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Short Title: Second Messenger Response to Steroid in Shock

ENHANCEMENT OF SECOND MESSENGER SYSTEM RESPONSE BY STEROID THERAPY IN HEMORRHAGIC SHOCK

by

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ABSTRACT

Hepatic and intestinal adenyl cyclase activity were measured after a single pulse injection of epinephrine or glucagon into normal dogs and into dogs subjected to hemorrhagic shock. The results indicated that hemorrhagic shock abolishes the increase in adenyl cyclase activity seen in normal animals following epinephrine and significantly reduces that induced by glucagon. These changes are reflected in the glucose production from the liver induced by these hormones. The response of adenyl cyclase to the in vitro addition of epinephrine or glucagon, as well as the nonspecific stimulator of adenyl cyclase, sodium fluoride, showed that it is the receptor site of the enzyme which is affected primarily by shock. The treatment of dogs with 30 mg/kg of Methylprednisolone following the reinfusion of shed blood significantly improved the response of adenyl cyclase to epinephrine in both liver and intestine, as well as to the in vitro addition of hormone, and these improvements were reflected in the glucose production by the liver in response to the hormone. Insulin levels were significantly increased in steroid treated animals. Plasma cyclic AMP levels were not altered by steroid therapy, although tissue phosphodiesterase was significantly reduced.

Steroids, therefore, enhance tissue response to hormonal stimulation in shock by restoring adenyl cyclase response and decreasing phosphodiesterase activity.

RESUME

L'activité de l'enzyme adényl cyclase du foie et de l'intestin grêle a été mesurée après un seul bolus intra-veineux d'épinéphrine ou de glucagon à des chiens normaux et à des chiens soumis à un choc hémorrhagique. Les résultats démontrent que le choc hémorrhagique abolit l'augmentation de l'activité de l'adényl cyclase suivant l'injection d'épinéphrine chez le group de chiens normaux, de même qu'il diminue d'une façon significative la réponse de l'enzyme à la stimulation par le glucagon. Ces changements induits par ces hormones provoquent une modification quantitative de la production du glucose hépatique. La réponse de l'adényl cyclase à l'addition "in vitro" d'épinéphrine, de glucagon ou bien d'un stimulateur non-spécifique de l'enzyme, le fluorure de sodium, a permis de démontrer que le choc hémorrhagic affecte principalement le site récepteur de l'enzyme. Le traitement des chiens avec 30 mg/kg. de méthylprednisolone injecté après la réinfusion du sang dont ils avaient été déflétés, a amélioré d'une façon significative la réponse de l'adényl cyclase à l'épinéphrine étudiée dans le foie et l'intestin. La même réponse a été notée à l'addition de l'hormone "in vitro". Ces améliorations de l'activité de l'enzyme ont de plus conduit à une production de glucose hépatique en réponse à l'hormone. Les taux d'insuline se sont élevés d'une façon significative chez les animaux traités aux stéroides. Les taux plasmatiques d'AMP cyclique n'ont pas été modifiés par la thérapie stéroidienne, bien que les taux de la phosphodiesterase tissulaire diminuèrent d'une façon significative.

Par conséquent, dans le choc, les stéroides augmentent la réponse tissularie à la stimulation hormonale en réactivant l'activité de l'adényl cyclase et en diminuant celle de la phosphodiesterase.

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LIST OF ABBREVIATIONS

A.C.A.	Adenyl Cyclase Activity
ATP	Adenosine Triphosphate
Cyclic AMP	Cyclic 3' – 5' Adenosine Monophosphate
DNA	Deoxyribonucleic Acid
M.P.S.S.	Methyl Prednisolone Sodium Succinate
NaF	Sodium Fluoride
PDE	Phosphodiesterase
RPM	Revolutions per Minute
S.E.M.	Standard Error of the Mean

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INTRODUCTION

SHOCK - A DEFINITION:

Shock is a state characterized by peripheral vascular insufficiency or peripheral vascular collapse. Shock may be associated with hemorrhage, trauma, myocardial infarction, acute pancreatitis, severe infection with gram negative bacteria and their contained endotoxins and gram positive bacteria with their elaborated exotoxins, intestinal obstruction, intestinal infarction, diabetic coma and many other diseased states. The precipitating events may have widely divergent effects upon physiologic and metabolic balance, however, there is general agreement (25, 143, 199, 218) that there is an ultimate common denominator in all forms of shock and that is - inadequate capillary and tissue perfusion. The tissues receive insufficient nutrients and oxygen to sustain normal cellular activity. In time, the peripheral tissues become anoxic and cellular metabolism and function are disrupted. This in turn aggravates the homeostatic attempts to restore adequate perfusion to these peripheral tissues.

SHOCK - REVERSIBLE AND IRREVERSIBLE:

The sympathoadrenal homeostatic response to moderate blood loss is a normal adaptive response. Losses of up to 1 litre or about 16 percent of the average adult blood volume can be tolerated in this manner and can be compensated for by the venous reservoir. An equili-

brium can be established so that the period of hypotension can be moderately prolonged, and retransfusion of the lost blood volume will restore the circulatory system to normal. This state of shock is termed reversible (143).

However, should the degree of hypotension become too severe or too prolonged, a state of irreversible shock results, and retransfusion of the shed blood volume cannot correct the syndrome. Vasodilation and increased capillary permeability occur, with accumulation of plasma and red blood cells in the capillary beds. The blood flow to the tissues is not sufficient to maintain their minimum biochemical needs. This stage is characterized by a decompensatory phase in which the vasculature becomes refractory to the effects of the high circulating levels of the catecholamines. This phase is recognized as the irreversible stage of shock whereupon replacement of the lost blood volume will not reverse the course of events leading to death. Many investigators have attempted to establish the sequence of events leading from reversible to irreversible shock.

Attention should be focused at the cellular level. Defects in the machinery of the cell are translated into total physiologic failure. Investigators must characterize the crucial changes in the cellular and subcellular processes induced by prolonged shock. Furthermore, a means to restore these damaged processes must be found before irreversible shock can be rendered reversible.

CELLULAR METABOLISM IN SHOCK:

The peripheral cells, namely those of the gastro-intestinal tract, liver, skin and muscle, gradually become anoxic due to the intense vasoconstriction and progressive shunting of blood away from these areas in the shock state. The ischemic anoxia (reversible stage) which later becomes stagnant anoxia (irreversible stage) has profoundly deleterious effects on the ultrastructure and function of these cells. The literature surveying the effects of hemorrhagic shock on metabolism in the peripheral organs has been derived from many different species of animals, using a variety of techniques. As a result, the differences in the effects of shock on metabolism are controversial. However, the common denominator of all forms of shock is cellular hypoperfusion.

Schumer and his associates have done extensive studies on cellular metabolism in shock (302, 303, 306, 308). Schumer defines shock as a "disorder of the molecules of the cells" (302). Normally, oxygen and nutrients such as glucose, amino acids, fatty acids and glycerol pass through the cell membrane with the aid of an energy transport system. The body's energy component is adenosine triphosphate (ATP) and it's energy storage molecule, creatine phosphate. Normally, most of the cellular ATP is derived from glucose breakdown to CO_2 and water. However, in the absence of sufficient amounts of oxygen, glucose can only be catabolized to pyruvate and lactate, reducing the production of ATP by 90%. Lactic acid accumulates within the cell reducing the intra-

cellular pH (305). The lactate continues to accumulate and leak into the plasma decreasing plasma pH and producing a lactic acidemia.

Normally, glucose is stored in the cytoplasm of liver and muscle cells in the form of glycogen. In the presence of increased circulating levels of the catecholamines coupled with a decreased availability of intracellular glucose for ATP production, the glycogen stores are broken down to form glucose. If anoxia is prolonged, the glycogen stores of the body become depleted and the body must use alternate pathways for glucose production. This is accomplished with the aid of the stress-induced increase in the circulating levels of cortisol (115, 126 Chapt. 24). Cortisol aids in stimulating the compensatory gluconeogenic process (308). This process uses any available lactate, amino acids, fatty acids and glycerol to manufacture glucose molecules for the energy (ATP) producing pathway. Oji and Shreeve (252) report that the amount of glucose available to the body in times of severe stress from the gluconeogenic process is about 10 times greater than that from the regular glycogen stores. However, despite the body's compensatory efforts to maintain ATP production and cellular function, should the anoxia continue, the ATP levels continue to fall. An 80% depression of ATP levels in shock has been noted in the intestinal mucosa (56, 133, 222, 280) and in the liver (50, 192, 308). As ATP levels decline and the cellular functions that it normally maintains begin to falter, the cell membrane sodium - potassium exchange pump fails, allowing

intracellular accumulation of sodium and water (15, 302, 323) and leakage of potassium to the extracellular space. Further derangements of cation balance occur as intracellular calcium levels increase (15, 48) while intracellular levels of bound magnesium fall and extracellular magnesium levels increase (3).

Associated with all these metabolic and electrolyte disturbances is an alteration in cellular membrane associated with an increase in permeability (255). In addition, the intracellular metabolic acidosis contributes to the weakening and eventual rupture of lysosomal membranes (120, 151). The release of the lysosomal hydrolases produces cell death, lysis and release of intracellular components into the already acidotic circulation.

The common pathway for all types of shock appears to involve a molecular type of derangement deriving from the basic defect in the energy forming pathways of the cell; and prolonged catecholamine - induced peripheral capillary ischemic anoxia appears to be the precipitating factor.

SHOCK - THE SPLANCHNIC ORGANS:

Shock is a syndrome of peripheral failure. It is consequent upon intense and selective peripheral vasoconstriction (128). The purpose of this homeostatic action is to shunt the remaining effective blood volume toward the vital organs - the heart and brain - at the

expense of other non-vital organs. In the dog, the sympathetically - induced vasoconstrictor response of the blood pressure regulating mechanism is selective (27, 131). It occurrs most noticably in the adrenergic sensitive splanchnic, followed by pulmonary and cutaneous beds. The blood flow in the hepatic artery and hepatic and portal veins is significantly reduced rendering the liver ischemic. The mesenteric blood flow has been reported (67, 141, 166, 328) to be significantly reduced to as little as 30 percent of normal in the hypovolemic state. The splanchnic organs have become well known as the "target organs" to the destructive vasoconstriction and ischemia associated with low flow states.

Hepatic function has been shown to be significantly impaired following severe shock and trauma (26, 48, 80, 97, 324). The damage incurred by the liver in shock is thought to be due directly to the decreased blood flow. Frank et al (104) and Hay and Webb (139) have found that protective perfusion of the liver with arterialized blood via the hepatic artery improved hepatic function and survival in dogs subjected to experimental hemorrhagic shock. Normally, the hepatic artery contributes one third of the flow to the liver, while the portal vein contributes the remaining two thirds. The hepatic artery is constricted during hemorrhagic shock (55), total hepatic blood flow is decreased 10 to 20 percent of normal (55), and oxygen levels in the portal blood are far below normal (80). The reduced flow coupled with intrahepatic vasoconstriction combine to produce ischemic anoxic damage to hepatic ultrastructure and function (104, 139, 151, 198).

Shoemaker and Fitch (324) reported diffuse congestion around the hepatic capillaries and venules in dogs subjected to the Wiggers' (355) procedure for hemorrhagic shock. Grossly, the liver appears distended and engorged. Histological studies by the same investigators and others (48, 151) showed marked congestion, hyperemia with disruption of the normal cellular and tissue architecture and increased loss of "definition of cellular detail". The mitochondria appeared swollen, glycogen stores depleted and distortion of the endoplasmic reticulum and hepatic lysosomes occurred.

The intestine as the "central lesion" in shock was reported in the classical studies of Lillehei in 1957 (197). Cross perfusion of the dog's intestine in hemorrhagic shock via the superior mesenteric artery with blood from a normal donor animal prevented death in 90 percent of the animals. In addition, the characteristic intestinal necrosis induced by ischemia, was prevented. Perfusion of the portal vein however, did not prevent death or intestinal necrosis.

There are numerous theories on the role of the intestine in the pathogenesis of irreversibility in shock. In 1942 Wiggers (356) found that in canine shock there was a resistance to portal flow created by the intense hepatic artery and intrahepatic vasoconstriction. This led to stagnation in the portal system contributing to intestinal mucosal congestion and pooling of blood in the intestinal capillary beds. The excessive vasoconstriction in the mesenteric bed (143, 169, 228, 247)

coupled with increased portal pressure produces anoxic damage to the intestinal mucosa. The intestine, like the liver, suffers severe metabolic, structural and functional alterations in hemorrhagic shock. Prolonged intestinal ischemia can be so detrimental that actual tissue necrosis and sloughing of the intestinal epithelium can occur (56). This necrosis has been called hemorrhagic enteritis and has been associated with irreversibility in shock (29, 197, 355, 367).

Evidence of severe metabolic depression in the intestinal epithelium in low flow states has been reported by several investigators (30, 32, 56, 225, 238, 279). This is associated with decreased mucosal ATP levels (56, 223, 225) and production (33, 221, 225). Furthermore, Chiu et al (56) found that morphological damage correlates well in time course and degree of severity with the metabolic depression in the intestinal mucosa in low flow states. The greater the reduction in flow to the intestine, the more severe the metabolic and morphological damage. Chiu et al (58) and McArdle et al (223) administered glucose intraluminally for its protective effect upon the failing ischemic intestinal mucosa. It was found that the ischemic damage to mucosal cells was diminished by the presence of glucose which was used directly by the tissue as substrate for energy production.

Several investigators (28, 96, 165, 315) have postulated that during prolonged hypovolemia, there is increased intestinal permeability to intraluminal substances which are normally retained. The depressed

hepatic function renders the reticuloendothelial system incapable of handling and detoxifying the increased circulating bacteria and toxins and thus contributes to irreversibility. However, these conclusions have been disputed by Zweifach et al (368) who reproduced the identical shock situation in germ-free animals. In addition, others (57, 59, 62, 261) believe that the observed increase in intestinal permeability in shock allows for leakage into the bowel lumen as opposed to increased absorption. Large amounts of plasma and eventually red blood cells leak into the intestinal lumen contributing further to a decreased circulating blood volume. Chiu et al (59) showed that absorption of toxins from the bowel lumen is not as integral to circulatory collapse and irreversibility in shock as is fluid loss into the intestinal lumen. Others have noted that the ischemic intestine becomes a secretory organ (145, 218, 220, 237). Similarily, in intestinal obstruction (170, 320, 321) and superior mesenteric occlusion (59, 218) there is up to 30 – 40 percent of the plasma volume lost into the intestinal lumen (57, 117, 261) often with an associated increase in hematocrit (170, 321).

There are several studies disputing the importance of the intestine as the site of irreversibility in shock. Partial (229) as well as total (113, 263) enterectomy produced little prolongation of survival time in canine hemorrhagic shock. Gergely and Nagy (113) pointed out that total enterectomy rendered the dogs incapable of tolerating hypotension.

Lefer and Glenn (188) have studied the role of the splanchnic myocardial depressant factor (MDF) in the pathogenesis of irreversibility in shock. MDF is thought to derive from the ischemic pancreas during bowel ischemia, hemorrhagic, endotoxic, cardiogenic and pancreatitis - shock. Lefer and Glenn indicate that MDF produces a negative ionotropic effect upon the heart, constricts the splanchnic resistance vessels, alters intestinal mobility and probably contributes to the reticulo-endothelial breakdown observed in shock. MDF is thought to work as a toxic factor by way of exacerbating the development of irreversibility.

BLOOD LOSS AND SYMPATHOADRENAL STIMULATION:

Loss of 10 to 20 percent of the blood volume in man and experimental animals produces a decrease in arterial blood pressure. This then produces a reduction in the afferent impulses from the baro-receptors in the carotid sinus and aortic arch, which in turn activates the sympathetic afferents to the heart and blood vessels. As arterial pressure falls, blood flow through the carotid and aortic bodies de-creases, stimulating the chemoreceptors to discharge. The increased chemoreceptor discharge coupled with a reduced baroreceptor discharge work together to stimulate the cardiovascular center (172) and sympathetic vasoconstrictor fibers. Bilateral vagotomy eliminates both chemoreceptor and baroreceptor responses resulting in severe drops in arterial pres-sure in response to small blood losses - thus indicating the importance

of these reflexes in maintaining circulatory homeostasis.

Activation of the sympathetic nervous system causes release of norepinephrine from the post-ganglionic nerve endings and release of norepinephrine and epinephrine from the adrenal medulla (82). Reviewing the data from several sources (349) indicates that lowering blood pressure to levels around 40 mm Hg can illicit 50 to 100 fold increases in circulating levels of epinephrine (55, 130) and 10 - 50 fold increases in norepinephrine (287, 349). Similar massive increases in circulating catecholamine levels have been noted in septic shock (97).

Minor increases in circulating catecholamines are beneficial in response to a small blood loss situation. However, massive and prolonged release in response to excessive blood loss upsets the normal and intricately balanced microcirculatory flow. Block et al (27) have shown that large doses of epinephrine (3 - 10 ug/kg) consistently cause marked peripheral vasoconstriction in man and dogs. The arterioles and precapillary sphincters of the viscera and skin are richly supplied with alpha adrenergic receptors which cause these vessels to constrict in response to the increased sympathetic nervous tone and high levels of catecholamines (2, 61). Visceral and cutaneous vasoconstriction produces a reduction in the vascular space, and total peripheral resistance is increased while blood flow to the brain and heart is maintained.

The normal reaction of the heart to the increased sympathetic tone is reflected in an increased heart rate and myocardial contractility.

The beginning of physiologic decompensation in shock is heralded by gradually decreasing catecholamine levels (16). Failure of the sympathetic homeostatic mechanism is characterized by a general vasodilatory response and resultant fall in total peripheral resistance. This refractory state may occur as a result of anoxia - induced release of vasodilatory substances from ischemic tissues, depleted catecholamine stores, a decreased effectiveness of the catecholamine action on the vasculature due to acidosis. Refractoriness has been demonstrated after prolonged epinephrine and norepinephrine infusions and may, in addition to other factors, be related to a desensitization of the vascular adrenergic response (106, 136).

The catecholamine - induced vasoconstriction occurs in the organs where it has been reported that so called alpha adrenergic receptors are highly concentrated. There are two major types of receptors that drugs or hormones can interact with to elicit a response in sympathetic effector cells - alpha and beta receptors.

