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Joshua M. Halper

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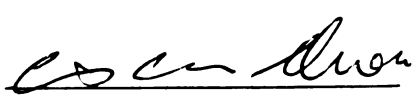

Major professor

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PROTECTIVE MECHANISMS OF INTESTINAL LUMINAL GLUCOSE-OLEIC ACID INSTILLATION IN HEMORRHAGIC SHOCK

By

Joshua Mark Halper

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ABSTRACT

PROTECTIVE MECHANISMS OF INTESTINAL LUMINAL GLUCOSE-OLEIC ACID INSTILLATION IN HEMORRHAGIC SHOCK

By

Joshua Mark Halper

Beneficial effects of perfusing solution containing 150 mM glucose and 40 mM oleic acid (G+O.A.) through the intestinal lumen of dogs during severe hemorrhagic shock were determined in anesthetized dogs. Aortic pressure was lowered to 35 mmHg for 3 hrs followed by reinfusion of all shed blood. All dogs were treated with a duodenal instillation of either G+O.A. or normal saline (N/S). Perfusion of G+O.A. increased survival rate (67%) in dogs. Blood glucose (BG) decreased to 20-40 mg% in N/S dogs and died within 8 hours. G+O.A. dogs absorbed 70% of perfused glucose and maintained normal BG for 26-41 hrs. Intestinal volume absorption was same for both treatment groups. Intestinal biopsies showed mucosal sloughing, congestion and hemorrhage in N/S dogs, while G+O.A. dogs had normal intact mucosal surface. The activities of cardiodepressants were unaltered in all samples for G+O.A. dogs but increased in all samples of N/S dogs.

DEDICATION

To my family and friends who gave me the strength and perseverance to complete this work.

ACKNOWLEDGEMENTS

I would like to thank Dr. William Frantz for serving on my committee. Special thanks to Dr. Robert Pittman for his valuable assistance and support. I especially would like to thank Dr. Ching-chung Chou for his guidance and wisdom during my study of physiology, research training, and in completion of this thesis.

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LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
C.O.	cardiac output
G+O.A.	Glucose-Oleic acid solution, bubbled with 95% oxygen - 5% carbon dioxide gas
Glc_A	arterial glucose concentration
hct	hematocrit
H.R.	heart rate
MABP	mean arterial blood pressure
MDF	myocardial depressant factor
N/S	Normal saline, bubbled with 95% oxygen and 5% carbon dioxide gas

INTRODUCTION

Chou, Kvietys, Post and Sit (1978) showed that instillation of chyme through the lumen of the small intestine markedly increased the local intestinal blood flow. Glucose (Chou, Burns, Hsien and Dabney, 1972) and oleic acid (Chou, Hsien, Yu, Kvietys, Yu, Pittman and Dabney, 1976) are the major contributing element of the physiological chyme that produces the hyperemic state. The nutrient-induced intestinal hyperemia is confined in the mucosal layer and not in the muscularis layer of the gastrointestinal tract (Chou et al., 1976). The rationale for the present study was that perfusion of glucose and oleic acid would increase local blood flow and oxygen delivery, especially to the mucosal tissues, and would supply energy substrates to the ischemic and hypoxic mucosa during hemorrhagic shock.

The purpose of this study was to elucidate the possible mechanisms of the beneficial effects of the glucose-oleic acid solution in prevention of irreversible hemorrhagic shock. Specifically we determined whether the treatment would prevent hypoglycemia, maintained blood volume by intestinal fluid absorption, prevent intestinal mucosal damage and cardiodepressant release.

I. LITERATURE REVIEW

A. Introduction

A basic feature of hemorrhagic shock is that blood flow through the vessels of the microcirculation is so impaired as to result in cellular damage. A contemporary question is whether the etiology of shock is due to poor cardiac performance and/or peripheral insufficiency.

Shock may exacerbate beyond the limits of control if hemorrhage is not corrected (Wiggers, 1950). The transition from reversible to irreversible status has been suggested by Crowell and Guyton (1961) to be due to an acute cardiac failure. It has been proposed that the "circulatory weak spot" in irreversible shock is the heart itself.

B. General Circulatory Sequence in Response to Hemorrhage

There are numerous ways to explain the phases of hemorrhagic shock. A simplified approach used by Guyton (1976) is based on the eventual outcome. With mild hemorrhage (less than 10-20% of total blood volume) the body will compensate, preventing deterioration of the circulation. This form of shock is called nonprogressive or compensated shock. The first line of defense is mediated through the baroreceptor reflexes. The reduction in mean arterial blood pressure (MABP) and pulse pressure during hemorrhage results in diminished stimulation of the baroreceptors located in the carotid sinuses and aortic arch. The diminished impulse decreases stimulation of the vasomotor center and results in an increase sympathetic drive to the heart and blood vessels (Berne and Levy, 1977). By further reduction in MABP below 50-60 mmHg, Neil (1962) demonstrated an increased

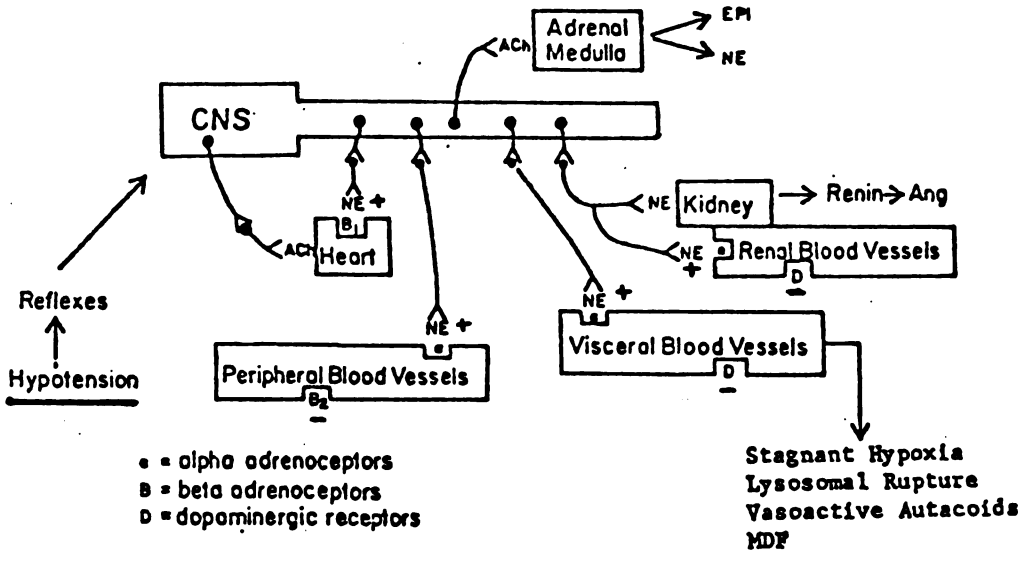
sympathetic drive via the chemoreceptor reflex. This reflex is also responsible for peripheral vasoconstriction, an increase in force and rate of respiration and an increase in venous return. However, the chemoreceptor reflex is not responsible for an increase in a cardiac sympathetic response (Downing and Siegel, 1963).

If the hemorrhage goes beyond 20% of one's total blood volume, the state of shock will worsen. Beyond a critical set point, shock becomes progressive. The events themselves will become a vicious cycle until complete deterioration of the circulation occurs.

Hypotension or hypovolemia causes activation of cardiovascular reflexes which result in a general increase in sympathoadrenal activity as shown schematically in figure 1. This figure also illustrates the deleterious positive feedback loop associated with circulatory shock. Hypovolemia results in sympathetic discharge stimulating release of epinephrine and norepinephrine from the adrenal medulla, and increased release of norepinephrine at sympathetic neuroeffector junctions of the heart, kidney and blood vessels. Release of these catecholamines cause increased myocardial contractility, heart rate, and peripheral vasoconstriction. Figure 1 also shows the splanchnic vasoconstriction and resulting visceral hypoxia and ischemia leading to breakdown of lysosomal membranes. The result is release of autolytic lysosomal enzymes into the circulation, formation of endogenous vasoactive substances, and possibly formation and release of a specific toxic factor(s) like myocardial depressant factor (MDF) (Adams and Parker, 1979).

Figure 1. Sympathoadrenal responses in cardiovascular changes associated with hypotension and circulatory shock. Alpha adrenoceptors associated with arteries and veins cause vasoconstriction, label with a (+). $Beta_1$ adrenoceptors associated with the heart causes increased myocardial contractility and heart rate; (+). $Beta_2$ adrenoceptors associated with arteries cause vasodilatation; (-). "Dopaminergic" receptors associated with mesenteric and renal arteries cause vasodilatation; (-).

Figure 1


Adrenal Medulla
EPI
NE
ACh
CNS
NE
Kidney
Renin→Ang
NE
Renal Blood Vessels
D
ACh
Heart
NE
Reflexes
Hypotension
NE
Visceral Blood Vessels
D
Peripheral Blood Vessels
α = alpha adrenoceptors
β = beta adrenoceptors
D = dopominergic receptors
Stagnant Hypoxia
Lysosomal Rupture
Vasoactive Autacoids
MDF

C. Cardiac Output and the Heart in Shock

The fundamental physiological defect in shock is a reduced cardiac output (C.O.) which may result from decreased myocardial efficiency or from reduced circulatory filling. Investigators have differing opinions of the relative importance of cardiac failure during the earlier stages of hypotension.

Hemorrhage reduces the rate of coronary blood flow and therefore tends to decrease ventricular function. The consequent reduction in C.O. due to hemorrhage leads to a further decline in arterial pressure, a classical example of a positive feedback mechanism (Berne and Levy, 1977). The myocardial ischemia results from inadequate coronary perfusion during severe hypotension (Carlson, Selinger, Utley and Hoffman, 1976; Hackel, Ratliff and Mikat, 1974). Studies by Kleinman, Krause and Hess (1979) and Jones, Smith, DuPont and Williams (1978) provided evidence for decreased coronary blood flow, subendocardial ischemia, necrosis, hemorrhage, and increased coronary vascular resistance in hemorrhagic shock. They demonstrated by tracer technique that regions of the left ventricle were totally unperfused in late hemorrhagic shock.

To prove cardiac hypofunction, Crowell and Guyton (1962) used cardiac output curves (plotting atrial pressure against the resulting cardiac output) to show progressive changes during the course of irreversible hemorrhagic shock.

Although C.O. is a useful index of myocardial function, Rothe and Selkurt (1964) used stroke work or minute work as a more inclusive factor for evaluating cardiac function. Rothe and Selkurt

showed an increase in heart rate following hemorrhage, however, at the last hour of hypotension, but prior to transfusion, there was a significant decrease in heart rate over the maximum hemorrhage (bleed out) period.

Myocardial depression is a factor in the development of irreversible hemorrhagic shock but the depression is not observed until at least 20% of the hemorrhage blood volume had spontaneously returned from the hemorrhage reservoir to the animal (Rothe, 1966). Early declines in cardiac output are due to the progressive reduction in cardiac filling. The moderate hemorrhagic hypotension and the cardiac/peripheral vascular damage could be corrected by fluid transfusions (Rothe and Selkurt, 1964).

D. Total Peripheral Resistance in Shock

Hemorrhagic shock generally has a greater effect on C.O. than arterial pressure. With a small blood loss (10% blood volume) the arterial pressure is maintained, while C.O. usually decreases. Many investigators using a canine model have found an increase in total peripheral resistance (TPR) after severe hemorrhage (Rothe, Love and Selkurt, 1963). With prolonged hemorrhagic hypotension, TPR generally decreases toward the prehemorrhage control levels. This gradual decline of TPR can be due to 1) a release of vasodilator materials into the circulation from ischemic organ(s), 2) the local accumulation of metabolites that cause vasodilation, 3) a decrease of sympathetic vasoconstrictor impulses, 4) a reduction of circulating catecholamines, and/or 5) the refractoriness of blood vessels to

catecholamines and possibly acidosis, which reduces vascular reactivity (Chien, 1967).

Another question arises concerning central nervous system depression. One possible explanation for the decline in TPR may stem from damage to the nerve pathway; whether the origin be central or peripheral, still remains unclear. Hinshaw (1971) has shown a suppression of autoregulation and spontaneous vasomotion, and Zweifach (1965) documented a diminished activity of the vascular smooth muscle during and after the hemorrhagic insult. During the later stages of hemorrhagic shock, the terminal vascular bed behaves more as a passive structure and no longer exhibits active adjustments to circulatory disturbances (Crowely and Trump, 1982).

E. Sympathoadrenal Responses in Shock

With a significant reduction in blood volume, cardiovascular reflexes are activated and result in a generalized increase in sympathoadrenal activity. This sympathoadrenal response causes the release of a large amount of activating catecholamines. Epinephrine and norepinephrine are released from the adrenal medulla. Norepinephrine is also released from adrenergic nerve terminals innervating blood vessels and the heart. These endogenous sympathetic mediators are active in the compensatory circulatory changes seen during the various stages of shock (Adams and Parker, 1979).

Freedman and Miller (1941), with infusions of epinephrine at low concentrations (3.4-16.4 ug/kg) produced shock and death in dogs. Poole and Watts (1959) showed that Freedman and Miller's lowest infusion rate produced epinephrine arterial blood levels of 39 ug/l.

This level produced shock and death in normovolemic animals. In 1964, Watts and Westfall were unable to detect catecholamines in a series of eight dogs whose arterial pressure was experimentally lowered from their prehemorrhagic control levels to approximately 80 mmHg (in 30 mmHg step reduction). Only after further reduction in pressure were significant levels observed. Watts (1956) documented a 5-10-fold increase in norepinephrine and a 50-100-fold increase in epinephrine during hemorrhagic shock in the anesthetized dog. Richardson (1965) and other investigators have verified Watts' observations and also have shown significant increases in arterial norepinephrine in the dog during shock.

The use of steroids will alleviate some effects of catecholamine. High levels of cortisol and corticosterone, are produced in excess during shock even when hypotension is severe, prolonged, and when blood flow to the adrenal cortex is compromised. Large doses of glucocorticoids have been shown to exert a beneficial influence in experimental shock only when administered within the first few minutes of the onset of shock and in the proper dosage (Griffiths, 1972). Generally, the beneficial effects of steroids may be related to vasodilation, increased production of adenosine triphosphate (ATP), increased conversion of lactic acid to glycogen, and stabilization of lysosomal membranes.

