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thesis entitled

CARDIOTOXIC FACTORS RELEASED FROM THE CANINE JEJUNUM

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Physiology

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Date November 14, 1980

CARDIOTOXIC FACTORS RELEASED FROM THE CANINE JEJUNUM

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1980

ABSTRACT

CARDIOTOXIC FACTORS RELEASED FROM THE CANINE JEJUNUM

By

John Thomas Senko

Plasma samples from dogs subjected to irreversible hemorrhagic shock were assayed for cardiotoxic substances in the Langendorff isolated guinea pig preparation. Blood samples were obtained from the femoral artery and jejunal vein of hemorrhaged animals (35 mmHg for 3 hours then retransfusion of shed blood) and sham shock control dogs. The guinea pig heart was a sensitive bioassay, detecting cardiodepressant activity in shocked canine plasma diluted 1:10 with Krebs-Ringer-bicarbonate perfusate. Depressive activities were not detected in either the arterial plasma (-0.07 $\pm$ 5.0 Cardiodepressant Activity, CDA) or the jejunal venous plasma (-9.0 $\pm$ 3.0 CDA) before hemorrhage. Significant increases of CDA were observed in the jejunal venous plasma (+20.0 $\pm$ 7.0 CDA, $p<0.01$) after the hemiorrhage proceedure but were not observed in the arterial plasma (+10.0 $\pm$ 4.0 CDA) after hemorrhage. Sham shock control dogs did not show significant changes in CDA throughout the experiment. Cardiotoxins as detected by the Langendorff guinea pig bioassay are released into the canine circulatory system during hemorrhage and the intestine may be one of the sources of these toxins.

DEDICATION

This thesis is dedicated to my mother who always has given me support when I needed it.

ACKNOWLEDGEMENTS

I would like to extend special thanks to the members of my committee: Dr. Bob Pittman for coordinating the project, Dr. Ching Chou for his special expertise, and Dr. Tom Emerson for his valuable suggestions.

I also thank my fellow graduate student, Rich Nyhof, for his assistance in the development of the techniques. Additionally, Don Weston lended valuable assistance and moral support.

Financial support for this study came from the Michigan Heart Association for which I am grateful.

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Introduction

A number of clinical and experimentally induced states, those involving acute or chronic injury, are characterized by a gradual erosion of the cardiovascular system. When these insults are of a severe intensity and of sufficient duration, a state is encountered in which corrective measures are no longer effective. The vital body functions are compromised to such an extent that the state becomes self-sustaining and culminates in complete collapse of the circulatory system (Zweifach and Fronek, 1975). This is the irreversible shock syndrome.

Hemorrhage has been widely used to induce a state of shock and if allowed to progress for sufficent time, ultimatly leads to death. The loss of circulating blood volume from hemorrhage sets into motion a sequence of compensatory reactions that ultimately results in underperfusion of body tissues. The body responds through humorally and neurally mediated compensatory mechanisms, which bolster the falling blood pressure in the short run but when sustained may exacerbate the shock syndrome (Chein, 1967). A relatively precise control of tissue prefussion and tissue ischemia is allowed by hemorrhage and, therefore, over the underlying initating factors of irreversible shock.

Three general concepts have been advanced to account for the development of irreversible hemorrhagic shock. These include impairment of capacitance function, decreasing cardiac performance and the effects of

cardiotoxic factors. The experiments in this study were conducted to determine if cardiotoxic substances are released from the shocked canine intestine.

I. Literature Review

A. Introduction

A number of reviews are available on the topic of hemorrhagic shock, and two symposia, one edited by Selkurt (1961) and the other by Hinshaw and Cox (1972), give good overviews. Wiggers book on the physiology of shock (1950), though somewhat dated by recent findings, is still one of the best analyses of experimental hemorrhagic shock and cardiovascular changes occuring during the course of hemorrhage. Chein (1967) offers a comprehensive review of the role the sympathetics play in shock. Zweifach and Fronek (1975) discuss the peripheral and central factors opperating in irreversible hemorrhagic shock. Finally, Lefer (1978) presents data for the involvment of cardiotoxins in irreversible shock.

B. Experimental Hemorrhagic Procedures

1. Types of Procedures

The procedures used to produce hemorrhagic shock fall into two broad categories, the "single withdrawal" model or the "Wiggers fixed hypotension" model. To produce shock by hemorrhage one needs to reduce the effective blood volume and blood flow through tissues to the proper degree for a sufficent peroid. As Wiggers (1950) stated, only in this way "can a slow and gradual wreckage of the cellular machinery be accomplished." If the bleed out is too fast, death results prematurely from respiratory or cardiac failure; if it is to slow, shock may not occur (Wiggers, 1950). The degree and length of the hypotensive period needed to produce irreversible shock depends on the experimental animal. The state of health, sex, age, etc. of the research animal can influence the

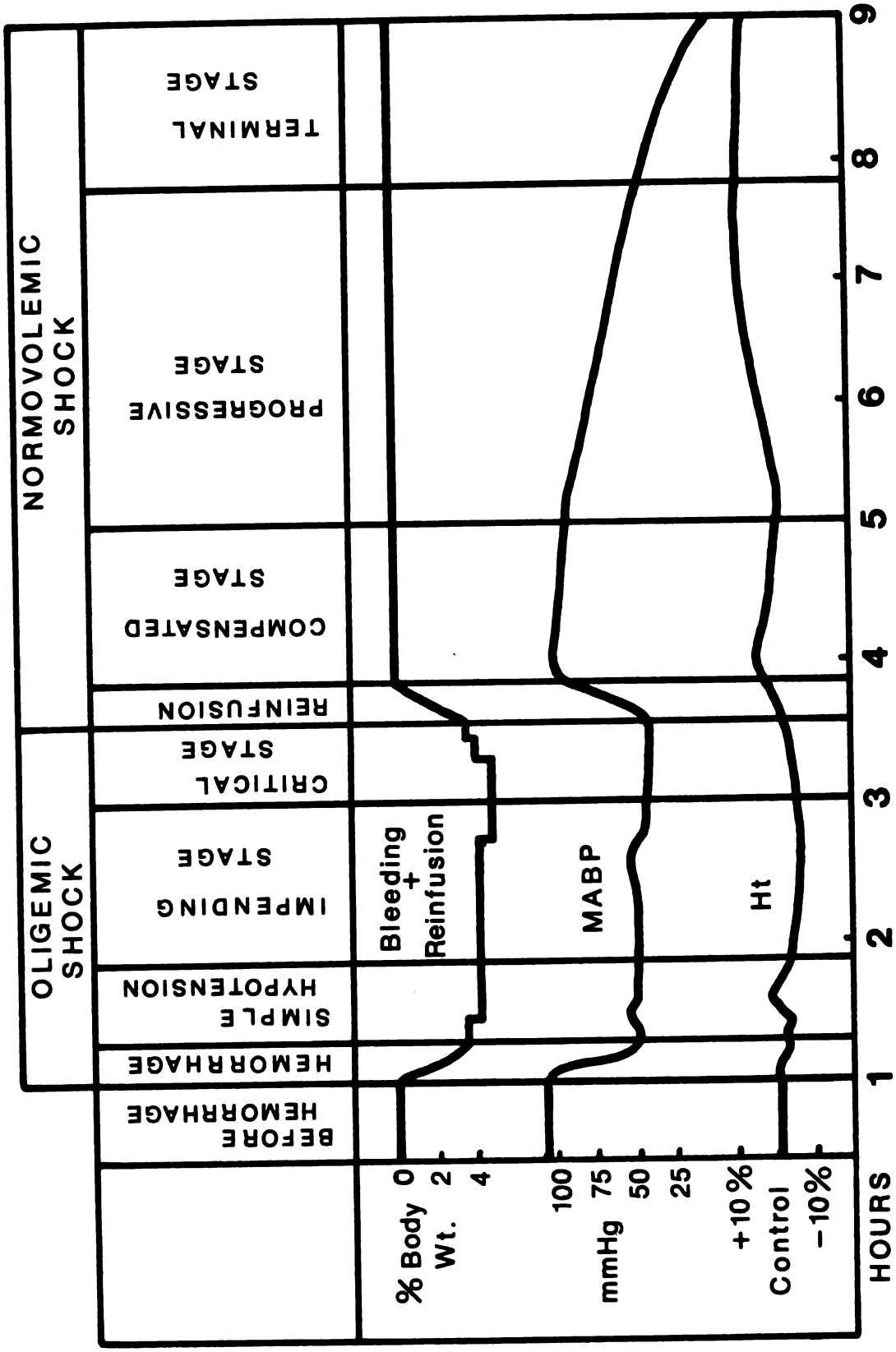
progression and severity of the shock state. The biological and physiological characteristics of the research animal need to be taken into account when choosing a hemorrahgic model in order to understand the relative importance of the mechanisms leading to irreversible shock (Zweifach, 1961).

The single-withdrawal model is an attempt to duplicate clinical conditions. A fixed volume of blood is withdrawn, usually an arbitrary predetermined percentage of the circulating blood volume, and then the animal is allowed to compensate (Walcott, 1945). In this model the blood volume removed is controled while the arterial blood pressure fluxates independently. The major disadvantage of the single-withdrawal model is a variable hypotensive peroid, the result of natural variations in the compensatory adjustments of research animals to the blood loss.

The Wiggers technique (Wiggers, 1942) or one of its modifications is the most common procedure used to produce hemorrhagic shock. In the Wiggers model, blood is withdrawn to reduce the mean arterial blood pressure to a predetermined level which is maintained by appropriate bleedings or transfusion of the shed blood. After a set period of hypotension the shed blood is returned to the animal. Lamson and DeTurk (1945) modified this bleeding technique by hemorrhaging into a reservior connected directly to the arterial system. The level of hypotension in the animal is set by the height of the reservoir. Fluxations of the arterial blood pressure are prevented by automatic bleed out or "take up" of blood from the resevior.

The stages of irreversible hemorrhagic shock as named by Wiggers (Wiggers, 1950) are summarized in Figure 1. Hemorrhage creates a shock state characterized by low blood volume and is named oligemic shock.

Figure 1. Diagram of the stages of experimental hemorrhagic shock as described by Wiggers (1950). Bleeding and reinfusion of blood (% of body weight), mean arterial blood pressure (MABP) in mmHg, and hematocrit (Ht) in % of control are shown from the inital hemorrhage to death of the animal.


NORMOVOLEMIC SHOCK
OLIGEMIC SHOCK
BEFORE HEMORRHAGE
HEMORRHAGE
SIMPLE HYPOTENSION
IMPENDING STAGE
CRITICAL STAGE
REINFUSION
COMPENSATED STAGE
PROGRESSIVE STAGE
TERMINAL STAGE
Bleeding + Reinfusion
MABP
Ht
% Body Wt.
0
2
4
mmHg
100
75
50
25
+10%
Control
-10%
HOURS
1
2
3
4
5
6
7
8
9

Oligemic shock was divided by Wiggers into three stages; (1) simple hypotension, (2) impending (preirreversible shock) stage and (3) critical or irreversible stage. The three stages indicate progressive increases in the severity of the shock state. The uptake of shed blood by the animal is referred to as retransfusion and signals that the animal is in the impending stage of oligemic shock. After a fixed percentage of the shed blood is taken up by the animal or after a set peroid of oligemic hypotension, all remaining shed blood is retransfused and the blood pressure allowed to fluxate freely. With the return of the shed blood the animal is in a state of normovolemic shock.

Normovolemic shock can also arbitrarly be subdivided into three stages. Initally, after retransfusion of shed blood, the animal is in a normovolemic compensatory stage in which the circulating blood volume is near normal and the blood pressure is stable but below control levels. Slowly the blood pressure begins to fall, marking the entrance into the progressive or declining stage which is a transition to the terminal stage characterized by a rapidly falling blood pressure, ending in the death of the animal. Wiggers (1950) defined irreversible hemorrhagic shock as a falling blood pressure with a near normal blood volume after the oligemic hypotensive peroid.

The Wiggers model is reliable and reproducable in producing irreversible hemorrhagic shock. The loss of blood volume by hemorrhage is the primary disturbance. By controlling the mean arterial blood pressure, the tissue prefusion and the degree of ischemia in tissues can be controlled as well. The Wiggers proceedure was designed to produce irreversible hemorrhagic shock and to observe what componets of the circulatory system have or are failing after return of the shed blood.

2. Indices measured in hemorrhagic shock

The most reliable criteria to judge experimental shock is the absolute survial of the animal after induction of shock. Relative "survival" measures the time from a point of reference, usually hemorrhage or reinfusion, to the death of the animal. Changes in this length of time, positive or negative, are a measure of changes in the severity of the shock state. Most results from hemorrhagic shock experiments are reported using relative survial (Zweifach and Fronek, 1975).

The most readly obtainable hemodynamic parameters measured in hemorrhagic shock are arterial blood pressure, central venous pressure, heart rate and hematocrit. More difficult measurements, but good indices with which to evaluate myocardial dynamics, are cardiac output and intraventricular pressure (Selkurt and Rothe, 1961).

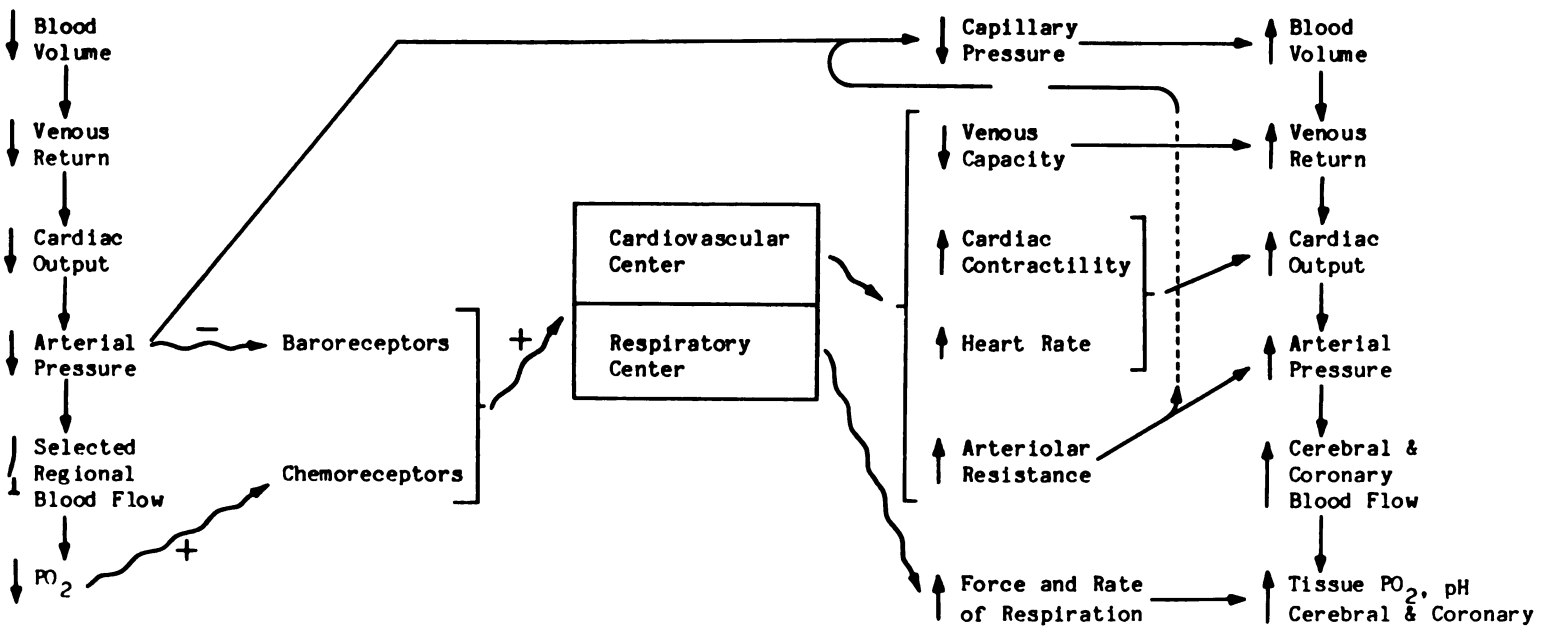
The close relationship between blood supply and cell metabolism during hemorrhagic shock are reflected in metabolic indices. The most commonly measured parameters are blood levels of enzyme activities, lactate-pyruvate ratios, pH, and pCO_2 (Mela, 1978). These parameters may give a measure of the type of metabolism, whether aerobic or anaerobic, and can give a indication of cellular death and degradation.