The alpha (α) and beta (β) receptors originally were conceptual structures proposed by Ahlquist in 1948 (2) to explain changes occurring in the precapillary and postcapillary arterioles and venules in the presence of catecholamines. Stimulation of the alpha receptors by epinephrine or norepinephrine produces vasoconstriction of both arterioles and venules, and hence reduced capillary perfusion and anoxia. Stimulation of the beta receptors which are located primarily in the striated

muscle, results in vasodilation. Beta receptors are found in the myocardium (and elsewhere) and stimulation of these receptors by the catecholamines results in the observed increase in force and rate of contraction.

There is strong evidence to suggest that prolonged and excessive increases in the circulating levels of the catecholamines may significantly contribute to the magnitude of tissue ischemia and subsequent irreversibility in most forms of shock. The role of the sympathetic nervous system in causing detrimental changes in prolonged shock have been emphasized by Fine (96) who noted that the vasoconstrictor response can be abolished and mortality improved by sympathectomizing certain areas such as the intestine and spleen. Drucker (77) states that the adrenergic response to hemorrhage is initially adaptive but when prolonged will compromise homeostasis. Lillehei (331 p. 7 - 15) and others (248, 307) have provided further evidence that catecholamine action contributes to irreversibility in shock. Lillehei (311 p. 7 - 15) has shown a correlation between circulating norepinephrine and epinephrine levels and the state of the circulation - greater catecholamine concentrations are associated with poorer tissue perfusion. A higher mortality rate has been observed (79) in patients with severe sepsis particularly if it is associated with hypotension and increased circulating catecholamine levels. Continuous infusions of norepinephrine or epinephrine (106, 136, 224) reproduces the shock situation in healthy dogs and is associated with a high mortality rate. The death usually being attributed

to lethal shock. Furthermore, if exogenous vasopressors such as metaraminol are added to boost a falling blood pressure, the microcirculation may deteriorate at an even more rapid rate, and the chance of survival may be further decreased.

Pre-treatment with an alpha - adrenergic blocking agent has been shown (135, 147, 347, 364) to blunt the vasoconstrictor effects of the catecholamines and thus protect the experimental animal from a potentially lethal hypovolemic episode. McArdle et al (224) have shown that both hepatic and intestinal hypoxia are significantly reduced in dogs pretreated with an α-blocking agent prior to a lethal Wiggers' shock procedure. In addition, α-blockade has been shown (106) to increase survival following epinephrine shock, norepinephrine shock (64, 98), endotoxic shock (347, 354), cardiogenic shock (27, 147, 199, 257) and hemorrhagic shock (135, 364). The most significant results have been obtained where the α-blocker was administered before the induction of shock. However, several studies (27, 199, 257) have shown that α-blockade coupled with adequate volume replacement following shock provide significant increases in survival.

TOLERANCE TO SHOCK:

A common feature of all types of shock is the viscero-cutaneous vasoconstriction and reduced blood flow and anoxia in response to the sympathoadrenal reaction to stress. Initially, this is a protective

response. However, if prolonged, it becomes deleterious and leads to eventual deterioration of the viscero-cutaneous microcirculation. Any measure that blunts the excessive sympathetic response appears to increase survival as seen in the studies with α-adrenergic blockade. In addition, development of epinephrine tolerance appears to blunt the effects of excessive sympathoadrenal activity in shock. Epinephrine - tolerant dogs have been shown to survive an otherwise lethal shock procedure (215). Normally, most dogs will die (acutely) if given a 1 - 2 mg/kg dose of epinephrine as an intravenous bolus (199). However, if the epinephrine is given three times a week in gradually increasing doses from less than 0.1 mg/kg to 2 mg/kg over a period of about 6 weeks - these animals will be tolerant to excessive doses of epinephrine.

The classic studies done on shock in 1964 by Lillehei and co-workers (199), showed that epinephrine tolerance conferred a significant (70 - 80 percent) protection against usually lethal hemorrhagic, endotoxic and cardiogenic shock episodes. The studies indicated that there is a basic hemodynamic disturbance which is common to all forms of shock. In addition cross tolerance studies showed that endotoxin - tolerant animals were protected from the excessive vasoconstriction and showed increased survival when subjected to lethal drum (traumatic) shock, hemorrhagic or cardiogenic shock. They stressed the potential value of modifying the sympathoadrenal response in shock to provide improved survival.

SHOCK AND OTHER HORMONAL ALTERATIONS:

The excessive catecholamine response, coupled with widespread disturbance of the vasculature and cellular metabolism are associated with alterations in function of all the endocrine glands. This is reflected by changes in circulating levels of other hormones.

Glucagon release, like insulin, is normally controlled by the interplay of gastro-intestinal food products and other hormones. Hypoglycemia is the most powerful stimulus for glucagon release (126 P. 1597 Chapt. 71). Unger (342) has suggested that glucagon may play a prominent role in catabolic states such as sepsis, diabetes, burns, trauma and malignancy. Hyperglucagonemia and hypoinsulinemia have been observed in several disease states associated with shock; severe trauma (205), extensive burns (358), endotoxic shock (97) and hemorrhagic shock (136). In addition, little or no change in glucagon levels were seen in major surgery unless accompanied by hypotension (204). It would appear that is is some factor of the shock itself, rather than the type of injury that stimulates glucagon secretion. Several investigators (272, 313) have postulated that plasma concentrations of glucagon increase when there is an increased requirement for endogenous glucose - such as in times of prolonged physiologic stress - and may be in part responsible for the transient hyperglycemia of shock. Gerich et al (114) have shown that increased levels of epinephrine stimulate glucagon release in man. On the other hand, Sheps and Maher (319) and others (294, 313) have shown

that glucagon can stimulate epinephrine release. In addition, increased cortisol levels have been associated with shock states (97, 189, 230) and also have been reported to stimulate glucagon release (97, 217).

Increased glucocorticoid release as a regular accompaniment to hemorrhage has been shown in dogs (111, 112) and in humans in surgical trauma (115). Cortisol levels increase in response to stress (115, 233, 310 P. 1 - 6) and even when adrenal glands are denervated (360). It has been proposed that the value of increased glucocorticoid release in stress lies in their capacity to play a permissive role in several of the effects of other hormones; i.e., the hepatic production of glucose from noncarbohydrate precursors (gluconeogenesis) (107, 127). Cortisol itself stimulates gluconeogenesis (250) and enhances the ability of epinephrine and glucagon to stimulate gluconeogenesis (161, 163). The glucocorticoids supply endogenous glucose in times of stress when normal stores are depleted.

Insulin secretion is normally regulated through the co-ordinated interplay between food products, gastro-intestinal hormones and other hormonal and neural stimuli. High circulating glucose levels, amino acids, fatty acids and ketone bodies stimulate insulin release (126 P. 1597 Chapt. 71). However, when circulating levels of the catecholamines are elevated, the predominant effect is usually one of decreased insulin release (6). Meguid et al (230) found that in shock there were inappropriately low circulating insulin levels associated with

hyperglycemia, hypercatecholaminemia and gradually increasing glucagon levels. Other investigators have reported depressed insulin release and hyperglycemia in several forms of shock in primates (240) and in humans (46, 171). On the other hand, Finley (97) has reported elevated insulin levels in post-operative patients who became septic. In addition, several others have also found elevated insulin levels associated with hyperglycemia in various forms of canine and human shock (17, 46, 76, 227). Baue et al (14) and others (52) have indicated that elevated glucose and insulin levels may be associated with a peripheral insulin resistance and decreased transport and utilization of glucose. The literature to date indicates a marked variation in insulin secretory responses in shock.

THE SECOND MESSENGER SYSTEM:

(i) Introduction:

Hemorrhagic shock, trauma and other low flow states are associated with dramatic hormonal and metabolic derangements. The circulating levels of the catecholamines, glucagon, and cortisol increase markedly during prolonged hemorrhagic shock. The catecholamine - alpha receptor interaction results in excessive vasoconstriction in the splanchnic organs reducing effective blood flow to the liver and intestine. If prolonged, ischemic damage to these organs is inevitable.

Hormonal regulation is fundamental to homeostasis. However, in shock, there is a "rude unhinging of the machinery of life" (132).

Most of the actions of all the hormones (or first messengers) are normally transmitted at the cellular level by the intracellular second messenger to the hormones, cyclic adenosine 3', 5' - monophosphate, or cyclic AMP.

Cyclic AMP was discovered by Rall, Sutherland and Berthet in 1957 (268). It was determined to be the intracellular compound capable of stimulating hepatic glycogen breakdown in response to epinephrine and glucagon. Epinephrine and glucagon increase the rate of hepatic cyclic AMP production, which in turn promotes the conversion of inactive phosphorylase b to active phosphorylase a (333) which then catalyzes glycogen breakdown (226) and glucose production and release by the liver.

Most of the known hormones have been shown to exert their influence in the target tissue by increasing or decreasing net production of cyclic AMP (282). Since cyclic AMP mediates the actions of most of the hormones, it's action consists primarily of regulating the rate of a number of intracellular processes. In fact, cyclic AMP is now recognized as an ubiquitous nucleotide which may be as crucial as ATP or AMP in controlling enzymatic reaction rates. However, as more in depth investigation into this system proceeds, the regulation and effects of net cyclic AMP production becomes more and more complex.

(ii) Adenyl Cyclase - Phosphodiesterase:

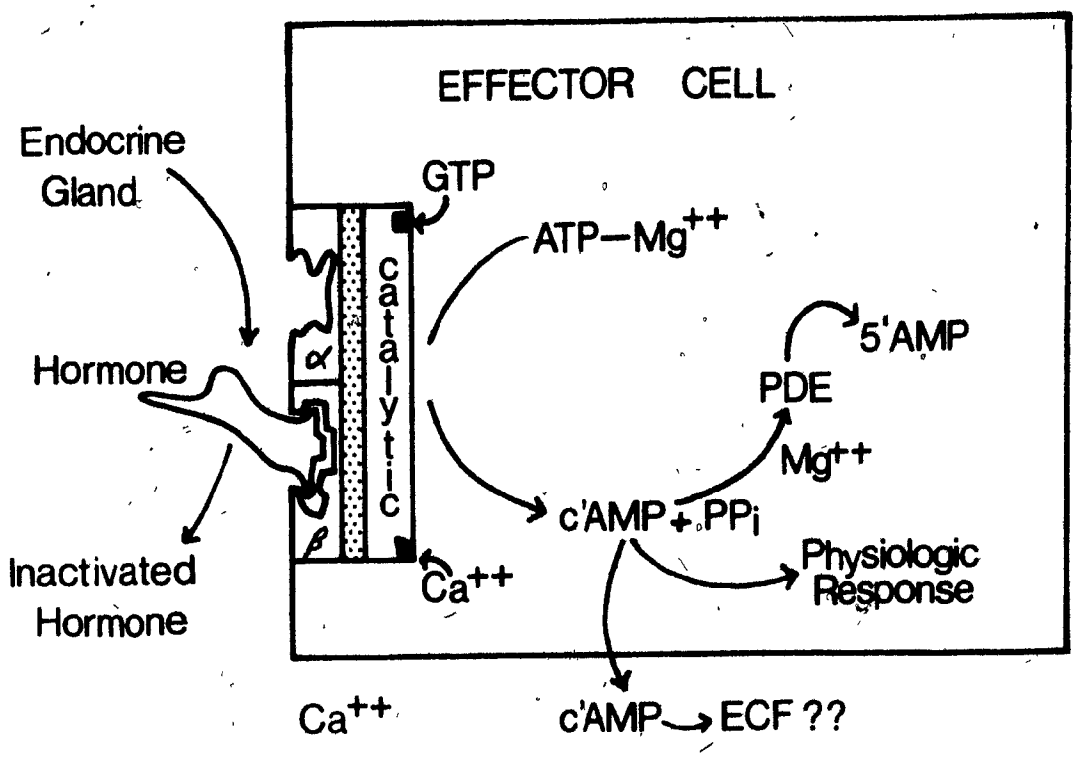
The intracellular level of cyclic AMP is dependent mainly on the relative activities of two enzymes, adenyl cyclase (AC) (282) and

phosphodiesterase (PDE) (335). Recently however, leakage of cyclic AMP from the cells into the extracellular fluid (ECF) has been shown to play an important role in the regulation of intracellular cyclic AMP levels. Cyclic AMP is formed from ATP (Figure 1) in the presence of magnesium (Mg^{++}) via the catalytic action of the membrane-bound enzyme adenyl cyclase (282). Epinephrine, glucagon and some of the prostaglandins stimulate AC activity as do several other polypeptide hormones, resulting in an increased intracellular accumulation of cyclic AMP (282). Insulin and some of the other prostaglandins are thought to exert a mild inhibitory influence upon adenyl cyclase, producing small decreases in cyclic AMP levels (283).

The degradation of intracellular cyclic AMP is catalyzed by phosphodiesterase (PDE), contained mainly in the cell cytoplasm. Cyclic AMP is rapidly hydrolyzed to 5' AMP in the presence of magnesium ions (335). PDE moderates cyclic AMP accumulation and thus plays a key role in terminating its action. PDE has been postulated to exist in up to 7 isoenzymatic forms (37, 239, 343).

There are numerous low Km (2 to 5 μM) and high Km (50 to 250 μM) forms of PDE in the liver (211). To date, little data is available on the intestinal mucosal content of PDE. Evidence seems to indicate that the low Km activity of the hepatic PDE enzyme is the most important for cyclic AMP metabolism <u>in vivo</u> (284 addendum Chapt. 4 p. 90). PDE, like AC may increase or decrease activity subsequent to hormonal or drug interactions. It's activity is increased by imidazole (53)

FIGURE 1


EFFECTOR CELL
Endocrine Gland
GTP
ATP—Mg++
α
catalytic
β
Hormone
5'AMP
PDE
Mg++
c'AMP + PPi
Physiologic Response
Inactivated Hormone
Ca++
Ca++
c'AMP → ECF ??

and is inhibited by methylated xanthines such as theophylline and caffeine (335), resulting in a decreased degradation and hence an increased net production of cyclic AMP.

(iii) The Second Messenger Concept:

Cyclic AMP has been found to exist in almost every animal tissue for which it has been assayed, including bacteria and unicellular organisms (284, Chapt. 2 p. 17). It is now considered the key intracellular mediator of the tissue responses to most hormones. These include those which influence hepatic and gastrointestinal function such as the catecholamines, glucagon, corticotropin, thyrotropin, vasopressin, insulin, growth hormone, leuteinizing hormones, parathyroid hormone, serotonin, angiotensin, the estrogens and prostaglandins (284, Chapt. 2 p. 17).

The concept of alpha (α) and beta (β) receptors initiated by Ahliquist (2) has since been expanded to include the adenyl cyclase - cyclic AMP - PDE system. The new concept (Figure 1) proposed includes the hormones as part of a two messenger system. The hormones, acting as the first messengers, are released from their cells of origin and travel via the circulation to their target tissues, where they interact with the membrane-bound adenyl cyclase to affect an alteration in the intracellular level of the second messenger, cyclic AMP, which in turn induces the affected cell to perform it's particular function. The α and β receptor theory proposed by several investigators (282) includes an expanded design of the adenyl cyclase enzyme. The enzyme is thought to be composed of at least three subunits (Figure 1). Two different outer

or regulatory subunits to which the hormones bind and an inner catalytic subunit which specifically catalyzes the breakdown of ATP into cyclic AMP. One of the outer regulatory subunits has properties associated with α receptors and the other with β receptors. Interaction between the α receptor and a sympathomimetic agent results in a decrease in adenyl cyclase activity. This α interaction leads to vasoconstriction in the splanchnic organs in response to epinephrine or norepinephrine (126, Chapt. 24, p. 485). On the other hand, activation of the β receptors leads to an activation in adenyl cyclase activity and (for example) an increase in glycogen breakdown in the liver. In tissues where α and β effects appear the same, the predominant α or β receptors may be bound to a catalytic subunit in such a way that interaction with, for example, the catecholamines, leads to a net α or inhibitory effect upon adenyl cyclase activity. Systemic vasoconstriction in dogs is a classical α response to the catecholamines (173). The overall effect of stimulation of both receptors in this case results in a predominantly α effect upon the vasculature. In addition, the differences in sensitivity of β receptors in different organs to β agonists of varied chemical structure are sufficiently great enough to differentiate two distinct types of β receptors - β_1 and β_2. Glucagon and epinephrine are both β agonists, but interact with separate and distinct β receptors in the liver to produce different responses (23).

The exact mechanism where by the binding of a hormone to it's particular receptor produces a change in adenyl cyclase activity is unknown. It is postulated (270), that changes in calcium permeability of the cell may signal the change in adenyl cyclase activity. Alpha stimulation increases

calcium entry into the cell to reduce adenyl cyclase activity, while stimulation reduces calcium entry to promote an increase in ACA. In addition, Rodbell et al (285 B) have recently observed that guanyl nucleotides (especially GTP) also modulate ACA and are able to potentiate the AC response to hormonal stimulation.

The inner or catalytic subunit function is more stable than the receptor subunit function. Rutenberg et al (289) and others (69, 243,) have reported disrupted receptor function associated with unchanged intrinsic or catalytic subunit activity. The intrinsic or catalytic activity of adenyl cyclase may be studied without regard to the receptor subunit influence with the aid of sodium fluoride (NaF). NaF is a non-specific stimulator of ACA activating the catalytic subunit of the enzyme only in broken cell preparations. The mechanism of NaF activation is not completely understood. It is thought that NaF releases an inhibition normally exerted upon the catalytic subunit. Hence, the 2 to 10 fold increase of AC activity (253) over basal in the presence of 10 millimolar (mM) NaF may represent a reversal of an inhibition (301); although it could be an artifact resulting from cell breakage. However, if the catalytic subunit of the enzyme is functioning normally, then no changes in the NaF - stimulated ACA would be expected regardless of changes produced in basal enzyme activity in the presence of α or β agonists. The ability of NaF to stimulate AC sometimes lasts longer than the ability of the receptor portion of the enzyme to bind or respond to hormones; but even this is very labile. The expression of basal adenyl

cyclase activity is also very labile and therefore great caution must be taken in preparation of tissue homogenates. It is therefore, with the use of in vitro additions of NaF and hormones, coupled with basal and in vivo hormonal stimulation of ACA, that precise alterations at any of the levels of the enzyme system may be studied.

