was a measure of splenic arterial pressure downstream from the flowmeter probe and clamp.

Changes in splenic weight were also recorded. The spleen was wrapped in gauze, soaked in warm saline solution and then wrapped in a thin polyethylene sheet. It was then placed on a weighing cradle suspended from a force-displacement transducer (Grass FTO3C) calibrated to record the splenic weight (see appendix 1). The signal from the transducer was fed into a Beckman dynograph (Type R). The height of the cradle was adjusted so that it hung freely just above the abdomen. At the end of every experiment the pedicle was tied and cut. A change

in recorded weight caused by this procedure was due to tension on the pedicle. This weight was, therefore, subtracted from the values obtained during the experiment. It was assumed that the pedicle tension remained constant throughout the experiment. This assumption was shown to be valid since if the splenic pedicle is tied but not cut there is no change in weight over several hours (Greenway, Lawson, and Stark, 1968).

2. Pressure-flow curves: In each experiment the relationship between splenic arterial flow and pressure was studied in a manner similar to that described for the intestinal vascular bed.

3. Hemorrhage: A Harvard constant infusion/withdrawal apparatus was used to withdraw blood through a cannulated femoral artery. Blood was removed for 20 minutes at the rate of 0.68 ml/min/kg body weight. Thereafter, no further blood was removed. Sixty minutes after the onset of hemorrhage the blood was reinfused intravenously. In some animals a second hemorrhage was induced.

4. Additional procedures: The following procedures were carried out in certain of the cats as described in the results.

The splenic vascular bed was denervated by ligation and section of the nerves and tissue surrounding the splenic artery. Cotton wool soaked in 1% procaine was placed round the artery. Adrenalectomy, nephrectomy, and hypophysectomy were carried out as described above.

C) Responses of the Intestinal, Splenic, and Hepatic Resistance Vessels to Infusions of Vasopressin and Angiotensin.

1. Preparations: In one group of animals superior mesenteric arterial flow was measured as described above (page 33). In a second group of

animals splenic arterial flow was measured also as described above (page 38). To measure the response of the hepatic arterial resistance vessels, a method was used similar to that described by Greenway, Lawson, and Mellander (1967). Figure 3 is a diagram of the preparation. The splenic vessels were ligated and the spleen removed. The splenic artery was cannulated so that the tip of the cannula lay close to the junction of the splenic artery with the coeliac artery. The left gastric artery was tied. The only remaining branch of the coeliac trunk was the common hepatic artery. The gastroduodenal branch of the common hepatic artery was tied. Flow through the coeliac artery was now a measure of hepatic arterial flow. Similar to the measurement of splenic arterial flow, the coeliac artery was exposed close to its origin at the aorta and an approximately 1 cm length of artery was dissected free from surrounding tissue. A non-cannulating electromagnetic flowmeter probe was placed around this portion of artery and a micrometer-controlled screw clamp was placed immediately downstream from the probe.

Occlusion of the common trunk of the hepatic artery at its point of division into the hepatic and gastroduodenal arteries gave the same zero flow reading as occlusion of the coeliac artery with the screw clamp. This confirmed that there were no untied branches between these two points. Branches from the hepatic artery to the gall bladder were not tied. Collateral circulation to the liver (e.g. phrenic arteries) was probably small since occlusion of the coeliac artery caused hepatic arterial pressure to fall below 20 mm Hg. Other studies have shown that less than 5% of the total liver

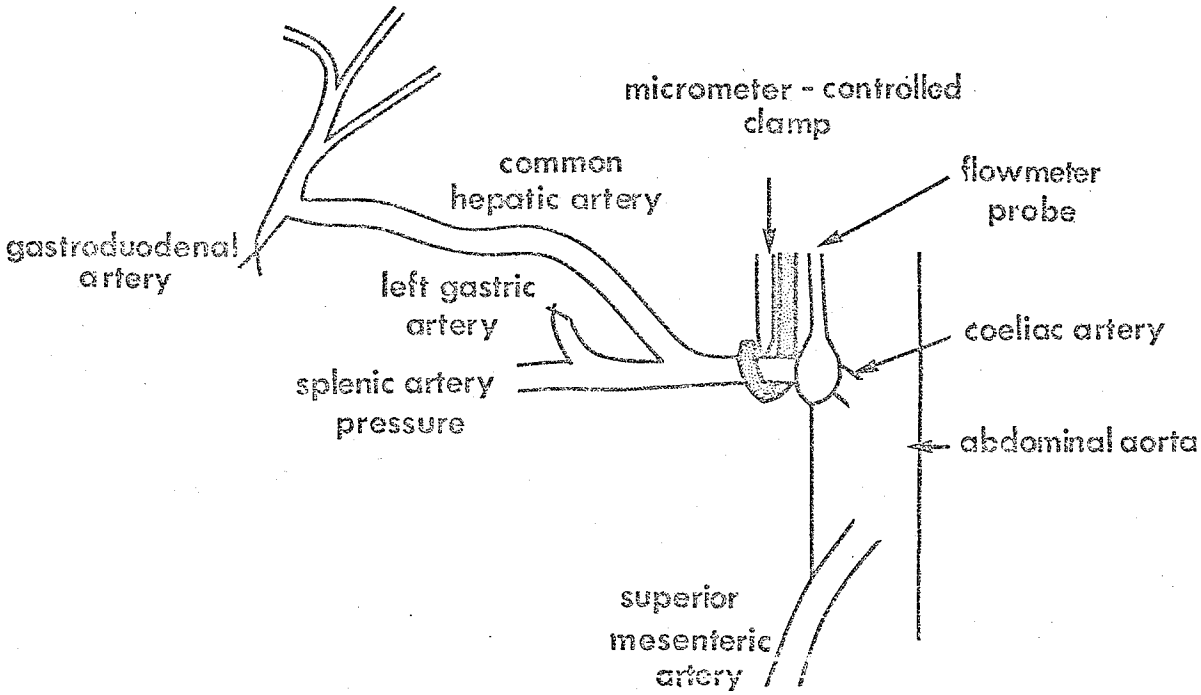


Figure 3. Diagram of the methods used to record hepatic arterial flow and pressure.

flow comes from vessels other than the hepatic artery and portal vein (Greenway and Lawson; 1966).

In each series of experiments, arterial pressure was recorded downstream from the probe and clamp. As arterial pressure increased during drug infusions, the clamp was progressively tightened to maintain arterial pressure in the vascular bed constant.

2. Infusions: Angiotensin (Hypertensin, Ciba Co., Ltd.) and vasopressin (Pitressin, Parke, Davis and Co., Ltd.) were diluted with 0.9% NaCl solution and infused into a cutaneous forelimb vein by a Harvard constant infusion/withdrawal pump. The infusions were continued for 6 minutes, by which time flow was steady at its new level.

3. Expression of results: In contrast to resistance, conductance is probably the more satisfactory method of expressing responses of a vascular bed in which blood flow changes (Stark, 1968). Conductance was calculated by dividing the flow through the vascular bed by the arterial pressure difference across it. It was calculated 1 minute before and at the 5 minute mark during each infusion. The conductance during infusion was expressed as a percentage of that before infusion began. In this way dose-response curves were obtained for both drugs on each vascular bed.

# SECTION I: RESPONSE OF THE INTESTINAL RESISTANCE VESSELS TO HEMORRHAGE

Pressure-Flow Relationships

In each animal the relationship between superior mesenteric arterial flow and arterial pressure was studied. Approximately one hour after the completion of surgery and 10 to 20 minutes prior to hemorrhage, a pressure-flow curve was obtained by graded clamping of the superior mesenteric artery as described in the methods. The recording from a typical experiment is shown in figure 4.

The absolute values and slopes of the pressure-flow graphs varied in the different animals. Therefore, in any one cat, the pressure-flow relationship during hemorrhage was compared to the pressure-flow curve obtained in the same cat. For this reason, a pressure-flow graph of mean values would have little significance. Therefore, as examples, three experiments from each group of animals were chosen by random numbers. The pressure-flow curves from these experiments for Groups 1 to 5 are shown in figure 5. The graphs from groups 1s, 2s, and POB are shown in figure 6. In general, the curves were concave to the pressure axis and usually flow ceased at pressures below 20 mm Hg. The second pressure-flow curve obtained one hour after reinfusion of the shed blood was usually very similar to the first pressure-flow curve.

Effect of Hemorrhage in Cats with Intact Organ Systems (Group 1)

The responses to 10 hemorrhages were studied in 5 cats in which the intestinal nerves, the adrenal glands, the kidneys, and the pituitary gland were all intact. Hemorrhage was induced by withdrawing blood rapidly as described in the methods.

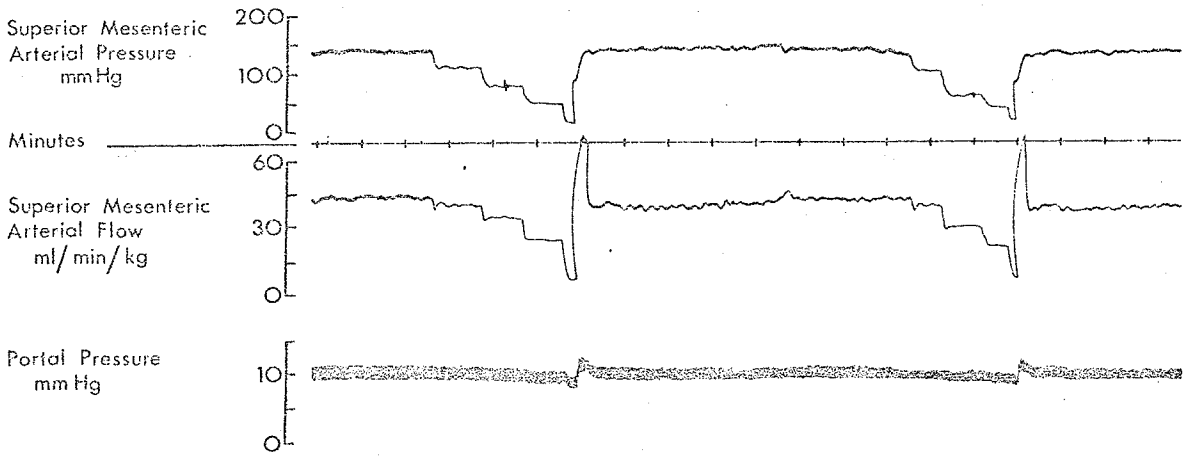


Figure 4. Recording from a typical experiment showing superior mesenteric arterial flow and arterial pressure during graded clamping of the superior mesenteric artery.

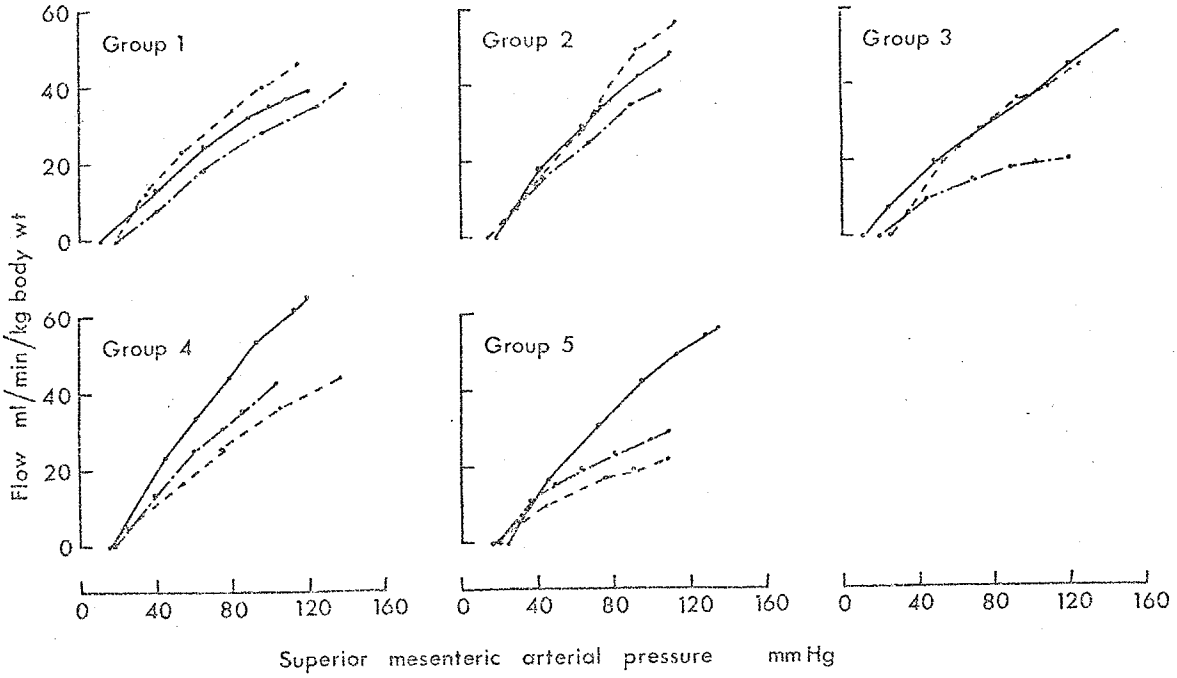


Figure 5. Examples chosen by random numbers of pressure-flow graphs. Group 1 - cats with intact organ systems; Group 2 - cats subjected to intestinal denervation, adrenalectomy, nephrectomy, hypophysectomy; Group 3 - cats subjected to intestinal denervation, adrenalectomy, nephrectomy; Group 4 - cats subjected to intestinal denervation, adrenalectomy, hypophysectomy; Group 5 - cats subjected to nephrectomy, hypophysectomy.

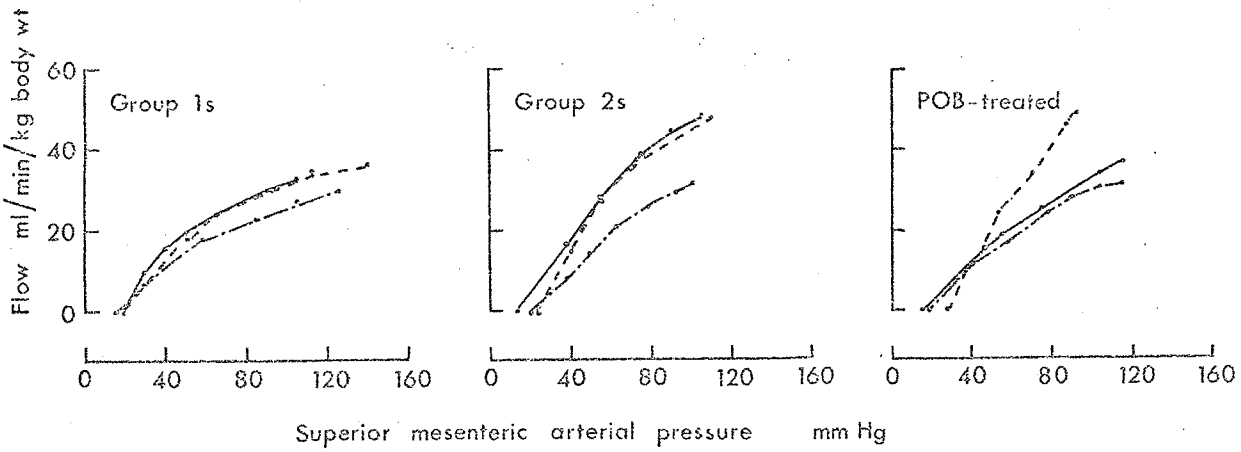


Figure 6. Examples chosen by random numbers of pressure-flow graphs. Group 1s - cats with intact organ systems; Group 2s - cats subjected to intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy; POB-treated - organ systems intact.

The recording from a typical experiment is shown in figure 7. Femoral arterial pressure and superior mesenteric arterial pressure are similar provided there is no obstruction by the flowmeter probe. The solid line is the actual flow recording. During the hemorrhage arterial pressure and superior mesenteric arterial flow decreased. When the withdrawal of blood was stopped, arterial pressure recovered towards the control level but flow recovered very little. Since a pressure-flow curve had been obtained prior to the hemorrhage, it was possible to calculate the flow which would be expected at the various arterial pressures which occurred after hemorrhage. This flow is shown by the broken line in figure 7 and is referred to as the passive local response of the vascular bed to hemorrhage. Since the actual flow response deviates substantially from this line, extrinsic factors must be causing the vasoconstriction. To quantitate the response, the actual flow was expressed as a percentage of the passive flow. These values are shown in the bottom of the figure. The value of 100% indicates that the actual flow at this pressure was the same as that expected from the pressure-flow curve and the extent of deviation from 100% represents the extent to which flow was altered by extrinsic factors. Following hemorrhage flow fell to 62%, then to 48% of that expected from the change in arterial pressure.

The responses to the second hemorrhage were not significantly different ($p > .3$ paired t-test) from those to the first hemorrhage and the data were pooled. The mean volume of blood removed from this group of animals in order to reduce the arterial pressure to 50 mm Hg and to maintain it there for one minute was $20.0 \pm 2.4$ ml/kg (mean $\pm$ S.E.) of body weight (Table 1).