C. Circulatory Sequence in Response to Hemorrhage

1. Early hemorrhagic shock

The responses of the cardiovascular system and the responses of the sympathetic nervous system to the inital blood loss are summarized in Figure 2. Decrease in blood volume immediately result in a fall of arterial pressure, and as regional tissue flow decreases, the tissue pO_2 decreases. The body responds to the severe blood loss and tissue ischemia

Figure 2. Responses of the circulatiory system to blood volume loss by hemorrhage



(Adapted from Milnor W. R. The Cardiovascular Control System. in V. B. Mountcastle ed. Medical Physiology vol 2, 1974)

by activating mechanisms which make compensatory adjustments to the decreased blood pressure. These adjustments may be temporally life saving, but they protect central body functions at the expense of the splanchnic and peripheral circulation.

2. Sympathetic system response to hemorrhage

Beginning with a loss of blood greater than 10 to 20% there is a narrowing of the pulse pressure and a fall in mean arterial blood pressure (Chein, 1961). The falling pressure reduces afferent nerve impulses from the baroreceptors in the carotid sinus and aortic arch. The medullary cardiovascular center released from baroreceptor inhibition, increases sympathetic efferent activity to the heart and blood vessels. Volume receptors in the large arteries and veins undoubtly play a role in severe hemorrhage, but the nature of this role is unclear (Milnor, 1974b). The predominate effect of the baroreceptor mediated sympathetic discharge is an increased heart rate and vasoconstriction.

The falling blood pressure also leads to a decreased blood flow through the carotid and aortic bodies. This in turn produces a stagnant hypoxia in these bodies. With decreases in pO_2 and increases in pCO_2, the chemoreceptors in the carotid and aortic bodies increase their rate of firing. The increased chemoreceptor discharge stimulates the respiratory center to a greater degree than the cardiovascular center. There is little increase in cardiac sympathetic impulses, but there is a stimulation of sympathetic vasoconstrictor fibers from chemoreceptor stimulation. The predominate effect of chemoreceptor stimulation is an increase in the respiratory rate (Berne and Levy, 1977).

The medullary cardiovascular center normally integrates baroreceptor and chemoreceptor reflexes and is the major site of integration for sympathetic discharge after hemorrhage. The cerebral cortex, hypothalamus, and spinal cord are also known to participate in the integration of sympathetic discharge and may function in hemorrhage (Milnor, 1974b).

The adrenal medulla is the major source of catecholamines from sympathetic activation, augmented by release of norepinephrine from nerve terminals. Farnebo et al. (1979), found by lowering the mean arterial blood pressure in anesthetized rats to 35 mmHg for 4 hours, the norepinephrine and epinephrine levels were raised from basal plasma levels of 0.97 and 0.54 nmol/l, respectively, to about 17 nmol/l at 4 hours for norepinephrine and 60 nmol/l at 4 hours for epinephrine.

Hemorrhage with less than a 10% loss in circulating blood volume does not significantly increase the heart rate. With a greater loss of blood volume, reflex tachycardia is produced primarly due to vagi inhibition and increases in sympathetic activity (Chein, 1961). Sympathetic nerve activation and released circulating norepinephrine cause an increased heart rate by acceleration of spontaneous depolarization in cardiac sinoatrial node pacemaker cells (Marshall, 1974). Cardiac sympathetic impulses also produce increased rates of tension development and fiber shortening in the ventricular myocardium which raises the stroke volume, and, together with the increasing heart rate, the cardiac output is raised (Marshall, 1974). The positive inotropic effects of sympathetic impulses are often masked by other changes in circulatory parameters during hemorrhage. Chein (1967) sites several factors which may operate to decrease myocardial contractility as severe oligemic hypotension is

maintained: (1) myocardial anaerobiosis from marginal coronary flow, (2) decreasing sympathetic impulses and myocardial catecholamine content, (3) decreased myocardial reactivity to circulating catecholamines, and (4) effect of cardiotoxic substances from the splanchnic circulation.

Sympathetic activity increases the resistance of vessels to blood flow and decreases the total capacity of the circulation. Non-neural factors such as increased blood viscosity (hematocrit changes), plasma protein concentration, and rate of blood flow can also increase resistance by passive mechanisms (Berne and Levy, 1977). In addition lowering blood pressure may increase resistance by lessening distending forces or may decrease resistance through local autoregulatory processes (Haddy et al., 1968). Therefore resistance changes after hemorrhage cannot solely be attributed to sympathetically mediated vasoconstriction.

The effect of hemorrhage on vascular capacity is most acutely exhibited in the venous system since approxmately three fourths of the total blood volume is contained in the veins (Milnor, 1974a). Hemorrhage causes the moblization of blood from the central blood volume by the combination of passive response of lowered transmural pressures and active sympathetic venomotor activation (Milnor, 1976b).

The differential vasomotor effects of sympathetic activation results in a redistribution of cardiac output. Generally in response to a large inital hemorrhage there is a decrease in flow and an increase in resistance within the skin and muscle (Haddy et al., 1968). Increases in resistance and decreases in flow are most marked in the renal and superior mesenteric beds (Fell, 1966). In anesthetized monkeys, Fursyth et al., (1970), reported that by removing 50% of the circulating blood volume, the flow in the kidneys decreased from a control flow of 961$\pm$265 ml/min/100g

to 108±81 ml/min/100g, and flow to the gastrointestinal tract decreased from a control flow of 105±20 ml/min/100g to 27±20 ml/min/100g. A varity of methods have shown a decrease in coronary resistance with falling arterial pressure as the coronary vessels attempt to maintain adequate flow (Chein, 1967). The cerebral flow, like the coronary vessels, autoregulates over a wide range of arterial pressure and is little influenced by the autonomic nervous system. The cerebral blood flow autoregulatory response can be abolished with mean arterial blood pressures below 50 mmHg and with extreme hypotension the cerebral blood flow decreases (Kovach and Sander, 1976).

The changes in splanchnic flow are very important in the pathophysiology of irreversible hemorrhagic shock. The resulting ischemia has been implicated as one of the causitive factors in the cellular disruption that results in the elaboration of cardiotoxic factors (Lefer, 1973). Acute rapid hemorrhage that decreased aortic pressure from 120 to 60 mmHg was found by Chou et al. (1976) to significantly decrease total wall flow in all segments of the G.I. tract from the stomach to the colon. They also found that with hemorrhage flow was redistributed away from the mucosa only in the stomach. Blahitka and Rakusan (1977) using microspheres in rats reported that after removing 45% of the circulating blood volume, the splanchnic flow was consistantly decreased, reaching a low of 29% of control 60 minutes after the end of hemorrhage. These same investigators found that the fraction of the cardiac output to the splanchnic region tended to decrease as the time after hemorrhage increased. Fell (1966) reported that hemorrhage to 35 mmHg for 3 hours decreased flow through the mesenteric circulation to near zero levels and produced more than a fivefold increase in resistance. With prolonged

oligemic hypotension the decrease in flow tends to become more severe and the elevation of mesenteric resistances more pronounced (Selkurt, 1958).

3. Late Hemorrhagic Shock (Decompensation)

With severe and prolonged oligemic hypotension, the increased cardiac output and vasoconstriction are no longer able to maintain the arterial blood pressure. The compensatory mechanisms of the animal begin to fail and the shed blood must be replaced or the arterial pressure falls. Wiggers (1950) stated that the continued uptake of blood is practically synonymous with irreversible shock. He concluded that the critical stage occurs "for one of two reasons: either the compensatory mechanisms fail or new conditions are produced that cause a further reduction of venous return and cardiac failure". Chen (1967) sited evidence that with the beginning of uptake there are decreases in sympathetic nerve impulses, circulating catecholamine concentrations, and total peripheral resistance; but he also stated that the decreases in cardiac output, total peripheral resistance, and plasma volume in prolonged hypotension may be due to humoral and metabolic factors.

4. Irreversible Hemorrhagic Shock

As has been previously stated, irreversible hemorrhagic shock was named because after prolonged and severe hypotension the lowered blood pressure of the animal is non-responsive to volume replacement, whereas earlier, the shock state can be reversed by retransfusion. The overriding feature of irreversible hemorrhagic shock is a falling blood pressure in face of replacement of the blood volume deficit. There are three general concepts that have been advanced over the last 40 years to account for

irreversible hemorrhagic shock. These are: (1) impairment of capacitance function or venous pooling, (2) decreasing cardiac performance or contractility, and, more recently, (3) metabolic disturbances and cardiotoxic factors.

From the 1940's into the 1950's the dominate emphasis shifted from peripheral mechanisms, involving stagnant hypoxia and decreased venous return, to central cardiovascular performance with decreasing myocardial contractility as the predominate influence responsible for irreversibility. Though Simeone (1961) sited electrocardiographic evidence, cardiac muscle circulatory depression, and derangement of myocardial metabolism as indicative of failure of the heart in oligemic hypotension, he still concluded that it was reasonable to doubt the heart as the major cause of irreversibility and gave decreasing venous return a greater importance in the development of irreversibility.

Crowell and Guyton (1962) determined cardiac output curves of normal dogs and cardiac output curves of dogs in successive stages of irreversible hemorrhagic shock. Early in shock, normal cardiac output curves were reported but; after retransfusion and the development of irreversiblity, curves indicating cardiac failure were always recorded, and successive curves indicated progressive development of cardiac failure. When cardiac output was kept constant by increasing the blood volume, right and left atrial pressures rose to extremely high levels as shock progressed, confirming the cardiac failure seen in the cardiac output curves. They stated that irreversibility was due to progressive cardiac failure but that the cause of the cardiac failure was unknown.

Using the Wiggers procedure, Rothe and Selkurt (1964) found in anesthetized dogs that just prior to retransfusion there was a significant

decrease in heart rate as compared to the heart rate at maximum bleedout, but at this stage they found no evidence of further cardiovascular impairment. They observed cardiac depression to be a significant factor only in severe prolonged hypotension. In moderate hemorrhagic hypotension they found reversible cardiac and peripheral vascular damage which when corrected by transfusions prevented the death of the animal from hypotension. They concluded that irreversible hemorrhagic hypotension is the result of decreased cardiac output from inadequate cardiac filling. Crowell and Guyton (1962) were partially correct, myocardial depression is a factor in the development of irreversibile hemorrhagic shock but the depression is not seen until at least 20% of the shed blood is returned (Rothe, 1970).

An increase in venous compliance resulting in decreased venous return has been a commonly stated hypothesis for the cause of irreversible hemorrhagic shock. Green (1961), summarizing the available literature, stated that the discrepancy between blood volume and vascular capacity was the cause of the decreased venous return and therefore cardiac output. A complicating factor in evaluating vascular capacity is the choice of the research animal. The dog has a sphincter-like muscular region in the effluent hepatic vein that is highly sensitive to vasoactive substances (Zweifach, 1961) which could result in splanchnic pooling in the dog. Rush (1972), reviewing the literature, found the question of blood pooling acceptable as a feature of hemorrhage but its importance in hemorrhagic shock unresolved. Although a number of investigators have sought to delineate the possible role of venous pooling in irreversible hemorrhagic shock, it has not been demonstrated that the venous capacitance system plays a significant role in the initiation of irreversibility during

either the hypo- or normovolemic phase of experimental hemorrhagic shock (Zweifach and Fronek,1975).

D. Origin of Cardiac Toxins

Evidence for cardiotoxic substances in the blood of animals in hemorrhagic shock began to appear in the literature in the 1940's and continued sporadically for the next 20 years. Katzenstein et al. (1943) reported that thoracic duct fluid from animals in tramatic shock could produce a fall in blood pressure in recepient dogs. Ravin et al. (1958) assayed for toxic factors in the plasma of shocked dogs with reversibly shocked rabbits. They reported that infusion of plasma from a dog in irreversible shock into shocked rabbits was lethal to the rabbits, whereas infusion of plasma from control animals lead to recovery of the reversibly shocked rabbits.

The dramatic changes in splanchnic circulation during hemorrhage had lead investigators to suspect these changes played a role in irreversible shock. Selkurt (1958), while investigating the role the liver plays in irreversibility, hypothesised that hemorrhage creates conditions in the intestinal bed that favor production and release of material toxic to the cardiovascular system. In 1966, Brand and Lefer reported that the plasma of cats in hemorrhagic shock depressed the contractility of isolated cat papillary muscles, and they named the suspected toxic substance "myocardial depressant factor" (MDF). Since these early beginnings, cardiotoxic factors have been reported in hemorrhagic, splanchnic ischemic, endotoxic, cardiogenic, tramatic, acute pancreatics, burn, and septic shock (Lefer, 1978).

1. Characteristics and Production

A varity of cardiotoxic factors have been identified and studied. There is remarkable consistancy among the factors with regard to sites of origin, size, and biological actions, which leads to the possibility that the different investigators are dealing with the same compound or family of compounds.

On the cellular level, the lack of adequate oxygen tension leads to a shifting to alternative modes of energy production, namily anaerobic glycolysis. Eventually prolonged hypoxia leads to a derangement of the energy producing mechanisms of cells (Schumer and Erve, 1975). An acute injury, such as hemorrhage, causes a decreased oxygen consumption and energy metabolism. Glycogenolysis is elevated and a hyperglycemia and lacticacidemia are observed, the extent of which may be directly related to the level of injury (Fleck, 1976). Crowell (1970) found that the decreased metabolism during hypotension was directly related to inadequate oxygen supply and not decreased oxygen demand. It is the difference between demand for oxygen and the availablity of oxygen which is the precursor for the tissue distruction seen in hemorrhage. Mela (1979) found that the primary metabolic injury to brain mitochondria with incomplete ischemia was a irreversible damage to the membrane system responsible for respiration and energy production. Ferguson et al. 1972 reported disruption of pancreatic lysosmes with hemorrhagic hypotension and the release of lysosomal contents was associated with cellular distruction. The disruption of lysosomal membranes was also implicated in splanchinc arterial occlusion shock (Glen and Lefer, 1970a). Due to these disruptive mechanisms, blood borne cardiotoxic subatances have been reported to contribute to the circulatory breakdown in hemorrhagic shock.