SHOCK AND THE SECOND MESSENGER SYSTEM:

There are such extreme changes in epinephrine, norepinephrine, glucagon and cortisol levels in shock, coupled with severe ischemic cellular structural and functional damage, that changes in the adenyl cyclase - cyclic AMP - phosphodiesterase system might be expected. Few experimental and even fewer clinical studies have been done in this specific area. However, of the experimental studies that have recently been initiated in this area, all of them indicate a depression in the normal function of the second messenger system in low flow states (224, 288, 289, Schofield - Jones, C.A., unpublished data, 1973). No such clinical data in patients suffering from hemorrhagic shock is available. However, studies done on dogs and pigs following hemorrhagic shock (224, 264) and in humans following major surgical trauma (115, Chiu C.J., McArdle, A.H., Hinchey, E.J., unpublished data), and myocardial infarction (332, 346) and sepsis (326) have shown increases in circulating plasma cyclic AMP levels when compared to levels in normal patients. The reports on shock - induced alterations in tissue PDE are inconclusive and controversial (118, Rutenberg, A.M., unpublished data). Stress - activated hormonal and associated metabolic

changes appear to be directly related to significant changes in the second messenger system. Inadequate or excessive response of the second messenger system could result in the decreased ability of the tissues to respond to homeostatic or therapeutic controls and therefore may contribute to irreversibility in shock.

THERAPY OF SHOCK:

(i) Introduction:

There are two major problems to correct in acute circulatory collapse - a deficiency in effective circulating blood volume and insufficiency in the peripheral vasculature presenting with stagnant anoxia. In other words, the integrity of the damaged microcirculation must be re-established in order to restore normal cellular metabolism.

Restoration of the circulating blood volume and return of normal blood pressure are of prime importance. Proper assessment of the degree of fluid loss must be attempted and appropriate losses (fluids, whole blood, plasma, etc.) replaced. Several experimental studies (59, 215) have shown that immediate short-term fluid therapy following an otherwise lethal period of superior mesenteric artery occlusion, confers 33 to 53% survival rates over controls with no treatment. If fluid therapy is delayed, changes typical of irreversible shock will ensue.

The microvascular and cellular metabolic derangements characteristic of stagnant anoxia often cannot be corrected by fluid replacement alone, as the exogenous fluid itself may accumulate within the stagnant

microcirculation increasing the interstital edema and further contributing to deterioration. The problem has long been recognized, although corrective measures lack complete success. The pharmacological agents that have been used in shock therapy can be divided into two basic groups, vasoconstrictors and vasodilators.

(ii) Vasoconstrictors:

Most clinicians have almost entirely abandoned the use of sympathomimetic agents with α-adrenergic (vasoconstrictor) action in refractory shock patients. Levarterenol is the synthetic equivalent of norepinephrine. It, like norepinephrine, has some beneficial effects upon myocardial contractility and rate, however, these benefits are far outweighed by the deleterious vasoconstrictor effects upon the microcirculation and tissue perfusion (137).

(iii) Vasodilators:

The primary aim of pharmacological therapy is to restore blood flow to the anoxic tissues. It is probably the initial phase of intense peripheral vasoconstriction and resultant tissue anoxia that perpetuate irreversibility in shock. In the presence of an α-adrenergic blocking agent, or vasodilator, the catecholamine - induced vasoconstriction, pooling of blood and decrease in flow are minimized. Associated with these changes is a decrease in the accumulation of metabolic waste and greater availability of oxygen to the anoxic tissues. The drugs used may produce vasodilation directly as a result of concentrated intravenous administration. They may also vasodilate by blocking α-adrenergic

receptors, or they may vasodilate by unknown means.

Nickerson, in 1955 (249), published preliminary observations on the clinical use of phenoxybenzamine (POB), a potent α-adrenergic blocking agent, in treating refractory shock. Since then, an abundance of experimental studies have provided data on the effects of POB in catecholamine (64), endotoxic (347, 354), hemorrhagic (98, 135, 208, 364) and cardiogenic (147) shock.

Most of the studies have shown improved survival with the use of POB before or soon after the induction of shock in a variety of animals. The increase in survival rate is usually attributed to the improved microcirculation and hence improved peripheral tissue metabolism.

Interest in α-adrenergic blocking agents led to studies with isoproterenol (91) which is a pure β-adrenergic agonist. Glucagon (181) and digitalis (73) have also been used in the therapy of shock. These agents are useful for their stimulatory effects upon the myocardium. Each of these agents also has side effects which must be considered, and if used accordingly and in conjunction with adequate blood volume replacement, may afford significant protection from the ravages of shock.

(iv) Glucocorticosteroids:

Melby et al described the use of aldosterone in septic shock in 1957 (232). Since then, there has been much controversy as to the efficiency of steroids in treating shock. Several investigators have found an increase in survival rate in animals given massive doses of glucocorticosteroids

before induction of catecholamine shock (110, 200), septic shock (194, 199), cardiogenic shock (194) or hemorrhagic shock (60, 134, 241, 353). Fukada (110) reported that pretreatment of rabbits with glucocorticosteroids increased survival by rendering the animals less susceptible to hypovolemic hypotension. Schumer (304) showed that administration of large amounts of dexamethasone at strategic intervals during bleeding, increased survival in splenectomized Rhesus monkeys subjected to hemorrhagic shock. Desmonts (70), Hakstian (134) and others (105, 179, 187) have found that administration of pharmacological doses of steroids after hypovolemia is not as effective as pretreatment however, does result in improved survival rates when compared to animals given no treatment.

Lillehei (143, p. 139) emphasized the importance of splanchnic hypoperfusion as a common denominator in the pathogenesis of various shock states. Corticosteroids have been reported to increase splanchnic flow (348) which may contribute to their salutatory effects in patients suffering from shock. However, Raflo et al (267) showed only a transient increase in splanchnic flow and no increase in survival in dogs pretreated with 60 mg/kg methylprednisolone (Solu-Medrol) prior to a lethal hypovolemic episode. Similarily, Replogle et al (276) was unable to demonstrate any significant improvement in hemodynamic or metabolic aspects in dogs receiving 5 mg/kg dexamethasone after ninety minutes of hypotension. Spink and Vick (330), Thomas and Brochman (339) and Reichgott and Melmon (273) also failed to show increased survival rates in shock with glucocorticoid treatment.

Desmonts (70) and Altura (7) have specified that when glucocorticoids are administered after shock is induced, the timing and dosage are crucial to improved survival. They postulated that perhaps failure to achieve increased survival with steroids might be due to inappropriately large or small doses of the steroid administered at the wrong time intervals.

Preliminary clinical studies by Schumer and Nyhus (309) of fifty patients in shock showed survival to be 20 percent greater in patients treated with steroids than those not treated with steroids. Lillehei, Motsay and Dietzman (200) believe that steroids prevent arteriolar and venular constriction in the viscerocutaneous circulation induced by the high circulating catecholamine levels in shock - thereby improving tissue perfusion. They have postulated that this effect may be due to α-adrenergic blocking properties of some glucocorticosteroids. They advocate one or more doses of 30 mg/kg of methylprednisolone or equivalent amounts of hydrocortisone or dexamethasone. Similarly, favourable results have been obtained with massive doses of hydrocortisone, methylprednisolone or dexamethasone for traumatic shock (310), gram negative septic shock (359) and for cardiogenic shock (72).

There are several side effects of prolonged steroid therapy that do not appear to be a problem in the acute use of steroids for shock therapy. Jama et al (167) report that if steroids are administered very early in clinical shock, and for only up to 48 hours, there is a decrease in the incidence of stress ulcers associated with low flow states.

Similarily, Dietzman et al (75) state that there is no increased frequency of gastrointestinal bleeding nor is there a slow down in wound healing following limited glucocorticoid therapy.

Feunfer et al (109) state that in vitro, there is no increased risk of infection with acute methylprednisolone treatment. These studies have been confirmed in vivo by Brothers et al (38).

Melby and Spink (233) have noted that adrenal supression is not a problem when pharmacological doses of steroids are given over a 48 hour period and that no replacement therapy is required.

It would appear, that when pharmacological doses of steroids are given for 48 hours or less the frequency of complications, due directly to the steroids, are no greater than in shock patients not receiving steroid therapy.

The mechanism(s) by which glucocorticosteroids preserve circulatory and cellular function are not fully understood. However, there are many theories and much data implicating steroid - induced benefits at all levels of cellular and tissue structure and function.

Glucocorticoids have been reported to produce peripheral vasodilation (74, 200) thus increase tissue perfusion, (308, 310, p. 7 - 15), decrease metabolic acidosis (309) and increase venous return to the heart. In addition to the hemodynamic improvements, glucocorticoids are reported to stabilize cellular and lysosomal membranes (4, 189, 302, 329). Therefore, the integrity of the capillaries, cell and subcellular membranes may be preserved in the face of anoxia.

Several reports have implicated lysosomal enzymes as being injurious to a variety of tissues (94, 119, 337). Hypovolemic hypotension produces tissue ischemia which results in an increased permeability of hepatic lysosomal membranes (143, p. 93 - 111) allowing release of lysosomal enzymes into the circulation (120) and resultant formation of the cardiotoxic peptide - myocardial depressant factor (MDF) (121). Corticosteroid therapy has been reported to prevent such elevations of plasma levels of lysosomal enzymes in several experimental shock states (120). Accordingly, Lefer (189) has shown that dexamethasone pretreatment decreases MDF formation compared to untreated controls.

Reversal of lactic acid into pyruvate and then finally glucose (gluconeogenesis) is an important mode of reducing metabolic acidosis and increasing energy (ATP) production. Schumer (309), has noted that glucocorticoids decrease lactic acidemia, aminoacidemia and the hyperphosphatemia of shock. Several others (75, 162, 252) have shown that glucocorticoids stimulate conversion of lactate to glycogen through an unknown metabolic pathway. With these facts in mind, Schumer (309) evolved the concept that corticosteroids stimulate gluconeogenesis, converting amino acids, fatty acids and lactate to energy - producing substances. This generates ATP. When more ATP is produced, there is a greater utilization of free phosphates.

Glucocorticoids have been reported to play a permissive role in the gluconeogenic process in the liver in response to epinephrine or glucagon (107, 127). In fact, glucocorticoids have been shown to

potentiate the effects of epinephrine, glucagon and norepinephrine on several cyclic AMP - mediated processes in normal and exercising dogs (160, 161, 162, 163). The most striking of which, is the potentiation of the effects of epinephrine and glucagon on hepatic glucose output. Up to 20 fold increases over control hepatic glucose output have been reported (160, 162, 163) in dogs challenged with epinephrine or glucagon after several days of treatment with methylprednisolone. The mechanism of this effect upon the cyclic AMP - mediated process is not fully understood. However, it has been postulated to be due to a sensitizing effect on the adenyl cyclase - cyclic AMP - phosphodiesterase system (163, 297, 316). Since the cyclic AMP - generating enzyme, adenyl cyclase, and perhaps one or more phosphodiesterase enzymes are integrated into the cell membranes in low flow states, then the damaging effects of ischemia and anoxia may be ameliorated by pharmacological doses of steroids.

MATERIALS AND METHODS

Preparation of Animals:

Fasted dogs of either sex, weighing 17 - 22 kg were anesthetized with 30 mg/kg sodium pentobarbital administered intravenously. The dogs were then intubated in order to maintain an adequate airway.

The animals were divided into three groups on the basis of the hormone they received, and each group was subdivided on the basis of treatment.

Group I: Twenty dogs received 0.3 mg glucagon administered intravenously as a single bolus. These animals were subdivided into four equal groups as follows:

A. Control animals.

B. Shock animals.

C. Shock animals treated with glucocorticoid.

D. Control animals pre-treated with glucocorticoid.

Group II: Fifteen dogs received 0.25 mg epinephrine administered intravenously as a single bolus. These animals were subdivided into 3 equal groups as follows:

A. Control animals.

B. Shock animals.

C. Shock animals treated with glucocorticoid.

Group III: Ten dogs received 0.1 mg norepinephrine administered intravenously as a single bolus. These animals were subdivided

into 2 equal groups as follows:

A. Control animals.

B. Shock animals.

Treatment of Animals:

A: Control:

Two hours following induction of anesthesia, a laparotomy was performed. Cannulas were placed in the femoral and portal veins. Blood samples were obtained from the portal and hepatic veins before, and at one, three, five, ten and fifteen minutes following a single pulse injection of hormone administered via the femoral vein. The samples of hepatic venous blood were obtained by direct puncture of the hepatic vein. A cannula was also placed in the femoral artery to monitor blood pressure throughout the entire experimental procedure. Wedge biopsies of liver were obtained from adjacent areas of the left ventral lobe. Intestinal biopsies were obtained from an area approximately 15 - 20 centimeters proximal to the ileocecal valve. Samples of liver and intestine were removed before hormone injection, and again two and fifteen minutes following hormone injection.

B: Shock:

Immediately following induction of anesthesia, the dogs were subjected to a modified Wigger's procedure for hemorrhagic shock. They were bled to a mean arterial blood pressure of 40 to 45 mm Hg which was maintained for three hours by removing or reinfusing

aliquots of blood as necessary. At the end of the three hour hypovolemic period, the dogs were reinfused their shed blood, and allowed to remain a further four hours without intervention whereupon they were challenged with a bolus injection of hormone. Tissue and blood samples were obtained in exactly the same manner as described for the control animals.

C: Shock Animals Treated with Steroid:

These animals were subjected to the same bleeding procedure described for shock animals. Immediately following reinfusion of shed blood, the dogs received 30 mg/kg Methylprednisolone sodium succinate (MPSS) (Solu-Medrol, Upjohn), and received a second 30 mg/kg dose 3 hours later. One hour following the second injection, the dogs were challenged with the respective hormone.

D: Glucocorticoid Treatment of Controls:

Five control animals were allowed to stabilize from induction of anesthesia for two hours. They were then given an intravenous bolus injection of 30 mg/kg body weight of MPSS over a one minute period. This was repeated again 3 hours later. One hour following the second injection of steroid, glucagon was injected and samples obtained exactly as specified for the untreated controls.

Blood Analyses:

A: Glucose:

Whole blood for glucose determination was collected in tubes containing fluoride, and glucose concentration was determined using the Boehringer Mannheim Test Kit for Glucose (Hexokinase Method).

B: Hematocrit:

Hematocrit was determined on whole blood by the standard technique of microcentrifugation.

C: Insulin:

Insulin was measured in serum by radioimmunoassay using the Phadebas Insulin Kit (Pharmacia, Canada Ltd.). Samples were run in duplicate and read off a standard curve which was set up for each group of samples. Serum insulin levels are expressed as microunits of insulin per millilitre (μ units/ml) of serum.

D: Cyclic AMP:

Whole blood was collected in tubes containing EDTA and immediately cooled to $0^{o}C$. The samples were centrifuged in the cold at 2,500 rpm. The plasma was collected and precipitated with an equal volume of ice-cold 0.6 N perchloric acid. The supernatant was decanted and frozen at -45^{o} C until analyzed for cyclic AMP content.

Tissue Analyses:

A: Adenyl Cyclase Activity (ACA):

The hepatic and intestinal biopsies obtained for biochemical examination were immediately immersed in ice-cold physiologic saline. The mucosal side of the intestinal biopsy was blotted on a piece of gauze and an epithelial cell preparation (221) was obtained by lightly scraping the surface with a scalpel. A portion of the intestinal and liver biopsies were then weighed on a precision torsion balance, then gently homogenized with 3 strokes in a Teflon homogenizer in 10 volumes

ice-cold 75 mM Tris-HCl buffer (pH 7.50), containing 25 mM $MgCl_2$. All samples were assayed for enzyme activity within 30 minutes.

(i) Basal ACA: Basal ACA was determined using a modification of the method of Krishna et al (182). The incubation medium consisted of 50 mM Tris-HCl buffer (pH 7.56), 5 mM magnesium chloride, 10 mM theophylline, 2 mM ATP, an ATP-regenerating system of 10 mM phospho-enol pyruvate with 250 micrograms/ml pyruvate kinase and an amount of mucosal homogenate containing 100 - 150 micrograms DNA or an amount of liver homogenate containing 35 - 40 micrograms of DNA. The final volume of the reaction mixture was 0.5 ml. All samples were run in duplicate. The reaction was initiated at 37° C by addition of the tissue homogenate and the incubation was carried out in a Dubnoff metabolic shaker. The reaction was terminated exactly 10 minutes later by placing the reagent tubes into a boiling water bath for 3 minutes. The tubes were then cooled and the clear supernatant collected by cold centrifugation and then stored at -45° C until assayed for cyclic AMP content. The precipitates were analyzed for their total DNA content.

(ii) Sodium Fluoride - Stimulated ACA (NaF - ACA): The effect of in vitro addition of NaF upon basal ACA was determined. 10 mM NaF was added directly into the incubation medium immediately preceding tissue homogenate introduction and initiation of the enzyme reaction. These samples were run in duplicate and exactly as described for basal ACA except for the in vitro addition of 10 mM NaF.

(iii) In Vitro Hormone - Stimulated ACA: The effect of in vitro addition of glucagon, epinephrine or norepinephrine upon basal enzyme activity was determined. Five nanomoles of either glucagon, epinephrine or norepinephrine were added directly into the incubation medium immediately before addition of the tissue homogenate. These samples were also run in duplicate in the same manner as that described for basal ACA except for the in vitro addition of hormone.

B: Phosphodiesterase Activity (PDE):

PDE activity in the liver and intestine was determined in duplicate from aliquots of the remaining Tris buffer tissue homogenates. Low Km (1 - 3 uM) was determined by a method developed in this laboratory. A constant amount of cold cyclic AMP (1×10^{-8} moles) was added into the incubation medium consisting of 50 mM Tris-HCl buffer (pH 7.60). The tissue homogenate was added to initiate the reaction which was carried out at 37° C in a Dubnoff metabolic shaker. The reaction was terminated after 5 minutes in the same manner as described for the AC assay. The clear supernatant was collected and stored at -45° C until cyclic AMP determination could be carried out. The precipitates were analyzed for their DNA content. The difference between the amount of cyclic AMP remaining in the supernatant and the standard amount initially added was considered as the amount of cyclic AMP destroyed by the enzyme.