F. Renal Response in Shock

With decreased perfusion pressure, blood supply to renal vascular beds will be temporarily maintained by the autoregulatory control. If the hemorrhagic hypotension persists, renal blood

flow is diminished and urine formation ceases when systemic arterial pressure falls to 50 mmHg (Bell, 1972). If blood pressure is not brought under control, an accompanying rise in renal sympathetic nerve activity causes the secretion of renin from the juxtaglomerular apparatus. Renin converts angiotensinogen to angiotensin I. A converting enzyme found in the lungs and other tissue converts this octapeptide into a very potent vasoconstrictor, angiotensin II, both forms of angiotensin aid indirectly in water conservation by the kidney. Angiotensin II has also been shown to stimulate the adrenal cortex in releasing aldosterone which acts to conserve body sodium and maintain blood volume (Guyton, 1976).

Hypotension has been shown by Logan, Jose, Eisner, Lilienfield and Slotkoff (1971) to cause an intrarenal redistribution of blood flow to the inner cortical nephrons. Several factors may play a role in the redistribution of intrarenal blood flow. The following alterations in renal hemodynamics may be related to hemorrhage: increased humoral release of norepinephrine, increased humoral release of angiotensin, enhanced adrenergic stimulation, and diminished renal perfusion pressure. The intrarenal infusion of either norepinephrine or angiotensin, as well as renal nerve stimulation, all increased renal resistance, but did not alter cortical distribution of blood flow (Rector, Stein, Bay, Osgood and Cerris, 1972).

G. Response of Intestinal and Splanchnic Circulation in Shock

Considerable interest has been focused on the intestine as a target organ in shock. Lillihei (1957) introduced the original concept of an "intestinal factor" in irreversible hemorrhagic shock.

The theory which implicates the intestine as a target organ was based on two unrelated findings: 1) cardiac depression occurred late in the shock state even in the absence of coronary insufficiency, pointing to possible production of circulatory cardiotoxic factor(s). Secondly, marked splanchnic hypoperfusion is a prominent feature of early shock and, if prevented, results in an improved survival (Lefer, 1978).

During the beginning stages of hypovolemia, autoregulation is a prominent feature in the splanchnic as well as the hepatic renal, dermal, and skeletal muscles (Brobmann, Underwood, McCoy, Price and Jacobson, 1970). As the hypotension continues, the autoregulatory mechanism wanes and flow is significantly reduced. The coronary and cerebral vascular beds are not hampered by the central vasoconstriction. The fraction of C.O. perfusing coronary and cerebral vascular beds is actually increased during shock (Roding and Schenk, 1970). Roding and Schenk observed in 92% of their experiments that during hypovolemia, the mesenteric fraction of the C.O. decreases significantly below control values. Therefore, an attempt is made to maintain the immediate function of the heart and brain at the long-term expense of many tissues.

During hemorrhage, many organ systems become ischemic and hypoxic. The most susceptible organ in the dog is the intestine. With the combined effects of a relatively high metabolism and greatly reduced blood flow, the small bowel becomes nonfunctional. At the point when ischemic necrosis of the mucosa occurs, the shock becomes irreversible (Longerbeam, Lillehei, Scott and Rosenberg, 1962).

Intestinal hypotension with regional arterial inflow pressure of 30-35 mmHg have the following effects: reduced total intestinal blood flow to about 40%, reduced muscularis blood flow to about 25%, and mucosal blood flow reduced to only 70% of the prehypotensive control value (Haglund and Lundgren, 1978). Reduced arterial pressure (40 mmHg) and sympathetic vasoconstrictor nerve stimulation, did not affect the blood flow in the intestinal villous (Lundgren and Svanvik 1973).

Longerbeam et al. (1962) used the electromagnetic flowmeter to measure blood flow in the carotid, superior mesenteric and renal arteries during shock. In comparison with the other arteries measured, the superior mesenteric showed the greatest decrease in flow. Following the onset of shock, flow in the superior mesenteric decreased to less than 20% of control values. Just prior to death, little to no flow was measured in this artery.

In 1957, Lillehei found that the mortality rate due to experimentally-induced hemorrhagic shock could be reduced from 90% to 10% by keeping the intestinal perfusion pressure constant. Constant perfusion of the inferior vena cava, liver, lower abdominal aorta or brain did not significantly alter survival rate.

Accumulation of blood and fluid in the intestinal vascular bed plus a large transcapillary fluid loss was evident after reducing perfusion of the gut to 20 percent of resting levels (Lillehei, Longerbeam, Bloch and Manax, 1964). Lillihei and associates proposed a causal relationship between the mucosal lesions and the collapse of the cardiovascular system after a period of low perfusion.

Johnson and Selkurt (1958) measured these intestinal fluid loss using a gravimetric method. Their work indicated that this volume only averaged 31 milliliters by the end of hypotension. Haglund and Lundgren (1978) observed during and after intestinal ischemia that the total intestinal accumulation of blood and fluid never exceeded 5% of the total blood volume of cats.

The intestinal vasculature possess the property of reactive hyperemia following ischemia (Folkow, 1949). Bean and Sidky (1957) observed an increased blood flow through isolated canine intestinal loops as a result of perfusion with hypoxic blood. They proposed that this increase in blood flow was due to an unknown circulating vasodilator rather than the passive autoregulatory factors affected by CO_2.

Selkurt (1959) suggested that the hypotensive conditions in the villous tip of the intestine created a favorable environment for production and/or release of a vasodepressor or toxic agent. This theory differs from that of ischemic shock where intestinal pooling of blood is thought to be the basic mechanism.

Haglund and Lundgren (1974) favor the view that the mucosal ulceration observed early during the course of hypotensive shock is not due to a mucosal vasoconstriction but rather to an increased "efficiency" of the intestinal mucosa countercurrent exchange mechanism. Reducing perfusion pressure from 100 mmHg to approximately 40 mmHg by partially occluding the superior mesenteric artery decreases total intestinal blood flow to 1/3-1/2 of control while villous blood flow remains almost unaltered (Lundgren and Svenvik, 1973). The

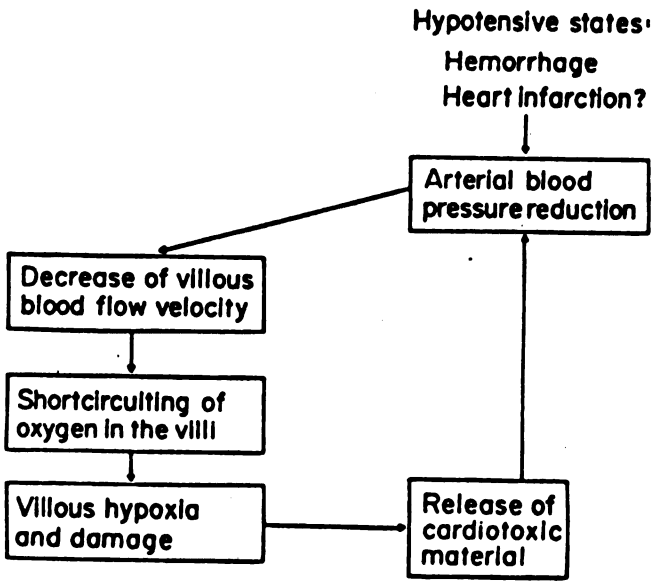
pathophysiological mechanism proposed by Lundgren and Haglund (1978) to be responsible for the development of the mucosal lesions in hypotensive states in the small bowel is illustrated in figure 2. Intraluminal perfusion with oxygenated saline will prevent mucosal lesions whereas perfusion with nitrogenated saline offers no clear-cut beneficial effects. This suggests that local tissue hypoxia causes mucosal ulceration. Haglund and Lundgren suggest that oxygen is short-circuited extravascularly in the intestine causing the villous tip to become severely hypoxic. This may explain why the mucosal lesions appear in the same location in numerous species. Studies by Bond and Levitt (1976) indicated that, in the dog, the villous circulation is relatively unaffected, which suggests that the countercurrent exchange mechanism is of no importance in this species. Lillehei et al. (1964) proposed that the mucosal lesions are secondary to hypoxia produced by an intense intestinal vasoconstriction in the dog. On the other hand, in the cat, the hemorrhagic hypotension does produce a vasoconstriction, although not as severe. If all intestinal venous blood was collected during the first 5 minutes following several hours of intestinal hypotension and replaced by blood from a healthy donor animal, systemic blood pressure remained unchanged. When the collected intestinal venous blood was returned to the animal, blood pressure fell rapidly (Haglund and Lundgren, 1978).

H. Interstitial Fluid Movement in Shock

Although interstitial fluid movement into the vascular compartment has been well documented (Haddy, Overbeck, Daugherty, 1968),

Figure 2. Schematic representation of the pathophysiological mechanism responsible for the development of mucosal lesions of the small intestine in a hypotensive state.

Figure 2


Hypotensive states:
Hemorrhage
Heart infarction?
Arterial blood pressure reduction
Decrease of villous blood flow velocity
Shortcircuiting of oxygen in the villi
Villous hypoxia and damage
Release of cardiotoxic material

intestinal fluid absorption as a compensatory mechanism for blood loss (volume replacement) is not as well established. It is presumed that hemorrhage stimulates precapillary vasoconstriction, and the resulting decrease in the mean capillary hydrostatic pressure causes a net inward movement of extravascular fluid (Mellander and Lewis, 1963).

Adolph, Gerbegi and Lepore (1933) bled animals rapidly, removing 20-35 ml/kg of body weight and estimated changes in plasma volume from changes in the concentration of plasma proteins. Results showed that the estimated plasma dilution from interstitial fluid movement was sufficient to restore approximately 35% of the plasma removed.

Jenkins, Hafaya, Morchioro, Montgomery and Swan (1961) reported that dogs bled over a 3-hour period for a total volume equal to 35% of their initial blood volume, did not show significant changes in blood pressure and plasma volume until approximately one-fourth of the total blood had been removed. Their data, along with other investigators, show hemodilution in hemorrhaged dogs is well correlated quantitatively and chronologically with the degree of blood pressure fall.

Marty and Zweifach (1971) produced severe hemorrhagic shock (30-45 mmHg) in nine rabbits and five splenectomized dogs. Within one hour after initiation of hemorrhage, hematocrits dropped 25 to 40% and colloid osmotic pressure dropped 30 to 33%. After the first hour of hypotension, little change in colloid osmotic pressure or hematocrit was observed after these initial changes.

I. Intestinal Fluid Absorption in Shock

The intestine plays a protective role during early hemorrhagic shock and represents an important site of early transcapillary fluid refill associated with hemorrhage shock. Net absorptive capacity of the jejunum for physiological saline is uninfluenced by severe hemorrhage (Van Liere, Northrop and Sluth, 1938; Goldberg and Fine, 1945). Hankes, Nelson, and Swan (1969) also showed that the jejunal capacity to absorb fluid (lactated Ringer's solution) from the intestinal lumen was not increased by hemorrhage.

Fromm (1973) showed (in the rabbit) that, in early stages of hemorrhagic shock, water and sodium absorption from the intestine increased more than 60% over control experiments. Sodium was found to be absorbed against its electrochemical gradient during shock. This suggests involvement of an active ion transport mechanism. In the above study, oxygen consumption in the ileal mucosa was measured during shock. The *in vitro* experiment showed oxygen to increase, which is consistent with an increase in active ion transport.

Hankes et al. (1969) observed *in vivo* a 30% decrease of water absorption in the canine jejunum during two hours of hemorrhage. While Bacalzo, Perkins, Miller and Parkins (1972) showed a 38% decrease in water absorption after a similar time period in the rat. Miles, Davies and Shields (1968) demonstrated that absorption of water and electrolytes from the intestine had ceased during irreversible hemorrhagic shock in dogs. Oligemia was aggravated by increased losses of fluid into the bowel. Whereas in early irreversible shock

the ileum absorbed water and sodium more rapidly than in control studies where dogs were not bled.

Gergely and Hagy (1969) found that normovolemic dogs subjected to surgical exclusion of the small intestine one week prior to hemorrhage were unable to tolerate severe hemorrhagic hypotension (30 mmHg). Most of their dogs died within one hour of hemorrhage. Eviscerated dogs tolerated hypotension for a longer period; however, less blood removal was required to precipitate circulatory collapse (Marty and Zweifach, 1971).

The process of fluid absorption during hypovolemia may result in a useful therapeutic maneuver. Oral administration of an isotonic solution containing necessary electrolytes into the gastrointestinal tract could temporarily replace lost volume during hemorrhage. Miller and Dale (1978) showed, in the dog, significant increases in the percent of plasma volume restored with enteral saline administration following hemorrhage. However, isotonic glucose treatment via the same route also increased plasma volume substantially, but to a lesser extent. Venous hematocrit and plasma protein concentration decreased along with the plasma volume expansion observed after fluid administration. However, Goldberg and Fine (1945) reported earlier that a limitation on fluid absorption from the gut was imposed by reduced blood flow through intestinal capillaries. Miller and Dale demonstrated a rapid and extensive volume recovery after fluid treatment. Their glucose absorption was not substantially impaired with hemorrhage as previously thought by Goldberg and Fine, and Cook, Wilson and Taylor (1971).

J. Glucose and Metabolism in Shock

As early as 1877, Claude Bernard observed the hyperglycemia of hemorrhage. His observations were verified by Wiggers (1950) and later by McCormack, Lien, Herman and Egdahl (1969). The hyperglycemic state was found to be accompanied by decreased liver glycogen and increased hepatic venous glucose concentration. Adrenal catecholamines release in response to decreased arterial blood pressure is responsible for hepatic glucose mobilization, general vasoconstriction, and tachycardia (Jorley and Watts, 1964). If animals are adrenalectomized, hyperglycemia is not observed during hemorrhage (Selye and Dosne, 1941). The demand for blood glucose surpasses glucose mobilization and glyconeogenesis in hemorrhagic shock. Arterial glucose levels will wane, resulting in terminal hypoglycemia and death.

The transition from reversible to irreversible phase of shock is associated with a decreased blood glucose, early morphologic changes in hepatic mitochondria and the spontaneous uptake of hemorrhaged blood from the reservoir in experimental hemorrhagic shock (Hift and Strawitz, 1961). Schumer (1968) reported in more severe forms of hypoglycemia that the supply of glycogen is catabolized very quickly and lasts for only 4 hours. Therefore, to support gluconeogenesis, the liver, kidney, and heart cells can absorb fatty and amino acids and convert them to the intermediates of the citric acid and glycolytic cycles. However, free fatty acids may be "toxic" to the myocardium. The preferred energy substrate is glucose (Opie, 1970). In addition, maintenance of blood glucose concentration has

been correlated with enhanced tolerance to hemorrhage (Drucker, Kaye, Kendrick, Hoffman and Kingsbury, 1954).