Lefer-Glenn's factor, myocardial depressant factor, is a small dialyzable peptide, molecular weight 800-1000 daltons, of pancreatic origin (Lefer and Martin, 1970a, 1970b) that has been identified in most forms of shock and from a range of research animals (Lefer, 1978). Myocardial depressant factor produces depression of myocardial contractility in isolated cat papillary muscles (Lefer et al., 1967) and a negative inotropic effect in isolated prefused cat hearts (Thalinger and Lefer, 1971). Myocardial depressant factor also constricts superior mesenteric arterial strips thereby establishing a positive feedback mechanism leading to the formation of greater amounts of the factor by further decreasing splanchnic blood flow (Glucksman and Lefer, 1971).

Reticuloendothelial depressing substance (RDS), Blattberg-Levy's factor, is a dialyzable and transferable substance formed during splanchnic ischemia and hemorrhage (Blattberg and Levy, 1962), which are characteristics in common with MDF. The main biological effect of RDS is a depression of the phagocytic ability of the reticuloendothelial system (Blattberg and Levy, 1966).

Nagler-Levenson's factor, passively transferable lethal factor (PTLF), is interesting in that it is not a small peptide and is not formed in the splanchnic region. Passively transferable lethal factor is a large molecule, MW> 10,000, formed in the blood stream by the action of white blood cell enzymes, and also depresses ventricular function (Nagler and Levenson, 1974).

A number of other researchers have also reported cardiotoxic factors. Haglund and Lundgren (1973), Okuda and Tamada (1974), David and Rogel (1976), and Goldfarb et al. (1978) have all found factors in the circulatory system originating primarly within the splanchnic region

during hemorrhagic shock having negative inotropic effects on cardiac muscle and are small peptides with molecular weights in the 500-1000 MW range.

The production of cardiotoxic factors can be modified by pharmacological means and in so doing allows insight into the mechanisms of formation and prevention. Glucocorticoids provide a degree of protection against cardiotoxic factors. When pharmacological doses of cortisol, dexamethasome, or methylprednisolone are given prior to hemorrhage, survial time after retransfusion of shed blood is increased, and the protection is correlated with low levels of MDF (Lefer and Martin, 1969, Glenn and Lefer, 1970a). Administration of cortisol after reinfusion of shed blood or high doses of mineralocorticoids given either before or after hemorrhage are ineffective in prolonging survival time and under these conditions, high plasma levels of MDF are measured (Lefer and Martin, 1969).

The naturally occuring glucocorticoid, cortisol, and the synthetic glucocorticoid, methylprednisolone, in both physiological and pharmacological concentrations failed to exert a significant direct systemic cardiovascular effect. Only the mineralocorticoid, aldosterone, showed direct positive inotropic effects on the heart (Jefferson et al., 1971). The protective actions of glucocorticoids, therefore, are acting through mechanisms other than a direct stimulatory effect on the heart.

Glenn and Lefer (1970a), reported a 300 to 400 percent increase in the plasma levels of lysosomal enzymes β-glucuronidase and cathepsins accompanied by elevated amounts of MDF in cats after two hours of splanchnic arterial occlusion (SAO). Animals, treated with methyl-prednisolone before SAO, did not show increases in plasma lysosomal enzyme

activities and consistantly showed enzyme activities below control animals (Glenn and Lefer, 1970a). Glenn and Lefer (1971) investigated alterations of lysosomal enzymes, cathepsins A-E, in plasma and tissues of cats during hemorrhagic shock. They reported that in late post oligemia there was significantly elevated activities of the cathepsins in the feline plasma which was associated with increased formation of MDF. Significant decreases in total lysosomal enzyme activity were found in the pancreas and liver which correlated with increased lysosomal size and vacuolization.

Methylprednisolone added to lysosomal fractions from pancreatic homogenates markedly increased the stability of the lysosomes as seen by increases in time to reach peak release of β-glucuronidase (Glenn and Lefer, 1970a), but methylprednisolone had no effect on the activities of freed cathepsins (Glenn and Lefer, 1971).

Other investigators have reported disruption of lysosomes in other forms of shock and in other tissues. Weissman (1964) reported evidence of in vitro stabilization of lysosomal membranes by glycocorticoids. The release of β-glucuronidase from incubated large liver granules from rabbits was decreased below control levels by cortisone and cortisol, indicating stabilization of lysosomal membranes. These results were later confirmed in the isolated prefused pancreas by Ferguson et al. (1972).

Okuda and Yamada (1973) reported striking pancreatic lysosomal abnormalities, as indicated by increased fragility and autophagic activity of lysosomes in dogs in prolonged cardiogenic shock. The changes in intestinal lysosomes following regional intestinal shock in the cat were investigated by Haglund et al. (1975). They report significant reduction in the activity of acid and alkaline phosphatases, and an increased ratio

of total to free activity in intestinal tissue with regional intestinal hypotension. Methylprednisolone treatment of cats prevented the lysosomal and cytoplasmic derangements in shocked intestine as refleted by near control values of lysosomal enzymes, acid phosphatase, and β-glucuronidase, and insignificant changes in intestinal tissue alkaline phosphatase. The intestinal mucosal lesions and cardiovascular derangements characteristic of regional hypotension in the cat were also prevented by treatment with methylprednisolone (Haglund et al., 1977).

Thus the beneficial effects of glucocorticoids in hemorrhagic shock seems to be through a stabilization of lysosomal membranes, preventing the release of their enzymes, and the beneficial effects are correlated with lowered levels of circulating lysosomal enzymes, a prevention of cytoplasmic abnormalities, and decreased MDF activities.

Lefer and Barenholz (1972) reported that with SAO shock in the cat, pancreatic activity of trypsin increased four times, and activities of pancreatic phospholipase A increased seven times. Aprotinin, an inhibitor of proteases (trypsin and phospholipasae A) from pancreatic zymogen granules, prolonged survival time when administered prior to hemorrhage, and prevented the appearance of MDF (Lefer and Barenholz, 1972). Lefer and Martin (1970) subjected ultrafiltrates of plasma from shocked cats to column chromatography, and measured the eludate for optical density at 230 nm. A series of six peaks are found. Peak D contains all the myocardial depressant activity of the original sample. They reported that ultrafiltrates of plasma from shocked cats pretreated with aprotinin were without peak D and the pattern of peaks was similar to the control pattern. Thus aprotinin by inhibiting the formation of trypsin and

phospholipase A from pancreatic zymogen granules prevents the formation of MDF.

Herlihy and Lefer (1974) using a variety of proteolytic enzyme inhibitors further eluded the mechanism of MDF production. They reported that cathepsin B from pancreatic lysomsomes and the pancreatic zymogen granule enzyme kallikrein have the best correlation with MDF production and hypothesize that kallikrein and cathepsin B may act in concert to produce MDF.

Lefer (1978) has hypothesized a model for the formation of MDF in the pancreas. Production of myocardial depressant factor in the pancreas appears to involve both lysosomes and zymogen granules. Hypoxia and ischemia, the primary effects of blood loss from hemorrhage, result in disruption of the membranes of pancreatic lysosomes and zymogen granules, allowing the release of proteolytic enzymes and acid hydrolases. Hypoxia and ischemia activate the released trypsin, kallikrein and phospholipase A, and phospholipase A alone, or with the activated trypsin and kallikrein, attack cellular membranes and release the compartmentalized protein substances. This action makes the proteins available to degradation by kallikrein and cathepsins. The peptide fragments thus produced contain MDF.

The model for the formation of MDF in the pancreas may be considered a paradigm for the formation of cardiotoxic substances in susceptable tissues under the proper forms of stress.

2. Role of the intestine

There have been several conflicting reports regarding the origin of cardiotoxins. Within the splanchnic region, the ischemic pancreas and the

ischemic intestine both have been reported as sites of cardiotoxin production (Lefer and Martin, 1970 and Lundgren et al., 1976).

Beardsley and Lefer (1974) reported that after two hours of splanchnic artery occlusion (SAO) in cats, the papillary muscles from SAO shocked animals were significantly depressed compared to papillary muscles from sham shocked animals. Beardsley and Lefer (1974) also reported the effect of plasma from shocked and nonshocked cats on isolated perfused cat hearts from both sham shocked and SAO shocked animals. Plasma from sham shocked cats did not significantly change contractility in either heart, whereas plasma from SAO shocked cats significantly decreased the force of contractility in both groups of hearts. Hearts from cats subjected to splanchnic artery occulsion were also more sensitive to the cardiodepressant effects of shocked plasma than hearts from sham shocked animals. The effect of shocked plasma on isolated papillary muscles, together with the difference between shocked and nonshocked hearts indicates a depression of cardiac function in cats subjected to splanchnic arterial occlusion. This suggests that the cardiac depression has its origins in disruptions within the splanchnic region.

Lefer and Martin (1970b) surgically prepared cats before hemorrhage by either: (1) complete ligation of the celiac, superior mesenteric, and inferior mesenteric arteries to produce splanchnic vessel occlusion (SVO), (2) sham splanchnic vessel occlusion, (3) splanchnic vessel occlusion with pancreatectomy, and (4) occlusion of superior and inferior pancreaticoduodenal arteries to produce isolated pancreatic ischemia. Blood samples were collected from the shocked cats and ultrafiltrates of plasma or the gel column fraction, peak D, were assayed for MDF activity on isolated cat papillary muscles. They reported that the greatest

depression of the papillary muscle was observed in plasma samples after splanchnic vessel occlusion and with isolated pancreas ischemia. Plasma obtained from animals with pancreatectomy prior to SAO had approximatly the level of activity of MDF as the sham splanchnic arterial occlusion animals. They concluded that the pancreas is the primary site of MDF production.

Haglund and Lundgren (1973) investigated changes in cardiac parameters of cats with simulated shock of the small intestine. Shock was simulated in the gut by local hypotension from vessel occlusion and by activation of sympathetic vasoconstrictor nerves leading to an isolated segment of the small intestine. The remainder of the intestine, spleen, greater omentum, and a major part of the pancreas of the cats were removed. The outflow of the intestinal segment was diverted around the liver and returned to the animal through the jugular vein directly to the heart. The heart, therefore, acted as an in situ bioassy organ since the heart was sympathetically denervated, the adrenals denervated, and the vagi blocked by atropine. They reported that, following a 2 hour period of simulated shock, cardiovascular derangements started rapidly only after the intestinal prefusion pressure was restored. The heart seemed to be the major cause of the cardiovascular deterioration which was characterized by decreases in aortic blood flow (cardiac output) and maximal dP/dt and a rising left ventricular end-diastolic pressure.

In a second set of experiments the intestinal outflow was collected for the first five minutes after release of hypotension. The blood that was collected was replaced by fresh blood and a stabilization of cardiac preformance was observed in the simulated shock animals. After sixty minutes the collected intestinal venous blood was returned to the animals.

This produced a dramatic fall in systemic arterial blood pressure, stroke volume, and external cardiac work. The exchange experiments seemed to suggest strongly that there can be release of blood borne cardiotoxic material which is at least in part from the intestine.

Lundgren, et al. (1976) tested plasma from cats in simulated intestinal shock on isolated rabbit papillary muscles and on isolated prefused rat hearts. In both preparations there was a significant decrease in contractile force, as measured by decreased developed tension or decreased systolic pressure. Since the time to peak tension development in the rabbit papillary muscle was uneffected, they concluded that during shock the feline intestine releases cardiotoxic material which produces a negative inotropic effect on the heart. Haglund, et al. (1975, 1977) further demonstrated the ability of the cat intestine to release toxic factors in simulated intestinal shock by showing the release of lysosomal enzymes from the intestine and the protection against cardiovascular deterioration and mucosal lesions with methylprednisolone.

E. Summary

The question of whether cardiotoxic factors exist and to what extent they contribute to irreversible hemorrhagic shock has been considered by a number of investigators. The role the intestines play in the development of irreversible hemorrhagic shock remains unclear and release of cardiac toxins from the intestine has not been clearly delineated. Experiments in this study were conducted to investigate the possible release of cardiotoxic factors from the intestine. A series of dogs were hemorrhaged. Blood samples were collected from a femoral artery and from the vein of an isolated jejunal segment. The samples were then assayed

for possible cardiotoxic activities using a Langendorff preparation of an isolated guinea pig heart.

II. Methods and Materials

A. Irreversible Hemorrhagic

1. Preparation of animals for hemorrhage

A series of seven mongrel dogs of either sex, weighing 10 to 20 kg, were fasted for 24 hours and anesthetized with sodium pentobarbitol (25 mg/kg). The dogs were placed in a supine position and a cuffed endotracheal tube was inserted. Artifical ventilation was maintained with a Harvard positive pressure respirator at a rate of 12 strokes per minute and tidal volume set at approximatly 250 ml per 10 kg of body weight.

A polyetheylene cannula (PE 320) was inserted through the left femoral artery into the abdominal aorta and a second cannula (PE 320) was inserted through the left femoral vein into the abdominal vena cava. The left arterial cannula was used to withdraw blood for sampling and to hemorrhage the animal, and the femoral venous cannula was used to supplement the anesthesia, to add heparin (500 units/kg), and to return blood to the animal. A third polyetheylene cannula (PE 240) was inserted into the abdominal aorta via the right femoral artery to moniter mean arterial blood pressure using a Statham pressure transducer (Model, P23 Pb) connected to a Hewlett Packard recorder (Model 7754 A).

A midline incision was made to expose the abdominal cavity and the duodenum located by identification of the ligament of Treitz. Following the natural mesenteric vascular pattern, a segment of the proximal jejunum, 10 to 12 cm in length, supplied with blood through a single artery and drained by a single vein was identified. The segment was externalized and placed on a flat platform positioned next to the abdominal opening.

Without disrupting the blood supply of the externalized segment, the venous drainage was exposed and after heparinizing the animal, the jejunal vein was cannulated with polyethylene tubing (PE 280). The outflow of the jejunal venous cannula was collected in a reservior containing 200 ml of heparinized saline and from this reservoir the jejunal venous blood was returned to the dog by a variable speed pump (Holter Company, Model RE 161) through the femoral venous cannula. The pump rate was set to match the venous outflow rate, returning the blood to the animal as it was shed by the jejunal venous cannula. This kept the circulating blood at a stable volume until the isolation of the jejunal segment was completed and hemorrhage initated.

Both ends of the segment were tied with suture and the mesentary cut to exclude collateral blood flow. Care was taken to leave the nerve supply to the jejunal segment intact. The naturally perfused in situ proximal jegunal segment was covered with plastic sheet to retard dessication, and the temperature of the segment maintained at 37^{o} C by a lamp.