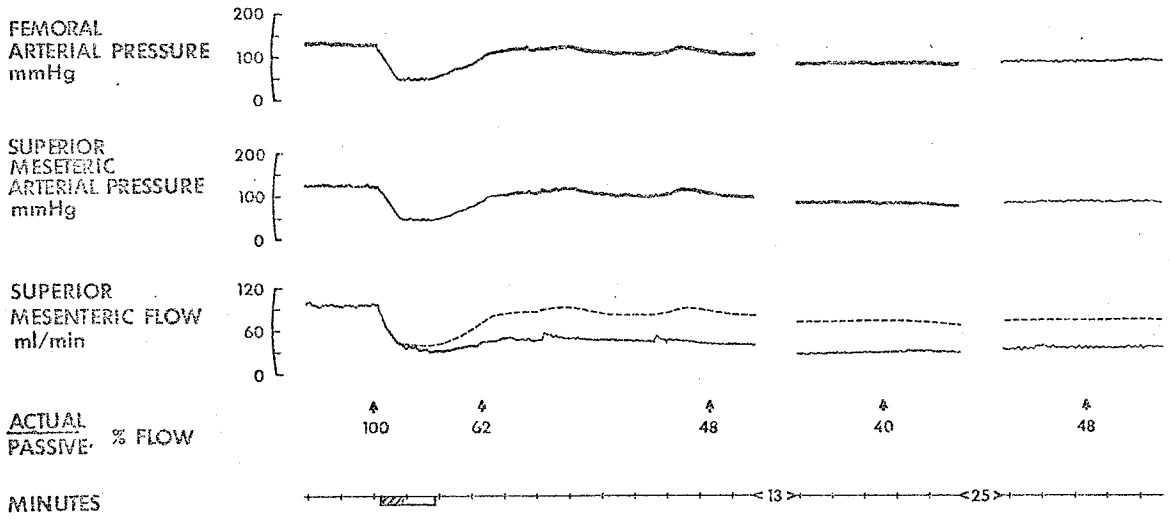


Figure 7. Response to rapid hemorrhage in a typical experiment in Group 1. Hatched signal represents period of blood removal to reduce arterial pressure to 50 mm Hg. Open signal represents period of blood removal to maintain arterial pressure at 50 mm Hg for one minute. The broken line represents the passive flow expected due to the decrease in arterial pressure, calculated from the pressure-flow curve for this cat.

TABLE 1

Means ± S.E. of the values for body weight, initial rate of blood removal, and volume of blood removed in the different groups of cats subjected to rapid hemorrhage.

| | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Body Weight (kg) | 2.8 ± 0.18 | 2.7 ± 0.21 | 3.4 ± 0.07 | 2.7 ± 0.27 | 3.1 ± 0.13 |
| Initial rate of blood removal (ml/min/kg) | 22 ± 2.5 | 19 ± 1.5 | 20 ± 1.7 | 23 ± 2.2 | 18 ± 2.2 |
| Volume of blood removed (ml/kg) | 20 ± 2.4 | 10 ± 1.0 | 19 ± 1.5 | 17 ± 1.7 | 11 ± 1.0 |

The mean arterial pressures and flows before and after hemorrhage are shown in Table 2. The arterial pressure prior to hemorrhage was 122 ± 3.8 mm Hg (mean ± S.E.). Following hemorrhage arterial pressure recovered to 87 ± 6.5 mm Hg within 10 minutes and 96 ± 6.5 mm Hg at 60 minutes.

The flow response of these animals to hemorrhage was calculated and the results are shown in figure 8. Within 5 minutes of the onset of hemorrhage, flow fell to 45 ± 4.7% (mean ± S.E.) of that expected from the decrease in arterial pressure as determined from the pressure-flow curve. Flow remained at the approximately 45% level for the remaining 55 minutes. At all times between 5 and 60 minutes, the values were significantly different from the pre-hemorrhage value measured at the time zero ($p < .001$, paired t-test).

Thus, following hemorrhage there is a marked vasoconstriction of the intestinal resistance vessels and this vasoconstriction is well maintained.

Effect of Hemorrhage in Cats Subjected to Intestinal Denervation, Adrenalectomy, Nephrectomy, and Hypophysectomy (Group 2).

The responses to 6 hemorrhages were studied in 6 cats which were subjected to denervation of the intestine, adrenalectomy, nephrectomy, and hypophysectomy. Hemorrhage was induced by withdrawing blood rapidly as described in the methods. The arterial pressures and flows before and after hemorrhage are shown in Table 2. The blood pressure prior to hemorrhage was 102 ± 5.4 mm Hg and this was significantly lower than in the animals of Group 1 ($p < .01$, unpaired t-test).

Table 2

Means ± S.E. of the pressures and flows in different groups of cats subjected to rapid hemorrhage.

| | | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|---|
| Arterial pressure | - before hemorrhage | 122 ± 3.8 | 102 ± 5.4 | 118 ± 3.0 | 118 ± 5.4 | 110 ± 5.4 |
| mm Hg | - 2 min after hemorrhage | 51 ± 0.6 | 50 ± 1.8 | 52 ± 1.4 | 52 ± 1.1 | 51 ± 0.4 |
| | - 60 min after hemorrhage | 96 ± 6.5 | 76 ± 8.0 | 91 ± 7.5 | 85 ± 6.2 | 73 ± 8.3 |
| Mesenteric flow | - before hemorrhage | 43 ± 2.7 | 47 ± 2.9 | 34 ± 4.2 | 48 ± 3.4 | 36 ± 5.4 |
| ml/min/kg | - 2 min after hemorrhage | 13 ± 2.0 | 19 ± 2.4 | 9 ± 0.9 | 16 ± 1.4 | 16 ± 2.0 |
| | - 60 min after hemorrhage | 17 ± 1.6 | 27 ± 3.5 | 12 ± 1.2 | 15 ± 1.9 | 18 ± 3.5 |

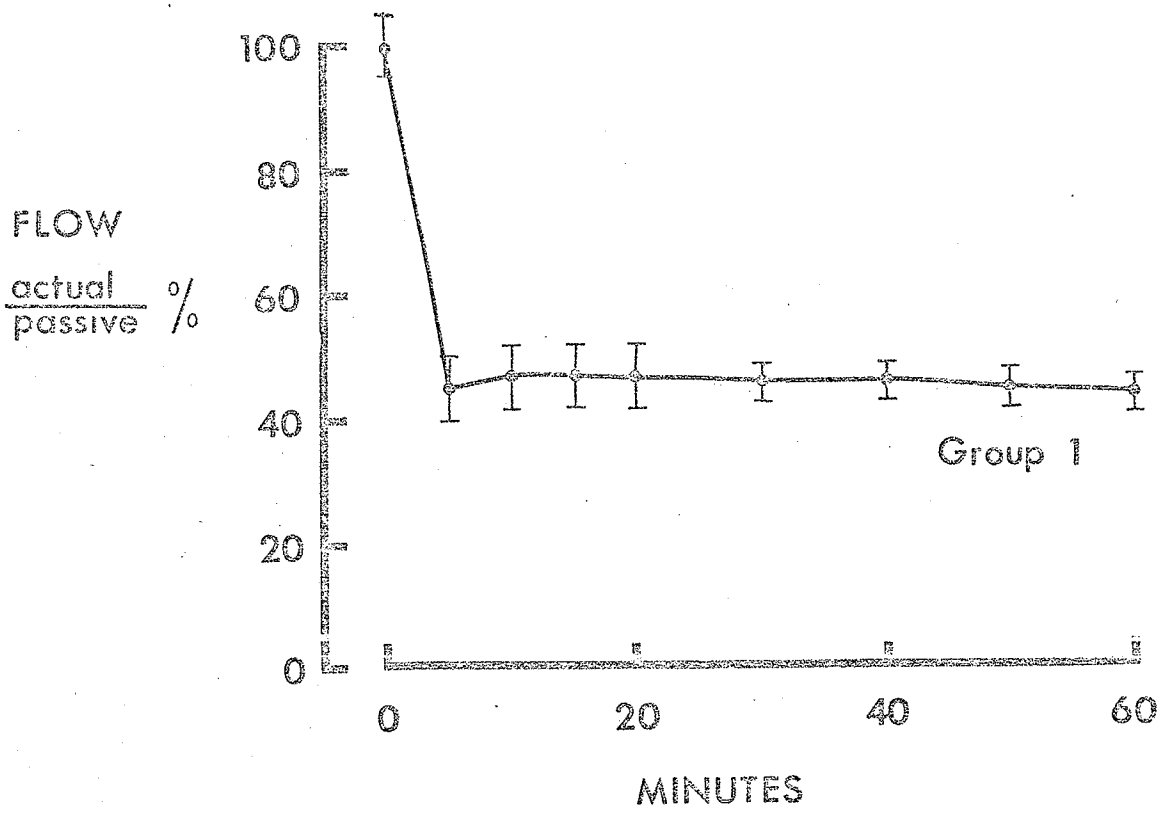


Figure 8. Response to rapid hemorrhage in cats with intact organ systems (Group 1). The response is shown as the means ± S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

During the period of hypovolemia, the arterial pressure recovered to 75 mm Hg after 10 minutes and 76 mm Hg after 60 minutes. This was a smaller recovery in arterial pressure than in Group 1.

The flow response to extrinsic factors was calculated and the results are shown in figure 9 (Group 2). Flow decreased and remained between 75% and 82% of that expected from the decrease in arterial pressure as determined from the pressure-flow curve. These values were significantly different from the pre-hemorrhage control value ($p < .025$, paired t-test). However, at all times between 5 and 60 minutes the values for Group 2 were significantly different from those for Group 1 ($p < .001$, unpaired t-test).

Therefore, although a small vasoconstriction remained, intestinal denervation together with adrenalectomy, nephrectomy, and hypophysectomy greatly reduced the vasoconstriction of the intestine following hemorrhage.

In this group of cats, the volume of blood removed in order to reduce the arterial pressure to 50 mm Hg and maintain it there for one minute was only 10 ml/kg body weight (Table 1). This was a significantly smaller volume of blood removed than in Group 1 ($p < .01$, unpaired t-test). It seemed most likely that this was the consequence of the inability of these animals to vasoconstrict. However, another explanation was possible; the smaller vasoconstriction in these animals may have been due to the smaller reduction in blood volume even though similar degrees of hypotension had been produced. To investigate this second possibility, more experiments were carried out in which similar volumes of blood were removed slowly and continuously.

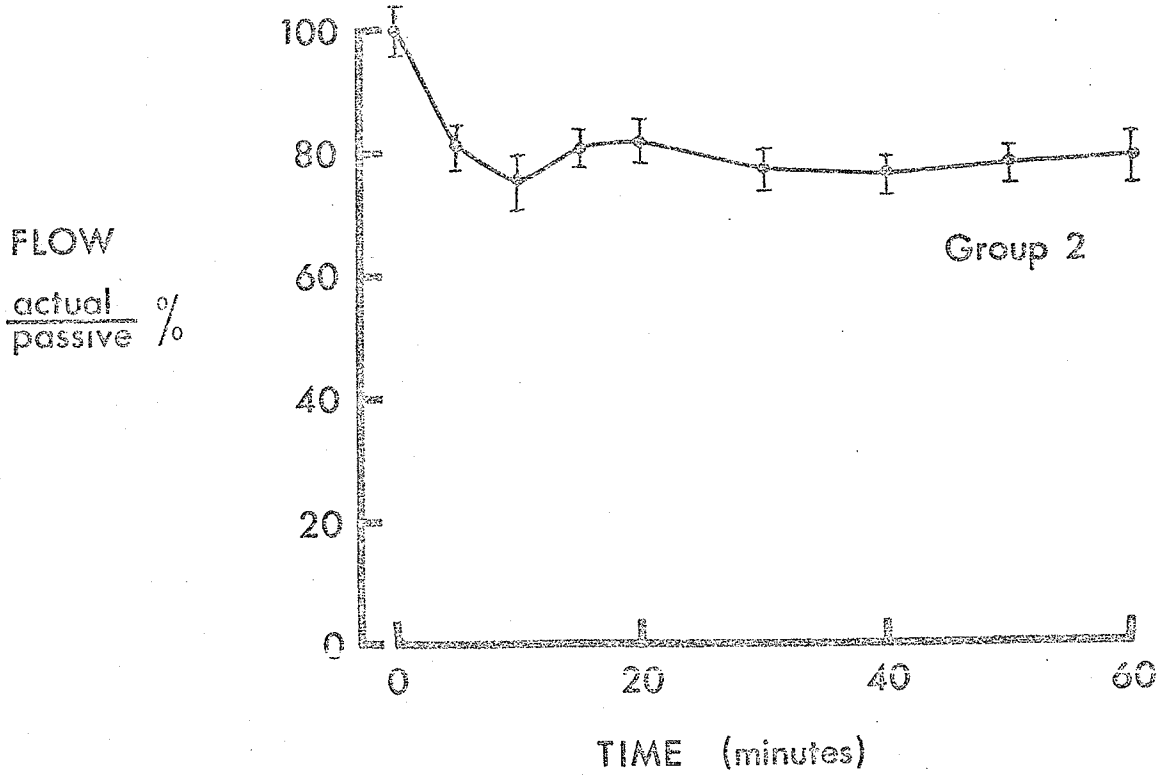


Figure 9. Response to rapid hemorrhage in cats subjected to intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 2). Response is shown as the means ± S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

Effect of Removing Similar Volumes of Blood in Cats with Organ Systems Intact (Group 1s) and in Cats Subjected to Intestinal Denervation, Adrenalectomy, Nephrectomy, and Hypophysectomy (Group 2s).

Two groups of animals were bled by removing blood at the rate of 0.6 ml/min/kg for 25 minutes as described in the methods. The arterial pressures and the superior mesenteric arterial flows before and after hemorrhage are shown in Table 3.

The responses to 6 hemorrhages were studied in 4 cats in which the intestinal nerves, the adrenal glands, the kidneys, and the pituitary gland were all left intact (Group 1s). Prior to hemorrhage, mean arterial pressure was 129 $\pm$ 6.8 mm Hg. During the 25 minute period of blood removal, arterial pressure decreased to 90 $\pm$ 8.5 mm Hg. Over the following 35 minutes, it decreased further to 78 $\pm$ 8.7 mm Hg. The vasoconstrictor response to extrinsic factors was calculated and the results are shown in figure 10. During the period of blood removal, flow decreased to 32 $\pm$ 5.7% of that expected from the decrease in arterial pressure. Flow remained between 32% and 36% during the following 35 minutes of hypovolemia. At all times between 5 and 60 minutes the values were significantly different from the pre-hemorrhage value measured at time zero ($p < .005$, paired t-test). Thus, removing blood slowly and continuously results in an intestinal vasoconstriction which is very similar to the vasoconstriction seen after rapid hemorrhage (Group 1).

In another 7 cats the intestinal nerves were divided and the adrenal glands, kidneys, and pituitary gland were removed (Group 2s). Prior to hemorrhage, mean arterial pressure was 101 $\pm$ 4.0 mm Hg. This

TABLE 3

Means ± S.E. of the values for body weight, pressures, and flows in different groups of animals subjected to slow hemorrhage.

| | | Group 1s | Group 2s | Group POB - treated |
|---|---|---|---|---|
| Body weight (kg) | | 3.2 ± 0.16 | 3.1 ± 0.29 | 3.0 ± 0.34 |
| Arterial pressure (mm Hg) | - before hemorrhage | 129 ± 6.8 | 101 ± 4.0 | 110 ± 6.9 |
| | - 25 min after hemorrhage | 90 ± 8.5 | 65 ± 7.0 | 73 ± 7.8 |
| | - 60 min after hemorrhage | 78 ± 8.7 | 71 ± 7.2 | 88 ± 12.9 |
| Mesenteric flow (ml/min/kg) | - before hemorrhage | 33 ± 1.7 | 43 ± 4.4 | 34 ± 3.4 |
| | - 25 min after hemorrhage | 7 ± 1.3 | 20 ± 3.0 | 11 ± 0.9 |
| | - 60 min after hemorrhage | 8 ± 0.6 | 23 ± 3.1 | 12 ± 1.1 |