Strawitz and Hift (1960) observed with starvation preceding hemorrhage that both the initial hyperglycemic response and the tolerance for the hypovolemia is shortened. In addition, glycogen depleted (fasted) animals were shown (by this group) to be less tolerant to hemorrhage in comparison with fed animals. However, the use of an "elemental" diet offered protection of essential organs and contributed to the enhanced survival of animals subjected to hemorrhagic shock (Bounous, Sutherland, McArdle and Gurd, 1967).

Hemorrhage is the primary factor for the development of cellular hypoxia during shock. The arterial hemoglobin is not deficient nor is there failure of tissue capability to extract oxygen. Nelson and Swan (1974) have suggested three determinants that lead to the lack of oxygen at the tissue level: 1) a reduced oxygen-carrying capacity of the blood due to decrease in red blood cells; 2) a diminished flow of red cells to all tissues; and 3) an uneven distribution of red cells to different tissues. Dogs subjected to hemorrhagic shock have an increased rate of oxygen consumption and glucose uptake after transfusion. As a result of prolonged shock, the liver does not maintain adequate production of glucose and blood sugar decreases during latter stages of normovolemic shock (Vigas, Hitery and Haist, 1971). Earlier reports by Wiggers (1950) and Smythe (1959) have suggested that hepatic malfunction during shock may be the consequence of reduced blood flow and attendant hypoxia. Damage to the liver may be responsible for reduced essential nutrient production,

clearance of metabolic by-products and an increase in circulating toxins.

Hypoxia has been thought to cause decreased intestinal absorption by damaging the intramucosal ATP resynthesis. Varro, Blaho, Csiernay, Jung and Szarvas (1965) demonstrated that local insufficiency of the circulation of a small intestinal segment affects the absorptive capacity of the mucosa through inadequate oxygen supply. The intestinal absorption of glucose is a process requiring energy through cellular activity. A significant correlation exists between the blood glucose level and changes in the respiratory control ratios of hepatic mitochondria (Rhodes, DePalma and Robinson, 1973). A subsequent study by Rhodes and associated found that loss of respiratory control (uncoupling of oxidative phosphorylation) was correlated with the need for infusion of blood. Damaged and uncoupled mitochondria have been blamed for the progressively inefficient utilization of substrate. Schumer, Das Gupta, Moss and Nyhus (1970) have shown that mitochondrial function in cardiac muscle, skeletal muscle, and liver subjected to hypovolemic shock depresses mitochondrial respiration and leads to the complete uncoupling of oxidation and respiration.

Beatty (1945) using dogs in hemorrhagic shock showed a progressive increase in lactate and a fall in glucose in venous vs. arterial blood. Beatty attributed the conversion of glucose to lactate to the prevailing anoxia in hemorrhagic shock. During hemorrhagic shock a rise in the lactate to pyruvate ratio occurs. This results from predominant anaerobic over aerobic carbohydrate metabolism. In

anaerobic metabolism, energy (ATP) is produced through glycolysis. This process alone is quite inefficient since approximately 8-14 times as much substrate is required to produce an equivalent amount of energy from anaerobic metabolism vs aerobic metabolism. The concomitant fall in blood glucose occurs as much more substrate is needed to produce ATP to maintain life-sustaining processes (Mela, Miller and Nicholas, 1972).

The intraluminal instillation of glucose into the small intestinal sacs was shown by Chiu, Scott and Gurd (1970) to protect the ischemic bowel. Administration of hypertonic glucose and sucrose during the beginning stages of physiological decompensation observed in shock were shown to cause transient expansion of plasma volume. Separate administration of glucose vs. sucrose was responsible for slightly longer survival time and maintained a better respiratory compensation (Moffat, King and Drucker, 1968). Spigelman and Ozeran (1970) using a modified Wigger's protocol, infused glucose intravenously late in the decompensation phase and noted a prolonged survival in dogs given insulin at the time of the glucose injection. However, Gump, Long, Wong and Kinney (1973) indicated that little of the administrated glucose was oxidized when dogs received glucose after a severe hemorrhagic hypotension. The exogenous glucose was cleared (or taken up by various organs) from the blood stream with, or without, the addition of insulin in their experiments. Normal dogs rapidly utilized the administered glucose and 90 minutes later the blood sugar concentration returned to fasting levels. Only a small percentage of the exogenous glucose was converted to lactate

and channeled to nonoxidative pathways in dogs subjected to hemorrhagic hypotension.

Hinshaw, Peyton, Archer, Black, Coalson, and Greenfield (1974) demonstrated that exogenous administration of 50 percent glucose (I.V.) infused at rates which maintain blood glucose concentrations at preshock values during the postendotoxin period prevented death. This same group of investigators showed that glucose infusion at an intermediate period in endotoxic shock when marked hypoglycemic blood levels were evident, resulted in a significantly increased survival rate. After a low endotoxin challenge of 0.25 mg/kg body weight Berk, Hagen, Beyer, and Gerber (1970) were able to increase survival rate significantly in the dog by maintaining the blood glucose level near control values with infusions of 50 percent glucose. The average amount given to each dog was 2.7 ml/kg body wt., while comparable volumes of normal saline were infused into control animals. Glucose administration had little to no effect with larger endotoxin doses of 0.5 mg/kg. While Cryer, Herman, and Sode (1971) subjected baboons to live E. coli organism shock, their treatment with a regimen of glucose-insulin-potassium was shown to extend the duration of the survival time over the nontreated shocked controls.

Repogle, Kundler, and Gross (1965) infused 25 ml of 50 percent glucose solution (I.V.) in hemorrhagic hypotension and produced a large increase in cardiac output and decreased total peripheral resistance. Similar infusions of 3 percent saline, whole blood or normal saline had no effect. Hypertonic glucose produced increases in superior mesenteric blood flow and oxygenation at low arterial

pressures (30 mmHg) during hemorrhagic shock (Bave, Tragus, and Parkins, 1967).

Long, Spencer, Kinney, and Geiger (1971) showed that injury and sepsis did not impair the ability of the body to oxidize glucose but that the glucose oxidation rate more than doubles. Numerous clinical cases and observations have been reported on the beneficial effect of glucose infusion. Combat casualties in hypervolemic shock were resuscitated in the usual fashion and, at the same time received, by random selection either nothing else or equal osmolar doses of 50 percent glucose, 25 percent mannitol or 30 percent saline. Glucose produced an increase in blood pressure and pulse pressure which was significantly greater and of longer duration than any of the other treatment groups (McNamara, Molot, Dunn, and Stremple, 1972). Critically ill patients maintained on 5 percent glucose infusions showed improvement over patients who received mannitol infusions. The beneficial effects of those patients who received the 5 percent glucose infusions were more pronounced than those patients maintained on 50 percent glucose therapy (Pindyck, Drueker, Brown, and Shoemaker, 1974).

K. Toxic Factors in Shock

Early reports by two groups; Blalock (1943) and Katzenstein, Mylon and Winternitz (1943), described a toxic substance in the thoracic duct lymph of dogs during tourniquet shock (Lefer, 1973). Then, in 1957, Lillehei proposed an "intestinal factor" in irreversible hemorrhagic shock. Since then, much attention has been devoted to the splanchnic region as one possible site for the origin of such

toxic factors. Selkurt (1959) and Baez, Hershey and Rovenstine (1961) proposed the presence of a toxic factor in splanchnic artery occlusion shock originating from the ischemic intestine. Dogs subjected to severe hemorrhagic hypotension (40 mmHg for 90 mins) released a humoral reticuloendothelial-depressing substance formed in the ischemic intestine (Blattberg and Levy, 1962).

Gomez and Hamilton (1964) attributed the myocardial depression late in shock to a cardiodepressant substance released in hemorrhagic shock. In 1964, Brand and Lefer showed that plasma taken from cats in the early stage of irreversible post-oligemic shock depressed contractility of isolated cat papillary muscles. Hence, the term, myocardial depressant factor or MDF was given to this substance(s). MDF was believed to originate from the feline ischemic pancreas. Similar myocardial depressant factor(s) were found in the plasma of both cats and dogs in hemorrhagic shock (Lefer and Martin, 1970).

Since Brand and Lefer's report of a toxic substance in plasma of shock animals, numerous investigators have reported a variety of "toxic factors" in many different forms of shock (Lefer, 1978). The general theory is that splanchnic hypoperfusion appears to be the common denominator in all of the forms of circulatory shock. Haglund and Lundgren (1978) proposed a direct and causal relationship between the mucosal lesions observed during shock and the cardiovascular collapse after intestinal hypoxia. This proposal may be due in part to release of toxic factors because exchange transfusions after intestinal hypotension proved to be beneficial. There is some skepticism over the site of origin for these "toxic factors". Hagland

and Lundgren (1972) could not attribute release of a myocardial depressant factor to the pancreas since in their experiment whole pancreases were surgically removed and minor remnants were never exposed to hypotension.

The general scheme which gives rise to MDF and other splanchnic toxins follows: the prolonged deficit of blood flow causes local ischemia, hypoxia, and acidosis in the splanchnic organs. Lysosomal membranes are disrupted and a variety of proteases and acid hydrolases are released. Cellular proteins are exposed to the hydrolytic action of the lysosomal proteases leading to the formation of a variety of biologically active peptides. These small peptides are believed to be cardiotoxic in nature. In addition, splanchnic lysosomal hydrolases themselves have been reported to circulate in shock, and may sensitize the heart to a variety of toxins (Parker and Adams, 1981). There is agreement that incomplete peptides may be formed during circulatory shock through tissue breakdown and release of lysosomal hydrolases. The question arises and skepticism continues over whether these peptides are really cardiotoxic and contain vasoconstrictor properties. No specific toxins have been identified and synthesized to this date.

L. Intestinal Pathology in Shock

Several groups of investigators have correlated the development of intestinal mucosa lesions with prolonged hypoperfusion and survival of the experimental animal. Wiggers (1950) observed congestion, edema, and hemorrhage in the intestinal mucosa after hemorrhagic hypotension in the dog. A useful six-step reference in grading the

morphological changes (damage) in the intestinal mucosa of dogs subjected to superior mesenteric artery occlusion was developed by Chiu, McArdle, Brown, Scott and Gurd (1970). By maintaining intestinal blood flow, Lillehei (1957) was able to reduce mucosal damage and mortality in dogs subjected to irreversible hemorrhagic shock. Lillehei proposed a causal relationship between the mucosal lesions and the collapse of the cardiovascular system after a period of low perfusion of the intestine.

Bounous (1969) attributed the mucosal damage to lowered tissue oxygen which, in turn, creates an environment ideal for enzymatic degradation. Lundgren and Haglund (1978), however, explained the development of mucosal lesions in shock in accordance with their theory of villous hypoxia; i.e., short-circuiting of oxygen through the countercurrent exchanger at the villous tip. The microscopic appearance of the mucosal lesions in all human patients observed in various forms of shock were identical with previously reported findings in the feline and canine small intestine after hemorrhage or local intestinal hypotension (Haglund, Hulten, Ahren and Lundgren, 1975).

The high metabolic rate in tissue of the intestine makes it vulnerable to changes in oxygen tension during low flow states. Haglund and Lundgren (1977) proposed that lowering of perfusion pressure is the important hemodynamic event in shock with regard to the development of the mucosal lesions. Perfusing the intestine during a period of regional hypotension with oxygenated saline

prevented a majority of mucosal damage, whereas the use of nitrogenated saline had no preventive effect. The use of a graded obstruction in an experimental shock model in the rat was shown by Haglind, Haglund, Lundgren, Romanos and Schersten (1980) to have a direct correlation with mortality rate and mucosal damage.

Glucose introduced into an intestinal sac at the time of occlusion of the superior mesenteric artery proved to be beneficial over adjacent sacs with no solution or nonabsorbable mannitol. Lactate levels in sac containing glucose were elevated suggesting that glucose acted as an energy source rather than just by an osmotic effect (Chiu, Scott and Gurd, 1970).

II. MATERIALS AND METHODS

A. General

Mongrel dogs of either sex (12-28 kg) were used in these investigations and all dogs were anesthetized with sodium pentobarbital (30 mg/kg). The dogs were intubated with a cuffed endotracheal tube and ventilated with a positive pressure respirator (Model 607, Harvard Apparatus, Dover, MA). They were placed in a supine position and ventilated with room air. Tidal volume and respiratory rate were adjusted to maintain normal arterial blood pH (pH = 7.38-7.45).

Polyethylene tubings (PE 320, i.d. = 2.69 mm, o.d. = 350 mm) were placed into the right and left femoral arteries and advanced to the level of the aorta. The left femoral vein was also cannulated with similar tubing. Measurements and sampling were made from the following vessels: 1) right femoral artery was connected to a low volume displacement pressure transducer (Statham p23 Gb, Hato Ray, Puerto Rico) for continuous monitoring of aortic blood pressure throughout the entire course of the experiment; 2) left femoral artery was connected to a calibrated experimental hemorrhage reservoir as described by Lamson and Deturk (1945) and also used for arterial sampling of glucose, cardiodepressants, pH, and blood gas determinations; 3) left femoral vein was used for sampling venous hematocrit, administration of supplemental anesthetic and an anticoagulant sodium heparin (500 μ/kg), and administration of supplemental 6% dextran 70 (medical grade dextran, Sigma Chemical Co.) in saline to replace lost fluids from surgery and sampling. Blood

pressure and heart rate were continuously recorded on a direct-writing oscillograph (Sanborn Model 7714-04A, Waltham, MA or Hewlett-Packard Model 7796A, Waltham, MA).

The abdomen was opened via a midline incision and the proximal duodenum was located and exteriorized. A rubber tube (Levin tubing, 12 French American Hospital Supply, McGraw Park, IL) was inserted into the duodenal lumen at approximately 10 cm distal to the pylorus and secured by a purse-string suture. The Levin tubing was connected to a Gilson Minipuls 2 pump (Gilson Medical Electronics, Inc., Middleton, WI) for infusion of the test solution. Proximal to the purse-string suture, a Bainbridge intestinal forcep was used to prevent fluid efflux through the pylorus into the gastric lumen.