C: DNA:

The total DNA content in the tissue precipitates from all the AC and PDE assays was determined by the method of Burton (41).

D: Cyclic AMP Determination:

(i) The cyclic AMP content in the supernatants of all the AC and PDE samples was determined by the Amersham Searle Cyclic AMP Assay Kit. The procedure follows the protein kinase binding method of Brown et al (39, 40) using a charcoal separation technique to bind free cyclic AMP, leaving the protein kinase-cyclic AMP complex in solution. Fifty microlitre aliquots of the supernatants were assayed directly, or diluted to within a 1 picomole to 8 picomole range with 50 mM Tris-HCl buffer. All samples were run in duplicate and cyclic AMP values read off a standard curve freshly prepared with each set of samples to be assayed. The total cylic AMP content of the supernatant fraction was divided by the total precipitate DNA content and ACA was expressed as picomoles of cyclic AMP generated per microgram DNA per 10 minute period. PDE activity was expressed as picomoles of cyclic AMP destroyed per microgram DNA per 5 minute period.

(ii) The cyclic AMP content of the tissues and plasma was determined as follows: aliquots of the tissue and plasma perchloric acid extract supernatants were thawed and passed through a Dowex 50 x 200 mesh columns (15 x 0.7 cm), then eluted with 0.1 N HCl. The resulting eluate was rapidly frozen and lyophylized, giving samples that were stable indefinitely at room temperature. The cyclic AMP content of these samples was determined by the protein kinase binding method of Gilman (116). The lyophylized samples were brought to volume with an appropriate volume of acetate buffer (pH 4.10), and run in duplicate

at two different concentrations. The cyclic AMP values were read off a freshly prepared standard curve ranging from 0 to 4 picomoles of cyclic AMP. Tissue cyclic AMP levels were expressed as picomoles of cyclic AMP per milligram wet weight of tissue. Plasma cyclic AMP values were expressed as nanomoles of cyclic AMP per litre of plasma.

Statistical Analysis:

The data obtained within each group at 2 and 15 minutes following hormone injection was compared to basal values using a paired-T-test. The differences between steroid-treated and untreated controls, and steroid-treated and untreated shock were analyzed by [redacted] Student's T test. Significance was obtained at $P = \text{or} < .05$.

EXPERIMENTAL: RESULTS AND DISCUSSION

Blood Glucose Response Curves - Control And Shock:

(i) Glucagon

Hepatic and portal blood glucose changes following *in vivo* glucagon injection into normal and shock dogs is presented in Figures 2 and 3.

The normal hepatic blood glucose response to glucagon injection (Figure 2) shows a sustained rise in glucose levels which becomes significant ($p < .05$) by 1 minute and remains so ($p < .01$) until 10 minutes where it levels off. In shock, the hepatic blood glucose response to glucagon is reduced but is still significant at 1 ($p < .05$) and 5 ($p < .05$) minutes.

In Figure 3, it can be seen that the control portal blood glucose response to glucagon shows a slower rise and reaches levels lower than those seen in the hepatic blood. In shock, this response is decreased compared to that seen in the hepatic blood.

(ii) Epinephrine

The hepatic and portal blood glucose response to epinephrine injection in normal and shock dogs is presented in Figures 4 and 5.

In Figure 4, it can be seen that the hepatic blood glucose level is increased significantly ($p < .01$) by one minute following epinephrine injection. It continues increasing to a peak at 10 minutes then falls slightly by 15 minutes. In shock, the basal pre-

FIGURE 2

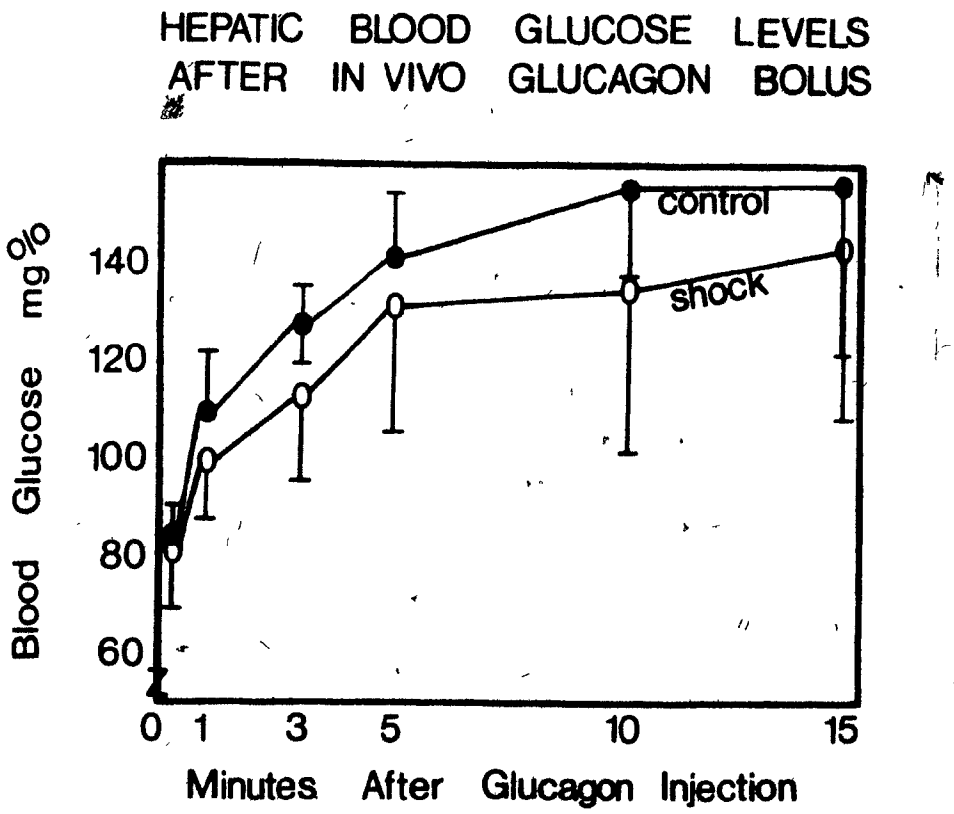
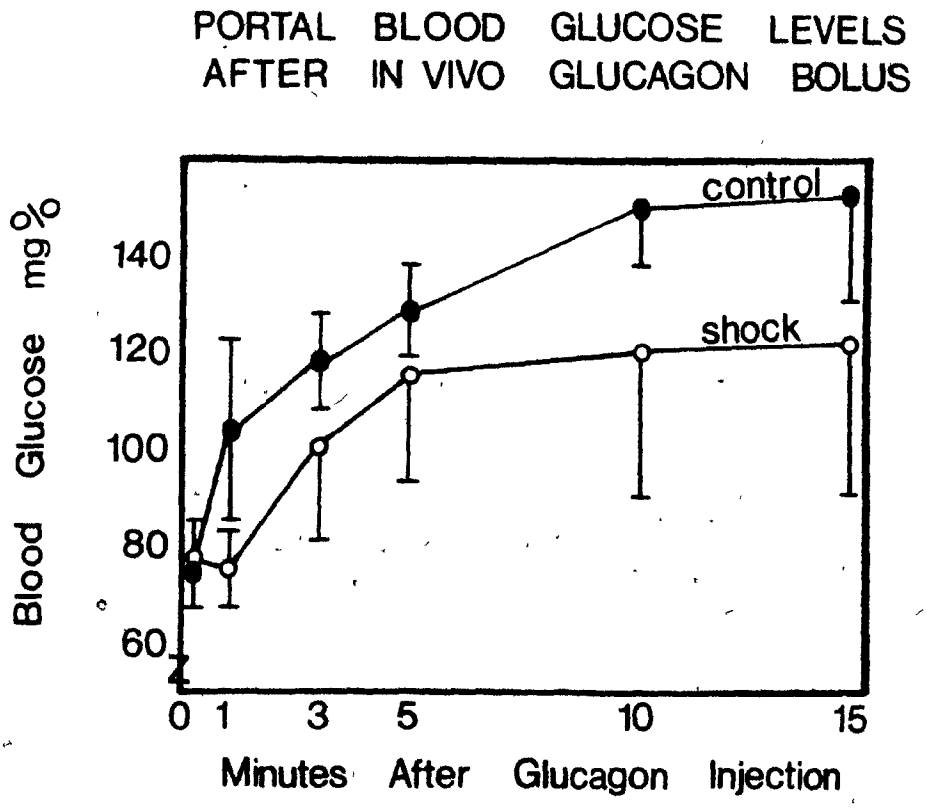

HEPATIC BLOOD GLUCOSE LEVELS
AFTER IN VIVO GLUCAGON BOLUS
Blood Glucose mg%
140
120
100
80
60
control
shock
0
1
3
5
10
15
Minutes After Glucagon Injection

FIGURE 3


PORTAL BLOOD GLUCOSE LEVELS
AFTER IN VIVO GLUCAGON BOLUS
Blood Glucose mg%
140
120
100
80
60
control
shock
0 1 3 5 10 15
Minutes After Glucagon Injection

hormone level is 19% lower than control levels. In addition, the response to epinephrine is completely abolished ($p < .005$).

In Figure 5, it can be seen that the control portal blood glucose response curve follows much the same pattern as that seen in the hepatic blood, however absolute levels are not as great. In shock, again the blood glucose response to epinephrine injection is abolished.

(iii) Norepinephrine

Hepatic and portal blood glucose changes following norepinephrine injection are presented in Figures 6 and 7.

Figure 6 shows that there is little change in hepatic blood glucose levels following norepinephrine. The increases are significant only at 5 ($p < .05$) and 15 ($p < .01$) minutes. In shock, there is less of a response, the only significant increase being at 15 ($p < .05$) minutes.

Figure 7, shows that the control portal blood glucose response to norepinephrine is similar to that seen in the hepatic blood. In shock there is no response.

Discussion:

Glucagon is a more potent hepatic glycogenolytic and gluconeogenic hormone than is epinephrine (23, 88, 210). The maximum hepatic blood glucose levels reached in this study following in vivo glucagon bolus in normal animals is 33% greater than those reached following epinephrine bolus (Figures 2 and 4). Similarily, Issekutz

FIGURE 4

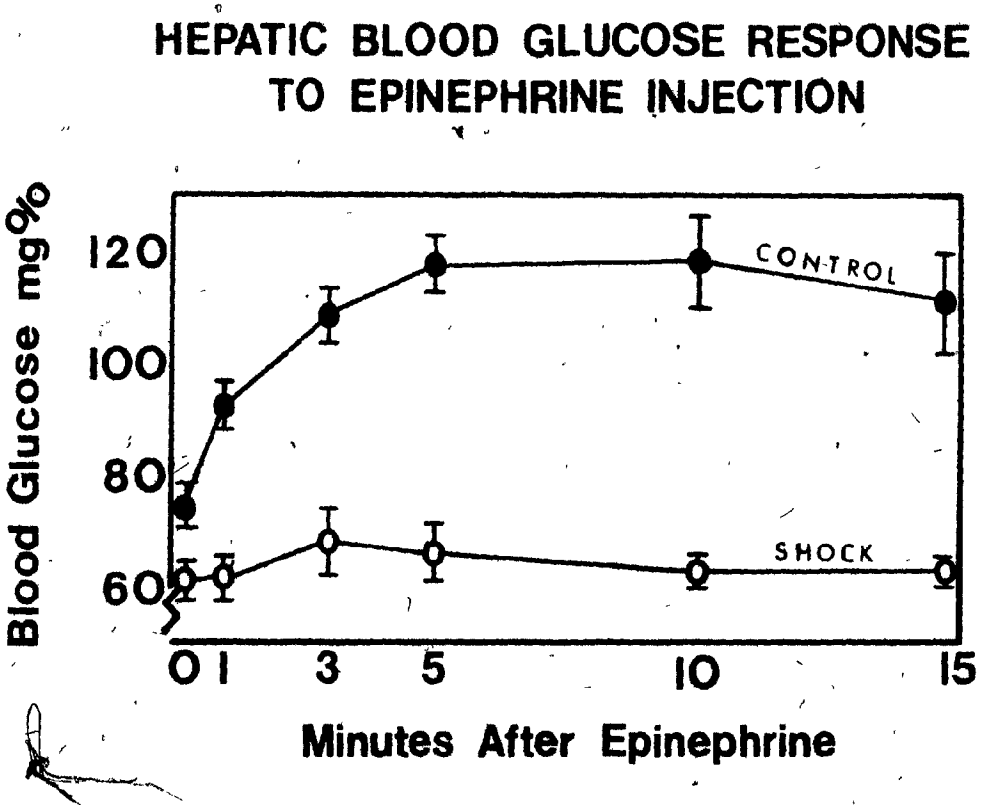

HEPATIC BLOOD GLUCOSE RESPONSE
TO EPINEPHRINE INJECTION
Blood Glucose mg%
120
100
80
60
CONTROL
SHOCK
0 1
3
5
10
15
Minutes After Epinephrine

FIGURE 5

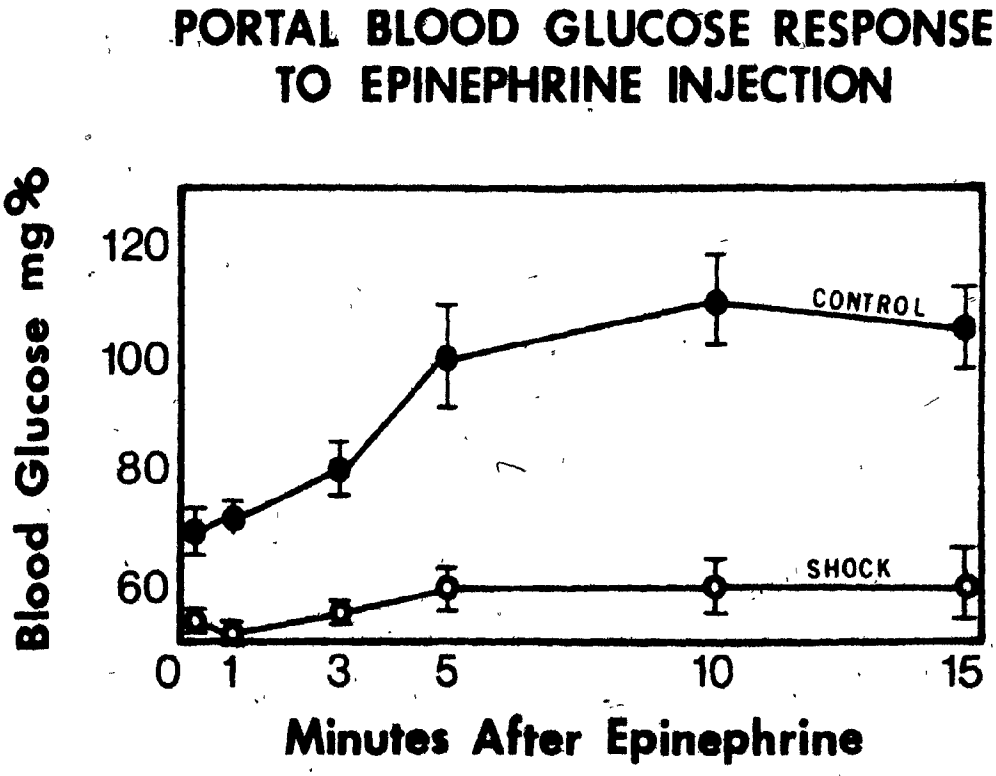

PORTAL BLOOD GLUCOSE RESPONSE
TO EPINEPHRINE INJECTION
Blood Glucose mg%
120
100
80
60
CONTROL
SHOCK
0
1
3
5
10
15
Minutes After Epinephrine