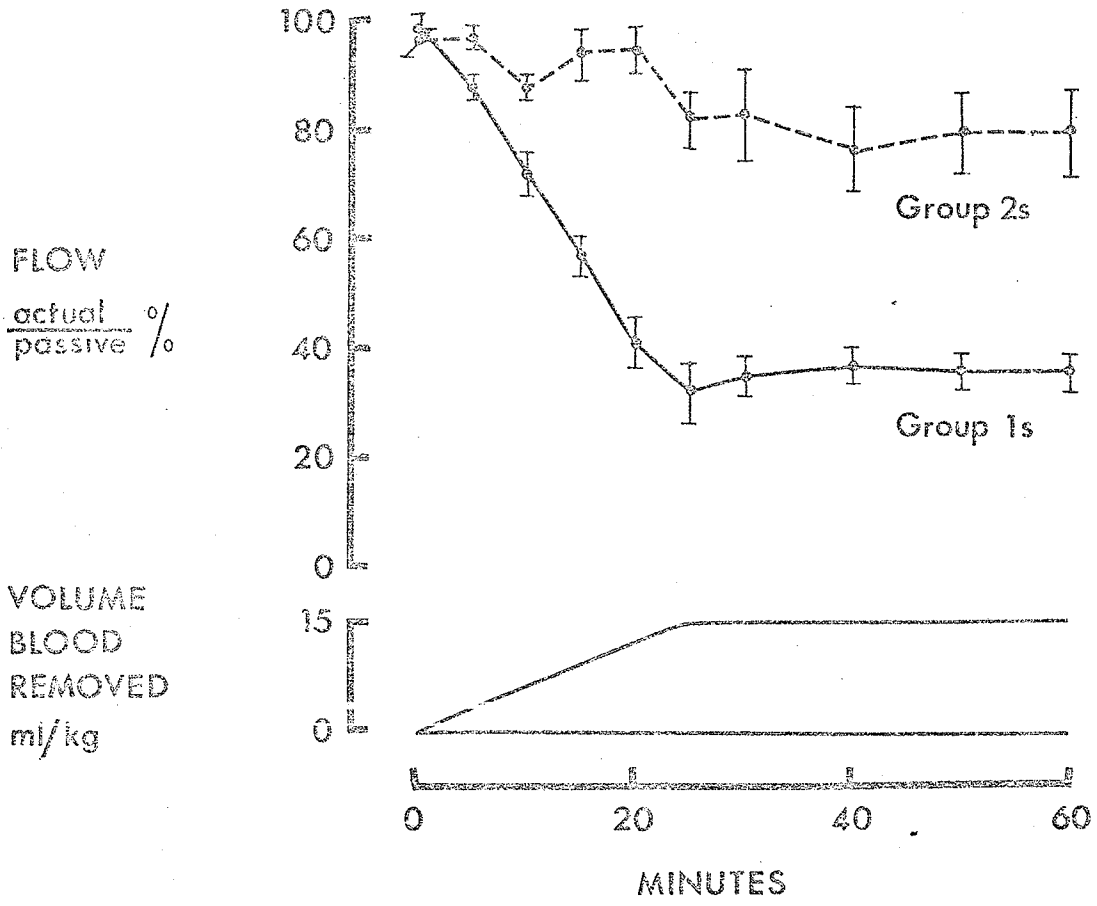


Figure 10. Responses during and after slow, continual removal of blood. Group 1s - cats with intact organ systems; Group 2s - cats subjected to intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy. The responses are shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

was a significantly lower pre-hemorrhage arterial pressure than in animals of Group 1s ($p < .005$, unpaired t-test). During the period of blood removal, arterial pressure decreased to 65 $\pm$ 7.0 mm Hg. This was significantly lower than the corresponding value in Group 1s ($p < .05$, unpaired t-test). Pressure then recovered slightly to 71 $\pm$ 7.2 mm Hg over the next 35 minutes. The vasoconstrictor response was calculated and the results are shown in figure 10. During the period of blood removal, flow decreased slightly to 82 $\pm$ 5.1% of that expected from the pressure-flow curve. This was significantly different from the pre-hemorrhage value ($p < .05$, paired t-test). Flow remained between 77 $\pm$ 7.5% and 83 $\pm$ 8.4% over the next 35 minutes. At all times between 5 and 60 minutes the extent of vasoconstriction in these cats was significantly less ($p < .025$, unpaired t-test) than in cats with intact organ systems (Group 1s). Therefore, when identical volumes of blood were removed from the two groups of animals, the degree of hypotension produced was greater and the vasoconstriction was much smaller in the group in which the intestine was denervated, and the adrenals, kidneys, and pituitary removed. These experiments suggest that in Group 2 (effect of rapid hemorrhage in cats subjected to intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy) the small volume of blood removed acutely to reduce the arterial pressure to 50 mm Hg was a consequence of and not the cause of the smaller vasoconstriction.

Summary: After hemorrhage there was a marked and sustained vasoconstriction of the intestinal resistance vessels. The vasoconstriction was greatly reduced by intestinal denervation, adrenalectomy,

nephrectomy, and hypophysectomy. The results were the same whether a given degree of hypotension was produced or similar volumes of blood were removed and whether the hemorrhage was rapid or slow.

These experiments did not provide any insight into whether one factor was responsible for the vasoconstriction or whether several were contributing to the overall response. Therefore, experiments were designed to investigate the role of each of the factors in the overall response.

Effect of Hemorrhage in Cats Subjected to Intestinal Denervation, Adrenalectomy, and Nephrectomy - Pituitary Intact - (Group 3).

The responses to 9 hemorrhages were studied in 6 cats in which the intestinal nerves were divided and the adrenal glands and kidneys removed; the pituitary gland was left intact (Group 3). Hemorrhage was induced by withdrawing blood rapidly as described in the methods. The volume of blood removed is shown in Table 1. The arterial pressures and superior mesenteric arterial flows before and after hemorrhage are shown in Table 2. Arterial pressure prior to hemorrhage was 118 $\pm$ 3.0 mm Hg. This was very similar to the corresponding value in Group 1. Following the removal of blood, the arterial pressure recovered to 85 $\pm$ 7.5 mm Hg at 10 minutes and then 91 $\pm$ 7.5 mm Hg at 60 minutes. The vasoconstrictor response was calculated and the results are shown in figure 11. Within 5 minutes, flow decreased to 33 $\pm$ 3.6% of that expected from the decrease in arterial pressure. Flow recovered slightly to 41 $\pm$ 5.0% during the remaining 55 minutes. At all times between 5 and 60 minutes, the values were not significantly different

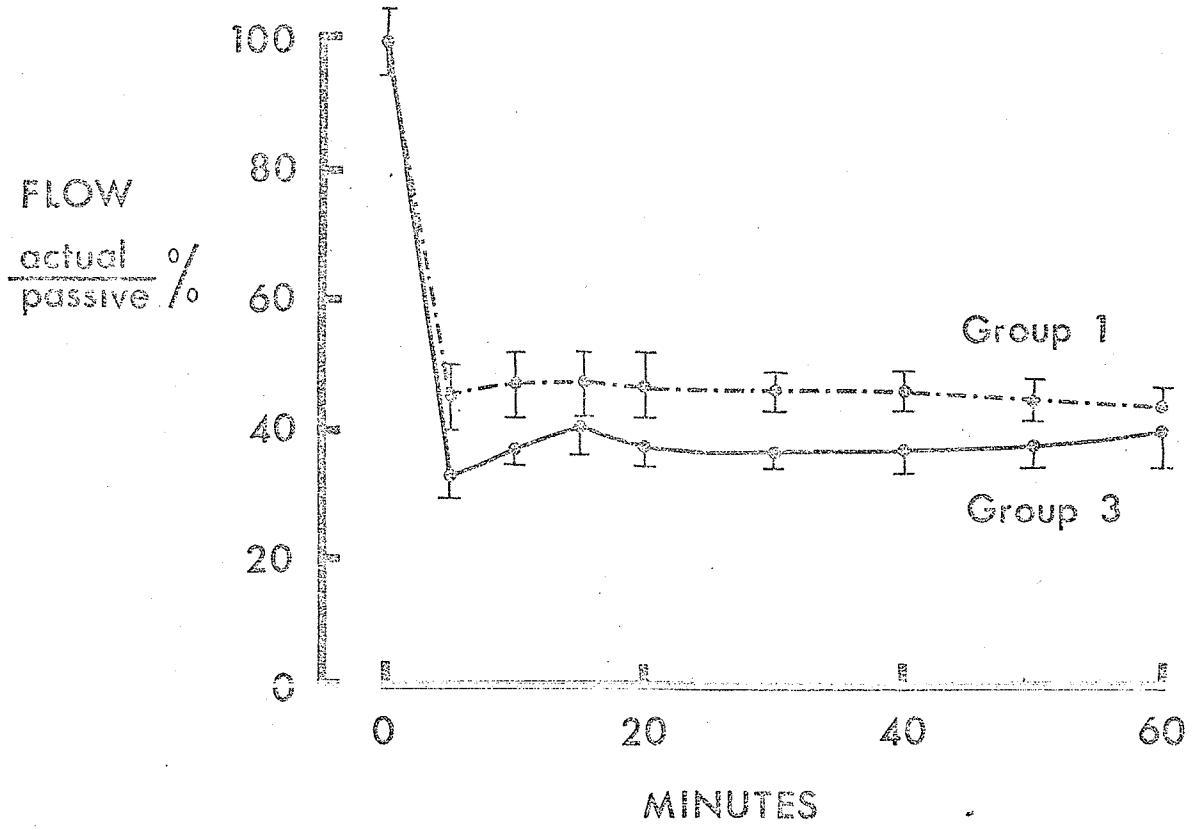


Figure 11. Response to rapid hemorrhage in cats subjected to intestinal denervation, adrenalectomy, and nephrectomy - pituitary intact - (Group 3) compared to that in cats with intact organ systems (Group 1). The responses are shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

from those of Group 1 ($p > .10 -> .50$, unpaired t-test). They were significantly different from those of Group 2 ($p < .001$).

Thus, the vasoconstriction following hemorrhage was not reduced by intestinal denervation, adrenalectomy, and nephrectomy. The presence of the pituitary alone was sufficient to ensure a maximal response to hemorrhage.

Effect of Hemorrhage in Cats Subjected to Intestinal Denervation, Adrenalectomy, and Hypophysectomy - Kidneys Intact (Group 4).

The responses to 6 hemorrhages were studied in 3 cats in which the intestinal nerves were divided and the adrenal glands and pituitary gland removed; the kidneys were left intact (Group 4). Hemorrhage was induced by withdrawing blood rapidly as described in the methods. The volume of blood removed is shown in Table 1. The arterial pressures and superior mesenteric arterial flows before and after hemorrhage are shown in Table 2. The arterial pressure prior to hemorrhage and the recovery of pressure following the removal of blood were similar to the corresponding values in Group 1 and Group 3. The vasoconstrictor response was calculated and the results are shown in figure 12. Within 5 minutes, flow decreased to 58 $\pm$ 8.6% of that expected from the pressure-flow curve. Flow decreased further to 47 $\pm$ 0.9% by 10 minutes and to 41 $\pm$ 3.7% by 60 minutes. At all times between 10 and 60 minutes, the values were not significantly different from those of Group 1 ($p > .50$, unpaired t-test). They were significantly different from those of Group 2 ($p < .001$, unpaired t-test).

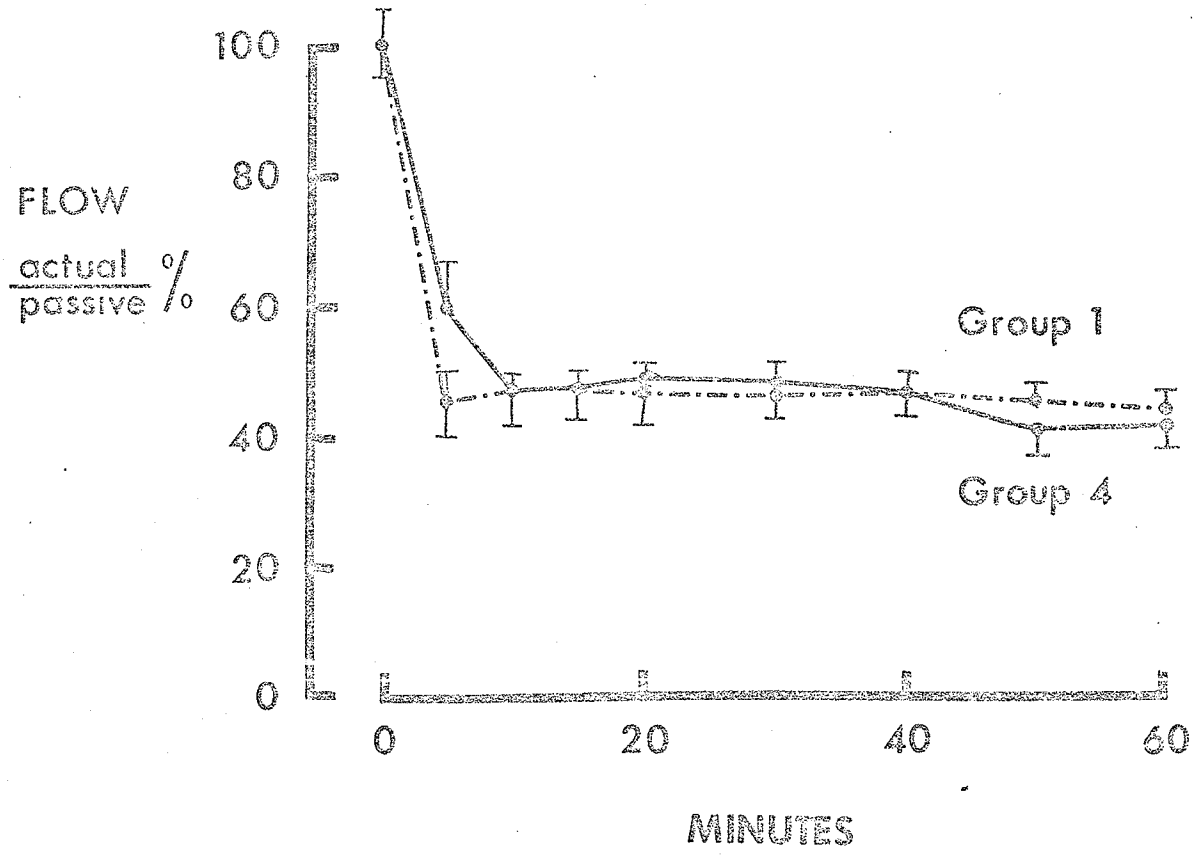


Figure 12. Response to rapid hemorrhage in cats subjected to intestinal denervation, adrenalectomy, and hypophysectomy - kidneys intact - (Group 4) compared to that in cats with intact organ systems (Group 1). The responses are shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

Thus, the vasoconstriction following hemorrhage was not reduced by denervation, adrenalectomy, and hypophysectomy. The presence of the kidneys alone was sufficient to ensure a maximal response to hemorrhage.

Effect of Hemorrhage in Cats Subjected to Nephrectomy and Hypophysectomy - Nerves and Adrenals Intact - (Group 5)

To investigate the role of the sympathetic nerves and adrenal glands, six hemorrhages were induced in 4 cats in which the kidneys and pituitary gland were removed; the innervation to the intestine and the adrenal glands were left intact (Group 5). Hemorrhage was induced by withdrawing blood rapidly as described in the methods. The volume of blood removed is shown in Table 1. The arterial pressures and superior mesenteric arterial flows before and after hemorrhage are shown in Table 2. Only 11 $\pm$ 1.0 ml/kg of blood were removed in order to reduce the blood pressure to 50 mm Hg and to maintain it there for one minute. This value was very similar to the volume of blood removed in Group 2 ($p > .40$, unpaired t-test) and was significantly smaller than the volume removed in Group 1 ($p < .02$, unpaired t-test). The pre-hemorrhage arterial pressure was 110 $\pm$ 5.4 mm Hg. Following the period of blood removal, arterial pressure recovered to 63 $\pm$ 6.3 mm Hg at 10 minutes and 73 $\pm$ 8.3 mm Hg at 60 minutes. These values were significantly different from those of Group 1 ($p < .05$, unpaired t-test), but were not significantly different from those of Group 2 ($p > .10$, unpaired t-test). The vasoconstrictor response was calculated and the results are shown in figure 13. During the first 5 minutes,

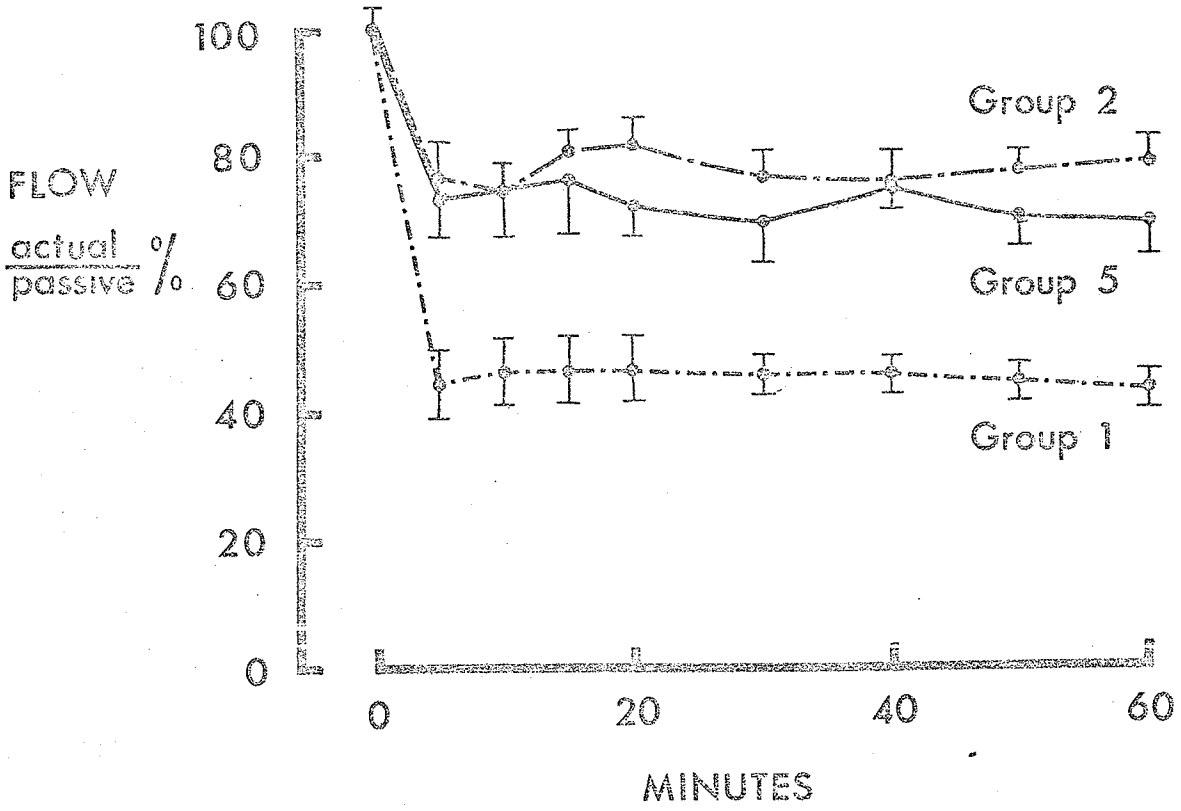


Figure 13. Response to rapid hemorrhage in cats subjected to nephrectomy and hypophysectomy - nerves and adrenals intact - (Group 5) compared to that in cats with intact organ systems (Group 1) and to that in cats subjected to intestinal denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 2). The responses are shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

flow fell slightly to 73 ± 2.8% of that expected from the fall in arterial pressure and it remained between 69 ± 5.0% and 76 ± 8.2% for the remaining 55 minutes. These values were significantly different from the pre-hemorrhage value ($p < .05$, paired t-test). They were significantly different from the corresponding values in Group 1 ($p < .01$, unpaired t-test), but were not significantly different from those of Group 2 ($p > .10$, unpaired t-test).