All dogs received one of three types of treatments via the duodenal tubings: The following 1) no treatment; 2) instillation of normal saline and bubbled with 95% oxygen - 5% carbon dioxide gas at 3 ml/min, and 35 ml/kg body weight (N/S); and 3) instillation of a solution, containing 150 mM glucose, 40 mM oleic acid and bubbled with 95% oxygen - 5% carbon dioxide gas, at 3 ml/min and 35 ml/kg body weight (G+O.A.). Table 1 shows the composition of the G+O.A. solution (Glucose and inorganic salts; Mallinckrodt Inc., Paris, KY). The osmolality and pH of the G+O.A. solution ranged 285 - 320 mOsm, and 7.20 - 7.35, respectively. In each series of experiments, treatment with the duodenal instillation began at one hour after the onset of hemorrhage and lasted as long as the infusate lasted, i.e., 35 ml/kg. The duration of instillation usually lasted 3½ hours.

Table 1. Composition of the Glucose-Oleic Acid Solution

Chemicals	mM	Grams/L
Glucose	150.0	27.0
NaOH	0.18	0.007
Taurcolic Acid[1]	6.01	3.1
NaCl	77.6	4.5
KCl	1.3	0.1
$NaHCO_3$	6.0	0.5
$MgCl_2$ $6H_2O$	0.53	0.107
NaH_2PO_4 H_2O	0.21	0.029
$CaCl_2$[2]	0.45	0.05
Oleic Acid[3]	40.0	11.0 (12.5 ml/L)

[1] Sodium salt, practical grade. ICN Nutritional Biochemicals, Cleveland, OH.

[2] The solution was stirring when $CaCl_2$ was added. Solution was then sonified and pH adjusted to 7.3.

[3] 90% grade, United States Biochemical Corp., Cleveland, OH.

B. Protocol for Hemorrhagic Hypotension

All dogs were allowed 45 - 60 minutes to recover from surgery before hemorrhage. A modified Wiggers hemorrhage method (Wiggers and Werle, 1942) was used. Each dog was hemorrhaged via the left femoral artery into a calibrated experimental hemorrhage reservoir in 100 ml increments until the mean arterial blood pressure fell to 50 mmHg. When the mean blood pressure reached 50 mmHg, the height of the hemorrhage reservoir was adjusted to further reduce the mean blood pressure to 37 ± 2 mmHg. The time course for bleeding was 30 ± 10 minutes. The blood pressure was then maintained at 37 ± 2 mmHg by adjusting the height of the reservoir for three hours. At the end of three hours, all the remaining shed blood was reinfused back to the animal. During the entire hypotensive period, the dogs were disconnected from the respirator.

C. Survival Series

The aim of this series was to determine whether instillation of G+O.A. through the small intestine would increase the survivability after hemorrhagic shock as described in general procedure (Section A). The experiments were performed under aseptic conditions and only one artery, i.e., the left femoral artery, was cannulated for arterial pressure measurement, hemorrhage and arterial sampling. A small midline incision approximately 8 - 10 cm in length was made. The proximal duodenum was exteriorized, the Levin tubing was placed into the lumen and secured by a purse-string suture. All incisions were closed immediately after the completion of surgical manipulations. A

mild antiseptic agent (Betadine ointment) was applied around the incised area.

After administration of sodium heparin (500 u/kg), the dog was subjected to three hour hemorrhagic hypotension. Seven dogs received no treatment, seven other dogs received a duodenal instillation of N/S, and nine dogs received a duodenal instillation of G+O.A. solution. Instillation was begun at one hour after bleeding for all treatments. The instillation rate of 3 ml/min and volume of 35 ml/kg body weight, were used in all series. Blood samples were obtained from the left femoral artery for plasma glucose, pH, blood gas determinations, and the venous blood was sampled for determination of hematocrits. All samples were taken every 30 minutes until termination of the experiment., Equivalent amounts of sterile dextran 70 were administered intravenously to replace blood loss from surgery and sampling. After the three hour hypotension, all remaining shed blood was reinfused. An antiheparin agent, Protamine sulfate (50 mg activity in 5 ml, Eli Lilly & Co., Indianapolis, IN) was administered 30 minutes later. Rumpon hydrochloride, an analgesic agent, was administered 0.3 mg/kg (I.M.) to the dogs before full recovery from barbiturate anesthetic to produce a quiet and restful recovery. Upon returning the dogs to their cages, Chloramphenicol 17 mg/kg and Procaine penicillin G, 2500 units/kg was administered intramuscularly.

Blood pressure and heart rate were continuously recorded until the dogs were partially recovered from the anesthesia. The dogs were then returned to their cages and observed for 26 to 41 hours or until death. Five dogs survived for longer than 26 hours. Before

sacrifice, the following variables were measured: arterial glucose, heart rate, mean arterial blood pressure, hematocrit, and rectal temperature.

D. Absorption and Cardiodepressant Series

Utilizing the surgical preparation and protocol described in Sections A and B, the amounts of fluid and glucose absorbed during the experiment were studied as follows: A large bore rigid plastic tube was inserted into the lumen of the ascending colon for collection of fluid from the small intestine during the experiment. At the end of the experiment, the entire small intestine and ascending colon was extirpated and all remaining fluid in this segment was collected. The volume absorbed was calculated by the following formula: Inflow - (Outflow + Luminal volume) = intestinal absorption.

Intestinal glucose absorption was measured as follows. Glucose concentration was first measured from a sample taken from the volume instilled into the duodenum (Initial). At the conclusion of the experiment the fluid collected from ascending colon tube and luminal contents were pooled. A second sample was obtained from the pooled fluid for glucose determination (Final). From the glucose concentrations and the volumes instilled and recovered, the amount of glucose absorbed was calculated in mg/kg body weight. Glucose concentration was measured using an enzymatic colorimetric glucose determination assay (kit no. 510-A, Sigma Chemical Co., St. Louis, MO).

In another series of experiments, a polyethylene tubing (PE 240 i.d. = 1.67 mm, o.d. = 2.42 mm) was placed into the splenic vein for

sampling of blood. Portal venous and arterial blood samples were obtained before the hemorrhage (pre-hemorrhage sample) and immediately after all the shed blood was reinfused back into the animal from the blood reservoir (post-hemorrhage sample). A paper chromatographic method for cardiodepressants described by Barenholz, Leffler and Lefer (1973) and Yamada and Pettit (1977) was used to assay the cardiodepressants in six dogs. The blood samples were centrifuged immediately at 2,000 g for ten minutes at 4°C. The plasma samples were deproteinized, extracted five times with ether saturated with water, and a 200 ul sample of processed plasma was applied to Whatman 3mm chromatographic paper. A 20 ul L-serine sample was also applied to the same chromatographic paper. Following 15 hrs of equilibration in the chromatographic tank with the appropriate solvent, each chromatograph was developed with a ninihydrin aerosol by incubating at 90°C for 15 min. The cardiodepressant dilute was found at a migration point roughly 1.6 to 1.8 times the distance that serine had migrated (Rs = 1.6-1.8).

E. Histopathology

Intestinal biopsies were taken from the duodenum, jejunum, and ileum prior to conclusion (termination) of six experiments. The tissue was fixed immediately in a 10% buffered formalin solution at 25°C. The specimens were embedded in paraffin and 5μ sections were prepared for light microscopic examination to determine if any changes or damage occurred during the three hours of hypotension with either the instillation of G+O.A. or N/S begun one hour after the initiation of hemorrhage. The following grading sequence was used to

quantify the morphological changes as described by Chiu and associates (1970).

1. Grade 0 - Normal mucosal villi.
2. Grade 1 - Development of subepithelial Gruenhayen's space, usually at the apex of the villi; often with capillary congestion.
3. Grade 2 - Extension of the subepithelial space with moderate lifting of epithelial layer from the lamina propria.
4. Grade 3 - Massive epithelial lifting down the sides of villi. A few tips may be denuded.
5. Grade 4 - Denuded villi with lamina propria and dilated capillarius exposed. Increased cellularity of lamina propria may be noted.
6. Grade 5 - Digestion and disintegration of lamina propria; hemorrhage and ulceration.

Statistical analysis

Statistical analysis was performed using Student's t test modified for unpaired replicates. Also two-way analysis of variance with an accompanying Duncan's multiple range test was used for plasma protein, blood gas and pH data. A p value less than 0.05 was considered significant.

III. RESULTS

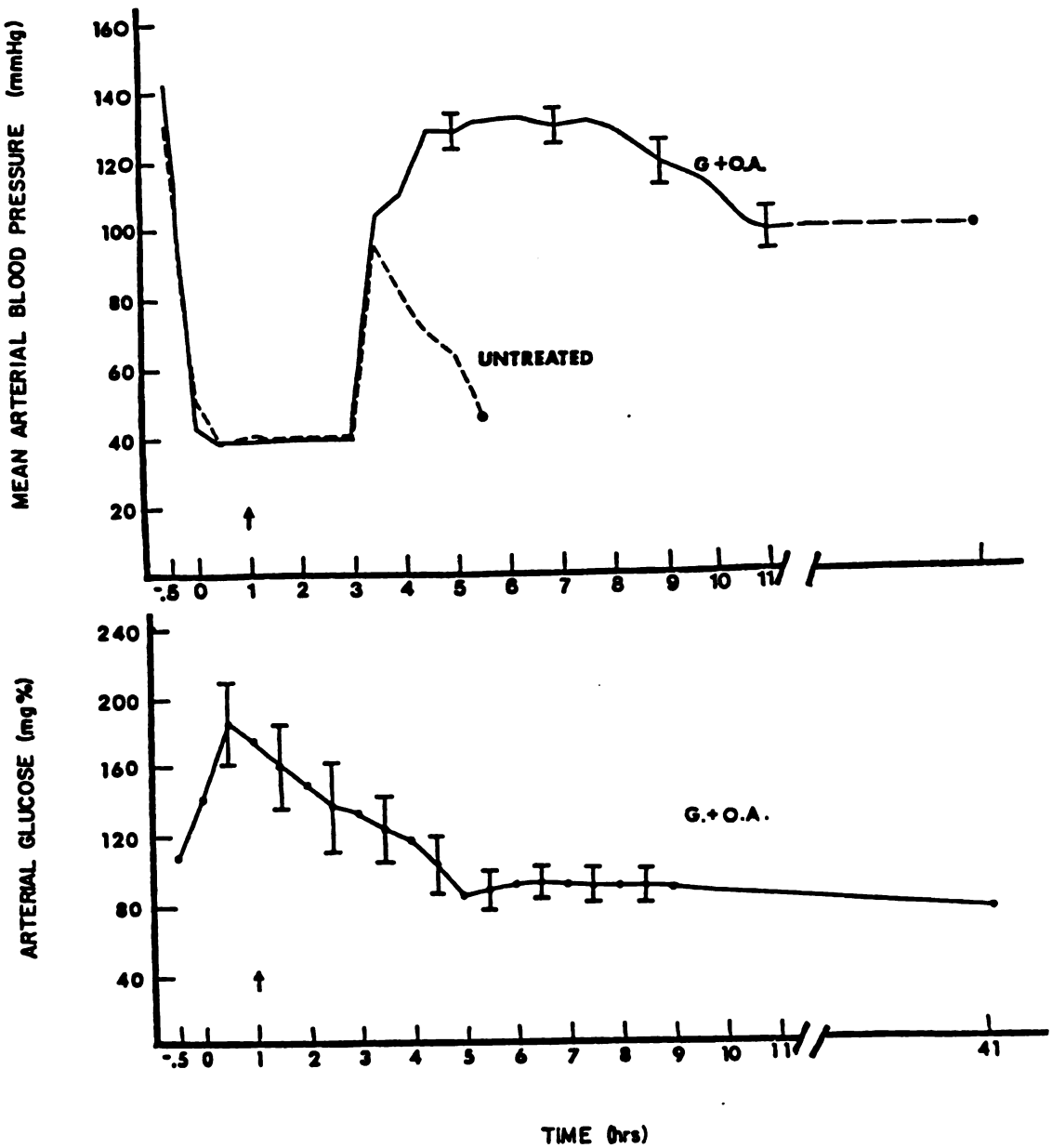
A. Survival series:

Eighteen dogs received a duodenal instillation of glucose-oleic acid - 95% oxygen and 5% carbon dioxide (G+O.A.) or normal saline (N/S) during hypotension and were observed for 26-41 hours after reinfusion of all remaining shed blood or until death. The outcome of the nine dogs treated with G+O.A. was as follows: Dog #2 survived for 10 hours after reinfusion of all shed blood (post-hemorrhage). Dogs #3 and #4 survived for 15 hours post-hemorrhage. Dog #8 survived for 18 hours post-hemorrhage and it was found at 17½ hours post-hemorrhage that there was bleeding in the region of the femoral catheters. Dogs #5, 6, 7 and 9 survived for 26 hours post-hemorrhage, at which time they were sacrificed. Dog #1 survived for 41 hours post-hemorrhage and, at the time of sacrifice, was eating solid food and drinking large quantities of water. The overall physical appearance of the dogs at the time of sacrifice was good. They were able to move about by their own locomotion and responded to attention by wagging their tails. Rectal temperature was measured before the time of sacrifice and all dogs were normal (39°C). All nine dogs treated with the duodenal instillation of N/S died by nine hours of the experiment, while 67% of dogs treated with G+O.A. survived.

Figure 3 shows the mean arterial blood pressure for two groups of dogs. The mean arterial blood pressure (MABP) for the G+O.A. treated group (n=9) was reduced from 142 ± 3.2 to 43 ± 1.9 mmHg after

Figure 3. Time-course of mean arterial blood pressure (upper panel) and glucose concentration (lower panel) in dogs treated with G+O.A. (solid line) and untreated dogs (dashed line). Arrow designates the initiation of G+O.A. instillation. n=9 for G+O.A. group and n=7 for untreated group.

Figure 3


MEAN ARTERIAL BLOOD PRESSURE (mmHg)
160
140
120
100
80
60
40
20
G+O.A.
UNTREATED
-.5 0 1 2 3 4 5 6 7 8 9 10 11
ARTERIAL GLUCOSE (mg %)
240
200
160
120
80
40
G.+O.A.
-.5 0 1 2 3 4 5 6 7 8 9 10 11 41
TIME (hrs)

bleeding in 30 ± 10 minutes (min). The untreated dog's (n=7) MABP was reduced from 134 ± 3.9 to 38 ± 1.1 mmHg after 30 ± 10 minutes of hemorrhage. The blood reservoir was set at a height to maintain a MABP of 37 ± 2 mmHg for three hours. After the three hours of hemorrhagic hypotension, all remaining shed blood was returned to the animal. The MABP of the treated group returned to 90 ± 10.9 mmHg upon blood transfusion and increased to 124 ± 5.9 and 126 ± 6.8 mmHg two and four hours after the transfusion, respectively. The blood pressure was continuously monitored for eight hours after the transfusion for all dogs. At the end of eight hours, MABP for G+O.A. group was 103 ± 7.0 mmHg. The MABP of the four dogs who were sacrificed at 26 hours was 112 ± 13.0 mmHg. Dogs #1 who was observed for 41 hours post-hemorrhage maintained a blood pressure of 100 mmHg at the time of sacrifice. In untreated dogs, MABP increased to 95 ± 8.9 mmHg upon transfusion but subsequently fell to 52 ± 4.3 mmHg at 2½ hours after the transfusion. All dogs died within 6 hours after blood transfusion.