FIGURE 6

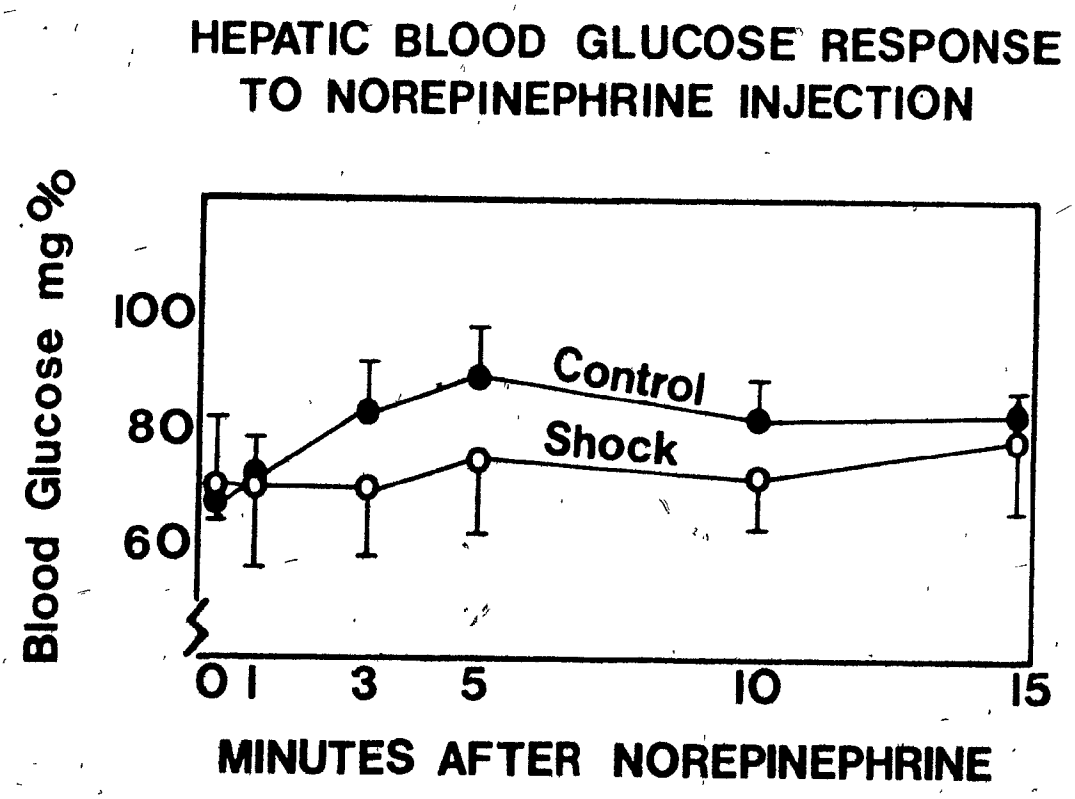
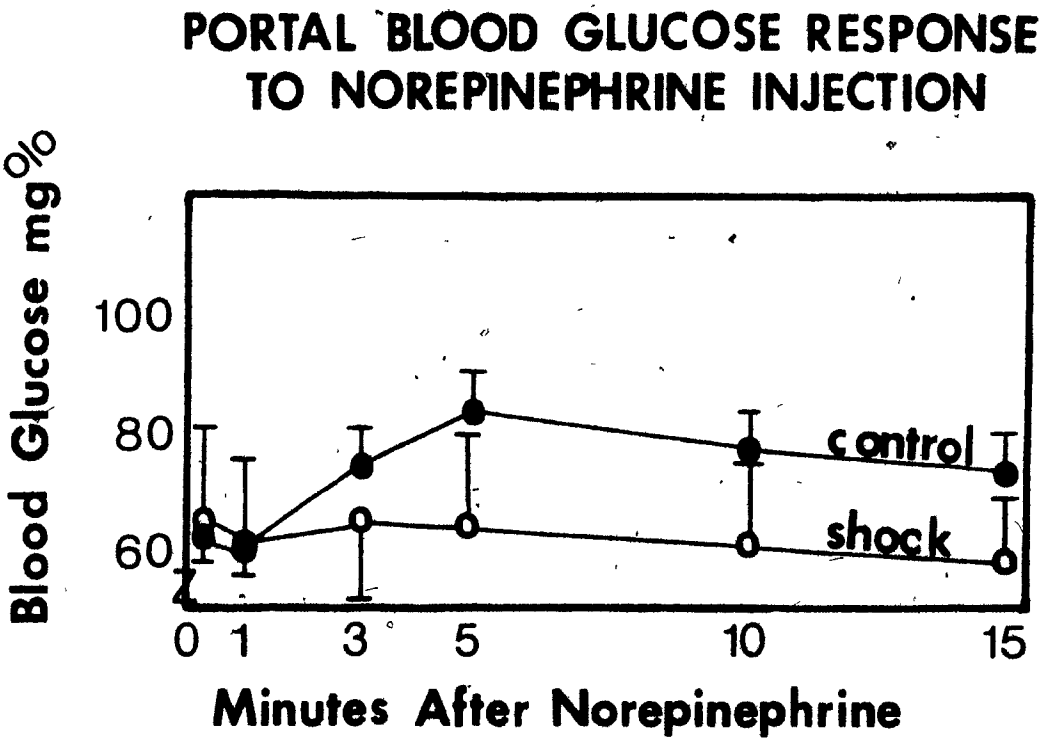

HEPATIC BLOOD GLUCOSE RESPONSE
TO NOREPINEPHRINE INJECTION
Blood Glucose mg %
100
80
60
Control
Shock
0 1 3 5 10 15
MINUTES AFTER NOREPINEPHRINE

FIGURE 7


PORTAL BLOOD GLUCOSE RESPONSE
TO NOREPINEPHRINE INJECTION
Blood Glucose mg%
100
80
60
control
shock
0 1 3 5 10 15
Minutes After Norepinephrine

et al (161, 163) and Altszuler and Morrison (5) report a 25 to 30% greater increase in hepatic blood glucose levels during intravenous infusion of glucagon compared with infusion of an equivalent dose of epinephrine in normal dogs.

Glucagon is a more potent mobilizer of glucose, however it has been shown that there is a lag period before the maximal effects of the hormone becomes evident (86, 88, 286). The maximum effect of epinephrine bolus in normal dogs in this study is obtained by 3 minutes, whereas with glucagon this is not observed until after 5 minutes.

Several sources (88, 126, Chapt. 24, p. 478, 161, 165) indicate that small doses of norepinephrine have virtually no effect upon hepatic carbohydrate metabolism in normal dogs. No change in hepatic blood glucose levels are noted following norepinephrine infusion (161) in normal dogs. This study shows that in addition to normal dogs, there also is no effect of norepinephrine on glucose output in hemorrhagic shock in dogs (Figures 6 and 7).

Since the time of Claude Bernard, hyperglycemia has been known to accompany injury, surgical trauma, and early hemorrhagic or endotoxic shock (4, 17, 46, 76, 154, 227). However, the later and irreversible stages of shock have been associated with both hyperglycemia and hypoglycemia. In this study, only in the shock - epinephrine group were both basal hepatic and portal blood glucose levels significantly reduced from their respective control values. When data

TABLE I

Baseline Blood Glucose Response
To Shock and Steroid Therapy

	HEPATIC BLOOD	PORTAL BLOOD
Control :	75.34 ± 4.03[+]	68.92 ± 5.06
Control - Steroid :	104.91 ± 9.50[*]	74.28 ± 5.73
Shock :	70.30 ± 6.63	63.77 ± 8.81
Shock - Steroid :	91.80 ± 16.90	79.13 ± 16.47

[+] All values represent mg % ± S.E.M.

[*] Significantly (p = or $<.05$) elevated from control (Student-T-Test)

on basal glucose levels were grouped (Table I) there were insignificant decreases in shock hepatic and portal blood glucose levels when compared to their respective grouped control levels.

Despite the fact that basal blood glucose levels are not significantly altered in hemorrhagic shock; the normal increases in blood glucose in response to the hormones are significantly altered in hemorrhagic shock.

The complete abolition of glucose response following epinephrine injection may be due to a depletion of liver glycogen stores - a well known effect of ischemia upon the liver (80). However, the fact that the hepatic blood glucose output in response to glucagon in shock is not abolished; only slightly decreased from normal and is the same as that obtained in the control dogs following epinephrine - suggests that perhaps hepatic glycogen depletion is not the sole cause of an altered glucose output following hormone injection in shock.

Basal AC Response To In Vivo Hormone Injection - Control And Shock:

(i) Glucagon

The effect of in vivo glucagon injection on hepatic ACA in control and shock animals is seen in Figure 8. The "0" time represents basal ACA - prior to hormone injection. The control tissues are represented by the dotted bars and shock tissues by the open bars.

The control basal ACA is 6.64 ± 0.69 picomoles of cyclic AMP generated per microgram of DNA per 10 minute incubation (pmols/µg DNA/10 mins.). There is a significant ($p < .005$) 72 percent increase in ACA at 2 minutes following glucagon injection. At 15 minutes the hepatic ACA is still elevated above normal basal levels.

Basal ACA in the shock tissue does not differ from that of the control. However, the response to glucagon is no longer significant and is reduced by 46% compared to the control response. The ACA measured at 15 minutes in the shock animals was also greater than basal levels but again reduced from respective control values.

The AC response in the intestine following glucagon injection is shown in Figure 9.

Control basal intestinal mucosal ACA is 2.21 ± 0.46 pmols cyclic AMP/µg DNA/10 mins., which does not change following glucagon injection.

The basal ACA in shock animals is not significantly different from control values. ACA increases significantly ($p < .05$) at 2 minutes in the shock tissue.

FIGURE 8

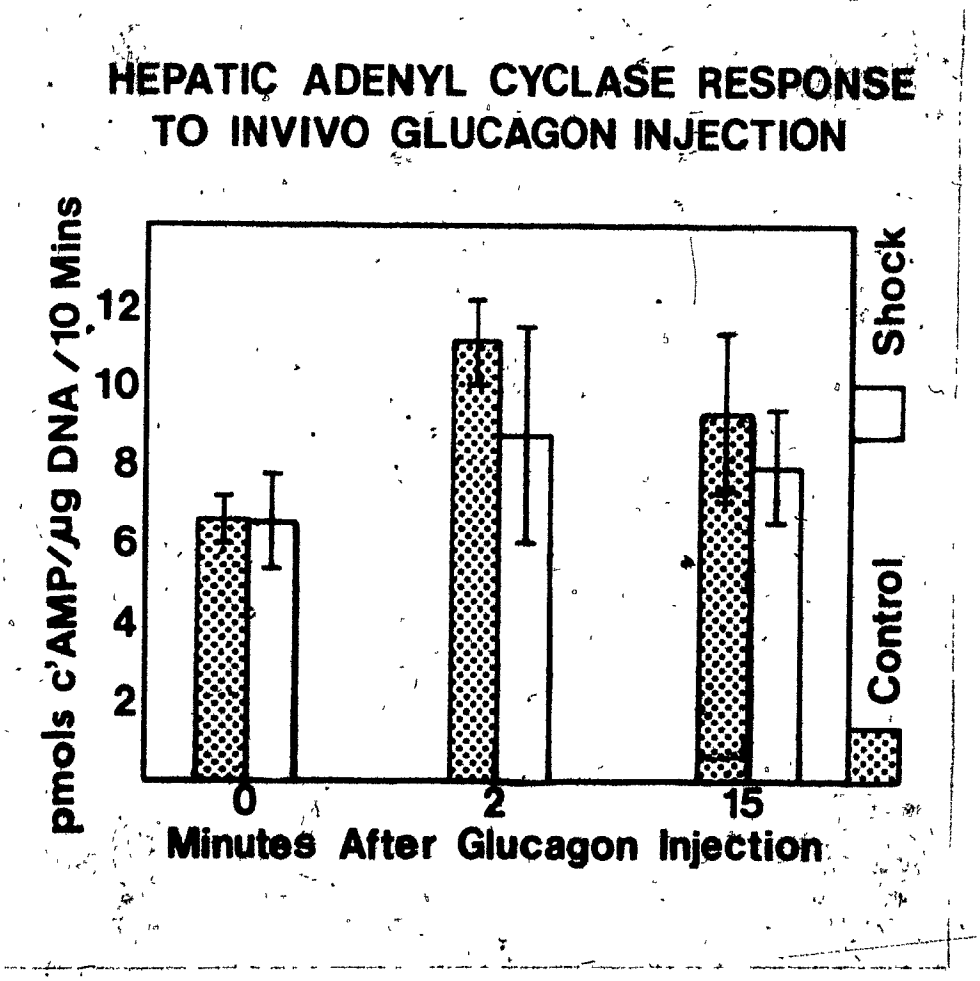
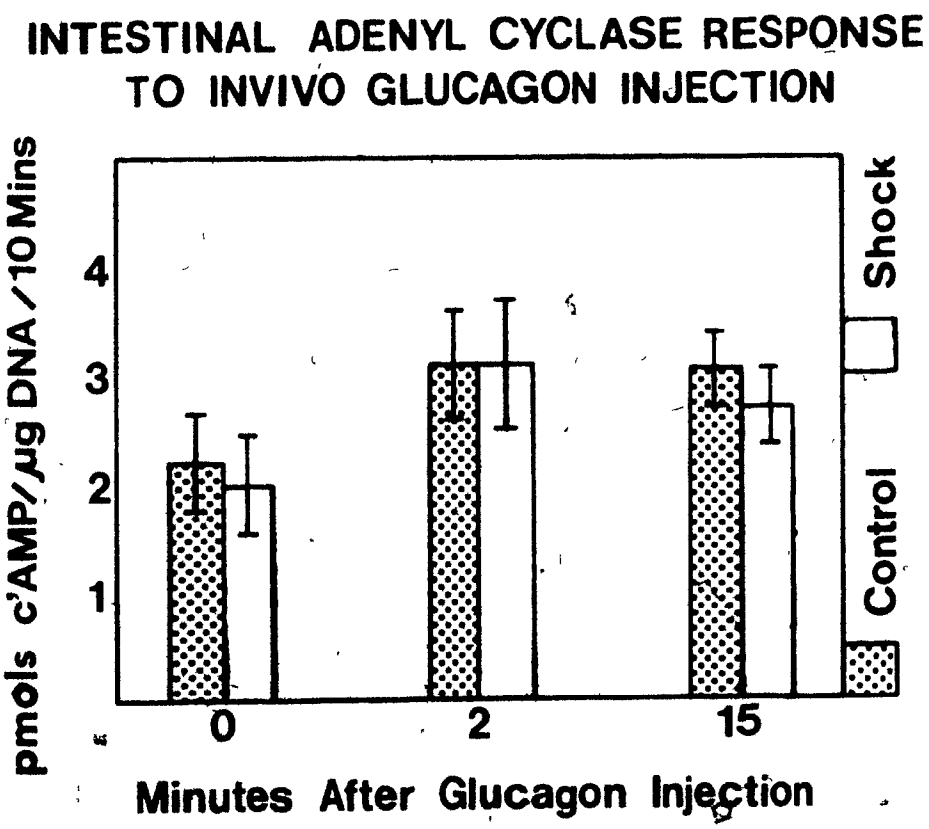

HEPATIC ADENYL CYCLASE RESPONSE
TO INVIVO GLUCAGON INJECTION
pmols c'AMP/µg DNA /10 Mins
2
4
6
8
10
12
0
2
15
Minutes After Glucagon Injection
Shock
Control

FIGURE 9


INTESTINAL ADENYL CYCLASE RESPONSE
TO INVIVO GLUCAGON INJECTION
pmols c'AMP/µg DNA/10 Mins
4
3
2
1
0
2
15
Minutes After Glucagon Injection
Shock
Control

(ii) Epinephrine

The adenyl cyclase response to *in vivo* epinephrine injection is presented in Figures 10 and 11.

There is a significant ($p < .025$) 27% increase in hepatic ACA over basal ACA at 2 minutes following epinephrine injection (Figure 10). ACA returns toward normal by 15 minutes.

Basal hepatic ACA in shock was not significantly different from normal basal ACA. However, the normal increase is completely abolished in shock ($p < .025$). There is no significant difference between control and shock by 15 minutes following epinephrine injection.

Changes in intestinal ACA following *in vivo* epinephrine pulse are seen in Figure 11.

Control ACA is increased by 27 and 15% respectively at 2 and 15 minutes after epinephrine.

Basal ACA in shock is not significantly different from control ACA. However, the response is abolished ($p < .01$) in the shock tissue.

(iii) Norepinephrine

The response of AC to *in vivo* norepinephrine injection into control and shock animals is presented in Figures 12 and 13. In Figure 12 it can be seen that there is no change in hepatic ACA following norepinephrine injection in normal animals. In shock, however, basal ACA is slightly reduced from control and shows a gradual increase to a significant ($p = .05$) 52% increase above basal

FIGURE 10

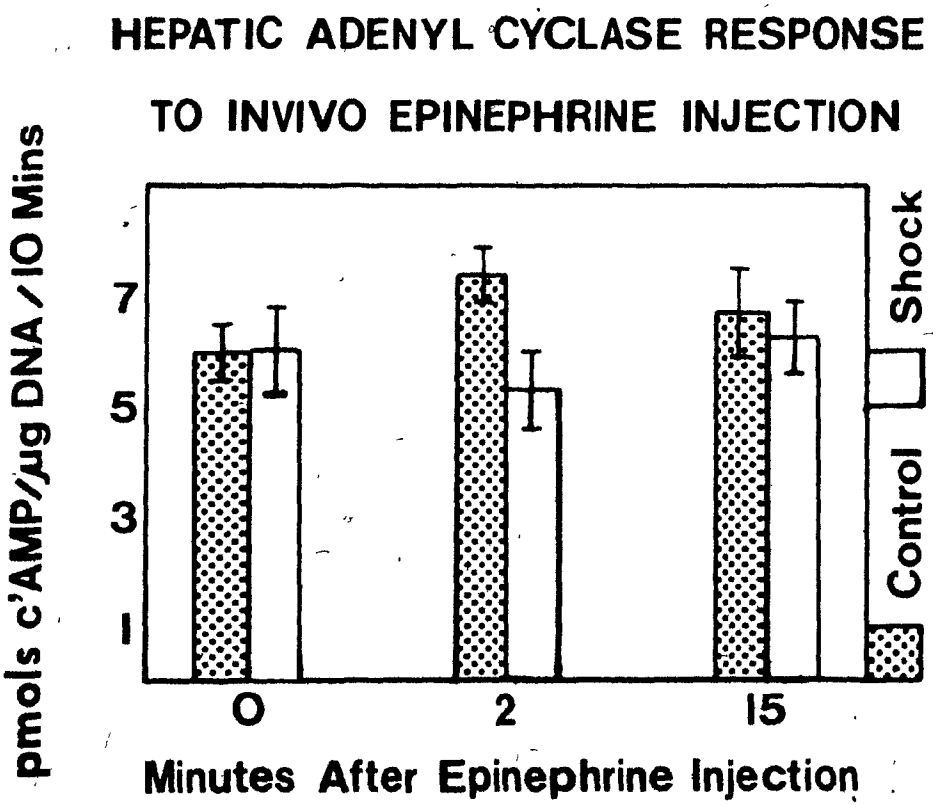

HEPATIC ADENYL CYCLASE RESPONSE
TO INVIVO EPINEPHRINE INJECTION
pmols c'AMP/µg DNA / 10 Mins
7
5
3
1
0
2
15
Minutes After Epinephrine Injection
Shock
Control