Thus, the small remaining vasoconstriction of animals subjected to denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 2) was not modified by the presence of the intestinal innervation and adrenal glands.

Effect of Hemorrhage in Cats Treated with Phenoxybenzamine.

Since the intestinal nerves and adrenals did not appear to contribute to the intestinal vasoconstriction following hemorrhage, one would expect that the α-blocking agent, phenoxybenzamine, would not block this response. To test this hypothesis, phenoxybenzamine was administered intravenously to 4 cats in which the intestinal innervation, adrenals, kidneys, and pituitary were all left intact. In 2 of the cats the dose of phenoxybenzamine was 5 mg/kg and in the other 2 cats the dose was increased to 10 mg/kg. After the phenoxybenzamine, 5% dextran (Rheomacrodex diluted with 0.9% NaCl, 10 ml/kg) was given. One hour later, hemorrhage was induced by removing blood at the rate of 0.6 ml/min/kg for 25 minutes as described in the methods. Since the responses in all 4 cats were very similar, the data were pooled. The arterial pressures and superior mesenteric arterial flows before and

after hemorrhage are shown in Table 3. Mean arterial pressure prior to hemorrhage was 110 ± 6.9 mm Hg. During the 25 minute period of blood removal, arterial pressure decreased to 73 ± 7.8 mm Hg. During the following 35 minutes, it recovered to 88 ± 12.9 mm Hg. The vasoconstrictor response was calculated and the results are shown in figure 14. During the period of blood removal, flow decreased to 46 ± 3.0% of that expected from the decrease in arterial pressure. Flow remained at 46% for the remaining 35 minutes. At all times between 5 and 60 minutes, the values were not significantly different ($p > .05$, unpaired t-test) from the corresponding values in cats which did not receive phenoxybenzamine (Group 1s).

Thus, phenoxybenzamine did not block the intestinal vasoconstriction following hemorrhage.

It was important to show that the doses of phenoxybenzamine were effective in blocking the intestinal response to nerve activity and circulating catecholamines. Therefore, before, and one hour after the administration of phenoxybenzamine, noradrenaline was injected into the superior mesenteric artery. The responses in a typical experiment are shown in figure 15. In all experiments the vasoconstrictor response was abolished or converted to a small dilatation. At the end of each experiment, the nerves surrounding the superior mesenteric artery were cut and the peripheral ends inserted through bipolar platinum ring electrodes. Supramaximal rectangular pulses (15 volts) of 2 msec. duration were supplied from a Grass SD - 5 stimulator. The frequency was varied from 2/sec to 8/sec. Figure 16 shows the response taken from a single experiment. Stimulation of the nerves resulted in either no change or a small increase in superior mesenteric arterial flow.

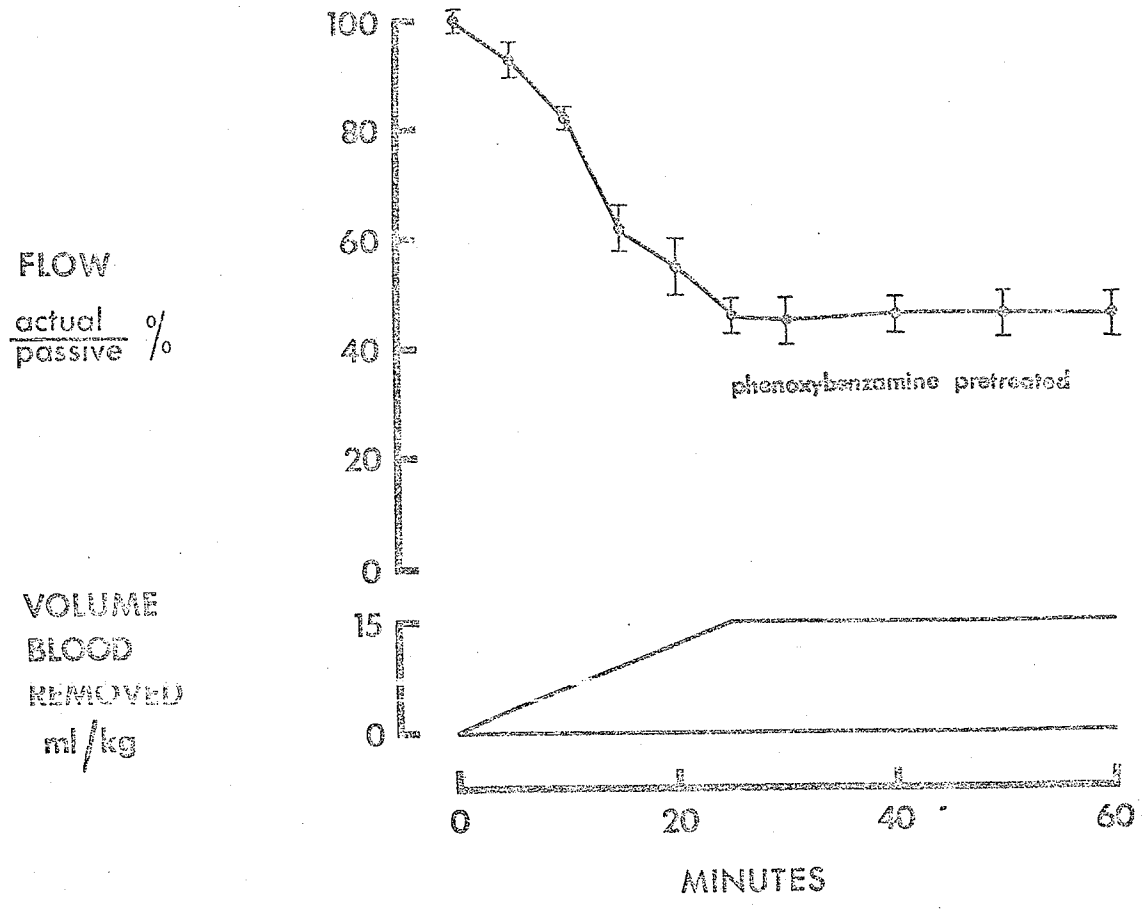


Figure 14. Response during and after slow removal of blood in cats treated with phenoxybenzamine (5 or 10 mg/kg). The response is shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

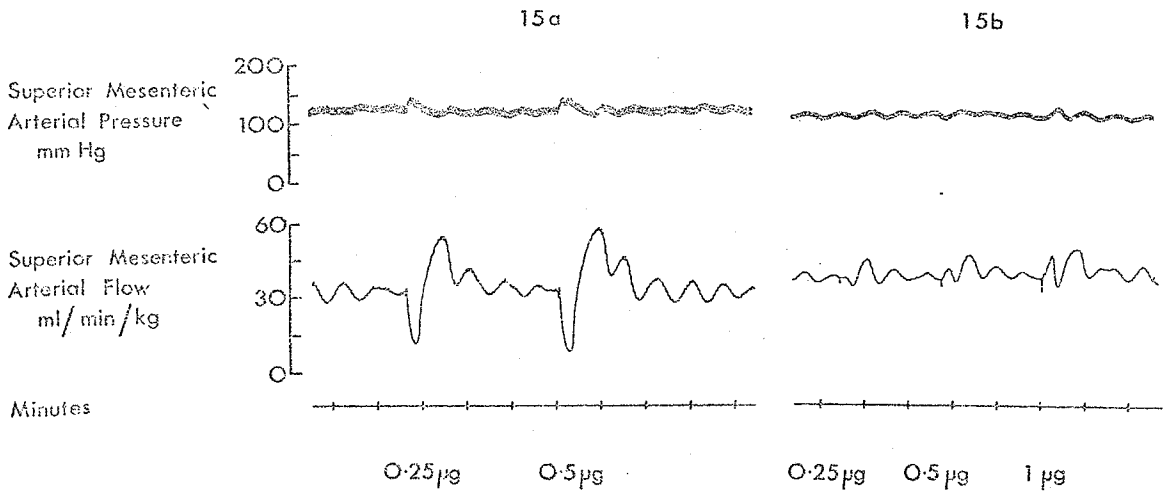


Figure 15. Response to noradrenaline injected into the superior mesenteric artery before (a) and one hour after (b) the administration of phenoxybenzamine (5 mg/kg).

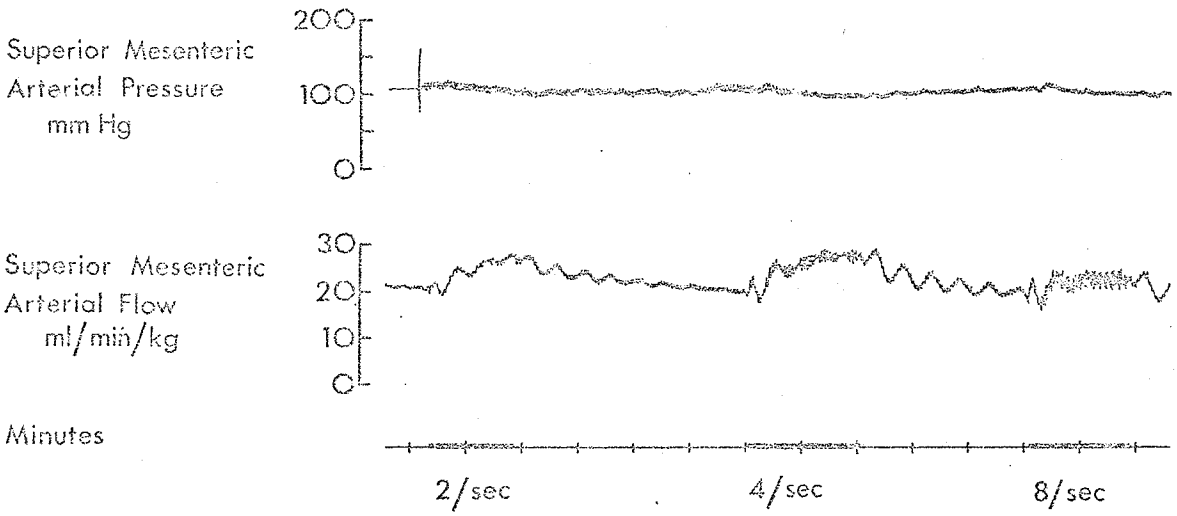


Figure 16. Response to stimulation of the intestinal nerves in animals treated with phenoxybenzamine.

Discussion

All experiments were done in cats anesthetized with sodium pentobarbital. A variety of studies in dogs has indicated that anesthesia with pentobarbital has negligible effects on the splanchnic vascular bed; splanchnic blood flow remains essentially unchanged and this has been associated with a slight decrease in splanchnic vascular resistance (Evringham, Brenneman, and Horvath, 1959; Gilmore, 1958; Katz and Bergman, 1969; MacCannell, 1969; Pratt, Holmes, and Sheid, 1952). Most cardiovascular studies are done in animals anesthetized with either pentobarbital or chloralose. Baroreceptor and chemoreceptor reflexes are somewhat depressed under pentobarbital anesthesia but they are exaggerated and less uniform under chloralose (Greisheimer, 1965; Brown and Hilton, 1956; Armstrong, Porter, and Langston, 1961; Strobel and Wollman, 1969; Clifford and Soma, 1969). In the cat, Greenway, Lawson, and Mellander (1967) found that the response of the hepatic arterial bed to carotid occlusion was similar under pentobarbital or chloralose anesthesia. A great deal of the previous work of the effects of hemorrhage on the splanchnic vascular bed has been done using pentobarbital (Greenway and Stark, 1971). Since much more is known about the pharmacological actions of pentobarbital than about chloralose and since pentobarbital tends to minimize rather than exaggerate the responses, pentobarbital was chosen as the anesthetic agent. The present results may be applied only to cats anesthetized with sodium pentobarbital. Further investigation is required to confirm these results in different species and in conscious animals.

In the dog, there is a marked vasoconstriction of the intestinal

resistance vessels following hemorrhage; however, the response in the cat was not clear (see introduction). In the present study, hemorrhage resulted in a marked intestinal vasoconstriction which was well maintained during the sixty minute period of hypovolemia. A few preliminary experiments indicated that the vasoconstriction was maintained during several hours of hypovolemia (see appendix 2, figure 24). Thus, the present results show clearly that in the cat, as in the dog, there is a marked and sustained intestinal vasoconstriction in response to hemorrhage.

Experiments were designed to investigate the mechanisms of the intestinal vasoconstriction following hemorrhage. It has usually been assumed that the sympathetic nerves are the most important factor in this response, but circulating vasoactive factors may also play a role (see introduction). Vasopressin is released and angiotensin is formed in response to hemorrhage and both of these substances are potent vasoconstrictor agents. The roles of these factors were investigated by removal of the major sources of these agents and by intestinal denervation. The results do not prove that the effects of hypophysectomy and nephrectomy were due solely to the absence of circulating vasopressin and angiotensin. However, since it is known that the concentrations of these agents in the blood increase markedly following hemorrhage, the results have been interpreted on this assumption.

In most experiments, hemorrhages were standardized by inducing a controlled degree of hypotension. Arterial pressure was reduced to 50 mm Hg for 1 minute. Under these conditions the degree of intestinal vasoconstriction and the volume of blood removed in order to produce a given degree of hypotension are both indications of the integrity of

the complex mechanisms by which the animal compensates for hemorrhage. To show that the responses were not due to the different volumes of blood which were removed, similar volumes of blood were removed in some animals.

Although the results of this investigation can be accurately assessed only by comparison of the pressure-flow relationship before and during hemorrhage as discussed earlier, the results may be summarized by comparing the calculated resistance to flow before and after hemorrhage. Figure 17 is a summary of the results. The resistance to flow (pressure difference across the bed divided by the flow through it) was calculated prior to hemorrhage and sixty minutes after hemorrhage. The increase in calculated resistance was expressed as a percentage of the pre-hemorrhage control value.

After intestinal denervation and removal of the adrenals, kidneys, and pituitary (Group 2) the intestinal vasoconstriction was greatly reduced. In addition, the volume of blood removed to produce a given degree of hypotension was less. The mechanism of the small remaining vasoconstriction was not investigated. Extrarenal sources of renin have been reported, namely in the uterus (Ferris and Mulrow, 1965, rabbit) and in the salivary glands (Bing and Farup, 1965, mouse; Takeda, DeBusk, and Grollman, 1969, mouse). Recently, Ganten et al. (1970) reported that, following hemorrhage in bilaterally nephrectomized dogs, renin was also released from the splanchnic area. However, a local effect of the renin-angiotensin system on these tissues is unlikely. It has been shown in dogs that "converting enzyme" activity in blood is too slow to account for the rapid in vivo conversion of

| | Increase Resist. above control (%) | B.V. removed ml/kg |
|---|---|---|
| Group 1 (intact) | 101 | 20 |
| Group 3 (pit. only) | 120 | 19 |
| Group 4 (kid. only) | 104 | 17 |
| Group 2 (all removed) | 32 | 10 |
| 5 (nerves & adr. intact) | 30 | 11 |
| Group 1s (all intact) | 169 | 15 |
| Group 2s (all removed) | 26 | 15 |

Figure 17. Summary of the responses of the intestinal resistance vessels to hemorrhage. The resistance to flow was calculated immediately prior to hemorrhage and after sixty minutes of hypovolemia. The increase in resistance is expressed as a percentage of the pre-hemorrhage control value. The volume of blood removed from each group of animals is expressed in ml/kg of body weight.

angiotensin I to angiotensin II and that the conversion *in vivo* takes place in the pulmonary circulation (Ng and Vane, 1967 and 1968). This conclusion has been confirmed for cats as well (Biron and Huggins, 1968). Thus, it appears that the renin-angiotensin system is designed as a circulating rather than a local hormone system. It is possible that the remaining intestinal vasoconstriction was due to angiotensin generation in the lungs subsequent to renin release from extrarenal sources. If this were the case, then the splenic resistance vessels should also constrict since the sensitivities of the splenic and intestinal resistance vessels to intravenous infusions of angiotensin are very similar (see section 3). This problem will be discussed further in the next section.