2. Hemorrhage protocol

The hemorrhage protocol used in these experiments was based on the hemorrhage model of Wiggers (1942). Dogs were hemorrhaged from the left femoral arterial cannula in 100 ml increments over a half hour peroid until the mean arterial blood pressure decreased to 35 ± 5 mmHg and the blood pressure was maintained at this level for three hours. At this point, the dog was disconnected from the respirator to more closely simulate clinical conditions. The start of the three hour oligemic hypotensive period was considered to begin when the mean arterial blood

pressure at 35 ± 5 mmHg. The blood removed during the inital large hemorrhage was collected in a seperate reservior and placed in a water bath maintained at 37° C.

Animals were hemorrhaged to this degree of hypotension to produce irreversible shock. Hypotension below 50 mmHg and maintained until a minimum of 20% of the shed blood has been returned was reported by Wiggers (1950) to produce irreversible shock in nearly 100% of the animals.

The blood pressure would usually stabilize within one and one-half hour of the start of the hypotensive period, then begin to decrease as the animal's physiological compensatory mechanisms could no longer maintain a stable blood pressure. Shed blood was then returned to the animal by the variable speed pump via the femoral venous cannula to maintain blood pressure at 35 ± 5 mmHg. Animals in which the venous outflow from the jegunal segment fell to one ml per minute or less were disconnected from the return pump and the shed blood returned to the animal, as in the Lamson and DeTurk model (1945), from a reservior connected directly to the femoral venous cannula. Otherwise the variable speed pump was used to return shed blood to the animal.

Shed blood was returned as needed until the three hour hypotensive period elapsed, at which time the remaining shed blood was returned. The remaining shed blood was retransfused within one half hour after the end of the oligemic hypotension period. The blood pressure was then allowed to fluxuate. The experiment was terminated when the mean arterial blood pressure fell to 55 mmHg which indicated that the possibility of the animal recovering was very low and irreversible hemorrhagic shock could be assured.

3. Blood sampling procedure

Blood samples (20-25 ml/sample) were collected simultaneously from the left femoral arterial cannula and from the jejunal venous cannula. Five samples were taken: (1) before hemorrhage which served as a prehemorrhage sample, (2) just before it was necessary to return shed blood to maintain mean arterial blood pressure at 35 $\pm$ 5 mmHg, the prereturn sample, (3) when 20% of the shed blood was returned which was approximately half way through the hypotensive period, the 20% returned sample, (4) after all shed blood was returned, the 100% return sample, and (5) when the mean arterial blood pressure had fallen to 55 mmHg, the terminal sample.

Blood samples were immediately placed on ice and centrifuged in a refrigerated centrifuge (Servall, Type SS4) at 7000 rpm for 10 minutes. The plasma was then pipetted into test tubes. A two ml alquiot of the plasma sample was set aside to be analyzed for electrolyte concentrations and the remainder of the plasma sample was assayed for cardiotoxic activity in the guinea pig bioassay to be described below. Plasma samples to be assayed the following day were refrigerated at 4° C; the plasma samples to be tested at a later date were frozen.

The electrolyte concentrations of Na^{+} and K^{+} in the plasma samples were determined, using the flame photometric method, with a Beckman Flame Photometer (Model 105) and the plasma concentrations of Ca^{++} and Mg^{++} were determined using a Perkin Elmer Atomic Absorption Spectro Photometer (Model 290 B). At each sample period, the arterial and jejunal venous hematocrits were determined using centrifuged heparinized capillary tubes.

B. Controls

1. Preparation and blood sampling procedure of sham shock animals

A series of six mongrel dogs of either sex, weighing 10 to 20 kg, were anesthetized with sodium pentobarbitol (25 mg per kg, i.v.). The surgical preparations performed on the control dogs were identical to those procedures performed on the hemorrhaged dogs. The sham shock animals were treated identically to the hemorrhaged animals through out the course of the experiment but were not bled. By examining the blood pressure record of each hemorrhaged animal, the time from the beginning of the hemorrhage proceedure to when each blood sample was taken could be determined. An average time to each sample period could, in this way, be calculated and these average times were used to take blood samples in the sham shock animals.

The beginning of the oligemic hypotension peroid in the hemorrhaged dogs was considered time zero for all the experiments. Blood samples from sham shock amimals were taken simultaneously from the left femoral arterial and the jegunal venous cannula at: (1) 0.5 hour prior to time zero for the prehemorrhage sample, (2) one hour 15 minutes after the beginning of sham hemorrhage for the prereturn sample, (3) two hours 15 minutes after time zero for the 20% shed blood returned sample, (4) three hours 30 minutes after time zero for the 100% shed blood returned sample, and (5) at five hours 15 minutes for the terminal sample.

The control blood samples were treated the same as the hemorrhaged blood samples. The blood samples were iced, centrifuged, the plasma pipetted to test tubes and tested for cardiotoxic activity in the isolated guinea pig heart preparation. Hematocrit was determined for arterial and jejunal venous blood samples at each sample period. Electrolyte

concentrations of Na^+, K^+, Ca^{++}, and Mg^{++} were determined for sham shock plasma samples as described for plasma samples from hemorrhaged animals.

C. Bioassay for Cardiodepressant Activities

1. The Langendroff preparation

A Langendorff preparation of an isolated guinea pig heart, following the method of Bunger et al. (1975), was used to assay for a humoral factor which depressed cardiac function. The preparation that was used did not recirculate the prefusate and the heart did not perform external mechanical work. The coronary arteries were perfused at a constant pressure of 55 mmHg. The height of the vessels containing the perfusate solutions was set to deliver a pressure of 55 mmHg and pressure head of the perfusate solutions was maintained as the perfusate was used by the use of Marriot tubes. A coronary perfusion pressure of 55 mmHg is within the range (approximatly 29 mmhg to 72 mmHg) in which the isolated guinea pig heart autoregulates its coronary flow (Bunger et al., 1975).

The assemblege (Figure 3) used to maintain a functioning isolated heart consists of: (1) water jacketed vessels used to contain the perfusate solutions, (2) a water bath and pump with tubing to maintain a constant temperature of the perfusate solution, and (3) a gas cylinder containing 95% oxygen, 5% carbon dioxide with tubing for aerating the perfusate solutions.

Three vessels were used to hold perfusate: (1) a 500 ml vessel for perfusate solution used during the initial isolation and stabilization of the heart, (2) a 500 ml vessel for the perfusate used when estabilishing baseline parameters of heart function, and (3) a 200 ml vessel to contain

Figure 3. A diagram of the modified Langendorff preparation of an isolated guinea pig heart. The diagram illustrates the water bath and pump, the gassing mechanism, and the mechanisms to record cardiac mechanical performance.

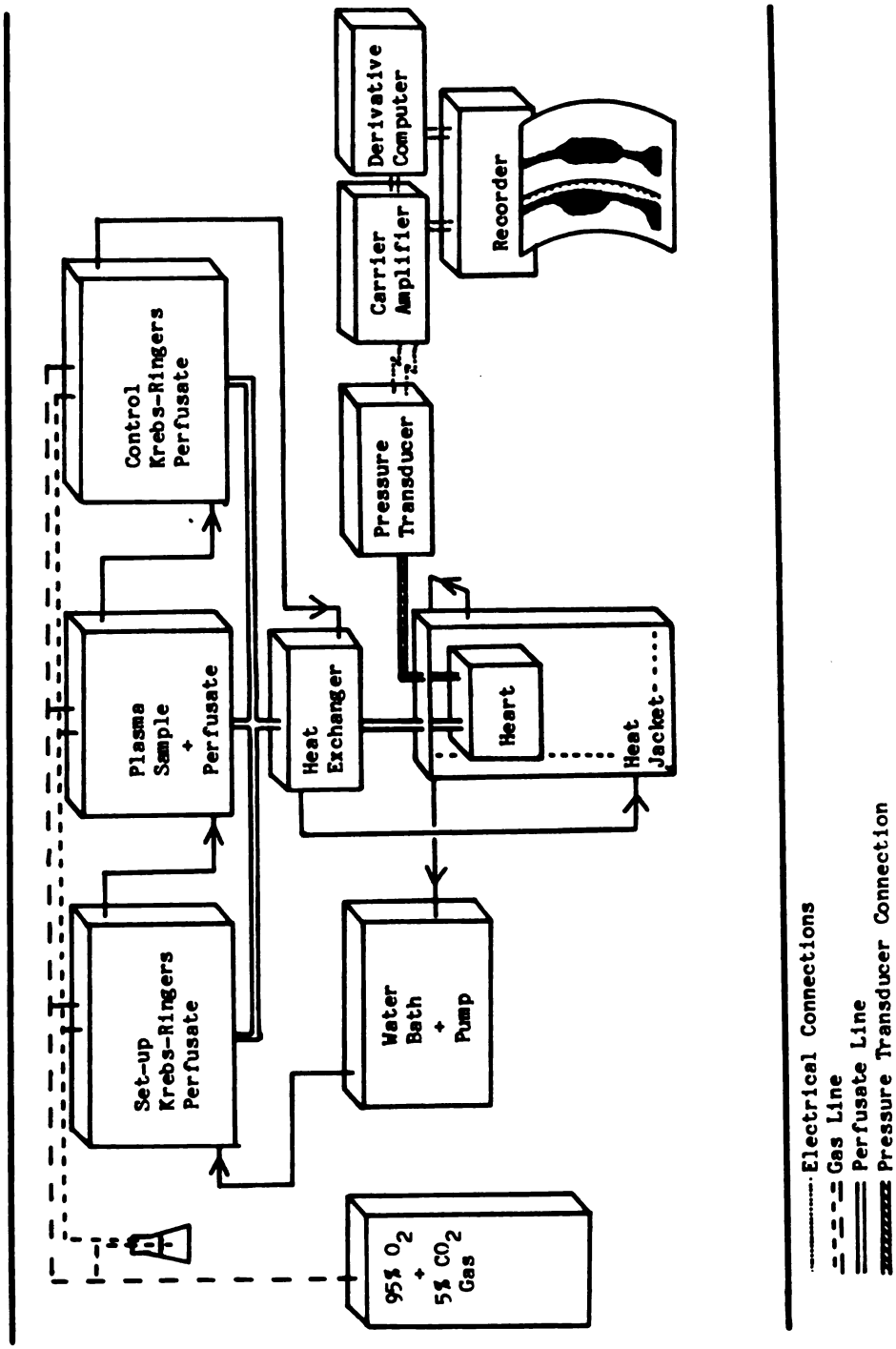


Figure 3. Diagram of the isolated guinea pig heart apparatus

the perfusate with a plasma sample to be assayed for cardiotoxic activity. Perfusate was conveyed to the heart through Tygon tubing.

A water pump with emersion heater, circulated heated water to the three perfusate vessels, a perfusate heat exchanger consisting of a spiral glass coil encased in a water jacket, and a water jacketed vessel which acted as a final heated air chamber surrounding the heart after isolation . This system maintained the temperature of all the perfusate solutions in the vessels at the temperature of the water bath (37° C - 39° C), and the heat exchanger located on the perfusate line just before the heart assured the temperature of the perfusate reaching the isolated heart was maintained within -0.5° C of the water bath temperature.

Vessels for the regular perfusate and the perfusate with a plasma sample were stoppered and each stopper equipped with the following: (1) a glass tube through which 95% oxygen, 5% carbon dioxide could be introduced to aerate the perfusate solutions, (2) a Marriot tube connected to a water trap which allowed the volume of perfusate used to be replaced with 95% oxygen, 5% carbon dioxide, and (3) a third opening which was used as a vent during the initial bubbling with the gas mixture.

The isolated heart was perfused with a Krebs-Ringer-bicarbonate solution modified by the method of Bunger et al. (1974) with the addition of pyruvate, glucose, and bovine serum albumin (Table 1). The perfusate solution was prepared daily, filtered through a 1.2 µm Milipore filter to remove particulate matter, aerated for 15 minutes with 95% O_2-5% CO_2, $CaCl_2$ added, and then aerated for another 15 minutes. After the $CaCl_2$ was completely dissolved, approximatly 400 ml of the perfusate was transfered to the larger vessels. Bovine serum albumin (1.25 gm of Fraction V, Sigma Chemical Co.) was dissolved in 50 ml of the perfusate

Table 1. Components of the modified Krebs-Ringer-bicarbonate solution used to perfuse isolated guinea pig hearts.

Compound	mM	Grams/L
NaCl	127.2	7.40
KCl	4.7	0.35
$CaCl_2$	2.5	0.28
KH_2PO_4	1.2	0.16
$NaHCO_3$	24.9	2.10
Na-pyruvate	2.0	0.22
Glucose	5.5	1.00
Bovine Serum Albumin	0.5 gm%	5.00
Tween 20	trace	5 µl/L

Aerated with 5% CO_2-95%O_2 to equilibrum at 38°C

and added with approximatly 5 µl of Tween 20 (polyoxyethylene sorbitan monolaurate, Sigma Chemical Co.) to each of the two larger vessels. Normal perfusate was then added to bring the volume in each vessel to 500 ml, and gassing was reinitated.

A 10 ml plasma sample to be tested for toxic activities was filtered through a 1.2 µm Milipore filter and added with perfusate and approximatly 1 µl of Tween 20 to bring the volume of the smaller vessel to 100 ml.

The Krebs-Ringer-bicarbonate perfusate was used in the initial isolation of the guinea pig heart and when establishing baseline cardiodynamic parameters before exposing the heart to samples of dog plasma. The plasma samples were diluted to allow reversible depression of cardiac function of the isolated heart during the testing proceedure. This permitted a single isolated heart to be used for more than a single test.

2. Isolating the guinea pig heart

Male guinea pigs, weighing 250-350 grams, were sacrificed by a sharp blow to the cervical vertebra and occipital portion of the cranium. The heart was exposed by incisions through the abdomen and diaphragm and quickly cooled by pouring iced saline over the heart. The thoracic cavity was completely opened by removing the anterior portion of the chest. The heart was isolated by retrograde cannulation of the ascending aorta with polyethylene tubing (PE 260), perfusion started and the heart dissected free of the surrounding tissue, taking care to avoid removing right atrial tissue.

A saline filled balloon made from flexible rubber was placed in the left ventricle of the isolated guinea pig heart by cutting an opening in the left atrium and inserting the balloon into the left ventricle through

the mitral valve. The balloon was connected to a Statham pressure transducer (Model P23Pb) which conveyed left ventricular pressure changes to a Hewlett Packard recorder (Model 7754A). The recorder was equipped with a carrier amplifier (Model 8805B) and a first derivative computer (Model 8814A) which recorded the left ventricular developed pressure (mmHg) and the rate of ventricular pressure change, dP/dt, (mmHg/sec) of the isolated heart. The isolated guinea pig heart did not perform mechanical work and the volume of the balloon was adjusted to give an end-diastolic pressure of 0 mmHg. Under these conditions of constant work load and constant end-diastolic pressure the left ventricular dP/dt is a reflection of the contractility of the myocardium (Katz, 1977).

The hearts were allowed to stabilize for 15 to 30 minutes. Hearts were discarded if they failed to develop a minimum left ventricle pressure of 75 mmHg or failed to develop a heart rate greater than 240 beats per minute.