FIGURE 11

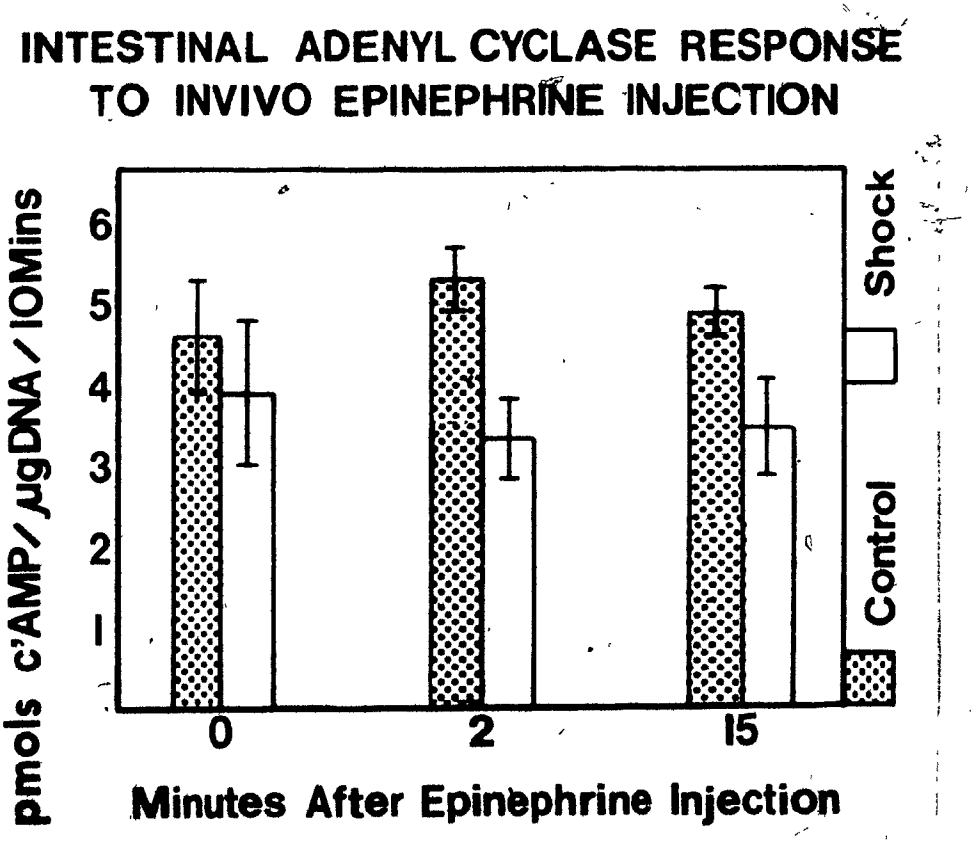

INTESTINAL ADENYL CYCLASE RESPONSE
TO INVIVO EPINEPHRINE INJECTION
pmols c'AMP/µgDNA/10Mins
6
5
4
3
2
1
0
2
15
Minutes After Epinephrine Injection
Shock
Control

FIGURE 12

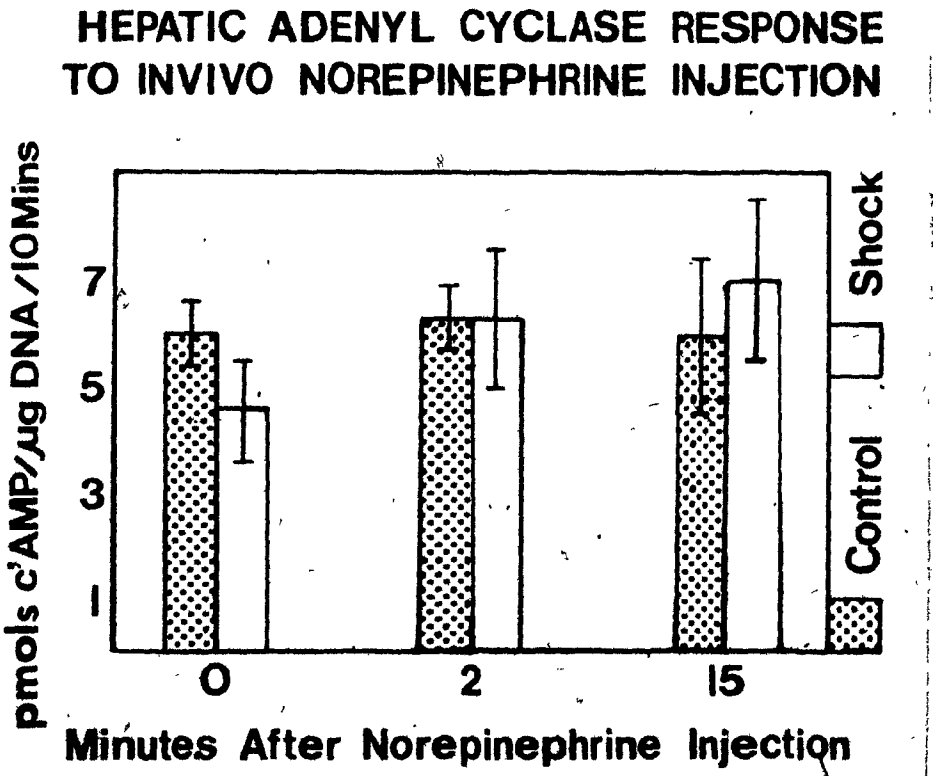
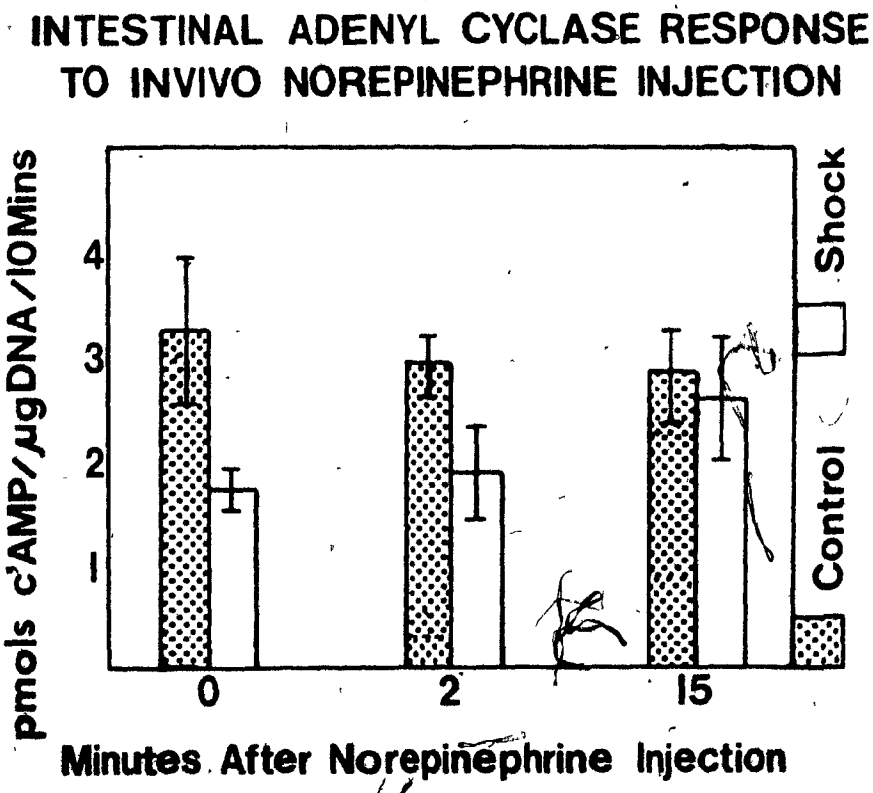

HEPATIC ADENYL CYCLASE RESPONSE
TO INVIVO NOREPINEPHRINE INJECTION
pmols c'AMP/µg DNA/10Mins
1
3
5
7
0
2
15
Minutes After Norepinephrine Injection
Shock
Control

FIGURE 13


INTESTINAL ADENYL CYCLASE RESPONSE
TO INVIVO NOREPINEPHRINE INJECTION
pmols c'AMP/µgDNA/10Mins
4
3
2
1
0
2
15
Minutes After Norepinephrine Injection
Shock
Control

levels.

Basal ACA and that seen at 2 minutes in the shock intestine (Figure 13) are significantly ($p < .05$) reduced from their respective control values. No pattern of response to norepinephrine appears in either control or shock intestinal tissue.

Discussion:

Glycogenolysis and hyperglycemia in response to epinephrine (87, 333) and glucagon (87, 333) are well known to be mediated by the adenyl - cyclase - cyclic AMP - phosphodiesterase system. Since both the liver (12, 36, 88, 196) and intestine (196, 224, 351) have been shown to be major sources of plasma cyclic AMP following epinephrine or glucagon infusion, the response of adenyl cyclase in these splanchnic organs following in vivo injection of epinephrine, glucagon or norepinephrine was studied in normal dogs. In shock, the hyperglycemic response to epinephrine was abolished while that to glucagon was attenuated - it was of interest to see if these changes were reflected by parallel changes in the AC response to these hormones in shock dogs.

The results obtained in the hepatic and intestinal adenyl cyclase assay are expressed as pmols cyclic AMP/µg DNA/10 minutes. Most investigators express ACA per mg of tissue protein. The reason for the use of DNA is primarily because mucus in the intestine and mucous discharge contributes significantly to the weight of the tissue. Mucus

is also protein - containing and therefore may not truly represent the cellular component of the tissue. The DNA, however, is constant in each tissue and always represents the cellular component of the tissues.

Basal ACA in both liver and intestine, when appropriate conversion factors are employed, compare favorably with values obtained in the same tissues by several other investigators (101, 175, 178, 260, 318).

Glucagon produces greater increases in hepatic glucose output than does epinephrine and it might also be expected that glucagon produces greater increases in hepatic adenyl cyclase activity than does epinephrine. Indeed, this is the case. The maximal increase in ACA following glucagon injection is almost 50% greater than that seen following epinephrine injection in normal animals (Figures 8 and 10).

Despite the fact that in shock the basal hepatic ACA is unchanged from control levels, the ability of this enzyme to respond to hormonal stimulation is reduced following glucagon and abolished following epinephrine (Figures 8 and 10). Thus, the reduction and abolition of the hyperglycemic responses to glucagon and epinephrine respectively are accompanied by parallel losses in the adenyl cyclase sensitivity to these hormones.

Rutenberg et al (289) have shown a significant 29% reduction in basal hepatic ACA in hemorrhagic shock. Indeed, in the shock group of the norepinephrine series in this study (Figure 12) basal hepatic ACA is significantly reduced by 24%. However, when data

from all three series were grouped (Table II), the decreases in basal ACA were not significant. Thus, an altered response may be associated with a normal - looking basal enzyme activity. It is crucial therefore, to determine the ability of the enzyme to respond to stimulation. This will indicate whether there is a serious alteration in biochemical and thus physiologic response.

Intestinal adenyl cyclase shows little response to glucagon injection in normal or shock (Figure 9) animals. Klaeveman et al (178) also failed to find any effect of glucagon upon normal human jejunal mucosa. Schwartz et al (312) have stated that there is lack of response because the intestine lacks receptors with any significant affinity for glucagon. On the other hand, there appears to be a significant number of receptors in the intestinal tissue for epinephrine. This is evident from the significant increase in intestinal ACA following epinephrine injection (Figure 11). The physiologic significance of this particular response is unknown at this time. However it may possibly be associated with a stimulation of the active secretory process for ions and water in the mucosa of the small intestine (95). In shock, the adenyl cyclase response is abolished (Figure 11) and might possibly be related to the decrease in intestinal absorption of ions and water that is known to occur in early hemorrhagic shock (144).

Catecholamines also have the ability to decrease the level of cyclic AMP in some cells. The receptors responsible for this type of effect have the characteristics of α-adrenergic receptors. The

possibility that α-adrenergic effects might be mediated by a decrease or unchanged ACA has been discussed by Robison (284, p. 225 Chapt. 6). Thus, the lack of hepatic or intestinal AC response to norepinephrine might be expected (Figures 12 and 13). The significant decreases in basal ACA in shock, may be related to damage of the AC enzyme induced by the ischemia of shock.

Adenyl Cyclase Response To In Vitro Hormone Addition - Control And Shock:

(i) Glucagon

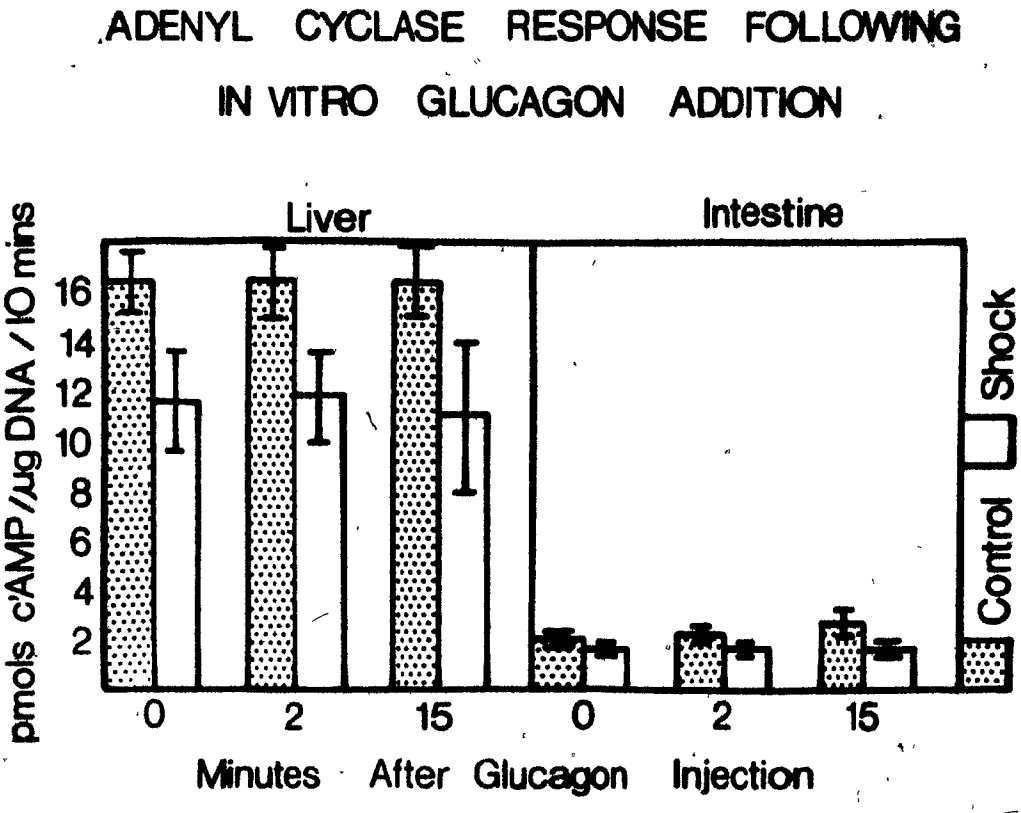
The response of hepatic and intestinal tissue to the in vitro addition of 5 nanomoles of glucagon is seen in Figure 14.

Addition of 5 nanomoles of glucagon in vitro to the incubation mixture containing hepatic tissue increases basal ACA from 6.64 ± 0.69 to 16.43 ± 1.27 pmols/µg DNA/10 minutes. Addition of 5 nanomoles in vitro into the incubation medium of tissue taken from animals at 2 and 15 minutes following in vivo glucagon injection still produces a final ACA of 16.34 ± 1.27 pmols/µg DNA/10 minutes. These results indicate that the in vitro addition of 5 nanomoles of glucagon is saturating all the available glucagon receptor sites in the 200 microlitre volume of hepatic homogenate. The effects of in vitro and in vivo glucagon are not additive.

The in vitro stimulation of basal ACA in shock is decreased from control by a significant 29%; that is from 16.43 to 11.63 ± 2.06 pmols/µg DNA/10 minutes. This 11.63 value appears to be the site saturation level in the shock tissue as there is no difference from this value even in tissues obtained at 2 and 15 minutes following in vivo glucagon injection.

There is no change in basal ACA when glucagon is added in vitro into intestinal homogenates from normal animals. In shock, however, the in vitro - stimulated intestinal ACA is significantly reduced from the control value both before ($p < .05$) and at 2 ($p < .025$) and 15 minutes ($p < .01$) after in vivo glucagon injection.

FIGURE 14


ADENYL CYCLASE RESPONSE FOLLOWING
IN VITRO GLUCAGON ADDITION
Liver
Intestine
pmols cAMP/µg DNA / 10 mins
16
14
12
10
8
6
4
2
0
2
15
0
2
15
Minutes After Glucagon Injection
Shock
Control

(ii) Epinephrine

The response of the liver and intestine to *in vitro* addition of 5 nanomoles of epinephrine is seen in Figure 15.

Addition of 5 nanomoles of epinephrine to control homogenates of hepatic tissue produces a 2.85 fold increase ($p << .001$) over basal adenyl cyclase activity. That is, from 5.92 ± 0.54 to 16.85 ± 2.07 pmols cyclic AMP/µg DNA/10 minutes. This value does not change when 5 nanomoles of epinephrine is added to hepatic homogenate obtained at 2 and 15 minutes after *in vivo* epinephrine injection.

The *in vitro* epinephrine - stimulated ACA in shock is significantly reduced ($p = .05$) by 40% from the respective control value. That is from 16.85 ± 207 to 10.08 ± 1.78 pmols cyclic AMP/µg DNA/10 minutes. This value does not change in tissues obtained following *in vivo* epinephrine injection.

Addition of 5 nanomoles of epinephrine to control homogenates of intestinal mucosa produces a 1.2 fold increase in ACA over basal activity. This remains unchanged in tissue taken following epinephrine injection.

The *in vitro* stimulated ACA in shock is significantly reduced ($p < .02$) by 26% from control levels. That is from 5.51 ± 0.73 to 4.07 ± 0.81 pmols cyclic AMP/µg DNA/10 minutes. This value remains unchanged when epinephrine is added to tissues obtained at 2 and 15 minutes following epinephrine injection.