After denervation, adrenalectomy, and nephrectomy (Group 3), the intestinal vasoconstriction was not significantly different from the response in the control animals (Group 1). This suggested the possibility that vasopressin was solely responsible for the vasoconstriction. However, when only the pituitary was removed, the response was not significantly reduced (see appendix 4). Thus, it appeared that either vasopressin or other vasoconstrictor factors could produce the response. After denervation, adrenalectomy, and hypophysectomy (Group 4), the intestinal vasoconstriction was not significantly different from the response in control animals (Group 1) nor from the response in animals subjected to denervation, adrenalectomy, and nephrectomy (Group 3). Thus, the results show clearly that either vasopressin secretion alone or angiotensin production alone caused similar responses to those elicited when both factors were acting simultaneously.

The relative contributions of vasopressin and angiotensin to the intestinal vasoconstriction following hemorrhage in animals with intact organ systems cannot be assessed from the present data. In the absence of one compensatory mechanism, the other may act more strongly. It may be argued that angiotensin plays a lesser role since tachyphylaxis to angiotensin has been reported in a variety of smooth muscles (Zaimis, 1968). However, the doses used in these studies were relatively large. Jonsson, Svanvik, and Vikgren (1967) used an arterial long-circuit technique to study the effect of intravenous infusions of angiotensin on the resistance vessels of the cat intestine. They reported that intestinal vasoconstriction was followed by marked tachyphylaxis. However, significant intestinal vasoconstriction occurred only at high doses. This may have been due to the use of an arterial long-circuit which is known to depress vascular reactivity (see introduction). In the present study, no evidence of tachyphylaxis was found when angiotensin was infused intravenously in amounts likely to be found in the blood after hemorrhage (see section 3). Another consideration is the fact that vasopressin is removed from the circulation by both the kidneys and the liver. Thus, it is possible that the rate of removal of vasopressin was slower in nephrectomized animals. However, further investigation is required to establish the relative roles of vasopressin and angiotensin in the intestinal response to hemorrhage. Determinations of the blood levels of vasopressin and angiotensin after hemorrhage would be useful in assessing their relative importance in the overall response.

After nephrectomy and hypophysectomy (Group 5) the intestinal vasoconstriction was greatly reduced. The small remaining vasoconstriction was not significantly different from that in animals with all organ systems removed (Group 2) suggesting that the sympathetic nerves and adrenal medullary secretions did not play a role in the response. It was possible that the nerves had been injured during placement of the flowmeter probe around the superior mesenteric artery. However, in a control experiment, the venous outflow response to stimulation of splanchnic nerves before and after placement of the flowmeter probe around the superior mesenteric artery indicated that the nerves were not damaged by this procedure (see appendix 3). Previous studies by others in normovolemic cats have shown that stimulation of the sympathetic nerves and infusions of noradrenaline do not cause sustained intestinal vasoconstriction and autoregulatory escape occurs (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a). Infusions of adrenaline cause intestinal vasodilatation (Greenway and Lawson, 1966a; Greenway and Lawson, 1968). Furthermore, when intestinal flow is reduced by partial occlusion of the superior mesenteric artery, the responses to sympathetic nerve stimulation are impaired (Folkow, Lewis, Lundgren, Mellander, and Wallentin, 1964a). In view of these reports, it is not surprising that the intestinal innervation and adrenal medullary secretions did not play a role in the intestinal vasoconstriction following hemorrhage, and that the response was unchanged after phenoxybenzamine.

During the administration of phenoxybenzamine, dextran (10 ml/kg) was given to prevent a large fall in arterial pressure. If

phenoxybenzamine had blocked the intestinal vasoconstriction, the results would have been inconclusive, since the hemorrhage would have represented a relatively smaller proportion of the animal's enlarged blood volume. However, since phenoxybenzamine did not reduce the response, the fact that the hemorrhage was proportionately smaller increases the significance of the observation.

When intense intestinal vasoconstriction occurred, the volume of blood removed to produce a given degree of hypotension varied from 17 to 20 ml/kg body wt. (figure 17; Groups 1, 3, and 4). On the other hand, when the intestinal vasoconstriction was small, the volume of blood removed was only 10 or 11 ml/kg of body weight (figure 17; Groups 2 and 5). It seemed likely that this was the consequence of the inability of these animals to vasoconstrict. However, another explanation was also possible. The smaller vasoconstriction of these animals may have been due to the smaller reduction in blood volume even though similar degrees of hypotension had been produced. However, the results were not altered when identical volumes of blood were removed (figure 17); intestinal denervation and removal of the adrenals, kidneys, and pituitary greatly reduced the intestinal vasoconstriction (Group 2s) which occurred in animals with intact organ systems (Group 1s).

This study was devised to investigate the overall resistance response of the intestinal vascular bed to hemorrhage; thus, it did not provide data on the distribution of blood flow to the component tissue layers of the intestine. As described in the introduction, Folkow and coworkers (1964b) reported that autoregulatory escape was associated with a maintained decrease in the capillary surface area

available for exchange and a diversion of blood flow from mucosal to submucosal structures. Thus, although the intestinal nerves and adrenal medullary secretions did not play a role in the overall resistance response of the intestine to hemorrhage, the possibility that they cause a redistribution of blood flow within the intestine cannot be excluded. Experimental data on this possibility would be of interest and of potential significance since conditions of prolonged sympathetic activity might lead to mucosal cell damage. However, until direct evidence is provided to confirm a redistribution of blood flow during sympathetic activation, the problem remains unanswered.

# SECTION II: RESPONSE OF THE SPLENIC RESISTANCE VESSELS TO HEMORRHAGE

Pressure-Flow and Pressure-Weight Relationships.

In each animal, the relationships of splenic flow and splenic weight to arterial pressure were studied. Therefore, approximately one hour after the completion of surgery and approximately 10 to 20 minutes prior to hemorrhage, a pressure-flow curve and a pressure weight curve were obtained by graded clamping of the coeliac artery as described in the methods.

The absolute values and slopes of the curves varied from one animal to the next. Therefore, in any one cat, the pressure-flow relationship and pressure-weight relationship during hemorrhage were compared to the pressure-flow curve and pressure-weight curve obtained in the same cat. The pressure-flow and pressure-weight curves from three experiments in each group of animals are shown in figure 18. An approximately linear relationship between splenic flow and pressure was seen under all conditions. Splenic weight remained near constant at all arterial pressures.

Effect of Hemorrhage in Cats with Intact Organ Systems (Group 6).

The responses to 6 hemorrhages were studied in 3 cats in which the splenic nerves, the adrenal glands, the kidneys, and the pituitary gland were all intact. Sham hypophysectomy was performed by exposure of the pituitary. In addition, the peritoneum over the kidney was divided and the perinephric fat was separated from the kidney. Blood was removed slowly and continuously as described in the methods. Mean arterial pressures, splenic flows, and splenic weights before and after hemorrhage are shown in Table 4. Prior to hemorrhage, mean

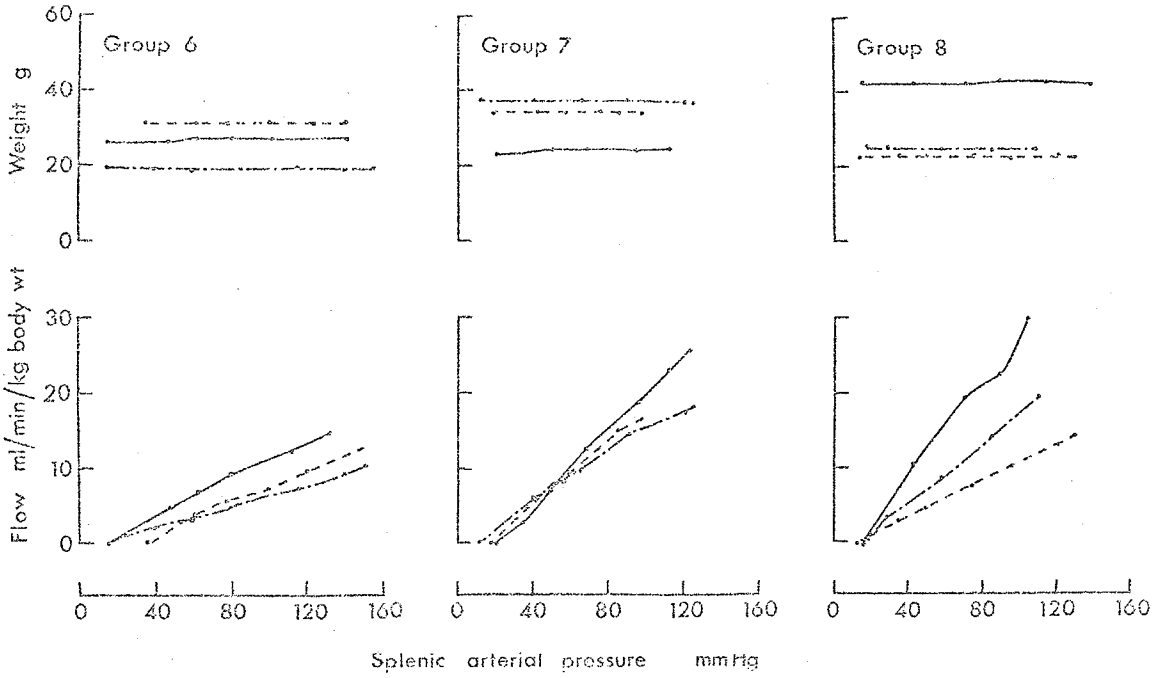


Figure 18. Examples chosen by random numbers of pressure-flow and pressure-weight graphs. Group 6 - cats with organ systems intact; Group 7 - cats subjected to splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy; Group 8 - cats subjected to nephrectomy and hypophysectomy.

TABLE 4

Means ± S.E. of the values for body weight, arterial pressures, splenic flows and splenic weights in different groups of cats subjected to slow hemorrhage.

| | | Group 6 | Group 7 | Group 8 |
|---|---|---|---|---|
| Body weight (kg) | | 2.9 ± 0.4 | 2.7 ± 0.3 | 2.8 ± 0.2 |
| Arterial pressures mm Hg | - before hemorrhage | 138 ± 7.5 | 116 ± 6.9 | 126 ± 3.2 |
| | - 20 min after hemorrhage | 107 ± 11.1 | 53 ± 2.3 | 90 ± 5.8 |
| | - 60 min after hemorrhage | 98 ± 6.9 | 62 ± 6.6 | 78 ± 4.6 |
| Splenic flow (ml/min/kg) | - before hemorrhage | 14 ± 3.0 | 21 ± 1.5 | 27 ± 4.7 |
| | - 20 min after hemorrhage | 5 ± 1.6 | 8 ± 1.1 | 12 ± 2.5 |
| | - 60 min after hemorrhage | 4 ± 0.9 | 10 ± 1.2 | 10 ± 1.9 |
| Splenic weight (g) | - before hemorrhage | 24 ± 1.8 | 32 ± 1.9 | 28 ± 3.7 |
| | - 20 min after hemorrhage | 21 ± 0.9 | 32 ± 1.9 | 24 ± 2.9 |
| | - 60 min after hemorrhage | 19 ± 1.7 | 30 ± 2.0 | 24 ± 3.0 |

arterial pressure was 138 ± 7.5 mm Hg. It fell to 107 ± 11.1 mm Hg during the period of blood removal and then decreased further to 98 ± 6.9 mm Hg over the following 40 minutes.

The flow response to hemorrhage was calculated and expressed in the same manner as that already described for the intestine. The results are shown in figure 19 (Group 6). During the period of blood removal, flow decreased to 39 ± 5.7% of that expected from the decrease in arterial pressure. Flow remained between 39% and 44% during the following 40 minutes of hypovolemia. At all times between 5 and 60 minutes, the values were significantly different from the pre-hemorrhage value measured at time zero ($p < .02$, paired t-test).

The splenic weight response was expressed in a manner similar to the flow response. Since a pressure-weight curve had been obtained prior to the hemorrhage, it was possible to calculate the weight which would be expected at the various arterial pressures after hemorrhage. The actual weights were expressed as percentages of the passive or expected weights. The splenic weight response in this group of animals was calculated and the results are shown in figure 19. During the period of blood removal, splenic weight decreased to 89% of that expected from the change in arterial pressure. It then decreased further to 76% over the following 40 minutes. At all times between 30 and 60 minutes, the values were significantly different from the pre-hemorrhage values measured at time zero ($p < .05$, paired t-test).

Thus, following hemorrhage, the spleen contracted and there was a marked and well maintained vasoconstriction.

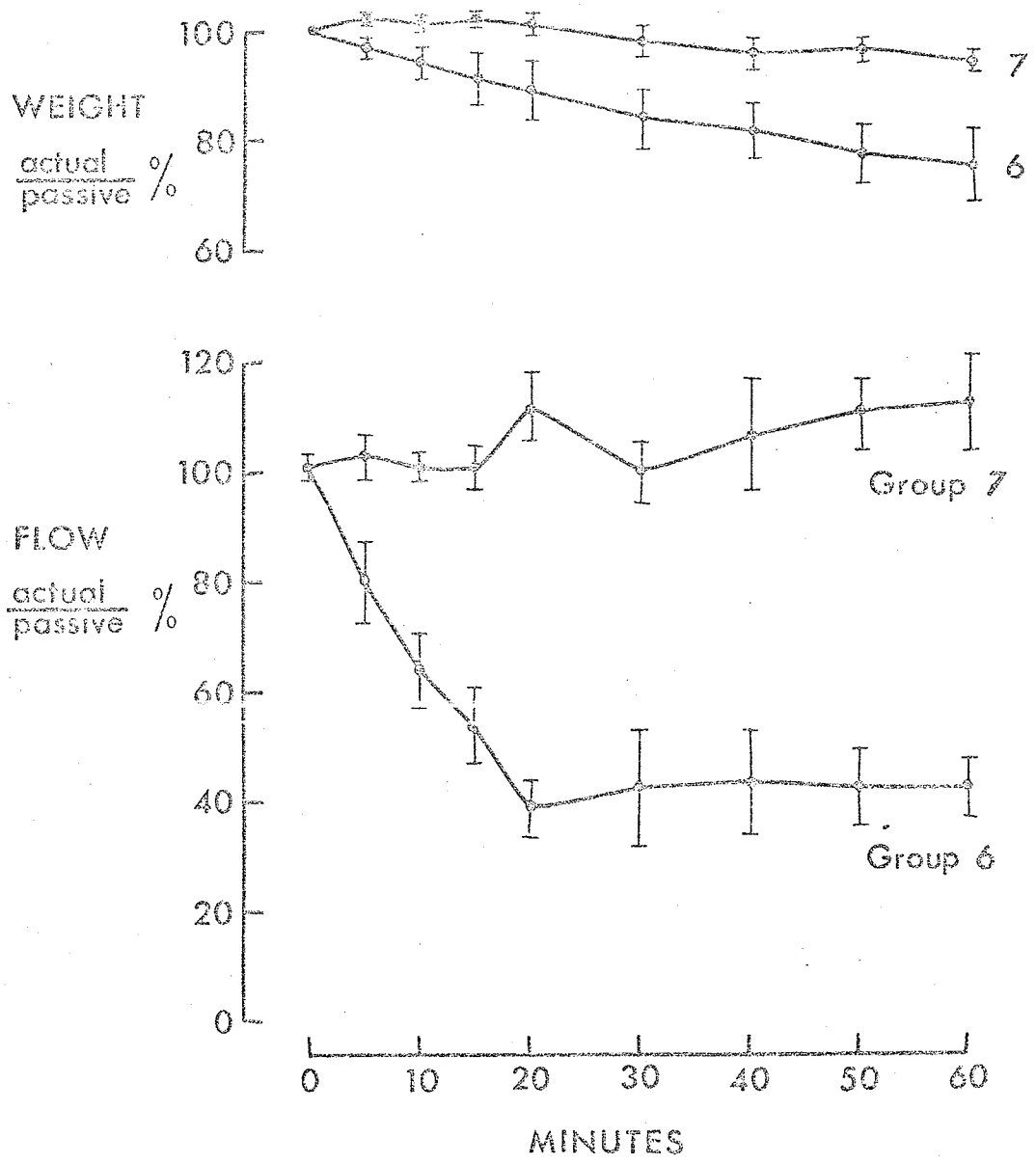


Figure 19. Responses during and after slow, continual removal of blood at the rate of 0.68 ml/min/kg for 20 minutes. Group 6 - cats with intact organ systems; Group 7 - cats subjected to splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy. The responses are shown as the means $\pm$ S.E. of the splenic arterial flows and splenic weights expressed as percentages of the passive flows and weights expected from the changes in arterial pressure.

Effect of Hemorrhage in Cats Subjected to Splenic Denervation, Adrenalectomy, Nephrectomy, and Hypophysectomy (Group 7).