3. Mechanical Performance of Isolated Hearts

A series of guinea pig hearts were isolated as described above and tested to establish baseline measurements of cardiac mechanical performance. The coronary flow was measured over a one minute period by collecting perfusate dropping from the heart in a graduated cylinder. The heart rate was recorded by counting the number of beats from the left ventricular pressure recording over a 10 second period. Left ventricular developed pressure and left ventricular dP/dt measurements were taken from the Hewlett Packard recorder. Baseline measurements were also taken after raising the coronary perfusion pressure to 75 mmHg. All baseline measurements of isolated hearts were taken in a steady state.

A second series of experiments were conducted to assess the effect of changing the perfusate calcium concentration on the mechanical performance of isolated hearts. Hearts were isolated as described and then exposed to perfusate that contained one and one half times the normal Ca^{++} concentration, three fourths of the normal Ca^{++} concentration, and one half the normal Ca^{++} concentration. When the hearts were in a steady state left ventricular developed pressure, left ventricular dP/dt, coronary flow and heart rate were measured as described.

4. Assaying for cardiotoxic activities in plasma

The plasma samples diluted with perfusate were assayed for cardiotoxic activity by switching the solutions perfusing the heart from the Krebs-Ringer-bicarbonate perfusate to the perfusate containing the plasma sample. Steady state values of left ventricular developed pressure, left ventricular dP/dt, heart rate, and coronary flow of isolated hearts were recorded, as described above, while maintained on Krebs-Ringer-bicarbonate perfusate to establish baseline levels of these parameters. The perfusion solution was then switched to the perfusate containing a plasma sample. When the hearts were in a steady state, values for left ventricular developed pressure, left ventricular dP/dt, heart rate and coronary flow were again recorded. In this way, a baseline measurement of cardiodynamic parameters of isolated hearts was determined before each test of plasma for cardiotoxic activity and from this baseline, changes in cardiac function produced by the dog plasma were calculated.

After exposing the isolated hearts to the perfusate with a plasma sample, the hearts were switched back to the Krebs-Ringer-bicarbonate

perfusate. Hearts were allowed to return to control levels before repeating the testing procedure. Hearts that failed to recover to near control levels were discarded. The plasma samples were each tested for toxic activities in duplicates. Plasma samples taken at the five seperate sample periods and from the artery and jejunal vein were tested randomly. Testing of the plasma from sham shock and hemorrhagic animals was also randomized.

5. Calculations

The cardiodynamic preformance of the isolated heart while prefused by the Krebs-Ringer-bicarbonate prefusate was considered the baseline control values for each individual test. For each test, the positive or negative change produced by the dog plasma, in left ventricular pressure, left ventricular dP/dt, heart rate, and coronary flow were calculated by subtracting the baseline values from the values obtained with the plasma perfusate.

The data were analysed to give mean values (absolute change or percent change $\pm$ standard deviation from baseline values) for the mechanical performance of isolated hearts. The data were analysed to give the results for the artery or the jejunal vein, by sample period, in each individual dog. The results from each sample period of the seven hemorrhaged dogs were summed and divided by the number of observations to give mean values $\pm$ standard error. In identical fashion mean values $\pm$ standard error were calculated for each sample period of sham shocked animals. The collected data was statically tested using Student's t-tests, modified for paired observations, to determine any significance. A p value of less than 0.05 was considered significant.

III. Results

A. Guinea Pig Heart Bioassay

A series of experiments were performed to determine whether the isolated guinea pig heart preparation, used as a bioassay in the experiment, produced acceptable levels of cardiac preformance. A second series of experiments was performed to determine if the isolated heart was sensitive to changes in the calcium concentration of the perfusate solutions. The results of these experiments are summarized in Table 2 and Table 3.

Measurements of coronary flow per gram of heart tissue, heart rate, left ventricular developed pressure (LVDP), and left ventricular dP/dt recorded from isolated guinea pig hearts are reported in Table 2. The data reported are the mean values plus or minus the standard error of the mean for baseline cardiodynamic parameters of the isolated hearts maintained on Krebs-Ringer-bicarbonate perfusate. These values correlate closely with similar baseline cardiodynamic parameters of isolated guinea pig hearts reported by other laboratories (Bunger, et al. 1975).

Raising the coronary perfusion pressure to 75 mmHg produced a variable change in the mechanical performance of the isolated hearts. LVDP and left ventricular dP/dt were usually raised but the coronary flow and the heart rate tended to remain near control levels with increased coronary perfusion pressure.

The responsiveness of isolated guinea pig hearts to changes in the calcium concentration of the perfusate is presented in Table 3. This table reports the mean value plus or minus the standard error of baseline cardiodynamic parameters of isolated guinea pig hearts maintained on the

Table 2. Mean values and standard error of baseline mechanical parameters of isolated guinea pig hearts perfused with Krebs-Ringer-bicarbonate solution. (N=12)

	Coronary Flow (ml/min/gm)	Heart Rate (beats/min)	LVDP (mmHg)	LV dP/dt (mmHg/sec)
Mean ± SE	8.02 ± 0.53	266.6 ± 6.16	83.52 ± 5.77	1455.6 ± 110.4

LVDP = Left ventricular developed pressure
LV dP/dt = left ventricular dP/dt
Mean ± SE = Mean values ± standard error of the mean

Table 3. Mean values and standard error of baseline mechanical parameters of isolated guinea pig hearts and the mean values and standard error of these parameters when the Ca^{++} concentration of the Krebs-Ringer-bicarbonate solution was changed from normal (2.5 mM/L) to: .5 times normal (1.25 mM/L), .75 times normal (1.88 mM/L), and 1.5 times normal (3.75 mM/L).

	Coronary Flow (ml/min/gm) N=9	Heart Rate (beats/min) N=23	LVDP (mmHg) N=22	LV dP/dt (mmHg/sec) N=23
X ± SE	5.29±0.48	241.5±7.0	94.3±4.1	1358.0±76.0
Change in X				
0.50 of Normal	-1.1±0.23*	-16.3±3.0*	-36.0±4.3*	-689±49*
0.75 of Normal	-0.65±0.12*	9.3±3.0*	-14.1±2.3*	-334±41*
1.00 of Normal	0	0	0	0
1.50 of Normal	0.12±0.09*	12.8±2.0*	3.0±2.4	332±43*

X ± SE = Mean values ± standard error of the mean
LVDP = Left ventricular developed pressure
LV dP/dt = Left ventricular dP/dt
* = $P < 0.05$ relative to values with normal Ca^{++} concentration

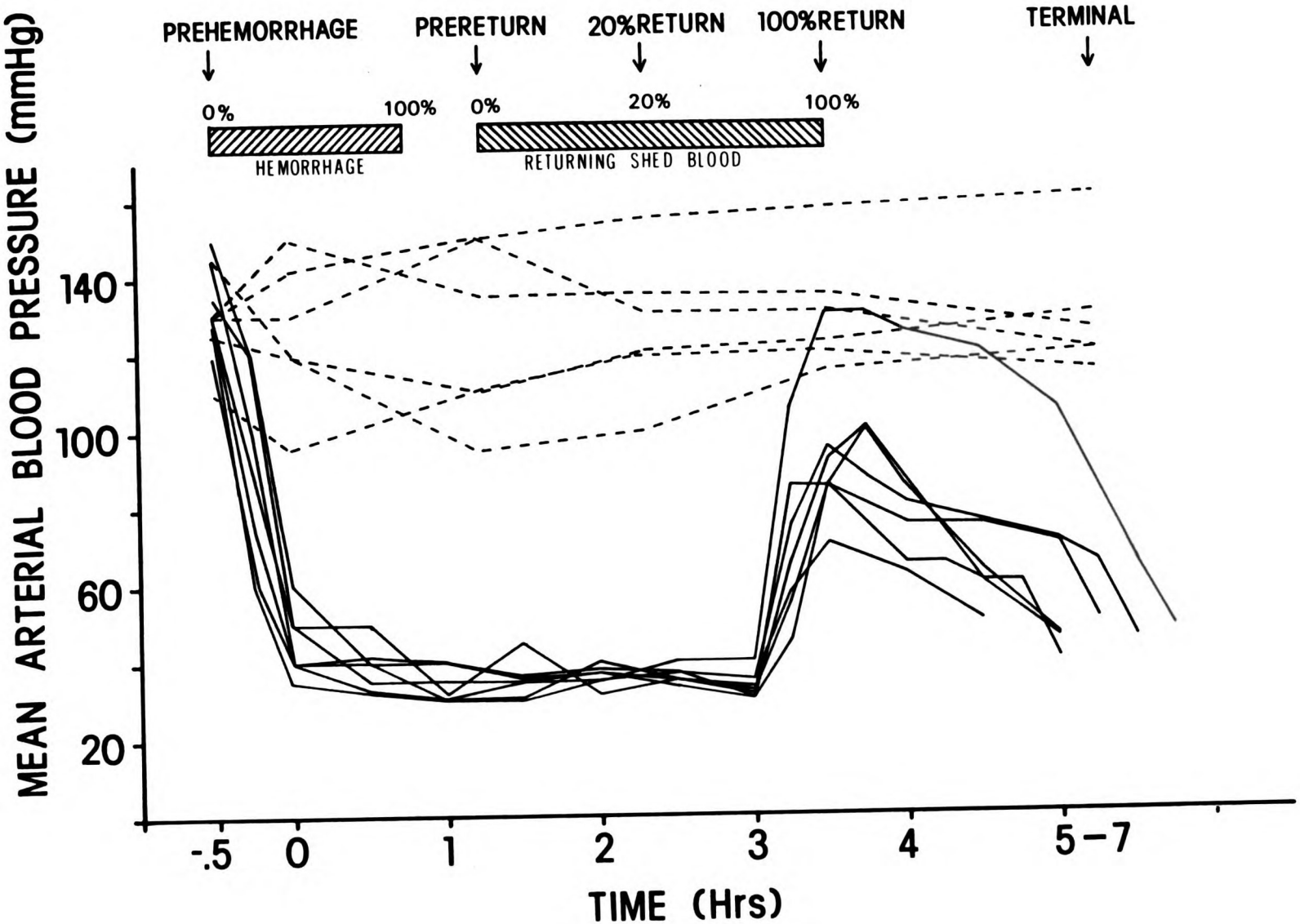
Krebs-Ringer-bioarbonate perfusate, and reports the changes in coronary flow per gram of heart tissue, heart rate, LVDP, and left ventricular dP/dt produced by altering the normal calcium conceration of the perfusate to 1.5 times normal, 0.75 times normal, and 0.5 times normal. The sensitivity of the isolated guinea pig heart to variations in the calcium concentration was reflected by changes in the LVDP and dP/dt. Removing one-half of the calcium in the perfusate significantly ($p<0.05$) reduced the LVDP in the isolated heart from 94.3 $\pm$ 4.1 mmHg by 36.0 + 4.3 mmHg and significantly ($p<0.05$) reduced the left ventricular dP/dt from 1358 $\pm$ 76 mmHg/sec by 689.1 $\pm$ 49.6 mmHg/sec. The guniea pig heart was more sensitive to decreases in the calcium concentration than an increase in the calcium concentration. As reported in Table 3, a 1.25 mM/L decrease in the calcium concentration produced a 689 $\pm$ 49 mmHg/sec decrease in the left ventricular dP/dt while a 1.25 mM/L increase produced a 332 $\pm$ 43 mmHg/sec increase in left ventricular dP/dt.

B. Hemorrhage and Sham Shock Dogs

The changes in mean arterial blood pressure of hemorrhaged dogs is shown in Figure 4. Figure 4 also shows the blood pressure changes in sham shock dogs and indicates the peroids of hemorrhage, retransfusion of shed blood, and denotes the mean times when blood samples were taken from the femoral artery and the venous cannulae of the isolated jejunal segment.

The mean arterial blood pressure of sham shock dogs was relatively stable over the total time of the experiment. The blood pressure of the hemorrhaged dogs dropped from an average of 134.3 $\pm$ 3.85 mmHg before hemorrhage to 38.4 $\pm$ 1.13 mmHg in 45 minutes. To reach a blood pressure below 40 mmHg and maintain this level of hypotension until reuptake of

Figure 4. Mean arterial blood pressure of hemorrhaged (solid lines) and sham shock (dashed lines) animals over the time of the experimental procedure. Indicated are the times of hemorrhage and retransfusion and the times when arterial and jejunal venous blood samples were taken.


PREHEMORRHAGE
PRERETURN
20%RETURN
100%RETURN
TERMINAL
0%
100%
0%
20%
100%
HEMORRHAGE
RETURNING SHED BLOOD
MEAN ARTERIAL BLOOD PRESSURE (mmHg)
140
100
60
20
-.5
0
1
2
3
4
5-7
TIME (Hrs)

blood began, dogs were bled at a mean rate of 57.12 ± 2.61 ml/kg of body weight. The blood pressure was then kept between 35 and 40 mmHg by retransfusion of shed blood until the three hour oligemic hypotensive peroid expired at which time all remaining shed blood was retransfused. The blood pressure of the hemorrhaged dogs recovered to a mean of 95.3 ± 8.09 mmHg then fell to a mean of 52.5 ± 4.25 mmHg one and one half hours later. Figure 3 also illustrates the varing ability of individual animals to tolerate the hypotension as shown by the variation in blood pressure and survial time after the shed blood was returned.

The electrolytes concentrations of Ca^{++}, Mg^{++}, Na^{+}, and K^{+} in arterial plasma samples at each sample peroid for hemorrhagic and sham shock animals are reported in Table 4. The arterial plasma electrolyte concentrations of Ca^{++}, Mg^{++}, Na^{+}, and K^{+} for sham shock animals were stable through out the experiment and did not show significant changes from the prehemorrhage sample peroid in any subsequent sample. There was no significant change in the arterial plasma concentration of Na^{+} in hemorrhaged dogs between prehemorrhage samples and the prereturn, 20% return, 100% return, or terminal sample. The K^{+} concentration was significantly ($p<0.05$) elevated in the arterial hemorrhagic samples between prehemorrhage and all the other sample peroids. A significant ($p<0.05$) increase in the plasma concentration of Mg^{++} was also observed in the arterial prereturn and terminal samples. A significant ($p<0.05$) decrease in the concentration of Ca^{++} was observed only in the terminal arterial sample.

Table 5 reports electrolyte concentrations of Ca^{++}, Mg^{++}, Na^{+}, and K^{+} in intestinal venous plasma samples from both groups of animals. As with the arterial plasma electrolytes, the jejunal venous electrolytes of sham

Table 4. Mean values and standard error of electrolytes concentrations in the arterial plasma samples of sham shock and hemorrhaged animals.