FIGURE 15

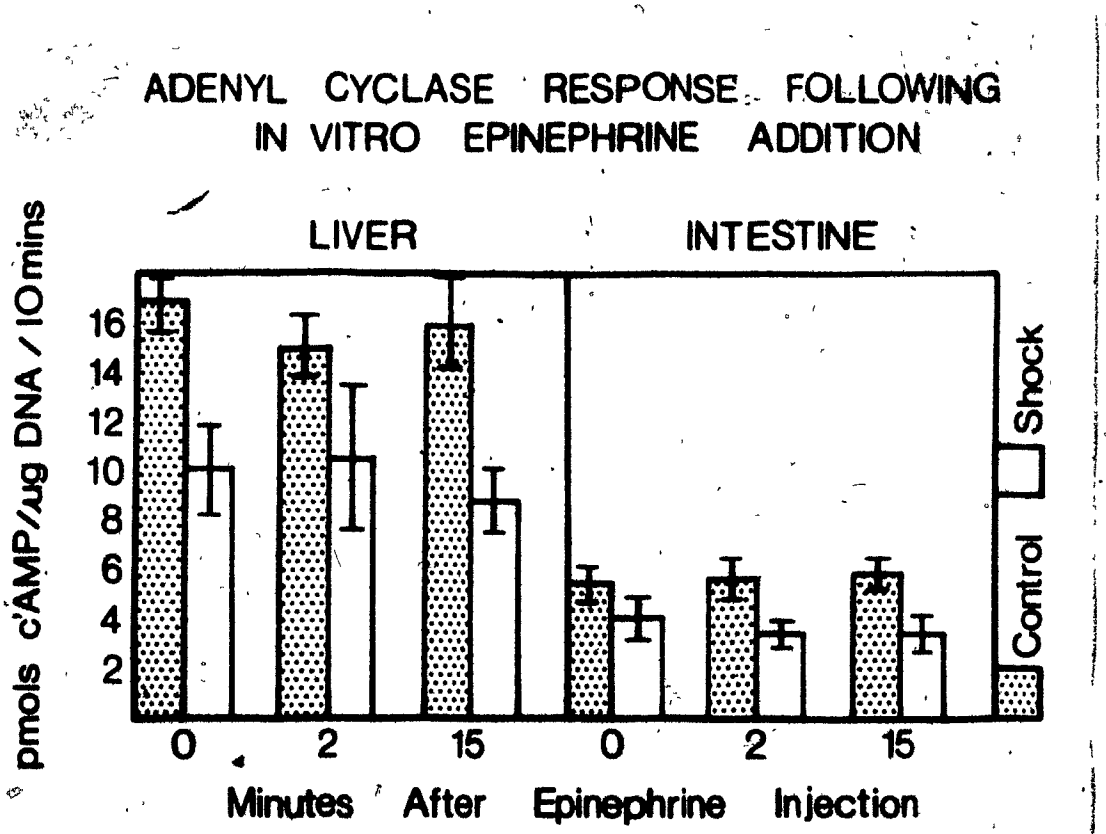
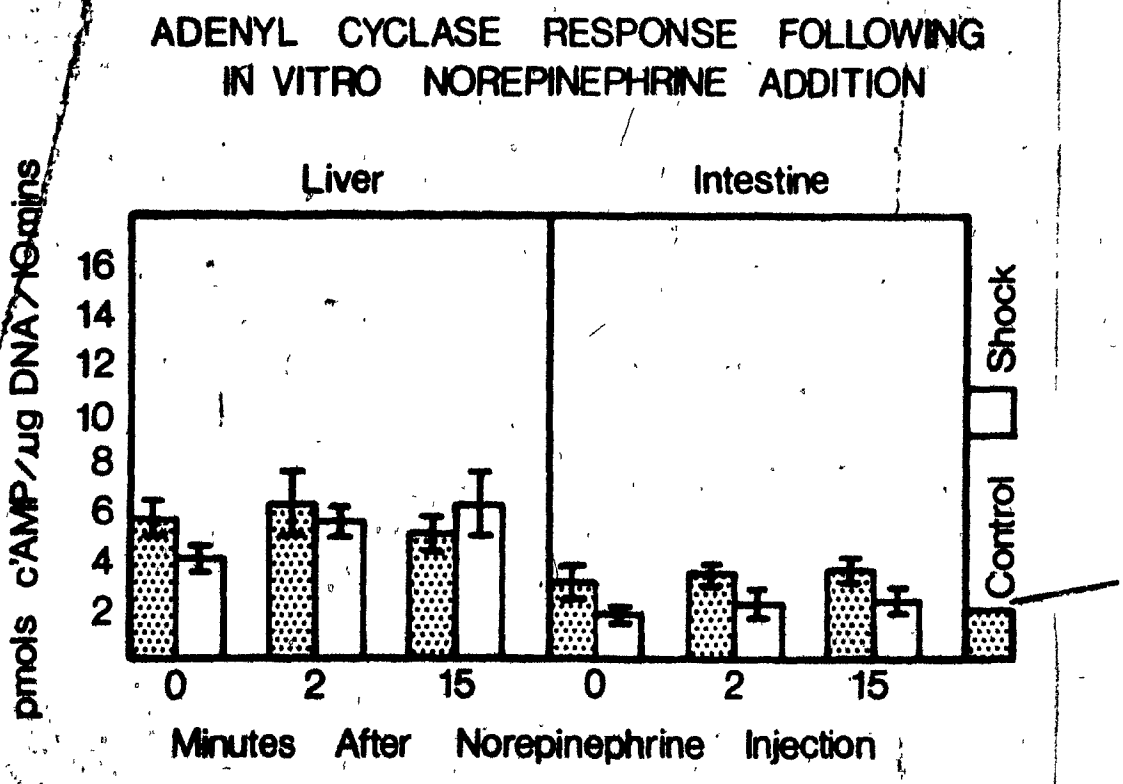

ADENYL CYCLASE RESPONSE FOLLOWING
IN VITRO EPINEPHRINE ADDITION
LIVER
INTESTINE
pmols c'AMP/ug DNA / 10 mins
16
14
12
10
8
6
4
2
0
2
15
0
2
15
Minutes After Epinephrine Injection
Shock
Control

FIGURE 16


ADENYL CYCLASE RESPONSE FOLLOWING
IN VITRO NOREPINEPHRINE ADDITION
Liver
Intestine
pmols c'AMP/µg DNA/30 mins
16
14
12
10
8
6
4
2
0
2
15
0
2
15
Minutes After Norepinephrine Injection
Shock
Control

(iii) Norepinephrine

Hepatic and intestinal responses to *in vitro* addition of 5 nanomoles of norepinephrine are seen in Figure 16.

Addition of *in vitro* norepinephrine to normal hepatic homogenates produces a 6% decrease in basal ACA. This response remains the same in hepatic tissue taken after *in vivo* norepinephrine injection.

There is no difference between control and shock tissue responses to *in vitro* norepinephrine addition.

A similar pattern is seen in the intestinal tissue, however *in vitro* stimulated ACA is significantly reduced from control in the shock tissue obtained before ($p < .05$) and 15 minutes ($p < .025$) after *in vivo* norepinephrine injection.

Discussion:

The data so far indicate a decrease in the ability of the splanchnic second messenger system to respond to hormonal stimulation in experimental hemorrhagic shock. The decreased response is reflected by the altered hepatic blood glucose response curve following glucagon and epinephrine injections.

The circulation to and within the splanchnic organs is severely compromised in hemorrhagic shock. The question arises as to whether the defects observed are a direct result of a decreased delivery of the hormone to the cells of the target tissue; or perhaps due to severe ischemic damage to the cellular membrane-bound enzyme.

Additional studies were therefore carried out in which the hormone to be studied was added directly into the tissue homogenates made from biopsies taken from the animals just prior to, and following hormone injection. The purpose was to investigate the adenyl cyclase response in normal and shock tissues when hormone contact with the target cell was assured in vitro. This was done by adding equal amounts (5×10^{-9} moles/500 µl incubation volume) (10^{-5}M) of glucagon, epinephrine or norepinephrine directly into the AC incubation medium.

The results obtained by others using similar methods of in vitro addition of hormones to hepatic tissue homogenates is variable. The % increase over basal ACA following in vitro addition of various concentrations of epinephrine ranges from 53 to 230% (100, 271, 291). In vitro addition of glucagon is associated with increases of 60 to 300% over basal AC levels (271, 285, 289).

Stimulation of ACA upon in vitro addition of epinephrine appears to be maximal (Figure 15); the stimulated ACA seen in animals who have received in vivo injection of epinephrine (Figure 10), does not increase to a level higher than that seen in the animals who have received the hormone in vitro. This, most likely, is due to the fact that the amount of hormone added in vitro is saturating all the AC receptors specific for epinephrine. Therefore further addition of epinephrine - in the form of an in vivo injection - will not alter an already maximally activated enzyme. The same is true for glucagon (Figures 14 and 8). Furthermore, Ray et al (271), using rat liver,

have indicated that concentrations of glucagon and epinephrine of 10^{-6} M and 10^{-5} M respectively are saturating for each hormone and will result in maximal ACA. Rodbell et al (285) indicate that for glucagon, the saturating concentration is even less, at $4 - 8 \times 10^{-8}$ M.

This also explains why the ACA is the same when equal amounts of either glucagon or epinephrine at saturating concentrations are added to an equal amount of tissue homogenate.

The fact that there is no change in basal intestinal ACA following in vitro (Figure 14) addition of glucagon is further evidence for the lack of glucagon receptors in the intestine.

Addition of epinephrine to intestinal homogenates is associated with a 28% increase in basal enzyme activity (Figure 15). This is the same % increase in basal ACA that was observed following in vivo injection of epinephrine (Figure 11). This would seem to indicate that 10^{-5} M epinephrine is a saturating concentration for intestinal as well as hepatic tissue. Furthermore an injection of 0.25 mg of epinephrine into a 17 kg animal would result in a dilution of the epinephrine to approximately 10^{-6} M. It may be that the saturating concentration of the intestine for epinephrine is less than that of the liver for epinephrine; since the in vitro addition of 10^{-5} M epinephrine produced a 185% increase, while in vivo injection produced only a 30% increase over basal ACA in the liver.

The discrepancy between the in vivo and in vitro responses to epinephrine in the liver may be explained by the fact that in vivo, the amount of hormone reaching the hepatic tissue is not as great

as that in the in vitro situation. In addition, the vasoconstrictor effects of epinephrine upon the vasculature may limit it's own delivery to the cells of the liver.

Glucagon has been shown to saturate hepatic adenyl cyclase at concentrations of $4 - 8 \times 10^{-8}$ Molar (285) which is within the range of concentration obtained by injection of 0.3 mg dose of glucagon into a 17 kg animal; and since glucagon has been shown to increase hepatic blood flow by 100% (180); the above explanations cannot account for the discrepancy between in vivo and in vitro responses of the hepatic AC system to glucagon (Figures 8 and 14).

The explanation may pertain to the fact that there is up to a 4 minute lag period before the in vivo glucagon - stimulated ACA reaches it's maximum (88, 286). This effect is dependent upon the concentration of glucagon in touch with the cell. The greater the glucagon concentration, the less the time lag (286). Therefore, since the tissue biopsy was obtained at 2 minutes following in vivo injection of glucagon, the ACA at this time, probably does not represent maximal activity. The in vitro measurement is obtained after 10 minutes of contact with a greater concentration of glucagon [than that in contact with the intact liver], and therefore represents a more close approximation of maximally stimulated activity.

There were no significant alterations in basal ACA in the liver or intestine following in vitro addition of norepinephrine (Figure 16). Furthermore, no changes in basal ACA were noted when norepinephrine was added in vitro to the tissues obtained following

in vivo norepinephrine injection. Norepinephrine in low doses, therefore, results in a nonspecific effect on AC in the liver and intestine. It may increase, decrease or produce no change in tissue ACA as seen in this study.

Shock is associated with significant depressions in both basal and norepinephrine *in vitro* - stimulated ACA before and after *in vivo* norepinephrine injection in the liver and intestine. The depressions in basal ACA in the liver and intestine of this group of animals may indicate that the extent of damage to the AC enzyme differs from that seen in the epinephrine or glucagon series of shock animals.

The response of the shock liver and intestine following *in vitro* addition of glucagon is (Figure 14) significantly reduced by 30 and 27% respectively. Rutenberg *et al* (289) have also reported a significant 50% reduction in the shock liver response to the *in vitro* addition of glucagon. There are no reports to date on the effects of *in vitro* addition of glucagon to shock intestine.

Hepatic and intestinal responses in shock to *in vitro* addition of epinephrine are significantly reduced by 40 and 27% respectively (Figure 15). These data indicate that even if the hormone is delivered in a normal fashion to the tissues in question, the ability of these tissues to respond normally to the hormones is significantly reduced in hemorrhagic shock.

Therefore the reductions in both the *in vivo* and *in vitro*

AC responses to the hormones are not totally a result of the compromised microcirculation, and reduced delivery of hormone to the tissues. However, the question arises as to why the membrane-bound enzyme is not functioning normally. Is there actual ischemic damage incurred by the enzyme? Is there a decreased affinity and therefore decreased binding of hormones to the β-receptors, providing less increase in adenyl cyclase activity?

Adenyl Cyclase Response To In Vitro Sodium Fluoride Addition - Control And Shock:

(i) Glucagon

The data obtained following the in vitro addition of 10 mM sodium fluoride (NaF) to control and shock tissue homogenates is presented in Figure 17.

Addition of 10 mM NaF to control hepatic homogenates before in vivo glucagon injection produces a 4 fold increase in adenyl cyclase activity over control basal ACA. This is from a basal ACA of 6.64 ± 0.69 to a NaF - stimulated ACA of 26.20 ± 4.69 pmols cyclic AMP/µg DNA/10 minutes. Following in vivo glucagon injection, there is little change in this value from the NaF - stimulated ACA obtained at "0" time.

There is a nonsignificant 30% decrease in the "0" - time NaF - stimulated ACA in shock. There is a slight increase in the NaF - stimulated ACA at 2 minutes following glucagon injection which falls slightly by 15 minutes.

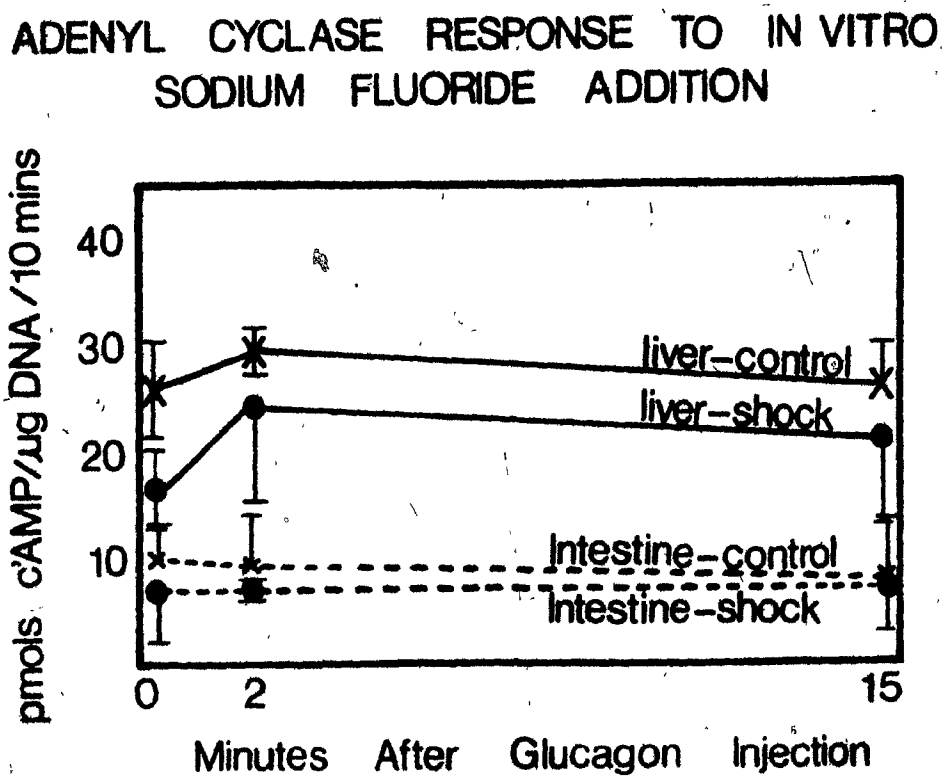
Addition of 10 mM NaF to control homogenates of intestinal tissue produces a 4.6 fold increase in basal ACA. This NaF - stimulated ACA does not change following glucagon injection.

NaF - stimulated ACA in the shock intestine (Figure 17) is reduced by 28% from the control value and remains only slightly lower (nonsignificant) than control values following glucagon injection.

(ii) Epinephrine

The response of AC to the in vitro addition of NaF in the epinephrine series of animals is presented in Figure 18.

FIGURE 17


ADENYL CYCLASE RESPONSE TO IN VITRO SODIUM FLUORIDE ADDITION
pmols cAMP/µg DNA /10 mins
40
30
20
10
liver-control
liver-shock
Intestine-control
Intestine-shock
0
2
15
Minutes After Glucagon Injection

The control hepatic tissue response to NaF addition to tissue obtained following epinephrine injection does not differ from that seen in the control glucagon series (Figure 17). However, in shock, the reductions in NaF - stimulated ACA are not as great in the epinephrine group as those seen in the shock - glucagon series; being significantly decreased ($p < .025$) at 2 minutes following epinephrine injection.

The response of the normal intestine to in vitro addition of NaF in the epinephrine series of animals (Figure 18) is not unlike that seen in the respective glucagon series. In shock however, the reductions in NaF - stimulated ACA are significant at 2 ($p < .025$) and 15 ($p < .025$) minutes following epinephrine injection.

(iii) Norepinephrine

The response of hepatic and intestinal tissue to in vitro addition of NaF in the norepinephrine series of control and shock animals is presented in Figure 19.

The normal liver responds to in vitro NaF addition in the same fashion as that seen in the glucagon and epinephrine series. In shock, NaF - stimulated ACA in the liver is significantly reduced from control levels before ($p < .025$) and at 2 minutes ($p < .05$) following norepinephrine injection.