The responses to 8 hemorrhages were studied in 4 cats in which the splenic nerves were divided, and the adrenals, kidneys, and pituitary removed (Group 7). Blood was withdrawn slowly and continuously as described in the methods. The arterial pressures, splenic flows, and splenic weights before and after hemorrhage are shown in Table 4. Prior to removing blood, mean arterial pressure was 116 $\pm$ 6.9 mm Hg. It then decreased to 53 $\pm$ 2.3 mm Hg during the period of blood removal and recovered slightly to 62 $\pm$ 6.6 mm Hg over the next 40 minutes. These values are significantly lower than the corresponding values in Group 6 ($p < .05$, unpaired t-test).

The vasoconstrictor response to extrinsic factors was calculated and the results are shown in figure 19 (Group 7). The changes in flow during the hypovolemic period were similar to those expected from the decrease in arterial pressure. At all times between 5 and 60 minutes, the values were not significantly different from the pre-hemorrhage values measured at time zero ($p > .10$, paired t-test). The values were significantly different ($p < .02$, unpaired t-test) from the corresponding values in animals with intact organ systems (Group 6).

It was concluded that all active extrinsic factors had been removed. Thus, the vasoconstriction of the splenic resistance vessels following hemorrhage was abolished by splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy.

The splenic weight decreased only slightly following hemorrhage and was not significantly different from the pre-hemorrhage value

($p > .05$, paired t-test). At all times between 10 and 60 minutes, the values were significantly different from the corresponding values in cats with intact organ systems (Group 6). Thus, splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy abolished the splenic contraction in response to hemorrhage.

Because of previous work by Greenway and Stark (1969) to be discussed later, it was not necessary to remove the sources of the various extrinsic vasoconstrictor factors in the same combinations as was done in the study of the intestinal vascular response to hemorrhage. These workers did not, however, investigate the role of the sympathetic nerves and secretions from the adrenal glands in the absence of the other factors.

Effect of Hemorrhage in Cats Subjected to Nephrectomy and Hypophysectomy (Group 8).

The sympathetic nerves and secretions from the adrenal medulla did not play a role in the intestinal vasoconstriction following hemorrhage. To investigate their role in the splenic vasoconstriction and splenic contraction, 8 hemorrhages were induced in 4 cats in which the kidneys and pituitary gland were removed; the splenic innervation and the adrenal glands were left intact (Group 8). Hemorrhage was induced by removing blood as described in the methods. The mean values of arterial pressures, splenic flows, and splenic weights before and after hemorrhage are shown in Table 4. Prior to hemorrhage, mean arterial pressure was 126 $\pm$ 3.2 mm Hg. It then decreased to 90 $\pm$ 5.8 mm Hg during the period of blood removal, and then decreased further to

78 $\pm$ 4.6 mm Hg over the next 40 minutes.

The vasoconstrictor response to extrinsic factors was calculated and the results are shown in figure 20. During the period of blood withdrawal, flow decreased to 71 $\pm$ 4.5% of that expected from the fall in arterial pressure. Flow remained between 65% and 71% during the remaining 40 minutes of hypovolemia. The values were significantly different from the pre-hemorrhage values ($p < .025$, paired t-test). At all times between 10 and 60 minutes, the values were significantly different ($p < .05$, unpaired t-test) both from those for cats with intact organ systems (Group 6) and from those for cats subjected to denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 7).

Thus, in contrast to the intestine, the sympathetic nerves and the secretions from the adrenal glands were capable of causing a significant splenic vasoconstriction, but this vasoconstriction was significantly smaller than that which occurred when the kidneys and pituitary were also intact (Group 6).

Splenic weight decreased to 86 $\pm$ 1.7% at 20 minutes and to 83 $\pm$ 1.0% at 60 minutes after the onset of hemorrhage (figure 20). These values were significantly different from the pre-hemorrhage values ($p < .01$, paired t-test). At all times between 5 and 60 minutes, the values were significantly different ($p < .005$, unpaired t-test) from those for cats subjected to denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 7), but they were not significantly different ($p > .4$, unpaired t-test) from those for cats with intact organ systems (Group 6). These results suggest that the sympathetic nerves and the secretions from the adrenal glands play an important role in the mechanism of the splenic contraction following hemorrhage.

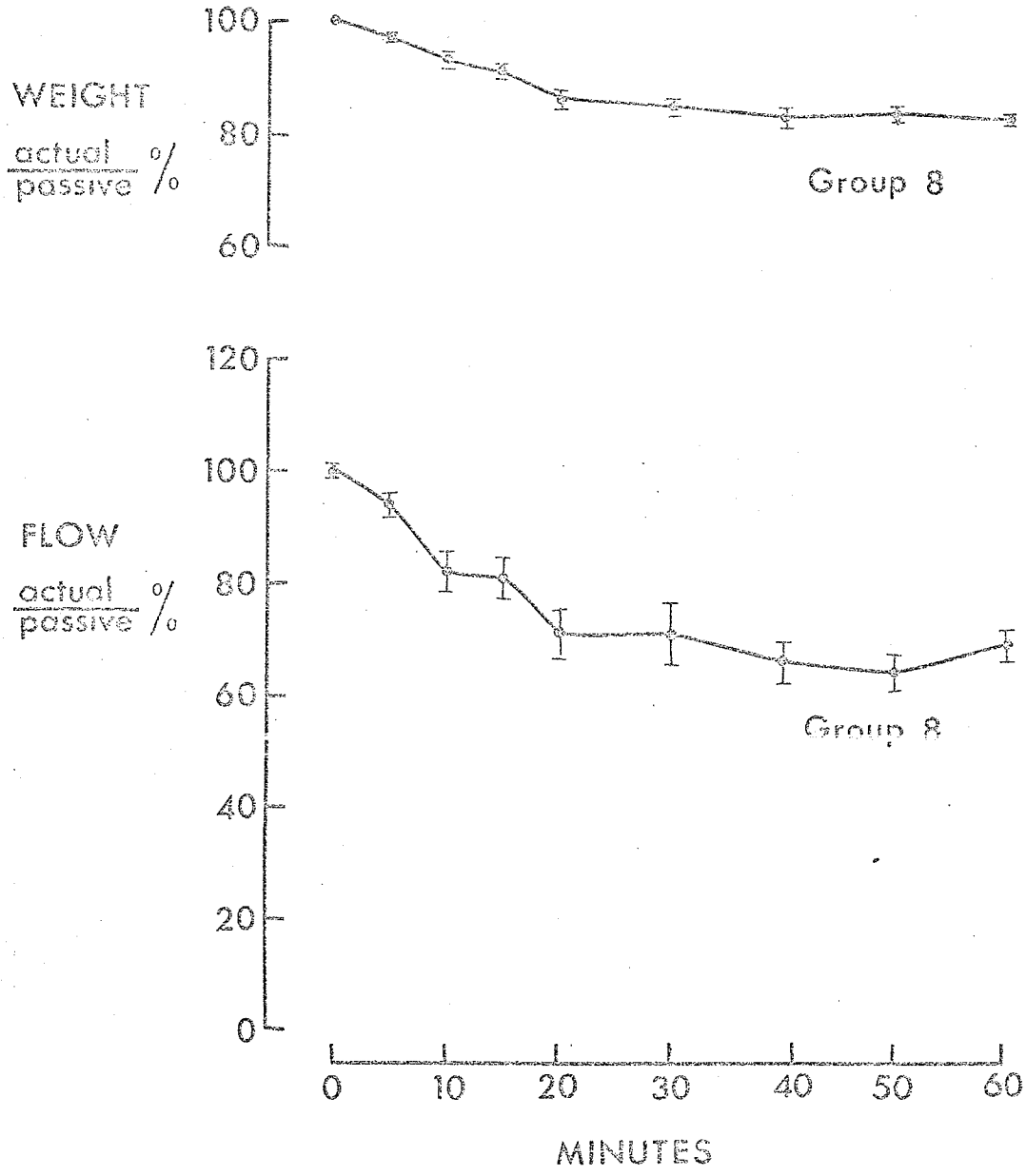


Figure 20. Responses during and after slow, continual removal of blood in cats subjected to nephrectomy and hypophysectomy (Group 8). The responses are shown as the means ± S.E. of the splenic arterial flows and splenic weights expressed as percentages of the passive flows and weights expected from the changes in arterial pressure.

Discussion

The approximately linear pressure-flow relationship in the splenic vascular bed was in contrast to the autoregulation of blood flow observed in the intestinal vascular bed. The absence of splenic autoregulation confirmed earlier studies in the dog (Frohlich and Gillenwater, 1963) and in the cat (Greenway and Stark, 1969). It is somewhat surprising that autoregulation does not occur since large oscillations in splenic flow, which are frequently seen, have been suggested to be caused by rhythmic myogenic activity of the arteriolar smooth muscle (Greenway, Lawson, and Stark, 1968). In addition, following occlusion and release of the splenic artery, there was a brief but marked reactive hyperemia. Since this was not seen after occlusion and release of the splenic vein (Greenway, Lawson, and Stark, 1968), it seems probable that the reactive hyperemia was a myogenic response of the smooth muscle to lowering of pressure within the vessels. Furthermore, Lutz and coworkers (1969) reported recently that elevations of venous pressure caused myogenic vasoconstriction of the splenic resistance vessels. Thus, myogenic responses appear to occur in the splenic arterial bed but the response to changes in arterial pressure is very limited.

Splenic weight was not affected by changes in arterial pressure. This observation was reported earlier by Greenway and Stark (1969) who concluded that the smooth muscle of the capsule and trabeculae did not show either pressure-induced myogenic contractions or passive responses to changes in arterial pressure.

The mechanism of the splenic vasoconstriction following hemorrhage was investigated in a manner similar to that described for the intestinal vascular bed. The roles of the various extrinsic factors were studied by removal of the various sources of these factors and by splenic denervation. However, because of previous work on this problem by Greenway and Stark (1969), it was unnecessary to remove the sources of the various factors in all of the combinations described for the intestine. Thus, the effect of hemorrhage was studied in only certain groups of animals.

It was previously shown that splenic denervation together with removal of the adrenal glands and kidneys significantly reduced, but did not abolish, the splenic vasoconstriction following hemorrhage (Greenway and Stark, 1969). Since denervation and adrenalectomy alone had little effect, it was concluded that angiotensin formation subsequent to renin release from the kidneys was the cause of part of the splenic vasoconstriction. The cause of the significant remaining vasoconstriction was not investigated.

The present results show that the removal of the pituitary gland in addition to denervation, adrenalectomy, and nephrectomy (Group 7) completely abolished the vasoconstriction. Thus, in addition to angiotensin, vasopressin also appears to play an important role in the splenic vasoconstriction following hemorrhage. Since the vasoconstriction was completely abolished, it was concluded that all extrinsic vasoconstrictor factors had been removed. This was in contrast to the intestinal vascular bed in which a small vasoconstriction still remained in animals subjected to intestinal denervation,

adrenalectomy, nephrectomy, and hypophysectomy (Group 2 and 2s). Since the sensitivities of the intestinal and splenic vascular beds to intravenous infusions of angiotensin were very similar (see section 3), it seems unlikely that the small remaining vasoconstriction of the intestinal vascular bed was due to extrarenal sources of renin as was suggested earlier. If this were the case, the splenic vessels also should have constricted. Thus, it would appear that some other unidentified vasoconstrictor agent which affects the intestine but not the spleen was involved.

After nephrectomy and hypophysectomy (Group 8), the splenic vasoconstriction was significantly different from both the response in control animals (Group 6) and the response in animals subjected to splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 7). Thus, in contrast to the intestinal vascular bed, the sympathetic nerves and/or secretions from the adrenal glands are capable of causing a splenic vasoconstriction after hemorrhage, although the vasoconstriction is smaller than that observed in animals with intact organ systems. The role of the sympathetic nerves in the splenic vasoconstriction is consistent with the observations that the extent of autoregulatory escape in the spleen is small and that there is little progressive impairment of the splenic response to nerve stimulation when the splenic flow is reduced for long periods by partial occlusion of the splenic artery (Greenway, Lawson, and Stark, 1968).

In two pregnant cats, splenic denervation, adrenalectomy, nephrectomy, and hypophysectomy did not completely abolish the splenic vasoconstriction following hemorrhage. These results suggest that

other vasoconstrictor factors may be important in the splenic vasoconstriction in pregnant cats. However, these were only preliminary experiments and further investigation of this interesting problem is required.

The relative contributions of vasopressin, angiotensin, and the sympathetic nervous system to the splenic vasoconstriction in animals with intact organ systems cannot be assessed from the present data. The problems in relation to this were discussed in section 1. Determinations of the blood levels of vasopressin, angiotensin, and circulating catecholamines, as well as an estimation of the rate of firing of the sympathetic nerves to the spleen, would be necessary.

The splenic weight response was abolished by denervation, adrenalectomy, nephrectomy, and hypophysectomy (Group 7). However, nephrectomy and hypophysectomy (Group 8) did not reduce the response. Thus, the presence of the nerves and adrenals was sufficient to cause the same response as when all factors were operating simultaneously. Previously, Greenway and Stark (1969) had shown that splenic denervation and adrenalectomy alone completely abolished the splenic contraction. Thus, the splenic contraction following hemorrhage appears to be mediated by the sympathetic nerves and adrenal medullary secretions, while vasopressin secretion and angiotensin formation appear to play little role. This conclusion is consistent with the effects of infusions of angiotensin and vasopressin. Vasopressin did not cause splenic contraction, while angiotensin caused contraction only in large doses (Greenway and Stark, 1970).

# SECTION III: RESPONSES OF THE INTESTINAL, SPLENIC, AND HEPATIC ARTERIAL RESISTANCE VESSELS TO INTRAVENOUS INFUSIONS OF VASOPRESSIN AND ANGIOTENSIN

Control Values.

For each group of animals, the mean values for body weight and control flows and arterial pressures are shown in Table 5. The weight range of the animals was small and there were no significant differences among the mean values in each series of experiments. The control flows and arterial pressures in these experiments were measured approximately one hour after completion of the surgery and were not substantially different from those previously reported for the hepatic arterial (Greenway, Lawson, and Mellander, 1967), the splenic (Greenway and Stark, 1969; this thesis, page 83) and the intestinal (this thesis, page 53) vascular beds.

Vasopressin Infusions.

The responses to intravenous infusions of vasopressin were studied in the intestinal, splenic, and hepatic arterial vascular beds as described in the methods. The infusion rates varied from 0.57 to 57.0 mU/min/kg body weight. These doses covered the range from that causing a minimal observable response to that causing cardiac arrhythmias. The dose-response curves are shown in figure 21 and each curve represents the mean $\pm$ S.E. of the responses in 5 to 7 cats. In the intestinal and splenic beds, vasopressin caused a marked decrease in conductance, and the corresponding values at each dose level were not significantly different ($p > .50$, unpaired t-test). On the other hand, vasopressin caused an increase in conductance of the hepatic arterial bed. The values at doses above 2.3 mU/min/kg were significantly different from the corresponding values for the intestinal and splenic

TABLE 5

Means ± of the values for body weight, arterial pressures, and control flows in the different groups of cats.

| | Group A (Hepatic arterial) | Group B (Splenic) | Group C (Intestinal) |
|---|---|---|---|
| Body weight (kg) | 2.7 ± 0.07 | 2.9 ± 0.19 | 2.8 ± 0.17 |
| Arterial pressure (mm Hg) | 120 ± 6.1 | 134 ± 8.0 | 121 ± 5.9 |
| Flows - (ml/min/kg) | | | |
| - hepatic arterial | 23 ± 3.8 | | |
| - splenic arterial | | 12 ± 2.2 | |
| - superior mesenteric arterial | | | 32 ± 2.1 |

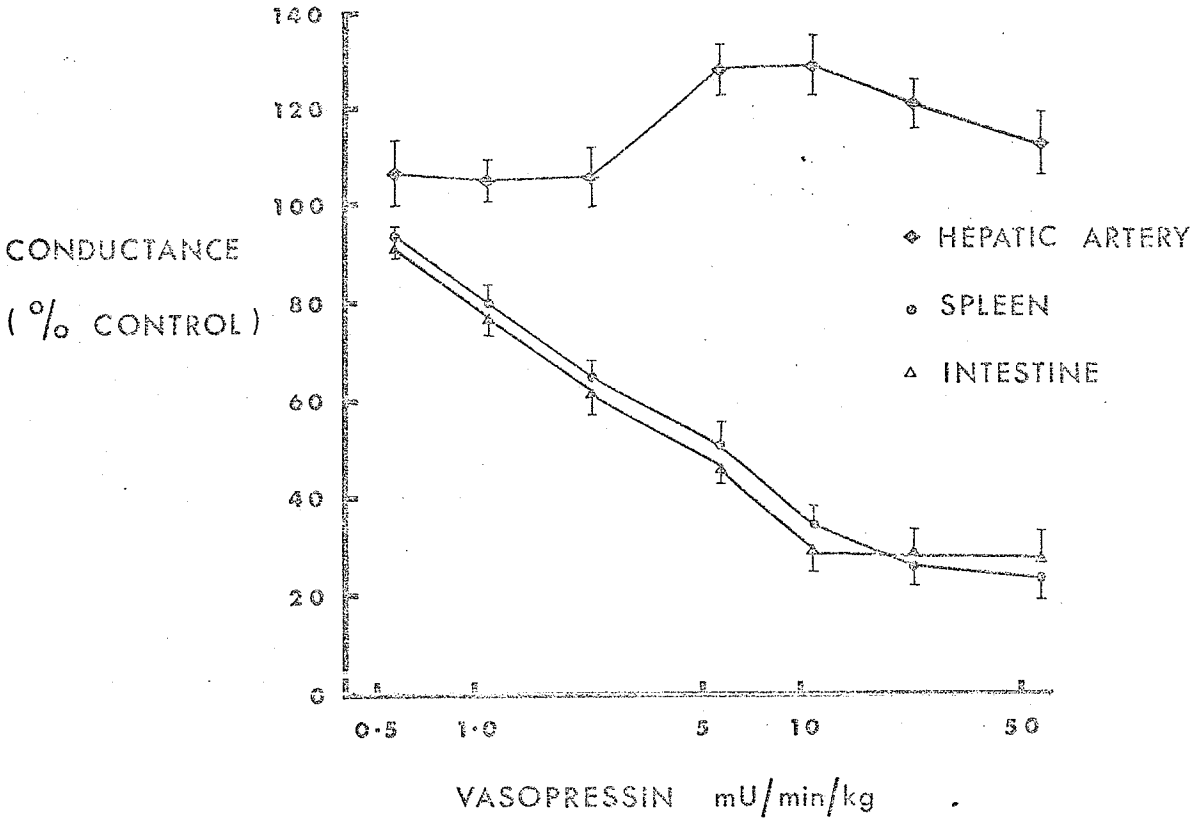


Figure 21. Responses of the hepatic arterial, splenic, and intestinal vascular beds to intravenous infusions of vasopressin. Each curve represents the mean $\pm$ S.E. of the responses in 5-7 cats.

vascular beds ($p < .01$, unpaired t-test).