Figure 3 also shows the mean arterial glucose concentration in mg/dl (GlC_A) for the nine dogs treated with G+O.A.. Prior to hemorrhage (control), GlC_A was 95 ± 5.1 mg/dl. With the onset of hemorrhage, GlC_A increased in all dogs. Arterial glucose concentration reached its peak during the first hour of hypotension. The GlC_A increased to 125 ± 24.7 mg/dl and gradually returned toward control levels fell during the hypotensive period. The GlC_A continued to fall after reinfusion of the shed blood to 88 ± 10.9 mg% 2 hours after the transfusion and remained at this level for another four

hours. At 3 and 6 hours after the transfusion GiC_A were 92 ± 9.2 mg/dl and 89 ± 10.9 mg/dl, respectively.

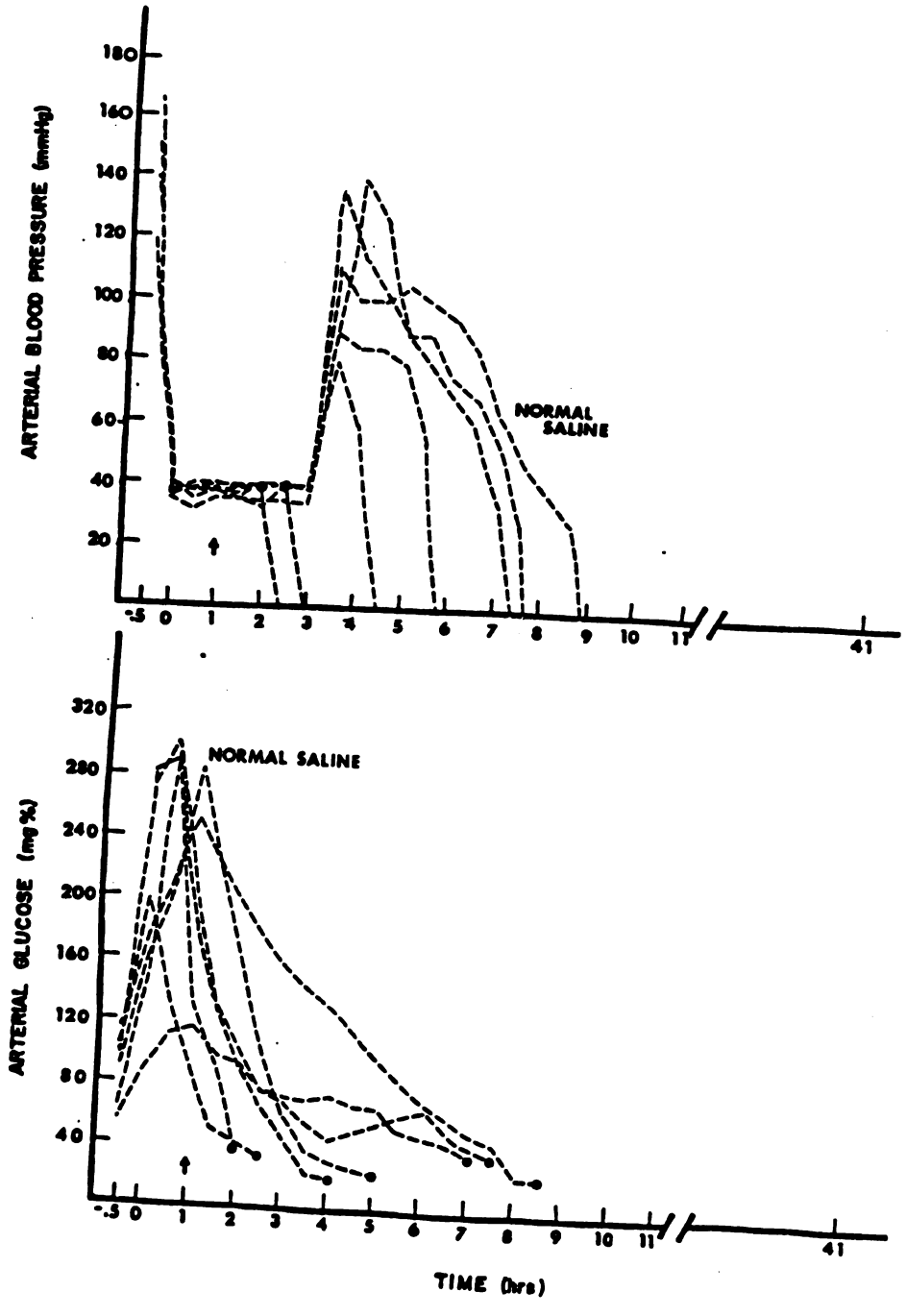
Figure 4 shows the absolute values of arterial blood pressure for 7 dogs treated with duodenal instillation of N/S solution. The MABP for seven dogs treated with the N/S solution before hemorrhage was 144 ± 4.6 mmHg and was reduced to 39 ± 1.0 mmHg in 30 ± 10 mins during initial hemorrhage. Dogs #5 and #6 died 2 and 2½ hours after the onset of hemorrhage, respectively. After three hours of hypotension, all remaining shed blood was returned. The arterial blood pressure of dogs #1 and #7 returned to pre-hemorrhage levels to 140 and 135 mmHg, respectively. All N/S treated dogs' blood pressure fell progressively during the six hours after reinfusion.

The lower panel of Figure 4 shows individual values of the arterial glucose concentration in the seven dogs treated with N/S. The arterial glucose concentration increased from a mean value of 96.5 ± 11.3 mg/dl to 188.9 ± 28.3 mg/dl one hour after the initiation of hemorrhage. The hyperglycemia occurred in all dogs and coincided with the first thirty minutes of hypotension. After the initial hyperglycemia, arterial glucose concentration fell to very low levels in the next eight hours. Comparing the two graphs of Figure 4, the death coincided with the very low glucose concentration in each dog.

The treatment with the duodenal instillation of either N/S or G+O.A. at a rate and volume of 3 ml/min and 35 ml/kg, respectively, had no significant effect on the hematocrit nor heart rate (Figure 5). There was no significant difference in hematocrit between the N/S and G+O.A. groups. The resting heart rates for the G+O.A. treated

Figure 4. Time-course of changes in arterial blood pressure and glucose concentration in seven dogs treated with normal saline instillation through the intestinal lumen. Arrow indicates the initiation of the instillation. n=7.

Figure 4


ARTERIAL BLOOD PRESSURE (mmHg)
180
160
140
120
100
80
60
40
20
NORMAL SALINE
-5 0 1 2 3 4 5 6 7 8 9 10 11 41
ARTERIAL GLUCOSE (mg %)
320
280
240
200
160
120
80
40
NORMAL SALINE
-5 0 1 2 3 4 5 6 7 8 9 10 11 41
TIME (hrs)

N/S treated groups were also not significantly different. The heart rate increase to the highest value of 194.7 ± 8.9 in G+O.A. group and 213.6 ± 8.2 in N/S group, one to two hours after the onset of hemorrhage. In both groups, heart rate returned to pre-hemorrhage rates one hour after the transfusion (4th hour on the figure). There was no significant difference in the changes in heart rate between the two groups over the entire course of the experiment.

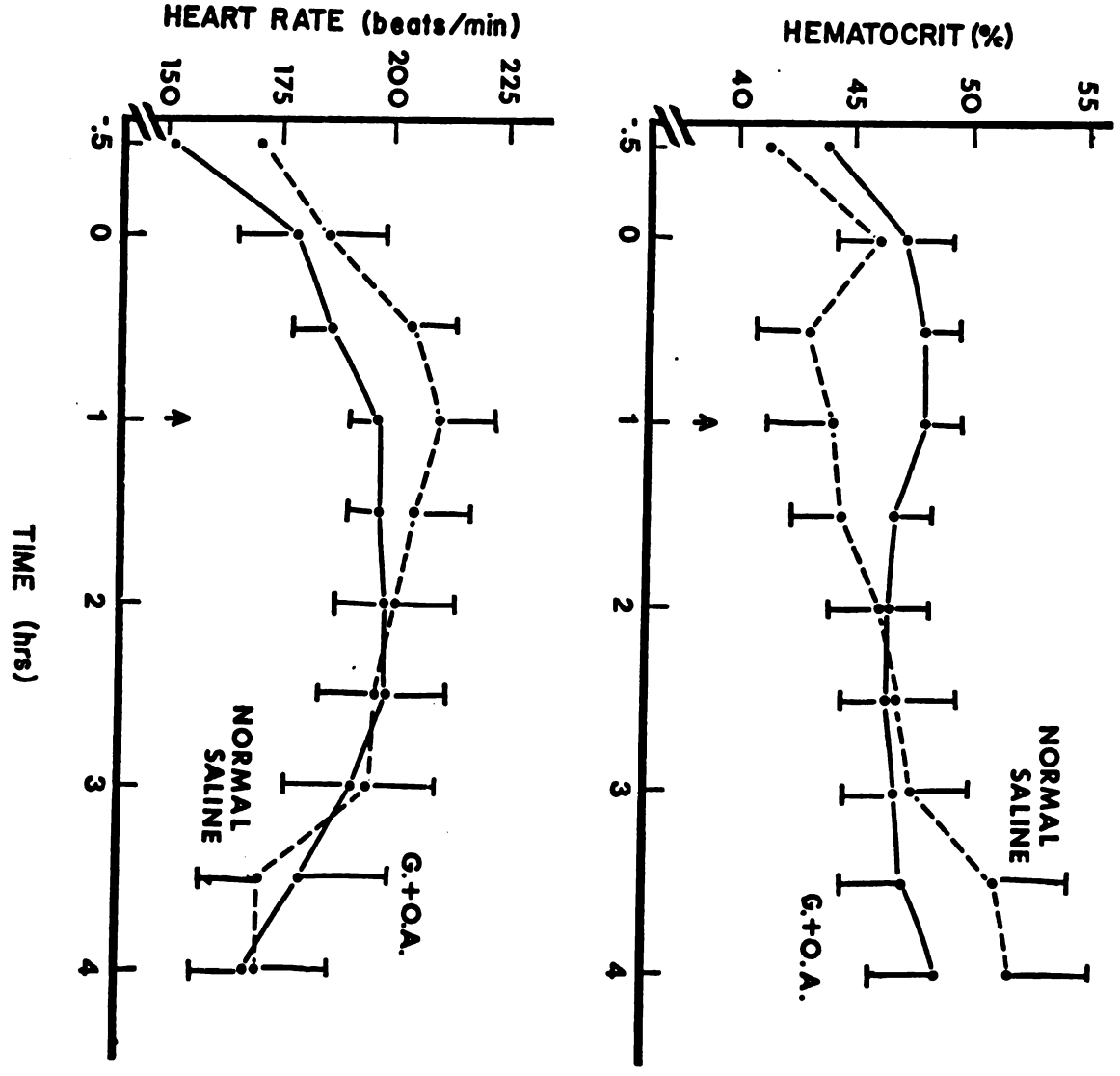
The volume of hemorrhaged (shed) blood in the calibrated blood reservoir was recorded to the nearest 25 milliliters every thirty minutes. A maximum shed blood volume of 49.4 ± 3.1 ml/kg was reached at 1½ hours after the initiation of hemorrhage in the G+O.A. treated group. The N/S treated group reached its maximum shed blood volume of 52.0 ± 3.1 ml/kg during the same time period. During the last 30 minutes of hypotension, the shed blood volume for the G+O.A.-treated group decreased to 42.2 ± 6.6 ml/kg, i.e., a spontaneous uptake of 14.5% of the shed blood volume from the reservoir. The N/S treated group's shed blood volume during the last 30 minutes of hypotension decreased to 31.9 ± 2.8 ml/kg, i.e., a spontaneous uptake of 38.6% of the shed blood volume from the reservoir. The 38.6% spontaneous uptake of blood from the reservoir to maintain blood pressure around 37 ± 2 mmHg in the N/S treated dogs was significantly greater than that observed in the G+O.A. group, i.e., 14.5% uptake.

B. Absorption series:

Control MABP for dogs (n=8) who were treated with duodenal instillation of G+O.A. (Group A) was 132 ± 9.1 mmHg and was reduced to 43 ± 2.5 mmHg in 30 ± 10 minutes during the initial hemorrhage

Figure 5. Time-course of changes in hematocrit and heart rate during the experiment in dogs treated with G+O.A. (solid line) and N/S (dashed line) in the survival series. n=9 for G+O.A., and n=7 for N/S group.

Figure 5


HEMATOCRIT (%)
40
45
50
55
-.5
0
1
2
3
4
NORMAL SALINE
G.+O.A.
HEART RATE (beats/min)
150
175
200
225
TIME (hrs)

(Figure 6). The control value of MABP for N/S treated dogs (n=8) (Group B) was 143 ± 10.1 mmHg and was reduced to 42 ± 2.0 mmHg during the initial hemorrhage period.

Figure 6 also shows the hemorrhage blood volume (ml/kg) recorded from the calibrated blood reservoir during the hypotensive period. Over the 2½ hours after the initiation of hemorrhage, the bleeding volumes of the two groups were not significantly different. However, during the last 30 minutes of hypotension the bleeding volume in the N/S group was significantly less than that in the G+O.A. group. The maximum shed blood volumes were 47.7 ± 3.8 ml/kg at 1½ hours for the G+O.A. group, and 48.5 ± 3.0 ml/kg at 1 hour for the N/S group. During the last 30 minutes of hypotension, the shed blood volume was 42.9 ± 3.4 ml/kg for the G+O.A. group, whereas that for the N/S group was 29.3 ± 3.3 ml/kg. This indicates that the N/S treated dog required spontaneous uptake of 40.0% of the shed blood from the reservoir during the last 30 minutes of hypotension to maintain blood pressure around 37 ± 2 mmHg. A third group of dogs (n=4) (Group C) who were also treated with an instillation of N/S had a spontaneous uptake of only 4.4% from the reservoir. The maximum shed blood volume for this group was 46.6 ± 4.3 ml/kg at 2½ hours after initiation of hemorrhage and decreased to 44.6 ± 4.0 ml/kg during the last 30 minutes of hypotension. The reason for the difference between this and the above N/S groups (Group B) was due to intestinal absorption of fluid as will be described below.