Sham Shock (N=6)					
Artery	Prehemorrhage	Prereturn	20% Return	100% Return	Terminal
Ca^{++}[a]	4.2±0.33[b]	4.4±0.47	4.1±0.39	4.2±0.36	4.0±0.29
Mg^{++}	1.68±0.31	1.77±0.19	1.62±0.14	1.65±0.18	1.67±0.20
Na^{+}	127.0±3.2	127.5±3.9	126.0±7.8	124.3±3.9	123.1±6.1
K^{+}	3.24±0.19	3.29±0.19	3.42±0.37	3.50±0.27	3.30±0.29
Hemorrhaged (N=7)					
Artery					
Ca^{++}	5.7±0.28	5.6±0.28	5.5±0.53	6.2±0.61	5.1±0.34*
Mg^{++}	2.55±0.33	2.95±0.13	3.38±0.22*	2.94±0.51	3.43±0.21*
Na^{+}	111.2±7.3	119.1±1.9	119.0±3.6	124.1±3.4	116.2±3.4
K^{+}	2.6±0.18	4.3±0.30*	4.1±0.30*	4.2±0.51*	4.4±0.45*

a = All electrolyes concentrations expressed in mEq/L
b = Mean values ± standard error of the mean
* = $p<0.05$ compared to prehemorrhage sample period

Table 5. Mean values and standard error of electrolytes concentrations in jejunal venous plasma from sham shock and hemorrhaged animals.

Sham Shock (N=6)					
Venous	Prehemorrhage	Prereturn	20% Return	100% Return	Terminal
Ca^{++}[a]	3.7±0.31[b]	4.1±0.38	4.3±0.32	4.1±0.30	4.0±0.27
Mg^{++}	1.72±0.32	1.57±0.13	1.86±0.25	1.85±0.28	1.77±0.19
Na^{+}	124.8±6.2	136.8±5.8	126.6±13.9	123.9±7.4	125.4±5.4
K^{+}	2.97±0.19	3.53±0.20	3.50±0.27	3.53±0.25	3.49±0.24
Hemorrhaged (N=7)					
Venous					
Ca^{++}	5.3±0.35	5.0±0.40	5.4±0.58	4.9±0.19	4.9±0.34
Mg^{++}	2.13±0.32	2.98±0.39	3.02±0.29	3.06±0.12*	3.22±0.14*
Na^{+}	129.0±6.2	119.5±3.4	121.6±3.0	124.1±3.5	122.6±2.8
K^{+}	2.9±0.30	5.0±0.51*	4.7±0.52*	4.7±0.41*	5.4±0.59*

a = All electrolyte concentrations expressed in mEq/L
b = Mean values ± standard error of the mean
* = $p<0.05$ compared to prehemorrhage sample period

shock animals were stable and did not show significant changes from the prehemorrhage sample in any subsequent sample. Significant ($p<0.05$) increases in the jejunal venous plasma concentration of K^+ were found in the prereturn, 20% return, 100% return, and terminal samples of hemorrhaged animals. A significant increase ($p<0.05$) was also observed in plasma Mg^{++} concentration in the jejunal venous plasma from hemorrhaged animals in the 100% return and terminal samples.

The hematocrit of arterial and jejunal venous blood samples was determined at each sample peroid for both hemorrhagic and sham shock animals. These values are shown in Table 6. The arterial hematocrit of hemorrhaged animals was significaltly ($p<0.05$) elevated between the prehemorrhage blood sample and all other sample periods. The arterial hematocrit rose from a mean of $40.0 \pm 2.18\%$ before hemorrhage to a peak of $53.3 \pm 2.27\%$ after all shed blood was returned. As with the arterial hematocrit changes, the jejunal venous hematocrit of hemorrhaged animals was significantly ($p<0.05$) elevated between the prehemorrhage sample and all other sample periods. The hematocrit of jejunal venous blood of hemorrhaged dogs rose from $40.0 \pm 2.04\%$ before hemorrhage to a peak of $55.5 \pm 2.31\%$ after retransfusion. Hematocrit values of both the arterial and venous blood samples then decreased slightly in the terminal blood samples. Hematocrit values from sham shock animals began with mean values of $41.3 \pm 2.73\%$ and $41.7 \pm 2.33\%$ in the prehemorrhage sample peroid for artery and vein, respectively, and tended to increase throughout the experiment, however, no significant increase occured.

Table 6. Mean values and standard error of hematocrit (Ht) for arterial and jejunal venous blood samples from sham shock (N=6) and hemorrhaged (N=7) animals.

	Prehemorrhage	Prereturn	20% Return	100% Return	Terminal
Sham Shock Ht, %					
Artery	41.3±2.73[a]	41.8±4.92	42.3±4.18	42.8±5.02	44.3±4.06
Venous	41.7±2.33	42.2±3.92	42.7±3.84	43.5±4.92	46.0±4.51
Hemorrhaged Ht, %					
Artery	40.0±2.18	43.1±1.60*	47.4±2.21*	53.3±2.27*	51.1±2.10*
Venous	40.0±2.04	46.9±1.62*	52.1±2.00*	55.5±2.31*	55.0±2.21*

a = Mean values ± standard error of the mean
* = $p<0.05$ compared to prehemorrhage sample period

C. Bioassay for Cardiac Toxins

The individual changes in left ventricular developed pressure (LVDP) of isolated guinea pig hearts after exposure to arterial and venous plasma samples is shown in Figure 5. Figure 5 shows the effects of diluted plasma from each of the hemorrhaged and sham shock animals in each of the blood sample periods on the LVDP. The arterial and venous plasma samples from hemorrhagic dogs had an increasingly depressant effect on LVDP as shock progressed, whereas, there was an absence of consistant depression by plasma from the sham shock animals. The actual changes in left ventricular dP/dt of isolated hearts are shown in Figure 6, and the left ventricular dP/dt changes are similar to the changes observed in the LVDP.

The positive or negative percent change in LVDP of isolated hearts, produced by the arterial and venous plasma samples from hemorrhaged and sham shock dogs, is reported in Figure 7. Figure 7 illustrates the increasing depressant activities in shock plasma as the hemorrhaged animals approached death and illustrates the random pattern of cardiac depression or stimulation from plasma of sham shock dogs. The prehemorrhage and prereturn plasma samples from hemorrhaged dogs show little percent change in LVDP. The 20% shed blood returned sample is the initial sample that showed a tendency for depressant activity in either arterial or venous samples. The percent change in LVDP of isolated hearts exposed to arterial plasma varies from $-1.5 \pm 4.2\%$ in the prehemorrhage sample peroid, to $-10.8 \pm 4.7\%$ in the 100% return sample, but these changes were not significantly different from the prehemorrhage sample. The percent change in LVDP, resulting from exposure to jejunal venous samples, began with a $2.8 \pm 3.0\%$ increase in LVDP with the prehemorrhage sample then fell in each succeeding samples, significantly ($p<0.05$)

Figure 5. Left ventricular developed pressure (mmHg) of isolated guinea pig hearts after exposure to plasma from sham shock and hemorrhaged animals. The open circles represent the left ventricular developed pressure before exposure to a plasma sample and the solid circle the left ventricular developed pressure after exposure.

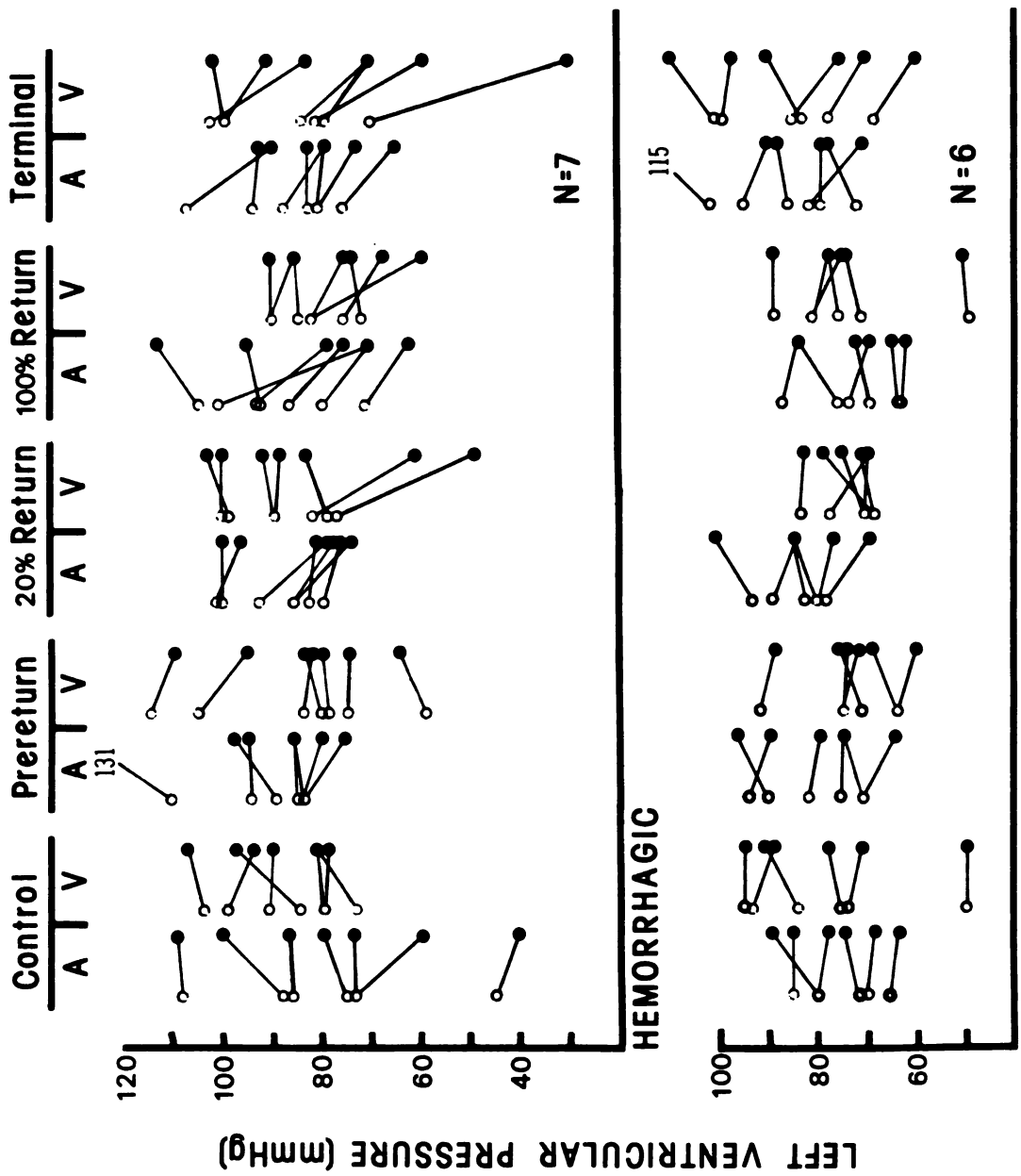

Control
Preretum
20% Return
100% Return
Terminal
A
V
131
115
N=7
N=6
HEMORRHAGIC
SHAM SHOCK
120
100
80
60
40
LEFT VENTRICULAR PRESSURE (mmHg)

Figure 6. Left ventricular dP/dt (mmHg/sec) of isolated guinea pig hearts after exposure to plasma from sham shock and hemorrhaged animals. The open circles represent the left ventricular dP/dt before exposure and the solid circles the left ventricular dP/dt after exposure to a plasma sample.

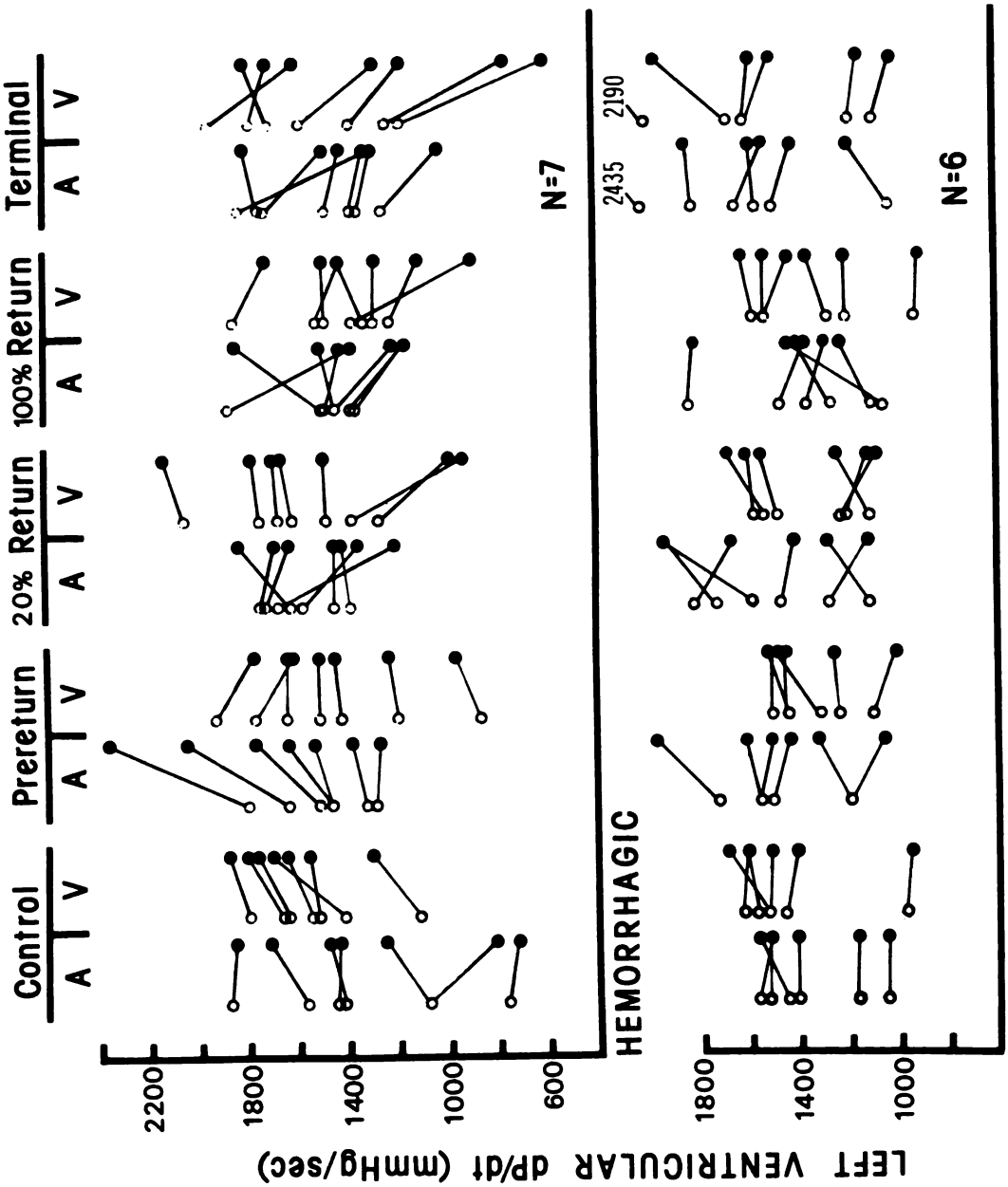
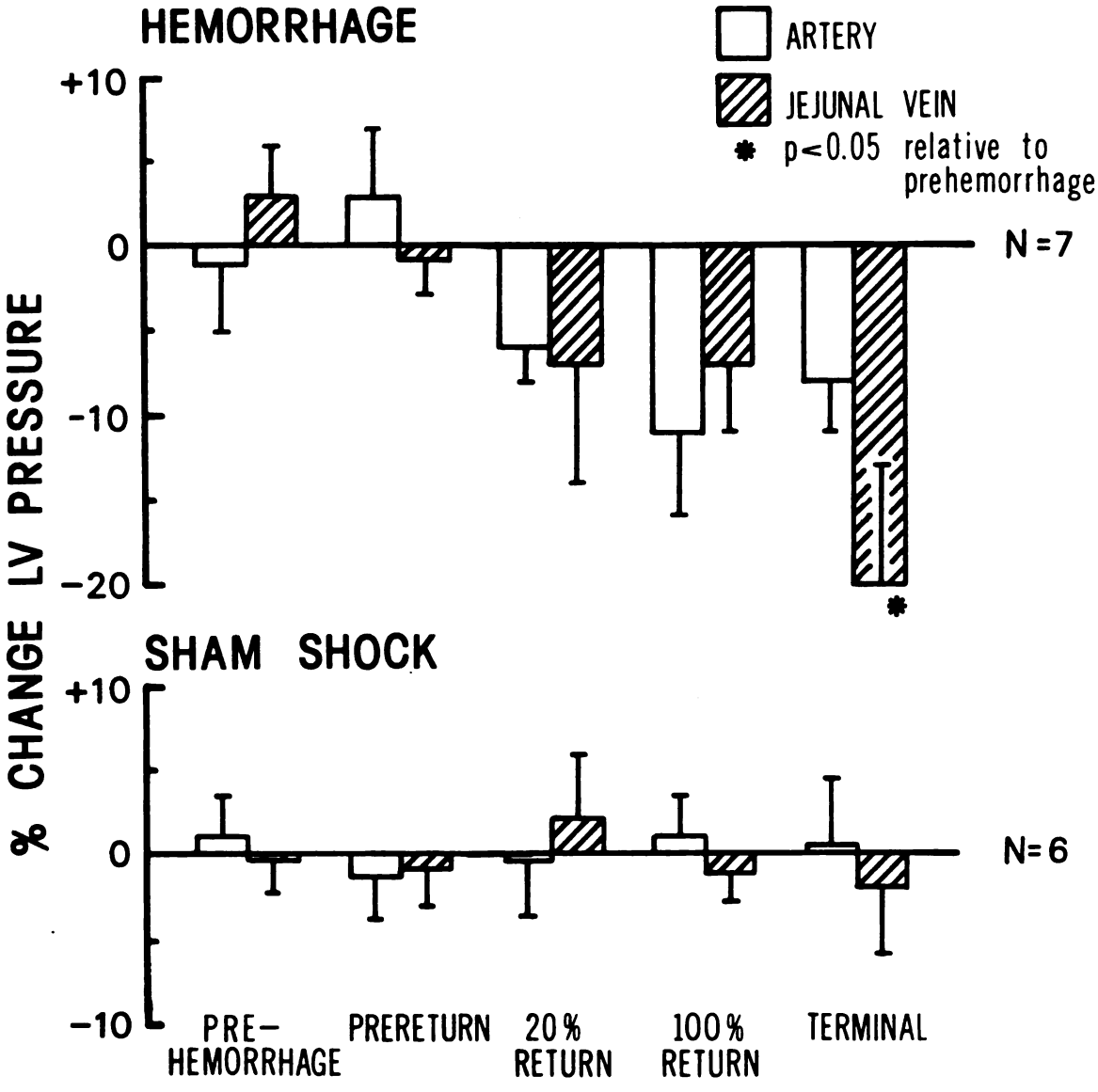