Thus, intravenous infusions of vasopressin cause intestinal and splenic vasoconstriction and hepatic arterial vasodilatation.

Angiotensin Infusions.

The responses to intravenous infusions of angiotensin were studied in the intestinal, splenic, and hepatic arterial vascular beds as described in the methods. The infusion rates varied from 0.004 to 0.40 μg/min/kg and covered the range from the dose causing a minimal observable response to that causing cardiac arrhythmias. The dose-response curves are shown in figure 22 and each curve represents the mean ± S.E. of the responses in 5 to 7 cats. Angiotensin caused a decrease in conductance in all three vascular beds. At all dose levels, the values for the intestinal and splenic beds were not significantly different ($p > .10$, unpaired t-test). At doses above 0.1 μg/min/kg, the response of the hepatic vascular bed was significantly less than those of the intestinal and splenic beds ($p < .05$, unpaired t-test).

Thus, intravenous infusions of angiotensin cause marked intestinal and splenic vasoconstriction and weak vasoconstriction of the hepatic arterial vascular bed.

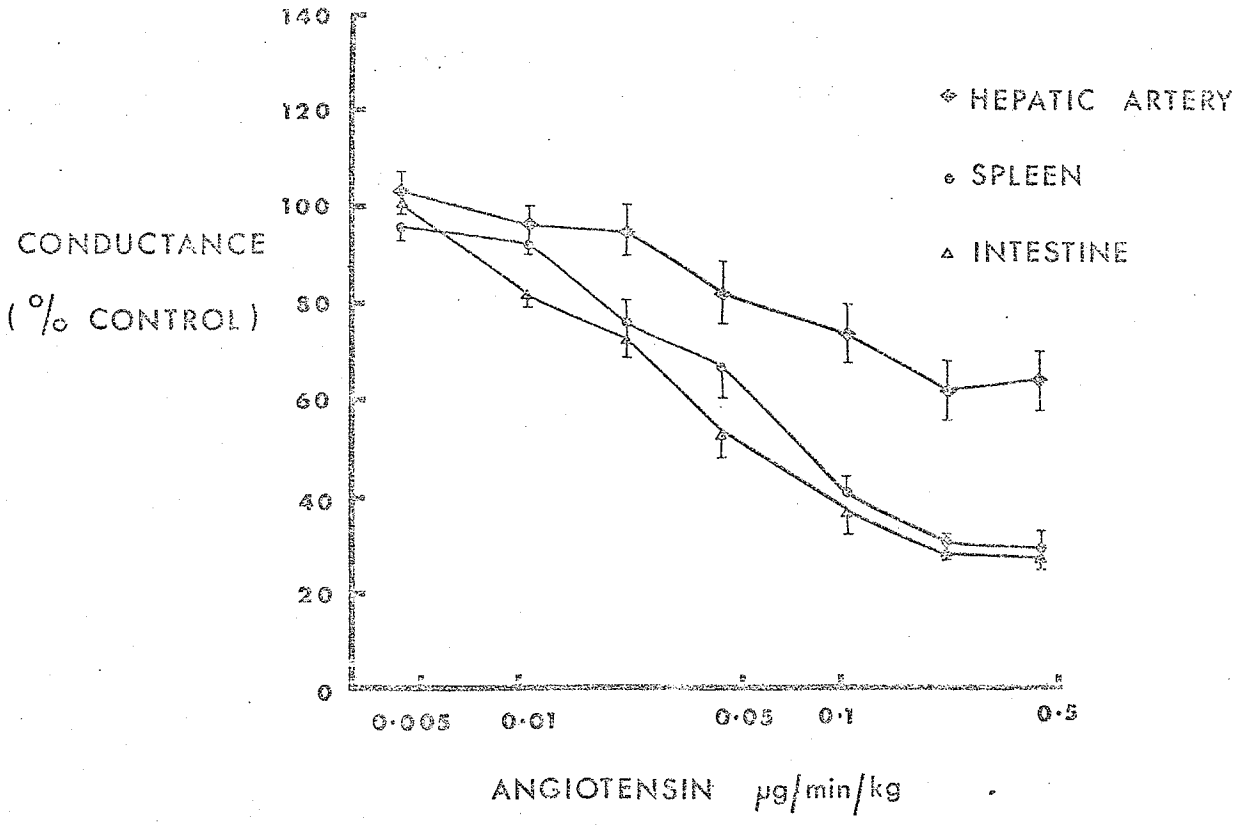


Figure 22. Responses of the hepatic arterial, splenic, and intestinal vascular beds to intravenous infusions of angiotensin. Each curve represents the mean $\pm$ S.E. of the responses in 5-7 cats.

Discussion

Following hemorrhage, the hepatic fraction of the cardiac output does not change. However, this response is the net result of intestinal and splenic vasoconstriction and hepatic arterial vasodilatation (see introduction). Studies of the mechanism of the intestinal and splenic vasoconstriction have suggested that vasopressin secretion and angiotensin production play important roles in these responses. Since hepatic arterial vasodilatation occurs following hemorrhage, it follows that the factors causing intestinal and splenic vasoconstriction either have no effect on the hepatic arterial resistance vessels or that their effect is counteracted and overridden by some other vasodilator mechanism. If vasopressin and angiotensin are important in the splanchnic response to hemorrhage, then intravenous infusions of these agents in amounts likely to be found in the blood during hemorrhage should cause a response similar to that which occurs after hemorrhage.

Under ideal conditions, the blood flows through each of the intestinal, splenic, and hepatic arterial beds should be recorded simultaneously. However, this requires three flowmeter probes with magnetic fields which do not interfere with each other when used in close proximity. These were not available so that each vascular bed was studied separately in groups of randomly selected cats of similar weight.

Intravenous infusions of vasopressin caused marked intestinal and splenic vasoconstriction and the sensitivities of these beds were very similar; the hepatic arterial bed dilated. Maximal responses were

obtained at infusion rates of 10 mU/min/kg of body weight. Assuming the blood volume of the cat to be approximately 50 ml/kg of body weight (Groom, Rowlands, and Thomas, 1965), infusions of vasopressin from 0.5 to 10 mU/min/kg for 5 minutes would be expected to produce blood concentrations of the drug from 50 to 1000 μU/ml in a 3 kg cat. This calculation assumes no inactivation of vasopressin during infusion. Following severe hemorrhage in the cat, Beleslin et al. (1967) reported that the concentration of vasopressin in the blood increased from 16 μU/ml to approximately 750 μU/ml. Clark and Rocha e Silva (1967) found that the blood levels of vasopressin in the cat rose from a control value of 47 μU/ml to 1020 μU/ml five minutes after hemorrhage and remained high at 763 μU/ml twenty minutes after hemorrhage. Thus, the amounts of vasopressin administered in these experiments are within the range found in the blood after hemorrhage, and the effects of intravenous infusions of vasopressin on the splanchnic vascular bed are consistent with the postulated role for vasopressin in the splanchnic response to hemorrhage.

Intravenous administration of angiotensin caused marked intestinal and splenic vasoconstriction, and the sensitivities of these two beds to the drug were similar. Angiotensin also constricted the hepatic arterial resistance vessels, but to a lesser extent than the intestinal and splenic vessels. Maximal responses to angiotensin were obtained at approximately 0.2 μg/min/kg of body weight. Assuming the cardiac output of the anesthetized cat to be approximately 130 ml/min/kg (Greenway and Lawson, 1966b), then intravenous infusions of angiotensin from 0.005 - 0.2 μg/min/kg would be expected to produce arterial

concentrations of 0.04 - 1.5 ng/ml. This calculation assumes there is no significant accumulation of angiotensin during the 5 minute infusion period. Hodge, Ng, and Vane (1967, dog) studied the rate of disappearance of angiotensin in various peripheral vascular beds. They found that 47-76% of infused angiotensin disappeared in one passage through most vascular beds. Only the lungs failed to remove angiotensin. These workers concluded that the half-life of angiotensin in the vascular system is approximately one circulation time. In view of the rapid removal of angiotensin from the circulation, it is unlikely that significant accumulation occurs during the infusions. This is supported by the observation that recovery of the flow response following cessation of an infusion was rapid. The formation of angiotensin during hemorrhage has not been studied as extensively as has the release of vasopressin. In dogs, Hodge, Lowe, and Vane (1966) reported that mild hemorrhage of 14-26 ml blood/kg causes an increase of 0.25 - 1.5 μg/min in the rate of generation and an increase of 0.1 - 0.33 ng/ml in the concentration of angiotensin in arterial blood. Scornik and Paladini (1964, dog) reported blood levels of 2.4 ng/ml after severe hemorrhage. Unfortunately, little work has been done in the cat. Assuming the data in the cats to be similar to that in the dog, the doses of angiotensin infused in the present experiments are not unlike the amounts found in the blood after hemorrhage. The responses of the intestinal and splenic vascular beds to intravenous infusions of angiotensin are consistent with the postulated role for this agent in their response to hemorrhage.

Since intra-arterial administration of either vasopressin or angiotensin causes hepatic arterial vasoconstriction (see introduction),

it seems likely that the effects of these agents on the hepatic arterial bed during hemorrhage and during intravenous administration must be counteracted by some vasodilator mechanism which opposes the direct action of these agents. Such a mechanism is established. As discussed in the introduction, a decrease in portal vein flow causes relaxation of the hepatic arterial resistance vessels and, as Hanson and Johnson (1966, dog) pointed out, the data is best explained on the basis of a myogenic response of the hepatic arteriolar smooth muscle to changes in sinusoidal pressure. Thus, it appears that there are no fundamental differences among the responses of the splenic, intestinal, and hepatic arterial vascular beds to vasopressin and angiotensin. However, in the hepatic arterial bed, the vasoconstrictor actions of these drugs are counteracted by a myogenic vasodilatation secondary to the decrease in portal vein flow. In the present experiments, the net result was a vasodilatation with vasopressin and a weak vasoconstriction with angiotensin. However, hepatic arterial flow was measured after the splenic vessels had been ligated and the spleen removed. Thus, the myogenic vasodilatator mechanism secondary to a decrease in portal vein flow was partially activated and it seems likely that the effectiveness of this mechanism in antagonizing the direct constrictor effects of vasopressin and angiotensin is probably greater in the intact animal than in the present experiments.

On the basis of previous work and the results described in this thesis it is possible to describe the overall response of the splanchnic resistance vessels to hemorrhage. Following hemorrhage,

portal vein flow decreases due to marked intestinal and splenic vasoconstriction. Vasopressin secretion from the pituitary gland and angiotensin production subsequent to renin release from the kidneys appear to play major roles in the mechanism of the intestinal and splenic vasoconstriction. The sympathetic nerves and adrenal medullary secretions may also play a role in the mechanism of the splenic vasoconstriction but they do not appear to be important in the mechanism of the intestinal vasoconstriction. In contrast to the marked vasoconstriction of the intestinal and splenic vascular beds following hemorrhage, the hepatic arterial resistance vessels dilate. This vasodilatation is probably a myogenic relaxation of the hepatic arteriolar smooth muscle in response to the decrease in portal vein flow. The hepatic arterial vasodilatation may play an important role in maintaining the oxygen supply to the liver. Under conditions where portal flow decreases, a quantitatively smaller increase in hepatic arterial flow would be sufficient to maintain the oxygen supply to the liver.

# APPENDICES

## Appendix 1: Recording Techniques

### A) Flow

Flow was recorded using a Nycotron (Oslo, Norway) type 372 electromagnetic flowmeter. A non-cannulating electromagnetic flowmeter probe with an internal diameter of 2 mm was chosen because this size provided a snug fit with the artery, yet did not offer a resistance to flow as judged by the pressure drop across the probe. The flowmeter probe was steadied by tying it to a micrometer-controlled screw clamp. Once properly positioned, the probe and clamp could be moved several millimeters in any direction without affecting flow or zero flow calibration.

The signal from the flowmeter was fed into either a Grass polygraph (Model 5C) or a Beckman pen recorder.

(i) Calibration of the flowmeter: At the end of each experiment, the flowmeter was calibrated as follows.

Heparin was administered and the superior mesenteric artery was cannulated downstream from the probe and clamp but proximal to any arterial branches. Therefore, flow through the cannula was equal to the flow through the flowmeter probe. Blood was collected in a graduated cylinder and the volume and time of collection noted. From these measurements the flow in ml/min could be calculated and this flow was represented by the deflection of the recorder. The calibration was usually expressed in ml/min/full scale deflection. Several determinations were made and the mean was used to calculate the flows during the experiment.

In experiments on the splenic vascular bed, the pedicle to the spleen was tied and blood was collected from a cannula in the hepatic artery (see figure 2).

In experiments on the hepatic arterial vascular bed, the common hepatic artery was tied and blood was collected from a cannula in the splenic artery (see figure 3).

(ii) Linearity: It was assumed that the flow passing through the flowprobe was linearly related to the deflection recorded by the flowmeter. This was checked periodically as follows.

Heparin was administered and the superior mesenteric artery was cannulated downstream from the flowmeter probe and screw clamp and proximal to any arterial branches as described above for the calibration procedure. A circuit had been constructed so that blood draining from the cannula passed through a graduated cylinder and then into a small reservoir. From the reservoir, the blood was pumped (Masterflex, Cole-Parmer, Chicago) back into the animal through the right jugular vein. The volume of the circuit was approximately 10 ml. When the flowmeter deflection was steady, the outflow from the graduated cylinder was clamped. The time elapsed to collect 5 ml of blood was noted and this provided a direct measurement of the flow rate which was represented by the flowmeter deflection. Controlled reductions in flow were made by graded clamping of the superior mesenteric artery. The flow was expressed as a percentage of the flow which gave a full scale deflection and the relationship of the flow to the flowmeter deflection is shown in figure 23. The amplification was varied and the flow ranged from 10 to 150 ml/min. Alterations of the hematocrit of the circulating blood from

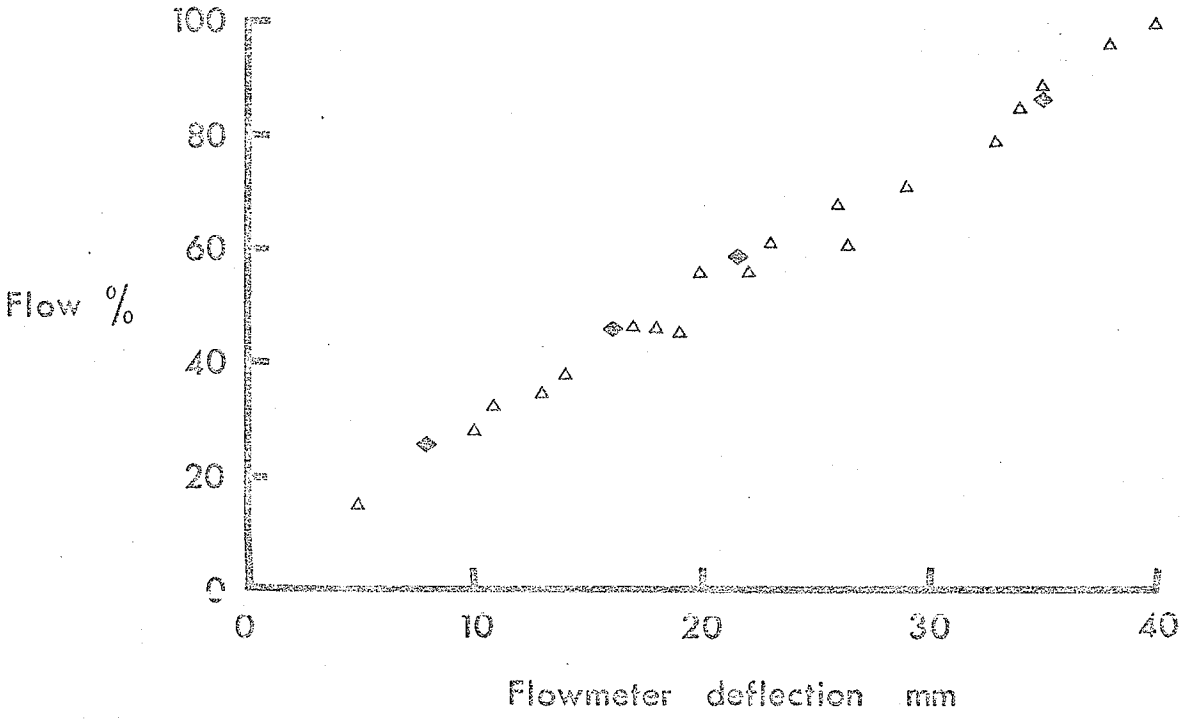


Figure 23. Relationship of superior mesenteric arterial flow rates to the deflection of the flowmeter. Flow rates were measured by collection of blood and expressed as percentages of the flow rates which gave a full scale deflection of the flowmeter. Open triangles - flow reduced by clamping superior mesenteric artery; closed diamonds - flow reduced by bleeding animal.