After 3 hours of hypotension, all remaining shed blood was returned. As shown in Figure 6, the MABP for G+O.A. group returned

Figure 6. Time-course of blood volume and mean arterial blood pressure in dogs treated with G+O.A. (solid line) and normal saline (dashed line) before, during, and after hemorrhagic hypotension for 3 hours. Arrow denotes the beginning of the treatments. The number in parenthesis indicates the volume of fluid absorbed from the intestine in ml/kg. n=8 for each treatment. Asterisks indicate values significantly different from the corresponding values of the normal saline treated dog.

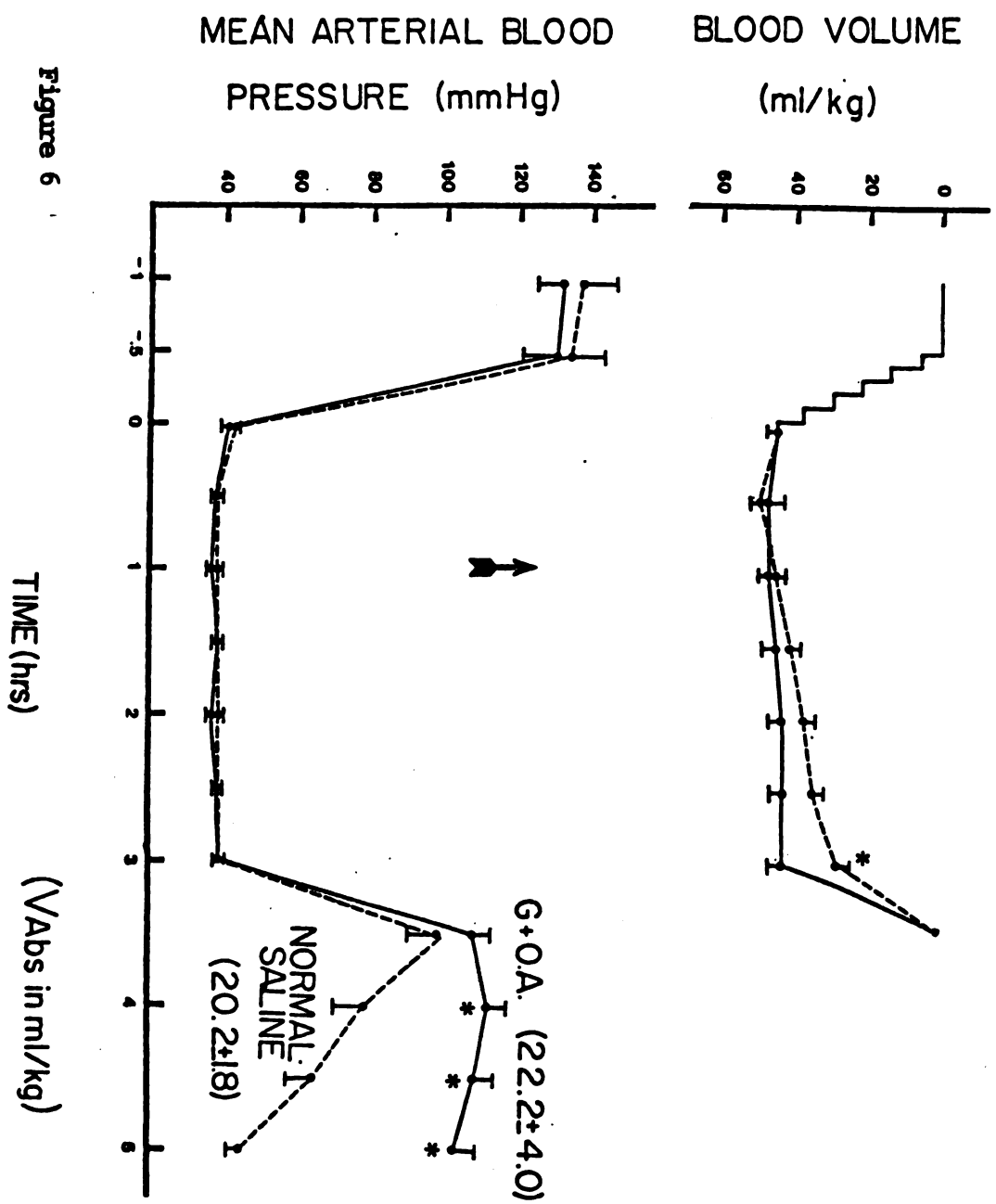

MEAN ARTERIAL BLOOD PRESSURE (mmHg)
BLOOD VOLUME (ml/kg)
TIME (hrs)
(VAbs in ml/kg)
G+O.A. (22.2±4.0)
NORMAL SALINE (20.2±1.8)

Figure 6 -

to 109 ± 4.7 mmHg upon blood transfusion and increased to 110 ± 5.4 mmHg at 4 hours and fell slightly to 103 ± 5.8 mmHg at 5 hours. The MABP of the N/S group (Group B) on the other hand returned to 95 ± 8.2 mmHg and fell to 78 ± 8.3 and 43.8 ± 2.7 at 4 and 5 hours, respectively. The MABP of the third group (N/S treated, Group C) returned to 100 ± 8.9 mmHg and increased to 103 ± 8.3 mmHg at 4 hours but fell only to 98 ± 3.2 mmHg at 5 hours.

Intestinal fluid volume and glucose (when applicable) absorption were measured in all three groups mentioned above and are expressed in Table 2. There was no significant difference in the volume of fluid absorbed in Groups A and B, but the volume absorbed by Group B and A was significantly less than that by Group C.

In the G+O.A. group (Group A), intestinal glucose absorption was measured. The G+O.A. solution contained 27 mg/ml (150 mM) of glucose; a total of 945 mg/kg of glucose was instilled at a rate of 81 mg/min. Thus, 70.8% of the infused glucose was absorbed.

Arterial glucose concentration was measured in all three groups. The GlC_A for Group A (G+O.A.) was 91.2 ± 8.0 mg% before hemorrhage. At 1½ hours of hypotension, the GlC_A increased to 196.8 ± 37.5 mg% and fell to 105.3 ± 22.4 mg% at the end of 5 hours. Control value for Group B (N/S treated) was 105.5 ± 5.0 mg% and increased to 204.3 ± 22.6 mg% at 1 hour of hypotension. During the hypotension and after reinfusion of shed blood, GlC_A fell progressively to 53.3 ± 9.8 mg% at the end of 5 hours. GlC_A for Group C (N/S treated) was 100 ± 10.4 mg% before hemorrhage and increased at 1½ hours of hypotension

Table 2: Fluid Volume and Glucose Absorption in The Dogs Treated With G+O.A and N/S.

	Volume Infused (ml/kg)	Volume Absorbed (ml/kg)	Glucose Absorbed (mg/kg)
Group A G+O.A. (n=8)	35	22.2 ± 4.0	669.5 ± 123.3
Group B N/S (n = 8)	35	20.2 ± 1.8	---
Group C N/S (n=4)	35	32.7 ± 0.5*	---

* $p<0.05$ relative to Group A and B.

to 220.0 ± 17.3 mg%. GlC_A fell to 77.2 ± 11.3 mg% at 5 hours of experiments.

The data for hematocrit and heart rate are shown in Figure 7. The changes in hematocrit in the two groups shown in Figure 5 were not significantly different. However, Group C (not represented) did show some hemodilution during the last hour of hypotension. The hematocrit in Group C increased to 50.0 ± 1.7% at 30 minutes of hypotension, which was similar to the other two groups represented in Figure 7. Over the next 2½ hours of hypotension, the hematocrit decreased to 43.5 ± 2.9%, whereas the other two groups showed an increase at the end of hypotension. There was no significant difference in heart rate among the three groups of dogs.

The data for plasma protein concentration are shown in Table 3 for six dogs. Plasma protein concentration did not significantly change from control values during or after hemorrhagic hypotension. Furthermore, the values for the G+O.A. and N/S groups were not significantly changed.

The arterial pH and blood gases (PO_2 and PCO_2) were measured in seven dogs. Table 4 shows the results from two treatment groups (G+O.A. and N/S). The control pH was between 7.44 - 7.45 for the two groups. The pH dropped to 7.40 in the G+O.A. treated group (n=4) and to 7.39 for the N/S treated group (n=3) at the beginning of hypotension. The pH was 7.26 and 7.19 for the G.+.O.A. and N/S treated groups, respectively, by the last hour of hypotension. Two hours after reinfusing the remaining shed blood, pH increased to 7.35 and 7.31 for the G+O.A. and N/S treated groups, respectively. Neither

Figure 7. Time-course of changes in hematocrit and heart rate during the experiment in dogs treated with G+O.A. (solid line) and N/S (dashed line) in the absorption series. n=8 for G+O.A. (Group A) and n=8 for N/S group (Group B).

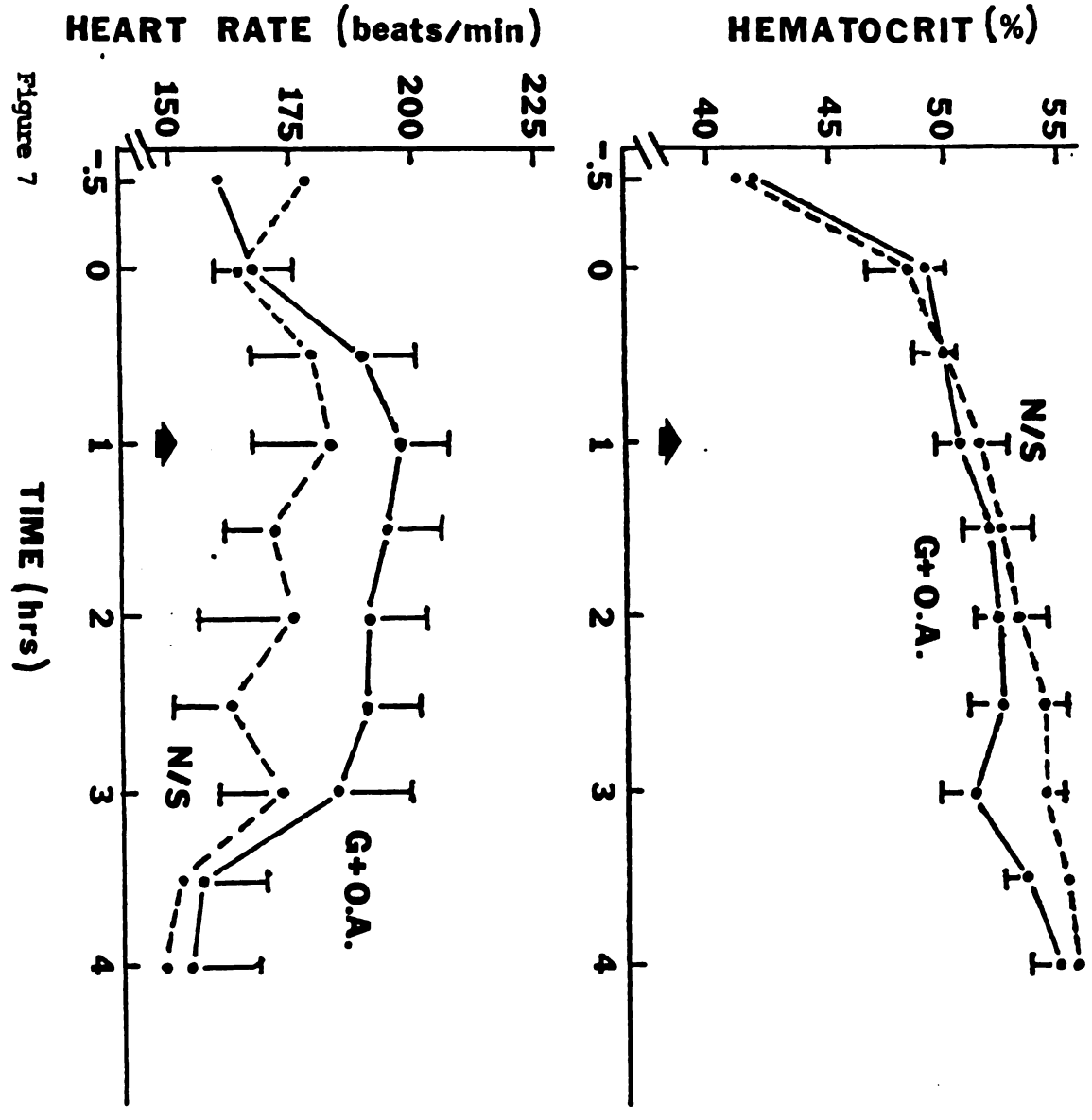

HEART RATE (beats/min)
150
175
200
225
-.5
0
1
2
3
4
TIME (hrs)
N/S
G+O.A.
HEMATOCRIT (%)
40
45
50
55
N/S
G+O.A.

Figure 7

Table 3. Time-Course of Plasma Protein Concentration (g/100 ml) in the G+O.A. and N/S Treated Dogs.

Treatment	Time (hours)					
	-.5	0[a]	1[b]	2	3[c]	4
G+O.A. (n=3)	5.9±0.6	5.2±0.3	5.1±0.4	4.9±0.3	4.9±0.4	5.9±0.3
N/S (n=3)	5.7±0.3	5.5±0.5	5.3±0.4	5.2±0.4	5.9±0.8	6.8±0.3

[a] Beginning of hypotension.
[b] Instillation begun.
[c] End of hypotension.

Table 4. Time-Course of pH and Blood Gases (mmHg) in the G+O.A. and N/S Treated Dogs.

G+O.A. (n=4)	Time (hours)						
	-.5	0[a]	1[b]	2	3[c]	4	5
pH	7.45±0.2	7.40±0.02	7.34±0.02*	7.27±0.05*	7.26±0.04*	7.31±0.02*	7.35±0.01*
PO_2	78.2±3.4	81.2±8.8	79.9±5.3	90.3±0.8	91.8±0.4	94.7±2.2	92.4±3.4
PCO_2	37.4±2.2	30.2±5.8	28.2±2.8	28.4±1.1	26.1±1.3*	28.7±1.9	28.3±0.6

Normal Saline (n=3)	Time (hours)						
	-.5	0[a]	1[b]	2	3[c]	4	5
pH**	7.44±0.01	7.39±0.02	7.30±0.04*	7.23±0.02*	7.19±0.02*	7.28±0.003*	7.31±0.02*
PO_2**	85.0±1.2	92.0±0.0	92.7±0.8	94.7±1.9	95.5±1.6	92.4±2.4	88.4±2.3
PCO_2	33.6±0.8	31.5±0.8	29.4±2.4	24.8±1.3	32.4±0.3*	28.7±1.8	30.0±2.3

[a] Beginning of hypotension.
[b] Instillation begun.
[c] End of hypotension.
* $p<0.05$ relative to time 0.
** $p<0.05$ relative to treatment with G+O.A.

group returned to control values at 5 hours but the N/S treated group showed a lower pH at the last hour of observation. Overall the pH between the two treatment groups is significantly different (F value = 7.06).