Control
Preretum
20% Return
100% Return
Terminal
A
V
N=7
N=6
2435
2190
HEMORRHAGIC
SHAM SHOCK
LEFT VENTRICULAR dP/dt (mmHg/sec)
2200
1800
1400
1000
600

Figure 7. Mean values and standard error of percent change in left ventricular developed pressure of isolated guinea pig hearts after exposure to plasma from sham shock and hemorrhaged animals.


HEMORRHAGE
ARTERY
JEJUNAL VEIN
* p<0.05 relative to prehemorrhage
+10
0
-10
-20
N=7
% CHANGE LV PRESSURE
SHAM SHOCK
+10
0
-10
N=6
PRE-HEMORRHAGE
PRERETURN
20% RETURN
100% RETURN
TERMINAL

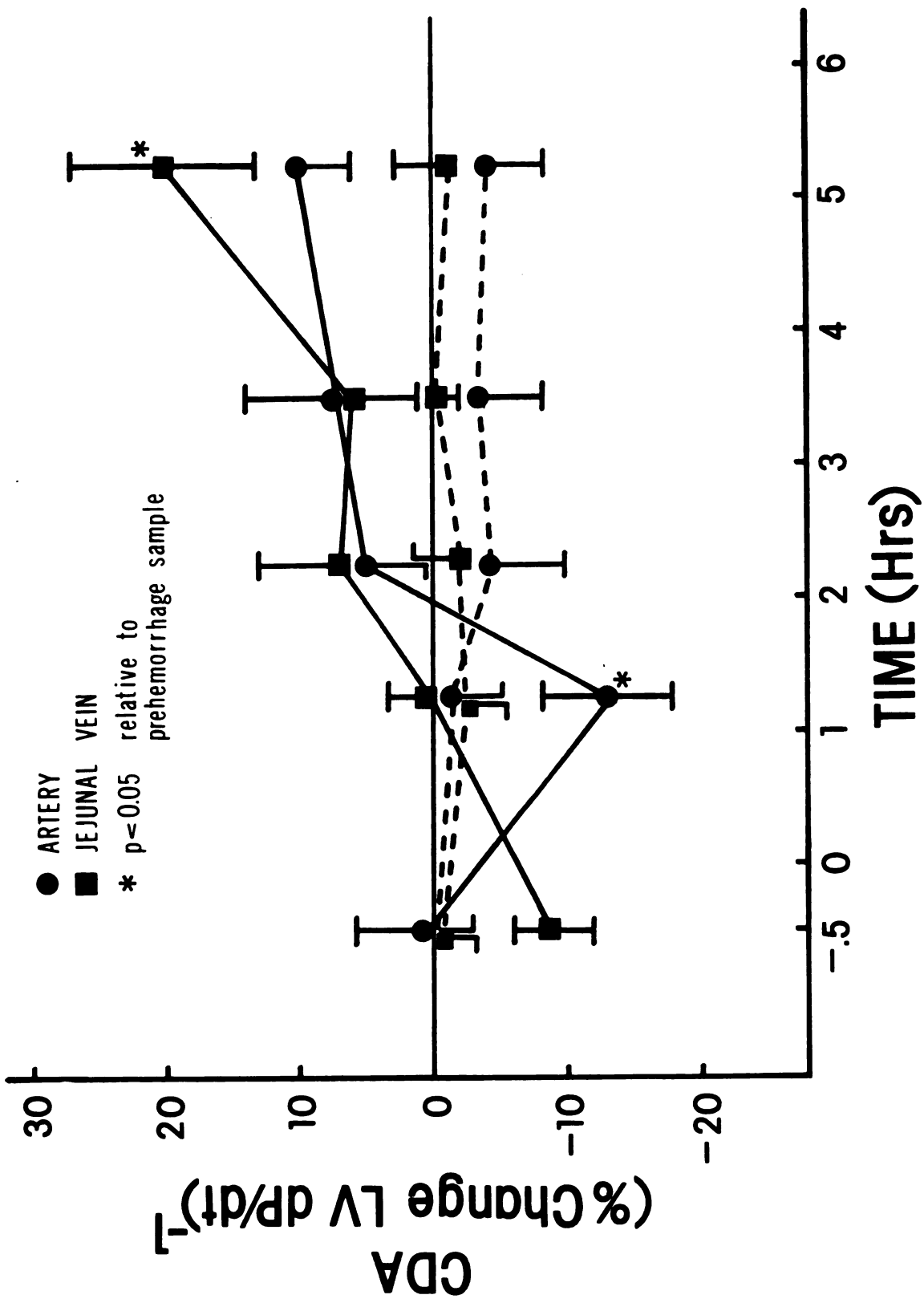
decreasing LVDP to -19.9 ± 7.4% by the terminal sample. Plasma from sham shock animals did not produce significant percent changes in LVDP when any of the sebsequent samples were compared to prehemorrhage samples.

An alternative method to express the depressant activity of plasma samples is the conversion of the percent change in left ventricular dP/dt of isolated hearts into units of cardiodepressant activity, (CDA). One CDA is equal to a one percent change in dP/dt of the isolated guinea pig heart, and negative percent changes in left ventricular dP/dt are expressed as positive cardiodepressant activities. Figure 8 shows the depressant activity of arterial and venous plasma samples expressed as cardio-depressant activity. Arterial plasma of hemorrhaged dogs did not show inital depression as reflected by 0.74 ± 4.7 CDA in the prehemorrhage sample. Significant ($p<0.05$) stimulation of contractility of the isolated guinea pig heart was observed in the arterial preinfuse sample with -13.1 ± 4.5 CDA. In subsequent arterial samples, significant depression was not evident but depressive activities did increase to 10.2 ± 3.9 CDA by the terminal sample.

Plasma samples from the jejunal vein of hemorrhaged dogs initially produced stimulation of contractility with -9.2 ± 3.4 CDA in the prehemorrhage sample peroid. In the next sample, the prereturn sample, stimulation of the isolated heart was no longer evident as shown by a CDA value of 0.14 ± 2.5. A significant ($p<0.05$) increase in the depression of the isolated heart was observed only in the terminal jejunal plasma sample with 20.0 ± 6.9 CDA.

The effects of plasma from sham shock and hemorrhaged dogs on the coronary flow, heart rate, % change in coronary flow, and % change in

Figure 8. Mean values and standard error of cardiodepressant activity (CDA) in (% change in LV $dP/dt)^{-1}$ of plasma from hemorrhaged animals and sham shock animals (dashed lines) for artery (●) and jejunal vein (■).


CDA
(% Change LV dP/dt)$^{-1}$
30
20
10
0
−10
−20
−.5
0
1
2
3
4
5
6
TIME (Hrs)
● ARTERY
■ JEJUNAL VEIN
* p<0.05 relative to prehemorrhage sample
*
*

heart rate of isolated guinea pig hearts are shown in Table 7 and in Table 8, respectively. When compared to the prehemorrhage sample, plasma samples from sham shock dogs (Table 7) did not produce signigicant changes in coronary flow or heart rate in any of the test periods for either the arterial or venous plasma samples. A significant decrease in the % change in coronary flow was produced only by jejunal venous plasma from the terminal sample period of sham shock animals.

Arterial plasma from the prereturn sample of hemorrhaged animals (Table 8) significantly increased ($p<.05$) the heart rate of the isolated hearts while heart rate changes produced by the other arterial plasma samples were not statistically different from the prehemorrhage sample. A significant ($p<0.05$) decrease in coronary flow was produced by jejunal venous plasma in the prereturn, 100% return and terminal sample periods. Jejunal venous plasma from hemorrhaged animals significantly ($p<0.05$) decreased the percent change in coronary flow and the percent change in heart rate in the 20% return and terminal sample periods. Significant decreases were also observed in the percent change in coronary flow with jejunal venous plasma in the 100% return sample.

Table 7. Mean values and standard error of coronary flow, heart rate, percent change in coronary flow and percent change in heart rate of isolated guinea pig hearts after exposure to plasma from sham shock animals.

	Prehemorrhage		Preinfuse		20% Return		100% Return		Terminal	
	Artery	Vein	Artery	Vein	Artery	Vein	Artery	Vein	Artery	Vein
Coronary Flow[a]	8.3±0.6[c]	8.0±0.7	7.8±0.4	7.3±0.7	8.1±0.7	8.2±0.7	7.3±0.5	6.8±0.4	8.0±0.5	7.4±0.4
Heart Rate[b]	276.7±12.6[c]	276.5±7.8	275.0±7.4	277.5±8.4	276.0±13.7	274.0±12.4	264.0±9.2	273.0±9.0	281.0±9.3	272.5±5.3
% Coronary Flow	-9.9±4.9[d]	-7.3±3.1	-5.8±3.7	-6.3±2.9	-4.6±4.2	-4.2±3.5	-8.1±5.5	-6.6±3.2	-9.7±4.6	-14.4±4.0
% Heart Rate	0.5±1.9[d]	-0.6±0.7	0.4±0.5	0.3±1.5	0.6±1.0	-1.3±1.2	-0.6±0.9	-0.8±0.7	3.2±2.7	1.7±2.55

a = Coronary flow in ml/min/gram of tissue
b = Heart rate in beats/minute
c = Mean values ± standard error of mean
d = % change ± standard error of mean
* = $p<0.05$ relative to prehemorrhage sample period

Table 8. Mean values and standard error of coronary flow, heart rate, percent change in coronary flow, and percent change in heart rate of isolated guinea pig hearts after exposure to plasma from hemorrhaged animals.

	Prehemorrhage		Prereturn		20% Return		100% Return		Terminal	
	Artery	Vein	Artery	Vein	Artery	Vein	Artery	Vein	Artery	Vein
Coronary Flow[a]	7.0±0.8[c]	8.2±0.6	7.6±0.4	6.7±0.4*	7.1±0.6	6.4±0.7	6.7±0.7	6.1±0.6*	6.2±0.7	5.4±0.8*
Heart Rate[b]	250.2±9.2[c]	279.4±11.6	261.3±5.4*	270.9±7.7	256.3±9.5	267.2±10.4	252.3±4.4	249.3±7.7	249.6±12.1	244.4±16.
% Coronary Flow	-8.7±5.0[d]	-0.1±4.7	-9.5±4.7	-13.6±4.2	-14.3±3.3	-15.7±4.2*	-22.5±4.9	-16.3±5.6*	-16.9±4.9	-28.9±8.3*
% Heart Rate	-1.0±1.2[d]	0.7±0.7	3.9±1.2	1.3±1.4	-2.2±1.1	-3.6±1.2*	-2.0±0.5	-2.3±1.4	-3.7±1.1	-6.8±2.9*

a = Coronary flow in ml/min/gram tissue
b = Heart rate in beats/minute
c = Mean values ± standard error of the mean
d = % change ± standard error of the mean
* = $p<0.05$ relative to prehemorrhage sample period

IV. Discussion

A decrease in cardiac function resulting from the actions of circulating cardiotoxic substances has been the most recent hypothesis advanced to explain the development of irreversible hemorrhagic shock. Substances in the plasma which have cardiodepressive activities have been proposed to originate primarily within the splanchnic region during prolonged hypotension (Lefer, 1978). One toxic substance, myocardial depressant factor (MDF) has been reported to originate in the ischemic pancreas (Lefer, 1978). Haglund and Lundgren (1978) and Blattberg and Levy (1962) have reported the ischemic small intestine also to be the point of origin of toxic substances.

Toxic factors formed within the pancreas are believed to enter the circulatory system primarly through the thoracic lymphatic duct (Glenn and Lefer, 1970b). Toxic substances formed in the intestine could also enter the circulatory system via lymphatic ducts or toxic substances from the intestine could be released directly into the splanchnic venous drainage. Theorically, intestinal toxic factors would then be more highly concentrated in the splanchnic venous blood. Once cardiotoxic factors enter the circulation they are suspected to exert a negative inotropic effect on the heart.