27% to 41% did not change the calibration of the flowmeter.

In animals subjected to hemorrhage, arterial pressure decreases and the geometry of the artery within the flowmeter probe may change. Therefore, in addition to reducing flow through the superior mesenteric artery by clamping the artery, flow was also reduced by stepwise removal of blood from the animal. Arterial pressure fell from 100 to 40 mm Hg. The relationship of the flow to flowmeter deflection is represented by the closed diamonds in figure 23.

It was concluded that flow through the flowmeter was linearly related to the deflection of the flowmeter. This relationship held whether flow was reduced by clamping of the superior mesenteric artery or by removal of blood from the animal.

B) Pressure

Mean pressures were measured using Statham pressure transducers (P23DC, P23AC). The signal from the transducer was fed into either a Grass polygraph or a Beckman pen recorder. All vessels were cannulated using thin-walled polyethylene tubing, the bore size of which was the maximum size permitted by the vessel. The cannulae were filled with 0.9% NaCl containing heparin (2 mg/ml). The zero reference point for all pressure measurements was at the level of the right atrium, approximately 3 cm above the operating table. The transducers were calibrated and linearity checked by comparison with the pressures on a mercury manometer.

C) Splenic Weight

Calibration: At the end of every experiment the splenic pedicle was tied and cut. A change in recorded weight caused by this procedure was due to tension on the pedicle. The spleen was removed leaving the cradle and wrappings of the spleen. Then series of weights were added to the cradle and a calibration was obtained.

Appendix 2: Effect of Long Periods of Hypovolemia on the Intestinal Vasoconstriction Following Hemorrhage.

The intense vasoconstriction of the intestinal resistance vessels following hemorrhage was well maintained for the sixty minute hypovolemic period (Groups 1, 3, 4, 1s, POB-treated). It was of interest to investigate the response over longer periods of time.

In 4 cats, blood was removed slowly and continuously until arterial pressure fell to 50 mm Hg. Thereafter, the arterial pressure was maintained between 35 and 50 mm Hg by either removing or returning small additional volumes of blood. In 3 of the animals with intact organ systems, the responses were recorded for 3, 3 1/2, and 4 1/2 hours following the onset of hemorrhage. The arterial pressures and volumes of blood removed are shown in Table 6. The vasoconstrictor response to extrinsic factors was calculated and the values for the means are shown in figure 24 (solid line). Vasoconstriction occurred during the initial period of blood removal and was well maintained for the entire period of study.

One of the animals was subjected to intestinal denervation, adrenalectomy, and nephrectomy. The pituitary was left intact. The responses were recorded for 8 hours. The arterial pressures and volumes of blood removed are shown in Table 6. The vasoconstrictor response was calculated and the results are shown in figure 24 (broken line). The vasoconstriction was well maintained for the 8 hour period.

It must be emphasized that these experiments are only preliminary and require confirmation. However, they do suggest that the vasoconstriction of the intestinal resistance vessels following hemorrhage is well maintained during several hours of hypovolemia.

TABLE 6

Values for arterial pressure and volume of blood removed in cats subjected to slow hemorrhage (Animals were bled until arterial pressure reached 50 mm Hg. Thereafter, arterial pressure was maintained near 50 mm Hg by either removing or returning small additional volumes of blood).

Cat 1, 2, 3 - intact organ systems; Cat 4 - subjected to intestinal denervation, adrenalectomy, nephrectomy.

| | Cat 1 | | Cat 2 | | Cat 3 | | Cat 4 | |
|---|---|---|---|---|---|---|---|---|
| Body weight (kg) | 3.5 | | 2.5 | | 2.9 | | 3.6 | |
| Time - after onset of hemorrhage | Art. P. (mm Hg) | Vol. Bled (ml) | Art. P. (mm Hg) | Vol. Bled (ml) | Art. P. (mm Hg) | Vol. Bled (ml) | Art. P. (mm Hg) | Vol. Bled (ml) |
| 0 | 138 | 0 | 140 | 0 | 108 | 0 | 103 | 0 |
| 30 | 83 | 53 | 58 | 55 | 50 | 48 | 50 | 36 |
| 60 | 75 | 92 | 50 | 83 | 50 | 71 | 50 | 52 |
| 90 | 50 | 125* | 50 | 98* | 45 | 87 | 50 | 55 |
| 120 | 50 | 125 | 55 | 76 | 55 | 87 | 50 | 60 |
| 150 | 50 | 105 | 60 | 68 | 45 | 92 | 50 | 62 |
| 180 | 50 | 90 | 50 | 70 | 50 | 95 | 50 | 64* |
| 210 | 50 | 81 | 50 | 67 | | | 45 | 60 |
| 240 | 50 | 74 | | | | | 35 | 62 |
| 270 | 50 | 64 | | | | | 35 | 59 |
| 300 | | | | | | | 40 | 49 |
| . | | | | | | | | |
| . | | | | | | | | |
| . | | | | | | | | |
| 480 | | | | | | | 35 | 29 |

* Maximum bleeding volume.

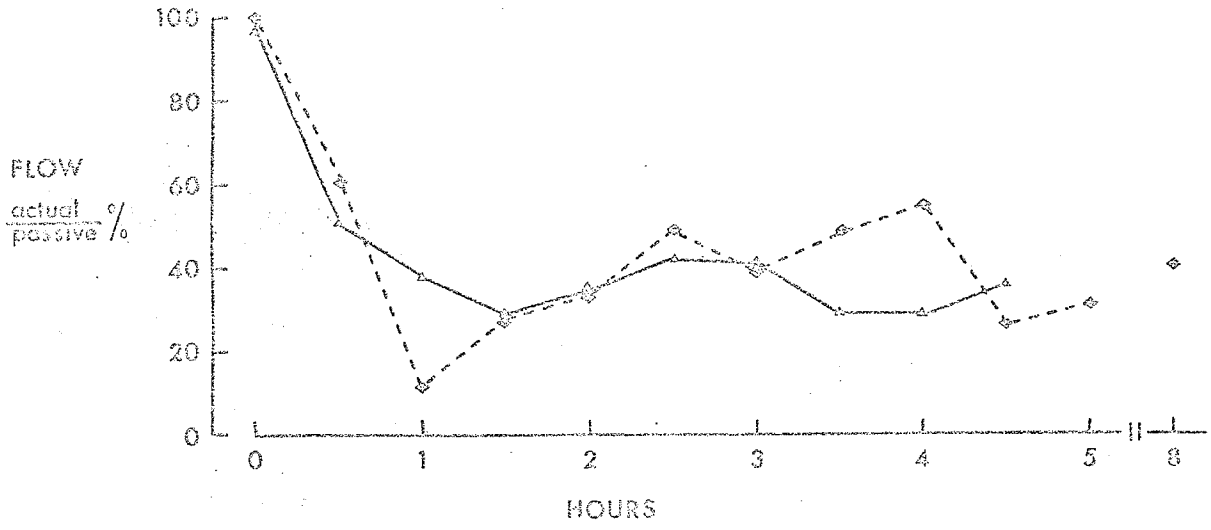


Figure 24. Responses during and after slow removal of blood in three cats with intact organ systems (solid line) and in one cat subjected to intestinal denervation, adrenalectomy, and nephrectomy - pituitary intact - (broken line). Superior mesenteric arterial flows are expressed as percentages of the passive flows expected from the changes in arterial pressure.

Appendix 3: Responses of the Intestinal Vascular Bed to Splanchnic Nerve Stimulation.

Following hemorrhage there was an intense vasoconstriction of the intestinal resistance vessels (Group 1); however, the intestinal innervation did not appear to play a role in this response (Group 5 and phenoxybenzamine treated group). It was possible, however, that the inability of the nerves to cause intestinal vasoconstriction was a result of injury to the nerves during placement of the flowmeter probe around the superior mesenteric artery (see methods). This possibility was investigated by recording the venous outflow response of the intestinal vascular bed to splanchnic nerve stimulation before and after placement of the flowmeter probe around the superior mesenteric artery. This was done in the following way.

Both adrenal glands were removed and hydrocortisone (5 mg/kg, Solu-Cortef, Upjohn Co.) was administered intramuscularly. The superior mesenteric vein was cannulated. A circuit had been constructed so that blood, draining from the cannula, passed through an extracorporeal flow-meter probe (3 mm diameter) and then into a small reservoir. A rotary pump returned the blood to the animal through the right jugular vein. Thus, the venous outflow from the intestinal vascular bed was measured and the superior mesenteric artery was undisturbed. The left splanchnic nerves were cut just beneath the diaphragm and the peripheral ends inserted through bipolar platinum ring electrodes. Supramaximal pulses (7-10 volts) of 2 msec. duration and at a frequency of 4/sec. were supplied from a Grass SD-5 stimulator. The flow responses to nerve stimulation are shown in figure 25a. Flow decreased markedly during

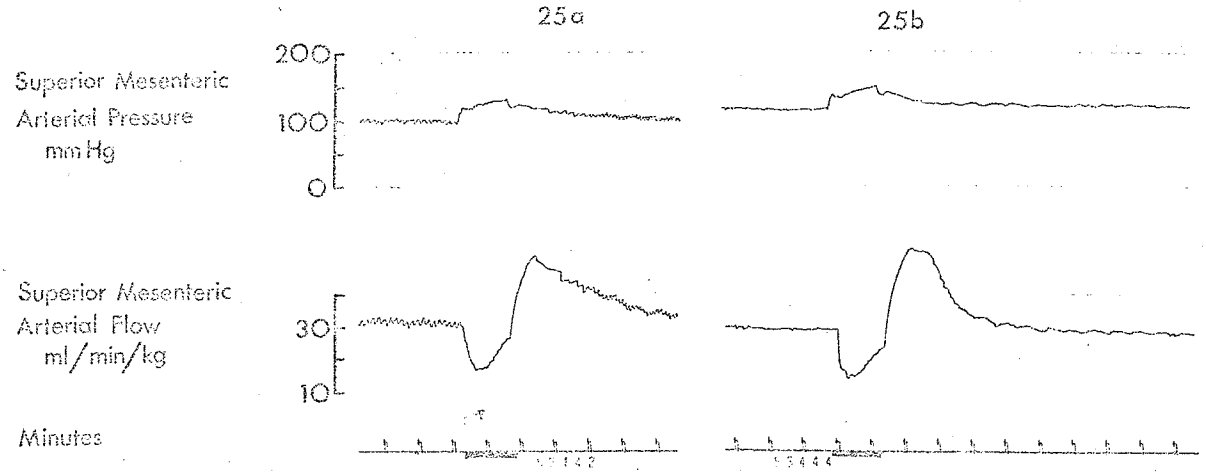


Figure 25. Response of superior mesenteric venous flow to splanchnic nerve stimulation before (a) and after (b) placement of the flowmeter probe around the superior mesenteric artery.

the first 30 sec. of stimulation and then "autoregulatory escape" occurred. The responses were similar to those reported by Folkow, Lewis, Lundgren, Mellander, and Wallentin (1964a). Then the superior mesenteric artery was dissected free from surrounding nerves and tissue and a non-cannulating electromagnetic flowmeter probe and screw-clamp placed around the artery. Nerve stimulation was repeated and the responses are shown in figure 25b. The responses were almost identical to those obtained prior to placement of the flowprobe around the artery.

Thus, the technique used to free the superior mesenteric artery from surrounding nerves and tissue and placement of the flowmeter probe around the artery did not appear to impair nerve transmission.

In the studies of the splenic vascular bed, it was unlikely that the splenic nerves were damaged since the flowmeter probe was placed on a section of the coeliac artery proximal to the point where the nerves from the coeliac ganglion joined the artery (see methods).

Appendix 4: Effect of Hemorrhage in Cats Subjected to Hypophysectomy.

During experimental work on the mechanism of the intestinal vasoconstriction following hemorrhage, it was observed that intestinal denervation together with adrenalectomy and nephrectomy did not reduce the vasoconstriction (Group 3); however, the addition of hypophysectomy to the other procedures did significantly reduce this response (Group 2). It was thought that the pituitary was the major factor causing the vasoconstriction and that removal of the pituitary would abolish the vasoconstriction.

Therefore, the responses to hemorrhages were studied in 3 cats in which only the pituitary gland was removed. The intestinal innervation, adrenal glands, and kidneys were all left intact. Hemorrhage was induced by withdrawing blood rapidly as described in the methods. Arterial pressure prior to hemorrhage was 127 $\pm$ 11.4 mm Hg. Following the period of blood removal, arterial pressure recovered to 94 $\pm$ 8.7 mm Hg at 10 minutes and 99 $\pm$ 5.9 mm Hg at 60 minutes. The vasoconstrictor response was calculated and the results are shown in figure 26. Within 5 minutes, flow fell to 55% of that expected from the pressure-flow curve and remained between 52% and 55% during the remaining 55 minutes. The values were not significantly different from those in Group 1 ($p < .05$, unpaired t-test).

Thus, besides the pituitary, other factors must have been important in the mechanism of the intestinal vasoconstriction following hemorrhage. Therefore, experiments were designed to examine the role of the kidneys (Group 4) and the role of the nerves and adrenals (Group 5).

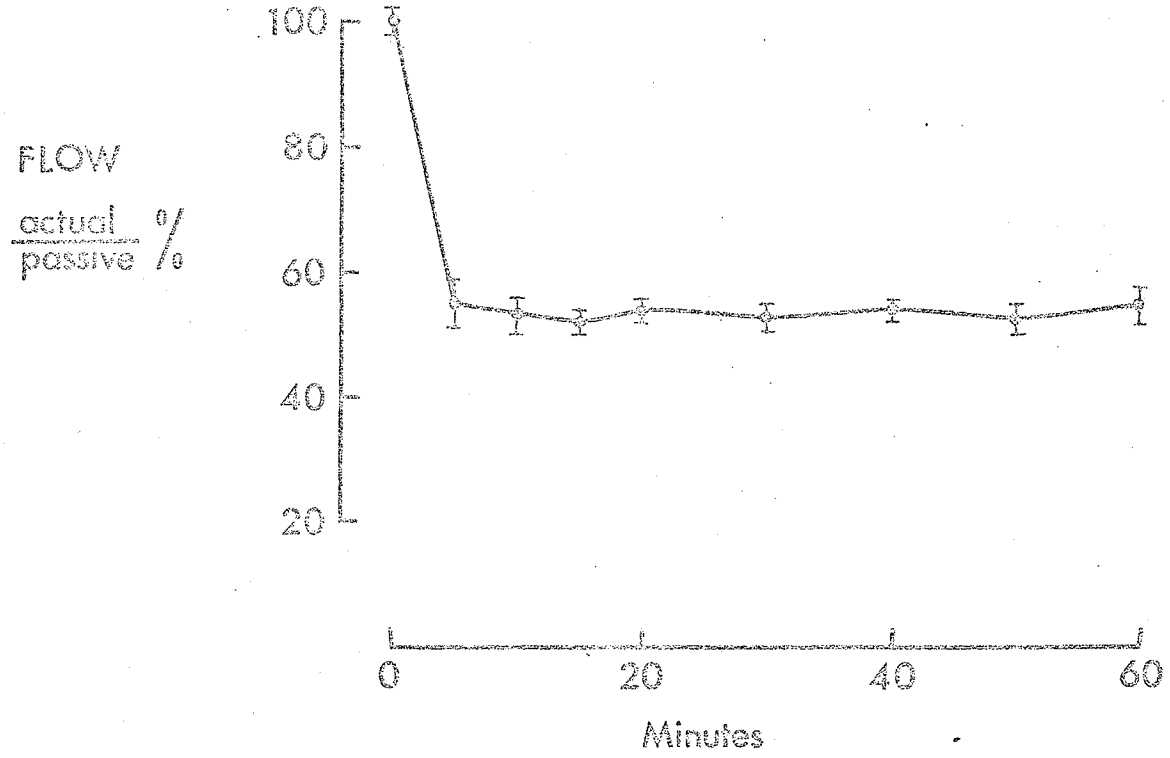


Figure 26. Response to rapid hemorrhage in cats subjected to hypophysectomy. The response is shown as the means $\pm$ S.E. of the superior mesenteric arterial flows expressed as percentages of the passive flows expected from the changes in arterial pressure.

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