The partial pressure of arterial oxygen (PO_2) was 78.2 mmHg for G+O.A. and 85.0 mmHg for N/S treated groups for the pre-hemorrhage period (-.5 hours). The arterial PO_2 continued to increase during the 3 hours of hypotension and fell slightly by 5 hours. The arterial PO_2 remained elevated but fell to 92.4 mmHg at 5 hours for the G+O.A. treated group. Arterial PO_2 of the N/S treated group returned within control values to 88.4 mmHg at the same time period. Overall the arterial PO_2 between the two treatment groups is significantly different (F value = 5.21).

The control partial pressure of arterial carbon dioxide (PCO_2) was 37.4 mmHg and 33.6 for the G+O.A. and N/S treated groups, respectively. In both groups, the arterial PCO_2 decreased during the hypotension. All animals were taken off from the respiration during entire hypotensive period. Rapid deep breaths were noted in all animals after the first hour of hypotension, thus contributing to the lowered arterial PCO_2 observed during latter hours of hypotension. The arterial PCO_2 was 28.3 mmHg for the G+O.A. group and slightly increased to 30.0 mmHg for the N/S treated group at 5 hours. There was a significant change by the 3rd hour of hypotension over time 0 within each group.

C. Cardiodepressants:

Results from two experiments are shown schematically in Figure 8. In both groups of dogs, blood samples were obtained from the blood reservoir (#1), femoral artery (#A) and portal vein (#V) before and after hemorrhage. In the N/S treated dogs, little or no cardiodepressants were present in the blood reservoir (#1) or those obtained before hemorrhage (#2A and 3V). The deproteinized samples taken from the N/S groups showed no spots in the pre-hemorrhage samples. After hemorrhage, spots were found in A, B, C, D and E (see legend of Figure 8 for explanation). The large dense lines of spot D represents the cardiodepressants (double arrows) that have a migration distance of approximately Rs = 1.7 to that of the serine standard (denoted by single arrow).

In the G+O.A. treated dogs, there were some cardiodepressants (indicated by less dense lines, letter D) present in deproteinized plasma samples taken before hemorrhage (#2A and 3V; pre-hemorrhage). No cardiodepressants were present in the samples taken from the blood reservoir (#1). Several other spots appear in pre-hemorrhage samples of glucose-oleic acid treated group at B, C and F. Hemorrhage, however, did not increase the amount of cardiodepressants in spot D (no lines) denoted by the double arrows. Other spots of letters B, C and F were smaller in the post-hemorrhage samples. Spot A is present in samples from the blood reservoir and post-hemorrhage samples from both G+O.A. and the N/S treated groups.

Figure 8. Representative paper chromatogram showing: 1) plasma from blood reservoir, 2) plasma from pre-hemorrhage femoral arterial sample (2A), 3) plasma from pre-hemorrhage portal venous sample (3V), 4) plasma from post-hemorrhage femoral arterial sample (4A), 5) plasma from post-hemorrhage portal venous sample (5V), and 6) serine standard. Rs values are shown and each spot is given a letter designate in order of increasing migration from the origin.* Darkest spots are the serine standard (C), followed by large dense lines (D), the dotted spots (A), and open faint spots (B, C, E and F). Double arrows indicate location of cardiodepressants and single arrow denotes the serine standard. The density of the lines in spot D represents the intensity of the spot, hence no lines inside the spot indicates less intense spot.

* The Rs value refers to the migration distance of any spot on the chromatograph in relationship to the distance that the serine standard has migrated.

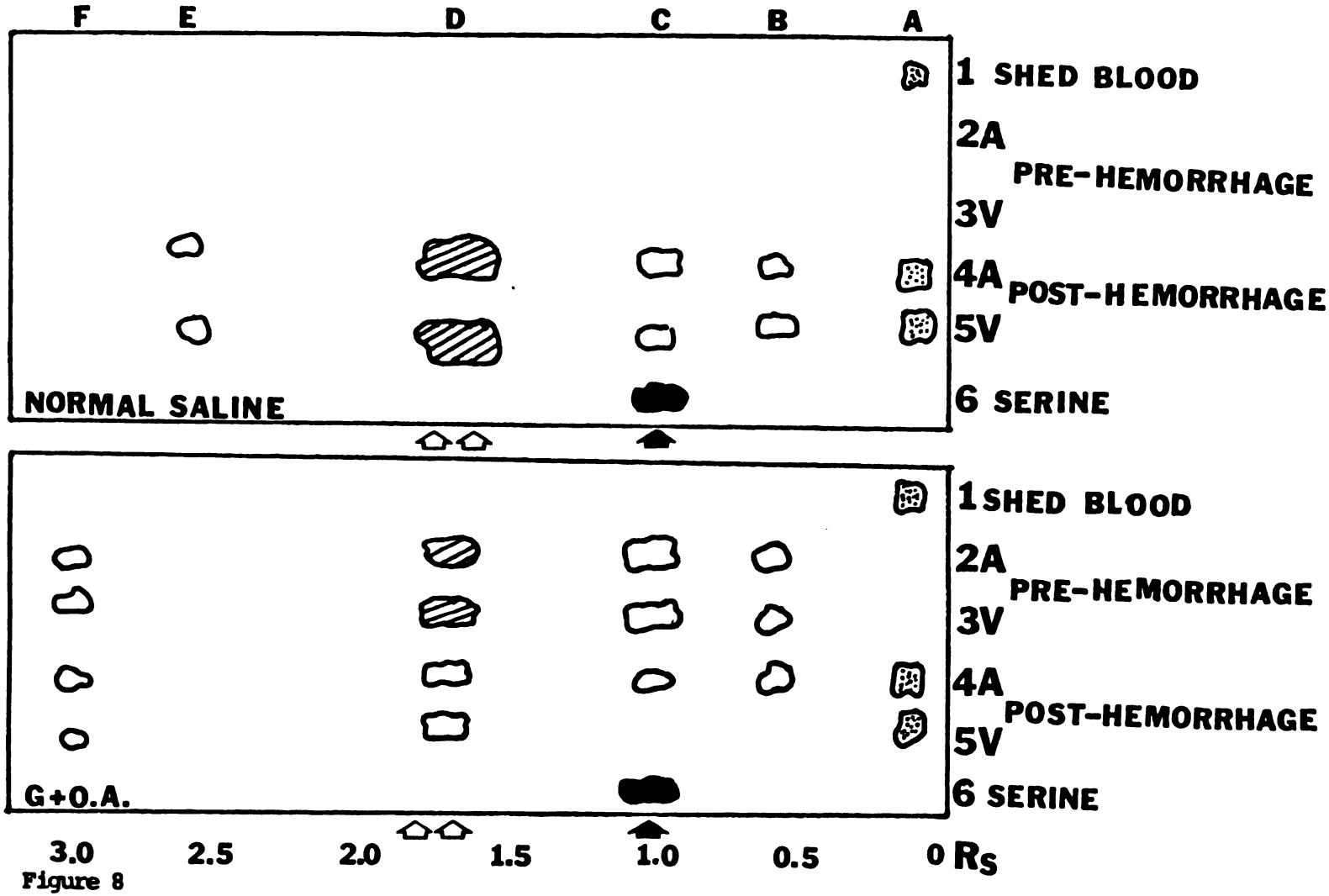

F
E
D
C
B
A
1 SHED BLOOD
2A
PRE-HEMORRHAGE
3V
4A
POST-HEMORRHAGE
5V
6 SERINE
NORMAL SALINE
1 SHED BLOOD
2A
PRE-HEMORRHAGE
3V
4A
POST-HEMORRHAGE
5V
6 SERINE
G+O.A.
3.0
2.5
2.0
1.5
1.0
0.5
0 Rs

Figure 8

D. Histopathology:

Tissue samples were collected for determination of any morphological changes (gross and histological) that may have occurred during the experimental procedure in both G+O.A. (n=3) and N/S (n=3) treated groups. According to Chiu et al. (described in methods), the three dogs who received N/S at one hour after hemorrhage showed histological damage in the range between grades two to four. Macroscopically, a common finding for dogs treated with N/S was mucosal sloughing, congestion and hemorrhage. In contrast, the three dogs treated with the G+O.A. solution only received a grading between zero to one upon histological observation. Macroscopic appearance showed a normal intact mucosal surface with no evidence of congestion but petechiae were found in the duodenum and ileum in one dog.

IV. DISCUSSION

Dogs subjected to hemorrhagic hypotension may succumb to a state of irreversibility if the shock is of sufficient degree and duration (Wiggers and Werle, 1942; Walcott, 1945). There are two commonly used methods to experimentally produce hemorrhagic shock. The first is known as the single withdrawal or the nonreservoir method (Walcott, 1945). The nonreservoir method consists of the removal of a predetermined quantity of blood and the immediate return of a fixed percentage of blood collected. The amount returned can be predetermined to produce hemorrhagic shock of varying degrees from the mildest symptoms to 100% fatalities. The second method is the graded hemorrhage method described by Wiggers and Werle (1942). The removal of blood at definite time intervals or proportionately smaller volumes of blood removed until the mean arterial blood pressure falls to a "shock level". A modified version of Wiggers hemorrhage method was employed in this study. Using the method described by Lamson and Deturk (1945) a reservoir was connected to a femoral artery catheter for bleeding. A graded hemorrhage of 35 mmHg for 3 hours was shown in our laboratory to produce irreversible shock and death in all dogs (Senko, 1980).

Brobmann et al. (1970) has demonstrated that autoregulatory mechanisms will sustain blood flow temporarily to all vascular beds in the beginning stages of hemorrhage. As the hemorrhagic hypotension progresses, only essential vascular beds (e.g., brain and heart) will receive sufficient blood flow. The intestinal vascular bed

among others suffer extensively in severe hemorrhagic hypotension. Roding and Schenk (1970) reported that the mesenteric fraction of the cardiac output is significantly reduced during a sustained hemorrhage. Marked splanchnic hypoperfusion is a prominent feature of prolonged hemorrhagic hypotension produced by graded hemorrhage (Reynell, Marks, Chidsey, 1955; Roding and Schenk, 1970). The splanchnic hypoperfusion no longer maintains adequate oxygenation of the highly metabolic mucosa. As a result, the ischemic splanchnic region liberates a number of toxic factors (Haglund and Lundgren, 1972; Lefer 1978). At autopsy, mucosal congestion and necrosis of the small and large bowel are common findings (Wiggers, 1950; Chiu et al., 1970; Haglund et al., 1975). These toxic factors contribute to the vicious deteriorating cycle observed during circulatory shock, ultimately leading to death. Lillehei (1957) reduced the mortality rate by 80% in experimentally-induced hemorrhagic shock through maintenance of intestinal perfusion pressure.

Our rationale was to maintain local intestinal mucosal blood flow and metabolism during the hemorrhagic hypotension by duodenal instillation of a glucose-oleic acid solution. The solution has been shown to increase blood flow and metabolism (Chou et al., 1972, 1976, 1978). As a control, normal saline was administered one hour after initiation of hemorrhage. The rate and volume of administration was standardized for all dogs to be 3 ml/min and 35 ml/kg body weight, respectively. The rate of volume infusion approximates normal gastric emptying and the amount of volume infused was found not to distend the intestine after completion of infusion.

Prolonged hypovolemia produces ischemic and hypoxic conditions of the intestine as shown by Selkurt and Brecher (1956); Haglund and Lundgrun (1974). Adequate perfusion should prevent irreversible damage from occurring.

Carl Wiggers (1950) described the classical response of arterial blood pressure after the return of hemorrhaged (shed) blood. Mean arterial blood pressure returns within prehemorrhage levels after retransfusion and progressively falls over the next several hours. The time interval between retransfusion and death is known as normovolemic shock (Ehrlich, Kramer, and Watkins, 1969). The MABP of the untreated group (Figure 3) and the individual blood pressures treated with normal saline (Figure 4) illustrate Wiggers' original description of the progressive stages of irreversible shock.

Prolonged survival for 26 to 41 hours after reinfusion of all shed blood occurred in dogs treated with a duodenal instillation of the glucose-oleic acid solution. We were able to demonstrate a 67% survivability in the dogs treated with G+O.A. Dogs untreated and treated with a duodenal instillation of normal saline showed 100% mortality within 3 to 6 hours after reinfusion of shed blood. The protective effects of an intraluminal glucose solution was shown by Chiu, Scott and Gurd (1970) to maintain the integrity of the intestinal mucosa. Hypertonic glucose not only acts as an energy substrate but also as an osmotic force (Moffat et al., 1968). In our study, instillation of the glucose-oleic acid solution improved the maintenance of the arterial glucose concentration. Maintenance of

blood glucose levels within the normal range throughout the hypovolemic and normovolemic periods coincides closely with improved survival of the animal (Figure 3). Hift and Strawitz (1961) have demonstrated a temporal relationship between blood glucose changes and morphologic damage in hepatic mitochondria. The tolerance to hemorrhage was also shown by Drucker et al. (1958) to be related to maintenance of blood glucose concentration. Survival was significantly prolonged in dogs who received an intravenous infusion of hypertonic glucose. Treatment was instituted when tolerance to hypovolemia began to decompensate by the need to reinfuse blood from the hemorrhage reservoir (Moffat et al., 1968). Initial stores of glucose may only last approximately four hours, forcing the body to utilize alternate energy sources during normovolemic shock. The predominately anaerobic metabolism during severe hemorrhagic hypotension significantly reduces the glycogen stores early in shock while only producing small quantities of much needed ATP. This lack of glucose forces glucocorticoid-stimulated gluconeogenesis to produce amino acids, fatty acids, and glycerol (Schumer and Erve, 1975). Wiggers (1950) and McCormick et al. (1969) have observed the hyperglycemic state early in hemorrhage.