A variety of experimental designs have been used to test the hypothesis that cardiotoxins are releaed from the splanchnic region. Rogel and Hilewtz (1978) conducted experiments using dogs in which the heart, maintained normotensive during hemorrhagic shock, served as an *in vivo* assay for toxic factors from the circulation. Beardsley and Lefer (1974) produced splanchnic arterial occlusion shock in the cat and then

assayed for cardiotoxic factors in the feline plasma with isolated cat papillary muscles. Haglund and Lundgren (1973) simulated shock in the feline small intestine by local intestinal hypotension and nerve stimulation, then used the heart of the same animal as an in situ bioassay to detect toxic substances from the shocked intestine.

The design of the present study differs from other hemorrhagic shock studies, in that dogs were subjected to total body hemorrhagic shock, blood samples were drawn from the general circulation as well as from the splanchnic region, and the plasma samples were assayed for toxic factors with an isolated guinea pig heart. In this experiment, arterial blood samples were taken to determine the presence of cardiotoxic factors in the general circulation throughout the course of shock. Jejunal venous blood samples were taken to assess whether the drainage of the intestinal bed contains higher cardiotoxic activities than the general circulation.

The mechanical function of isolated guinea pig hearts from this experiment was found to be similar to the mechanical function of isolated hearts reported by other investigators. The coronary flow, heart rate, left ventricular developed pressure (LVDP), and left ventricular dP/dt found in this experiment (Table 2, page 43) were sightly higher than values reported by Bunger et al. (1975) in the identical preparation but were lower than the values reported by Neely et al. (1973) and Steenberger et al. (1977) in paced working rat hearts. Although a direct correlation may not be possible between the guinea pig heart and the rat heart, the isolated hearts perform similarly regardless of the preparation. Thus the isolated guinea pig heart bioassay used in this experiment was cardiodynamically viable and any changes in cardiac mechanical performance

produced by plasma samples would not be an artifact resulting from the Langendorff preparation.

In addition to producing acceptable levels of cardiac function, the isolated guinea pig heart was sensitive to changes in the calcium concentration of the perfusate solutions (Table 3, page 44). Sensitivity to calcium was expected as calcium is a vital factor determining contraction of cardiac muscle (Katz, 1977). The calcium concentration in the plasma of sham shock and hemorrhaged animals was measured in order to assess if calcium could be a factor in cardiac depression of isolated hearts.

Wangensteen et al. (1973) raised the question of whether the depression of isolated papillary muscles, exposed to shock plasma, was the result of toxic substanaces or an artifact of the experimental procedure. They repeated the methods of Lefer to isolate MDF and reported evidence that the depressive activities in feline plasma were due to excess sodium ions rather than a specific cardiotoxic substance. Goldfarb and Weber (1977), using incubated pancreatic homogenates, found that cardio-depressant activity could be associated with two plasma fractions. One of the fractions consisted of peptidic material and the other fraction was made up of extremely high concentrations of sodium, potassium, and chloride ions. They reported that when raising the sodium chloride concentration of solutions bathing an isolated cat papillary muscle, depression was not noted until the sodium concentrations were above 140 mEq/L. Goldfarb et al. (1978) reported that when the calcium concentration of the bathing solution was reduced below 2.50 mM/L there was a depression of the contractile tension of isolated cat papillary muscles.

In this experiment, the concentration of sodium ions found in arterial plasma (111.2±7.3 mEq/L to 124.1±3.4 mEq/L) and jejunal venous plasma (119.5±3.4 mEq/L to 129.0±6.2 mEq/L) of hemorrhaged animals were not in the range which produces depression, and the calcium ion concentration (4.9±.19 mEq/L to 6.2±.61 mEq/L) was in the range that produces stimulation. The effect of electrolyte changes on the isolated heart were further precluded by the dilution of the plasma samples 1:10 with the perfusate solution prior to the testing procedure. Therefore, the electrolyte influences on the bioassay do not appear to be a cause of depression.

The mean arterial blood pressure changes of hemorrhaged animals (Figure 4, page 47) illustrates the classical description of experimentally induced irreversible hemorrhagic shock. After retransfusion of shed blood the animals were returned to a normovolumetric state. The blood pressure was observed to recover but did not return to the prehemorrhage levels. The blood pressure then fell over the next hour, which was described by Wiggers (1950) as characteristic of the progressive stage of irreversible shock. Five hours after the beginning of the hypotensive period the animals entered the terminal stage and the blood pressure rapidly decreased until the death of the animals.

The results of the electrolyte analysis of plasma from hemorrhaged animals (Table 4, page 49 and Table 5, page 50) confirms the severity of the shock state that was produced by the hemorrhage protocol. In both the arterial and jejunal venous plasma samples from hemorrhaged animals, significant increases were observed in the concentrations of potassium and magnesium. Because of the greater intracellular concentrations of potassium and magnesium compared to plasma, these increases are indicative

of cellular death with release of intracellular contents into the general circulation (Rush, 1972). Similar increases in the potassium were found by Okuda et al. (1974) in arterial blood of dogs in cardiogenic shock, by Bond et al. (1977) in dog skeletal venous plasma during hemorrhagic shock, and by Lundgren and Haglund (1978) in feline arterial plasma with simulated shock of the small intestine.

The hematocrit data from hemorrhaged animals (Table 6, page 52) support the results of the electrolyte analysis indicating that a severe state of shock was produced by the hemorrahge protocol. A significant increase was observed in the hematocrit in both the arterial and the jejunal venous plasma in all samples after the inital hemorrhage. Hemoconcentration during.hemorrhage appears to be the result of a greater postcapillary resistance than precapillary resistance, raising the capillary filtration pressure, thereby, favoring filtration of fluid out of the capillary and concentrating blood cells (Haddy, et al., 1968). This effect predominates late in hemorrhage while the reverse occurs early in hemorrhage. The increases in the hematocrit in this experiment were similar to the findings of other investigators. Rothe and Selkurt (1964) reported significant increases in arterial hematocrit in the post-transfusion stage of hemorrhage, and Coleman et al. (1975) reported that the hematocrit of arterial blood rose significantly from 52.4 ± 2.0 % in the control period to 60.5 ± 1.6 % 2 hours after retransfusion of shed blood.

Electrolyte concentrations in the arterial and venous plasma samples from the sham shock animals (Table 4, page 49 and Table 5, page 50) were stable and did not show significant changes from the prehemorrhage sample period. This indicates that the isolation of the jejunal segments was not responsible for the electrolyte changes observed in experimental animals.

The changes in electrolyte concentrations of hemorrhaged animals, therefore, were the result of the blood loss.

In the present study, decreases in the coronary flow of isolated hearts were found whenever the hearts were exposed to plasma samples (Table 7, page 64 and Table 8, page 65). Attempts were made to seperate the changes in the LVDP from the changes in coronary flow, produced by plasma samples, by varing the coronary perfusion pressure. The isolated guinea pig heart autoregulates its coronary flow when the coronary perfusion pressure ranges from approximatly 29 mmHg to 72 mmHg (Bunger, et al. (1975). Separation of the changes in LVDP from coronary flow changes was not possible by soley changing the coronary perfusion pressure, for coronary flow tends to remain near control levels as the prefusion pressure was varied. The decreases in coronary flow may have caused the decreased LVDP and the decreased left ventricular dP/dt but the values of coronary flow after exposure to shock plasma, as listed in Table 8, (page 65), were still within the values reported by Bunger et al. (1975) for normal isolated hearts.

In this experiment, jejunal venous plasma samples taken before hemorrhage produced a stimulation of the contractility of the isolated hearts (Figure 8, page 62). Hyperemia of the jejunal segment was commonally observed during the isolation procedure. Chou and Grassmick (1978) reported simple manipulation of the gut will cause an active hyperemia. Biber et al. (1974) induced hyperemia by mechanically stimulating the jejunal mucosa, and the hyperemia was reported by Biber et al. (1971) to be dependent on the release of 5-hydrozytrypamine (5-HT). Since manipulation of the gut induced a 5-HT mediated hyperemia, theoretically other substances may also be released, and one of these

substances could be responsible for the increased contractility that was produced by the jejunal venous plasma.

Significant stimulation of the contractility of the isolated heart also was produced by the arterial plasma from the prereturn sample period (Figure 8, page 62). The enhanced contractility may be the result of increased plasma concentrations of norepinephrine which stimulate the isolated heart when exposed to the prereturn arterial plasma sample.

There have been implications that the pancreas is the only source of myocardial depressant factor (MDF). Lefer and Martin (1970) were unable to find MDF in the plasma of dogs that had the pancreas removed prior to splanchnic vessel occlusion. The plasma obtained from these animals showed approximately the same levels of MDF as were observed in sham vessel occluded animals.

Logically, if the pancreas were the only source of cardiotoxic factors, experiments that remove the pancreas prior to hemorrhage should produce prolonged survival times. This does not appear to happen. Animals subjected to splanchnic vessel occlusion and pancreatectomy did not survive longer than intact animals (Lefer and Martin, 1970b). This would indicate that either the loss of the pancreas contributed to shock or the remainder of the splanchnic region is also responsible for changes leading to irreversible shock. Removal of the pancreas could be interfering with glucose metabolism through the loss of the ability to produce and release insulin. The animals, then, may be less able to withstand the trauma of hemorrhage, or cardiotoxic factors could be produced in other areas of the splanchnic region. If the pancreas were the only site of production and release of cardiotoxic factors, the depressive activities measured in the jejunal venous samples in this

experiment would have to have originated from factors that had traversed the jejunal capillary network, and then appeared in the jejunal venous blood.

A second hypothesis which could explain the toxic activity appearing in the jejunal venous plasma could be the production and release of cardiotoxic factors from the ischemic intestine. The bioassay would then be detecting either toxic factors from the intestine alone or a combination of pancreatic cardiotoxic factors and those cardiotoxic factors formed in the intestine.

The results of the bioassay for toxic factors in this experiment indicate that cardiotoxic factors were produced by the shocked intestine. Jejunal venous plasma from dogs in irreversible hemorrhagic shock depressed the contractility of isolated guinea pig hearts as shown by a significant increase in cardiodepressant activity (CDA) (Figure 8, page 62). In addition, the cardiodepressant activity was only detected in terminal plasma samples from the venous drainage of the jejunal segments. Arterial plasma samples failed to show significant cardiodepressant activities at any time. There also was nearly twice the CDA value for jejunal venous plasma than for arterial plasma from the terminal sample. The lack of cardiodepressant activities in the arterial plasma samples indicate that the arterial plasma could not have been the source of the toxic activities observed in the jejunal venous plasma. The intestine must have been the source of the cardiotoxic activities in jejunal venous plasma.

The results of this experiment are in aggrement with the findings of Haglund and Lundgren (1973) who reported cardiotoxic factors from the feline small intestine. The results also fulfill, where applicable, the

criteria of Lefer (1973) to determine whether a true shock factor was released from the intestine. Lefer's criteria for shock factors are the following:

1) a toxic substance should not be present in animals not in shock, 2) a toxic factor should be produced by different forms of shock, 3) a toxic factor should be able to be isolated in a purified form, 4) the toxic factor should exert a severe pathophysiological effect, and 5) the toxic factor should be present in clinical situations as well as in experimental animals.

Plasma from sham shock animals in this experiment did not show depressive activities when exposed to isolated hearts (Figure 7, page 59 and Figure 8, page 62). Plasma from animals that were hemorrhaged also did not produce depression of the isolated heart before hemorrhage and in the early stages of hypotension. Significant cardiodepressive activities were only observed with plasma samples from the jejunal vein and only after the animals showed signs of irreversible hemorrhagic shock (Figure 8, page 62).

This experiment investigated the possible release of toxic factors from the intestine resulting from hemorrhagic shock. Toxic factors that have similar cardiodepressant properties have been found in other forms of shock. Lefer (1979) reported myocardial depressant factor in plasma after prolonged endotoxic shock, Ferguson et al. (1977) reported cardiotoxic factors with acute pancreatis, and Hawkins et al. (1980) reported release of cardiac depressants after whole body irradiation.

Cardiotoxic factors from the intestine were not purified in this experiment. The chemical nature of toxic intestinal factors was eluded by Lundgred and Haglund (1978) who found two heat stable compounds with

negative inotropic effects from the feline small intestine. One factor was water soluble with a molecular mass between 500 and 1000 daltons and a second lipid soluble factor with a mass greater than 10,000 daltons.

Venous plasma from the jejunal segment in this experiment exerted a pathophysiological effect on the isolated hearts as seen by a significant decrease in the LVDP (Figure 7, page 59) and left ventricular dP/dt (Figure 8, page 62) of the hearts after exposure to shock plasma. Lundgren et al. (1975) reported that blood-borne toxic factors from the small intestine of cats in simulated shock significantly decreased peak isometric tension of isolated rabbit papillary muscles and significantly decreased the LVDP of isolated rat hearts. Glucocorticoids which have been theraputically successful in other forms of shock can also reduce the formation of toxic substances in the intestine. Haglund et al. (1977) reported that treatment of cats with methylprednisolone early in regional intestinal shock attenuated the release of intestinal lysosomal enzymes, prevented the mucosal lesions common in intestinal shock, and lessened the cardiovascular derangement usually found in intestinal shock. The mucosal lesions observed in the intestine with shock can also be prevented by lavage of the intestinal lumin with oxygenated saline, and the cardiovascular deterioration can then be largely prevented as well (Haglund and Lundgren, 1974). These results would seem to point to the intestinal villi as the probable sourse of cardiotoxic factors from the intestine.

The last critera of Lefer has been examined in a study by McConn et al. (1979) in which cardiotoxic factors in plasm from patients in septic shock were almost identical to cardiotoxic factors from animal studies.

The release of toxic factors from the intestine is not limited to

cardiotoxins. Blattberg and Levy (1962) reported that a reticuloendothelial depressing substance (RDS) could also be produced by intestinal ischemia. RDS activities, as measured by the depression of the clearance of carbon particles from the blood, increased in animals as the result of regional intestinal hypotension.

There is little doubt that toxic substances can be produced in the shocked intestine and exert a depressant effect on the cardiovascular system. The results of this experiment indicate the production of cardiotoxic factors in the canine jejunum with irreversible hemorrhagic shock.

V. Summary and Conclusions

Arterial and jejunal venous plasma samples from dogs subjected to irreversible hemorrhagic shock were assayed for cardiotoxic activities using a Langendorff preparation of isolated guinea pig hearts.

Major findings:

1. Cardiodepressant activity can be detected by the isolated guinea pig heart in shocked canine plasma diluted 1:10 with Krebs-Ringer-bicarbonate solution.

2. Depression of the isolated heart was not observed in plasma samples from the early stages of hemorrhagic shock, but stimulation of contractility was observed with the jejunal venous prehemorrhage sample and with the arterial plasma sample just prior to the beginning of retransfusion of shed blood.

3. Significant cardiodepressant activity was found only in the jejunal venous plasma from the terminal sample period.

4. It is postulated that hemorrhage ultimatly leads to the production and release of cardiotoxins and the intestine may be one of the sourses of these toxins.

5. In our preparation, changes in coronary flow of the ioslated guinea pig heart did not follow changes in dp/dt in a predictable fashion when perfusion pression was increased.

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