Sympathoadrenal mechanism is primarily responsible for the hepatic glucose mobilization (Jorley and Watts, 1964; Adams and Parker, 1979). Blood glucose was maintained for all dogs who received the glucose-oleic acid (Figure 3). The arterial blood pressure coincides well with the maintenance of blood glucose. Blood glucose returns to near control values as does the arterial blood

pressure after retransfusion. The instillation of glucose-oleic acid solution maintains blood glucose throughout the hypovolemic and normovolemic periods. In contrast, treatment with normal saline, although possibly acting as a temporary plasma expander, did little to maintain arterial glucose concentrations and survival in this group of dogs who received the instillation of normal saline at one hour of hypotension (Figure 4). The close correlation between arterial blood pressure and arterial glucose concentration represents the need for maintenance of blood glucose levels for prolonging survival. All dogs in this group treated with normal saline were hypoglycemic prior to death.

The fall in arterial glucose concentration below normal levels reduces the pump capacity for transport of gluconeogenic substrates to the cells. This, combined with a lack of oxygen, causes an increase in lactic acid, producing intracellular acidosis and an extracellular acidemia (Schumer and Erve, 1975). Markov, Oglethorpe, Young and Helkens (1981) showed that glycolysis is interrupted during severe metabolic acidosis. Phosphofructokinase is a multivalent enzyme that catalyzes the rate-limiting reaction of fructose-6-phosphate phosphorylation. The intracellular acidosis seen during shock decreases the catalytic action of phosphofructokinase, reducing the amount of ATP produced from the Embdem-Meyerhof pathway. The plasma pH of the dogs treated with the glucose-oleic acid solution fell significantly during the period of hypotension from time 0 through 5th hour. The fall in pH was also significant within the N/S treated group during the hypotension and the two hours after.

Markov's group (1981) using a similar hemorrhage protocol of 35 mmHg for 3 hours showed similar reduction in arterial pH.

All dogs showed hyperventilation indicating signs of respiratory stress throughout the hemorrhagic hypotension. The arterial PO_2 (Table 4) for both groups were increased due to the persistent hyperventilation. Overall there was a significant difference in pH and PO_2 but not for PCO_2 between the two treatment groups. It is reasonable to assume that the dogs in the normal saline group were suffering from a metabolic acidosis. The instillation of the glucose-oleic acid solution starting at the first hour of hypotension possibly contributed to the better respiratory compensation during the 3rd, 4th and 5th hours after initiation of hemorrhage. Through the maintenance of blood glucose levels, the glucose-oleic acid solution prevented the severity of a metabolic acidosis by making available adequate levels of substrate for oxidative phosphorylation. Moffat et al. (1968), by administering hypertonic glucose during the physiological decompensation of shock, has reported better respiratory values for those animals as compared to others receiving hypertonic sucrose.

Compensatory mechanisms were considered to be operating maximally one hour after initiation of hemorrhage. The influence of fluid treatment at this time, to support the compensatory mechanisms, would supplement interstitial fluid shifts and aid in restoring fluid loss by hemorrhage (Miller and Dale, 1978). Previous investigators (Van Lieve et al., 1938; Goldberg and Fine, 1945; Miles et al., 1968) have indicated that intestinal fluids do not participate in plasma

volume restoration after hemorrhage in the dog. These investigators agree in part with Miller and Dale in that normal saline absorption is maintained and enhanced to some degree during shock. Miller and Dale used a hemorrhage procedure that approximated 35% of measured blood volume. This hemorrhage volume was sufficiently below the LD_{50} blood volume of 42 to 43% (Swan, 1965). The hemorrhage insult used by Miller and Dale was of sufficient magnitude to activate compensatory mechanisms involved in plasma volume restoration, but not to such a degree as to cause 100% mortality. Van Lieve and associates used 3.2% of body weight to approximate hemorrhage volume and Goldberg and Fine used a systolic pressure of 50 mmHg for several hours to induce shock.

Results from our study showed no significant difference in volume absorption between the glucose-oleic acid (Group A) and normal saline (Group B) treatment groups (Table 2). There was a third group of dogs (Group C) who also received an instillation of normal saline. These animals not only survived the hemorrhage insult but showed significant absorption of fluids over the glucose-oleic group (Group A) and their normal saline counterparts; Group B. Our findings follow those of Miller and Dale's in that a sizeable amount of fluid was absorbed in all treatment groups. This suggests that placement of oxygenated fluid (i.e., normal saline) will assist in the continual maintenance of the absorptive capacity in the small bowel during severe hypotension. Group C did not succumb to the hemorrhagic shock and was able to continue and enhance absorption of normal saline.

Several possible explanations exist for the larger or equivalent fluid absorption in the saline groups as compared to glucose oleic acid group. First, a limited amount of work (energy) is required for absorption of normal saline than for the absorption of glucose. Secondly, a depletion of salt (i.e., chlorides) throughout the tissues of the body due to hemorrhage. When sodium chloride was placed in the intestine there was a higher diffusion gradient, and salt passed into the bloodstream more readily. Thirdly, severity of the hemorrhage protocol may be a factor in absorption of normal saline. Jenkins et al. (1961) and Ehrlich et al. (1969) found that compensatory mechanisms began to deteriorate 3 hours after a hemorrhage. After hemorrhage exceeds 40% of blood volume, the experimental animal becomes unresponsive to therapeutic maneuver (i.e., administration of normal saline) (Ehrlich et al., 1969). Jenkins et al. (1961) also showed that plasma dilution in hemorrhaged dogs is well correlated quantitatively with the degree of blood pressure experimentally reduced.

The group of dogs who received the duodenal instillation of the glucose-oleic acid solution absorbed 71% of the glucose administered. In our study, not only did glucose improve survival, but it enhanced and maintained arterial glucose levels, prevented the severe decompensation phase seen in Group B and maintained better respiratory compensation. Glucose also contributes directly to the metabolic maintenance of the myocardium and extracellular volume (Opie, 1970).

A large amount of experimental data has revolved about whether glucose is "good" and/or free fatty acids (FFA) are "bad" for the

ischemic myocardium. A number of changes accompany myocardial ischemia: depressed insulin response, augmented catecholamines, corticosteroids and growth hormone. Also, increased glycogen levels occur and are responsible for a variety of metabolic alterations seen during shock. All these endocrine changes are responsible for the relative glucose intolerance and increased FFA levels (Kones, 1975). The "glucose hypothesis" discussed by Opie (1970) postulates that glucose is "good" for the heart based upon the following predictions. Glucose availability enhances the rate of anaerobic glycolysis, reverses ion losses, alters impaired membrane electrophysiology, effects extracellular volume, decreases plasma FFA concentrations, and alters plasma osmolarity. The FFA are supposedly "toxic" to the ischemic heart (e.g. during severe hemorrhagic hypotension) in that arrhythmias are more likely to occur and contractility is depressed. However, Wildenthal (1971) explains the well-oxygenated myocardium shifts from FFA as the preferred substrate to glucose during ischemia or anoxia and depends upon the glycolytic pathway for energy production.

The plasma protein concentration (ppc) for the two treatment groups fell from control values in the initial stages of hemorrhage (Table 3). The fall in ppc continued for the first 2 hours during hypotension. Haddy, Overbeck and Daugherty, Jr. (1968) have reported that an increase in the precapillary resistance will predominate early in hemorrhage, favoring absorption of excellular fluid. The reverse is true late in hemorrhage, filteration predominates due to proportionately higher post-capillary resistance. Also, the increase

in capillary permeability may be one explanation for the loss of fluid late in shock, both before and after reinfusion of the shed blood. The gradual change observed in ppc for the glucose-oleic acid treated group correlates with the change in hemorrhaged blood volume. This correlation is also exemplified in the normal saline treated group where the ppc falls gradually during the first two hours of hypotension and then increases above control for the 3rd hour. Although no significant difference was evident between the two treatment groups for ppc.

The "spontaneous uptake" of blood from the blood reservoir represents one of the earliest signs of cardiovascular decompensation (Hollenberg, Waters, Toews, Davies and Nickerson, 1970). A spontaneous uptake of 40% of the shed blood from the blood reservoir was observed in the normal saline treated group (Figure 6, Group B), while the glucose-oleic acid treated group (Group A) spontaneously returned only 10% for the same time period. The amount of the return of blood from the reservoir to maintain the hypotensive pressure was shown to be significantly different between groups A and B (Figure 6). Rothe (1970) has suggested that, at 40% uptake of the maximum shed blood, there is significant depression of myocardial function. Similar response might occur in our normal saline treated group (Group B) and the response might have contributed to the fall in MABP during the normovolemic period and eventually lead to death of all dogs in this group. The third group of dogs, Group C, while also receiving the normal saline instillation, returned only 4% of the hemorrhaged blood volume. Group C absorbed significantly larger

amounts of fluid (Table 2) as opposed to Group B, but the maximal amount of hemorrhaged blood (measured in the reservoir) for Group C occurred 1 hour and 30 mins later than that of Group B. In group C, normal saline was absorbed in significantly larger quantity and may have acted as a temporary plasma expander to maintain circulating blood volume late in the hypotensive period.

Rothe and Selkurt (1964) suggest that an increase in hematocrit is due to a discharge of erythrocytes from the spleen. Further loss of fluid from the vascular system may continue to concentrate the red blood cells and plasma proteins. Results from our study indicate an increase of hematocrit for both treatment groups (Figure 5 and 7). Marty and Zweifach (1971), using splenectomized dogs, reported a drop in hematocrit of 25 to 40% from control values during the first hour of hypotension, with little change after the first hour. Our hematocrit data was similar to findings by Rothe and Selkurt who reported a significant increase in hematocrit ratios by the time the blood pressure was brought to 35 mmHg. The hematocrit increased at the beginning of hypotension but dropped suddenly, remaining below the initial increase until the second hour of hypotension. Coleman, Kallal, Feiger and Glaviano (1975) showed an 8% increase in hematocrit 2 hours after reinfusion of shed blood. Significant increases were evident for both saline treated groups series.

The highest heart rate recorded (Figure 7) coincides with the point of maximal hemorrhaged blood volume (Figure 6). Bond, Manning and Peissner (1979) also showed a maximal heart rate of 217 beats/min and a peak shed blood volume of 47 ml/kg at 1½ hours after hemorrhage

was initiated. Rothe and Selkurt (1964) showed a significant decrease in heart rate between periods of maximal blood volume and time just prior to transfusion, suggesting some degree of central nervous system depression. Our data show similar trends to that of Rothe and Selkurt, except the difference between these two periods are not significant. Rather than using a fixed time interval for hypotension, Rothe and Selkurt maintained the hypotensive pressure until there was 30% uptake over the maximal blood volume.

The paper chromatographic technique described by Yamada and Pettit (1977) offers a simple and highly specific assay for cardiodepressants. Only 200 ul of deproteinized plasma sample is required, eliminating the need for volume replacement. Serial sampling of several milliliters is required for analysis in papillary muscle preparations and other bioassays. The paper chromatographic technique is clean, reproducible, requiring small sample size, and particularly useful in serial studies involving small animals.

The cardiodepressants in our study were found to appear in the post-hemorrhage samples taken from the normal saline treated dog (Figure 8). Cardiodepressants were located by comparison of the migration distance or Rs value to a serine standard (#6). This spot, designated D in our study, should appear at a migration distance between Rs 1.6-1.8 times the distance that serine had migrated. This spot corresponds to similar data presented by Barenholz et al. (1973), Yamada and Pettit (1977); Rogel and Hilewitz (1978). Yamada and Pettit found that a large elution volume (85-105 ml plasma) prepared and removed from a paper chromatograph having a migration

distance between Rs 1.6-1.8 contained the highest depressant activity in papillary muscle preparation. This elate contained 42 units of higher depressant activity compared to a spot eluted with a migration distance of Rs 2.0-2.4.

Some cardiodepressants were present in the pre-hemorrhage sample taken from the glucose-oleic acid treated dog, probably due to surgical manipulation. However, hemorrhage did not increase the amount of cardiodepressants in arterial nor portal venous blood. Spot A may represent the large amount of circulating catecholamines present in the shock plasma only from samples taken from the blood reservoir (#1) and samples taken after hemorrhage (4A and 5V).

Haglind, Haglund, Lundgren, Romanus and Scherstern (1980) have correlated the amount of intestinal mucosal damage and mortality with the degree of graded decreases in blood flow to the intestines. The degree of mucosal damage correlates significantly with mortality. Using Chiu et al. (1970) scheme for morphologic characterization of the intestinal mucosa (see Material and Methods), Haglind and associates showed a high mortality rate in animals with histological damage between grades 3-5. The animals with grades between 0-2 had a low mortality rate. Our study showed the instillation of normal saline offers little to no protection of the mucosa during hemorrhage. In 3 dogs, the histological damage was between grades 2-4. Chiu et al. reported the placement of sodium chloride (0.9% and 1.8%) solutions into intestinal sacs of dogs. The histological analysis of these sacs after 60 minutes of ischemia showed some protection, receiving a grade between 2-3. Although the experimental design in

Chiu's study differs from the modified Wiggers' protocol, the analysis of mucosal damage correlates fairly well. The 3 dogs in our study who received the instillation of the glucose-oleic acid solution were given a grading between 0-1. The protective effects of glucose alone has been shown by Chiu, Scott and Gurd (1970) and Chiu, McArdle, Brown, Scott and Gurd (1970) to offer complete protection. Macroscopic observation reveals a normal mucosa with an occasional spot of petechiae after treatment with the glucose-oleic acid solution. Mucosal sloughing and hemorrhage was present throughout the small intestinal tract of those dogs who received the instillation of normal saline.

V. SUMMARY AND CONCLUSIONS

The use of a hemorrhage protocol that induces total body hemorrhage demands replacement of lost fluids temporarily until whole blood is made available and, most important, provisions of a readily utilizable energy substrate (such as glucose) as suggested by Opie (1970).

The instillation of a solution containing 150 mM glucose, 40 mM oleic acid and bubbled with 95% oxygen-5% carbon dioxide gas into the intestinal lumen of dogs subjected to irreversible hemorrhagic shock had the following beneficial effects:

A. The glucose-oleic acid solution prevented irreversible hemorrhagic shock and increased survival rate (67%) for 26-41 hrs.

B. Reduced the need for spontaneous uptake of shed blood from the hemorrhage reservoir to maintain the low pressure at 37 mmHg throughout the 3 hr hypotensive state.

C. Maintained arterial glucose levels between 87-127 mg% after reinfusion of all shed blood and throughout normovolemic stage of shock.

D. Prevented histopathological damage of the mucosa in the small intestine.

E. Prevented the release of cardiodepressants from the splanchnic region.

F. While fluid volume resorption was not different between the glucose-oleic acid and the normal saline treated dogs.

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