The response of the intestinal tissue to the in vitro addition of NaF (Figure 19) in both the control and shock situations does not differ from that seen in the glucagon and epinephrine series. The

FIGURE 18

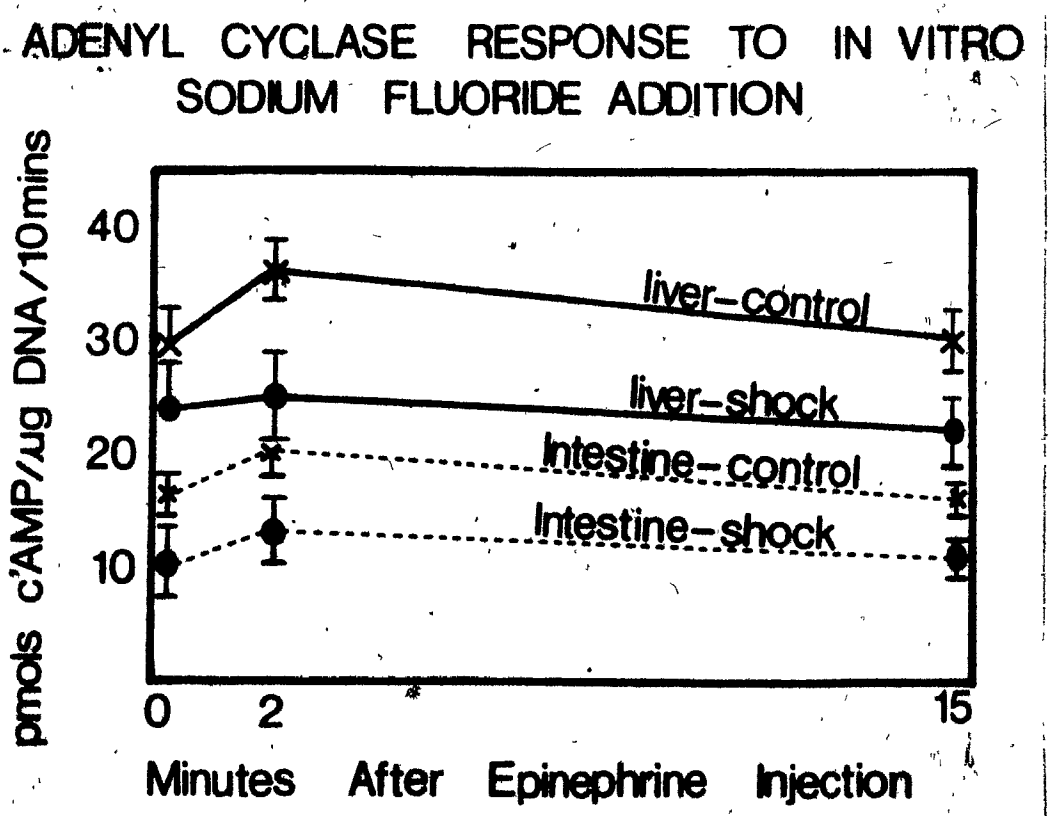
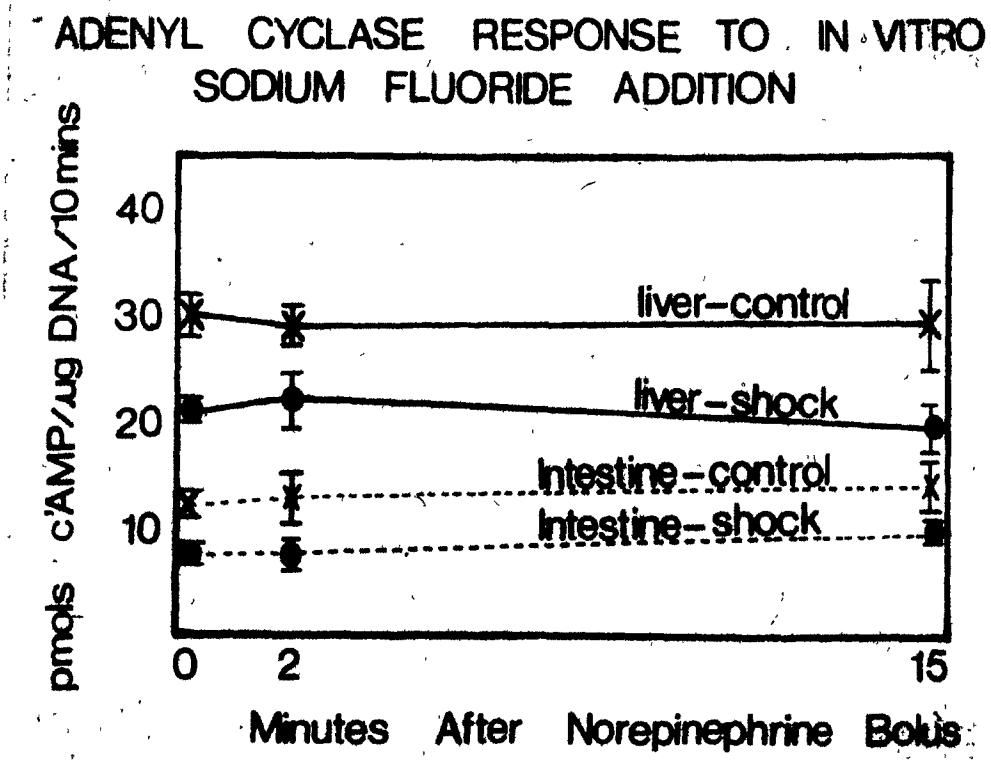

ADENYL CYCLASE RESPONSE TO IN VITRO SODIUM FLUORIDE ADDITION
pmols c'AMP/ug DNA/10mins
40
30
20
10
liver-control
liver-shock
Intestine-control
Intestine-shock
0
2
15
Minutes After Epinephrine Injection

FIGURE 19


ADENYL CYCLASE RESPONSE TO IN VITRO SODIUM FLUORIDE ADDITION
pmols c'AMP/ug DNA/10 mins
40
30
20
10
liver-control
liver-shock
Intestine-control
Intestine-shock
0
2
15
Minutes After Norepinephrine Bolus

NaF - stimulated ACA is significantly reduced before ($p < .005$) and 15 minutes ($p < .05$) after norepinephrine injection in the shock animals.

Discussion:

The membrane-bound adenyl cyclase enzyme is composed of two subunits; the receptor subunit and the catalytic subunit. It was of interest to study the defective AC response using this characteristic of the enzyme. In the face of impaired receptor function, the intrinsic ACA or the catalytic subunit function may be unchanged. This property of the enzyme may be studied without regard to receptor subunit influence with the aid of sodium fluoride (NaF) (20). This compound is a nonspecific stimulator of maximal ACA and works by stimulating only the catalytic subunit of the enzyme and only in broken cell preparations (336). Thus, the NaF - stimulated ACA represents the total amount of enzyme present (176). For example, increased tissue growth is associated with induction of AC synthesis and increases in NaF - stimulated ACA (243); whereas total NaF - stimulated ACA is not significantly different when added to hormone stimulated and unstimulated tissues (20).

Birnbaumer et al (20) found that in vitro addition of 10 mM. NaF to liver homogenates produced maximal stimulation of ACA - a 7 fold increase over basal hepatic ACA. Others (13, 20, 101, 268, 285(b), 289, 301, 345) have found between 3 and 10 fold increases over basal ACA when 10 mM NaF is added in vitro to various tissues. In this study 10 mM NaF was found to produce a consistent 5 fold

increase over basal hepatic ACA and a consistent 4 fold increase over basal intestinal ACA - data well within the limits reported by other investigators.

No change in the tissue response to NaF addition would be expected if the catalytic subunit is functioning normally, regardless of changes induced by shock upon the receptor subunit of the enzyme. A corollary to this statement is that, if in fact the intrinsic enzyme activity is intact, while receptor subunit - stimulated activity is altered in shock, the basal unstimulated enzyme activity may remain unchanged. Furthermore, Rethy et al (278) state that most investigators have observed that the fate of the NaF - stimulated ACA parallels that of the basal rather than the hormone - sensitive ACA.

Indeed, this is what was found. In the shock tissues where there are nonsignificant decreases in NaF - stimulated ACA, the basal ACA is unchanged from control values. However, when NaF - ACA is significantly reduced in shock (norepinephrine series - liver and intestine) so the basal ACA in the liver and intestine is also significantly reduced. The reductions in basal and NaF - stimulated ACA are comparable in the liver (24% decrease in basal, 29% decrease in NaF - stimulated ACA) and less so in the intestine (45% decrease in basal, and 35% decrease in NaF - stimulated ACA). It would appear then that only when adenyl cyclase function is very severely disrupted is the intrinsic or catalytic activity of the enzyme disturbed. This is evident in both the epinephrine and nor-

epinephrine series. In the glucagon series, where function is only slightly altered, there is no significant alteration in the catalytic function of the enzyme as determined with the in vitro addition of sodium fluoride. The structural integrity of the cell membrane has been altered in such a fashion as to result in significant losses of tissue responsiveness to hormonal stimulation at the receptor subunit level. However, the significant depressions in basal and NaF - stimulated ACA represent losses in the total amount of functional AC - representing more severe damage which includes both receptor and catalytic subunit function of the AC enzyme.

The data so far have shown that the response of the splanchnic tissue adenyl cyclase enzyme to both in vivo and in vitro hormonal stimulation is significantly reduced in hemorrhagic shock. Further investigation of the enzyme has revealed that of the two subunits of which AC is composed, there appears to be a defect in the hormone receptor site, while the catalytic subunit function remains normal. However, when hormone - stimulated receptor function is totally abolished, the catalytic function of AC also is altered.

General Discussion:

Speculation about the nature of the adenyl cyclase defect in hemorrhagic shock can lead in numerous directions.

ATP is the substrate for the adenyl cyclase reaction. The well documented (50, 152, 176) decreases in tissue ATP levels in shock might account for the observed reductions in ACA.

McArdle et al (224) and Rutenberg et al (288) have shown that there is a parallel decrease in intestinal and hepatic ATP and cyclic AMP levels in hemorrhagic shock in dogs. Since the ATP used for cyclic AMP production could be derived from a restricted pool (compartmentalized) (203) which may be more susceptible to fluctuations than total tissue ATP (277) - the possibility that substrate depletion accounts for decreases in ACA cannot be ignored. However, the Km of the adenyl cyclase enzyme for ATP is $1 - 5 \times 10^{-4}$ M (13). This is at least one order of magnitude lower than the concentration of ATP normally found in the liver or intestinal tissue (140). It is possible therefore, that the minimal decreases in tissue ATP observed in this study would not compromise the Km for AC. In addition, the particular assay for AC utilized in this study employs an in vitro addition of 2 mM ATP coupled with an ATP - regenerating system which allows for recovery of 80% of the initially added ATP at the termination of a 10 minute incubation period (21). The possibility that the crude tissue homogenates employed in this study are contaminated with ATP'ase - another source for ATP reduction - should also

be considered. However, it has been shown (280) that ischemia in the intestine is associated with a significant decrease in homogenate ATP'ase levels. Although it has not been studied, hepatic ATP'ase levels may also be reduced during ischemia.

Thus, the decreased tissue ATP and AC function may represent concomittant metabolic derangements which are characteristic of shock tissues.

Liver glycogen levels are relatively depleted in hemorrhagic shock (24, 151). A depleted glycogen store might exert a form of product inhibition upon hormone - stimulated increases in ACA. However, this cannot account for the abolished intestinal epinephrine - stimulated AC response, since the intestine does not contain glycogen stores. In addition, hepatic AC function is not only aimed at glycogen breakdown and glucose release. Therefore, it seems unlikely that a product inhibition could totally abolish epinephrine - stimulated increases in hepatic ACA. Furthermore, the hepatic blood glucose levels rose following glucagon injection in shock.

Changes in the intracellular ionic environment are well known to occur in shock tissues. As the mitochondria fail, and ATP levels fall, there is intracellular accumulation of such ions as sodium, chloride, calcium and water (48, 151, 295, 323) with an associated loss of potassium and magnesium (231, 344). These changes are accompanied by an increased intracellular pH (336, 345) and cellular swelling.

Beecher et al (18) have determined that there is a direct relationship between increased extracellular magnesium and the severity of shock in humans. Furthermore, Rodbell et al (286) state that if ATP is not complexed with magnesium, it may in fact inhibit adenyl cyclase. Excessive intracellular calcium has also been shown to exert an inhibitory influence upon ACA (260, 270, 327). Thus, massive losses and gains of intracellular magnesium and calcium respectively, in shock, may effect severe reductions in in vivo AC responsiveness.

These factors have been taken into account; 5 mM $MgCl_2$ is added in vitro into the assay for AC, thus providing the necessary magnesium. In addition, the Tris buffer is used in order to buffer the pH changes within the tissue homogenates. Therefore in the in vitro assay of AC most of the ionic and pH derangements are minimized and probably do not account for the decreased hormonal responsiveness.

GTP (Guanosine triphosphate) has been shown by Rodbell et al (286) and Salomon et al (292) to be a regulator, modulator or allosteric effector of hormone - stimulated ACA. Thus, in the absence of GTP, epinephrine and glucagon stimulation of ACA is not expressed maximally. In addition, GDP (Guanosine diphosphate) has been shown to inhibit ACA (286). In shock, GTP and GDP levels have not specifically been measured. Changes in these nucleotide levels therefore may also contribute to the altered AC response in shock.

The observed AC defect in shock appears initially to be associated with an alteration in the epinephrine and glucagon or β-

receptor sites in the hepatic and intestinal tissue. Others (14, 51, 52, 288, 289, 290) have made similar observations in that the shock tissues studied appear to be "resistant" to hormonal stimulation. Baue et al (14) noted the decreased transport and utilization of glucose in shock muscle, despite the high circulating levels of glucose and insulin. Rutenberg et al (288, 289) also found decreased hepatic AC response to in vivo injection and in vitro addition of glucagon or epinephrine in shock rabbits. However, Baue et al (14) have determined that the attachment or binding of the hormones to the receptor site is not impaired in shock. Furthermore, it has been shown by Lefkowitz (190) and Levey (193) that solubilization of the cell membrane phospholipids, destroys hormonal sensitivity leaving basal and NaF - stimulated ACA essentially unchanged. However, it was determined that reduced hormone sensitivity caused by exposure to phospholipases, is due, in part, to a reduced ability of the hormone to bind to the β-adrenergic receptors as assessed using (—) - (^{3}H) alprenolol (201). It would appear, that the structure of the receptor subunit is in some way altered so as the transducer effect following hormone binding is reduced. Whether or not the binding is altered for epinephrine and glucagon in shock has not been assessed.

Altered hormonal responses may reflect damage incurred by the cellular membrane structure induced by ischemia. Damage to structure and enzymatic function of cell membranes is well documented in shock (31, 68). It is also well known that cellular swelling and

increased permeability occur in shock and trauma (19, 151).

Adenyl cyclase is a membrane-bound enzyme and it is dependant upon the structural integrity of the membrane for optimum enzymatic function. Structural and functional membrane changes induced by cellular ischemia, swelling and altered permeability could therefore induce abnormalities in the function of AC.

In a related study done in this laboratory (170) and subsequently in another laboratory (Robinson, J.W.L., Personal Communication), reductions in ATP and cyclic AMP levels coupled with AC malfunction were observed in the hypoxic proximal portion of the obstructed intestine. The structural and/or functional changes in cellular membranes induced by hypoxia and ischemia do in this case produce abnormalities in the membrane-bound AC enzyme. This then, would thwart attempts on the part of the hormones to maintain or regain homeostasis in shock.

These changes, however, do not account for the differences in the extent of change in the AC response between epinephrine and glucagon. The fact that in shock, the AC response is more reduced following epinephrine injection than it is following glucagon injection may be related to the phenomenon of desensitization. Tolerance, desensitization or subsensitivity to certain drugs and hormones has long been observed in both clinical and laboratory settings (125). Ho and Sutherland (150) and others (22, 33, 69, 274, 340) have reported that exposure to high levels of a β-adrenergic drug renders a tissue less responsive to the same stimulator several hours later. Observations

in man (35, 196) and experimental animals (43, 65, 88, 174, 209, 224, 259) have shown that there is a tendency for hepatic cyclic AMP production to decline from an earlier peak during sustained epinephrine or glucagon infusion. Similar findings were obtained with thyroid tissue and TSH (325), adipose tissue and epinephrine (300) and myocardial tissue with epinephrine and norepinephrine (11). Furthermore, in rats with persistent hyperglucagonemia induced by infection, hepatic cyclic AMP response to exogenous glucagon is blunted (363), with no change in basal or NaF - stimulated ACA (191, 236, 245, 275). These findings are similar to what was seen in this study. Furthermore, the reductions in cyclic AMP levels were associated with a blunted AC response to subsequent epinephrine or glucagon challenge. Reports indicate up to 70% reductions in epinephrine - responsive ACA (236) and 30 - 40% reductions in glucagon responsive ACA (69).

Throughout the hypovolemic period and then again in late refractory shock when the blood pressure is again falling, the epinephrine receptors are bombarded with up to 100 fold increases in the circulating levels of epinephrine. Glucagon receptors, however, are not exposed to such excessive amounts of glucagon. Since epinephrine and glucagon have distinct receptors in the liver and only epinephrine receptors in the intestine, it is conceivable that such reductions in responsiveness to further hormone challenge could occur.

Ho et al (150) have recently postulated that a feedback regulator is responsible for the phenomenon of hormonal desensitiza-

tion. Repeated addition of epinephrine and glucagon or ACTH to liver or adrenal glands produced between 50 and 70% decreases in subsequent responses to these hormones. Basal ACA remained unchanged. The inhibition of subsequent responses was found to be due to the intracellular generation of a so-called feedback regulator during initial stimulation. Feedback regulator has since been found in the circulating blood of humans (149). It is thought that the feedback regulator acts at a site beyond that of the actual hormone binding (perhaps it acts upon the phospholipids of the cell membrane).

Other possibilities must not be excluded such as: accumulation of unidentified extracellular inhibitors of hormone action (150); local generation of peptide inhibitors of ACA (21); intracellular or extracellular formation of adenosine (93); increased local hormonal degradation (285) and numerous others.

Selective alterations in tissue responsiveness may serve to modulate cellular cyclic AMP generation and alter hormonal sensitivity during periods of excessive and sustained hormonal stimulation.

Undoubtedly, a compromised circulation such as that seen following hemorrhagic shock reduces normal cellular activity in the splanchnic organs. Delivery of nutritional substances, hormones or drugs may be prevented, contributing further to the ischemic damage incurred by the cell membrane; the structural integrity of which optimum adenyl cyclase activity depends. It appears that the enzyme system is defective in tissues from shocked animals since the response

to both *in vivo* and *in vitro* hormone stimulation is markedly depressed. Thus, hormonal sensitivity of the splanchnic second messenger system is significantly reduced in shock, reflecting the impaired response to homeostatic control at the cellular level.

Glucocorticoids and Glucose Response Curves:

(i) Glucagon

The effect of glucocorticoid administration upon the hepatic blood glucose response curve following in vivo glucagon injection is presented in Figure 20.

There are nonsignificant elevations in the normal hepatic blood glucose response curve following glucagon injection into normal animals treated for 4 hours with pharmacological doses of glucocorticoid. However basal or resting blood glucose levels are significantly increased ($p < .05$) by 24%.

Glucocorticoid treatment in shock produces a significant ($p < .02$) elevation in the baseline blood glucose level over untreated shock. However the response curves are not significantly different.

The effect of glucocorticoid administration on the portal blood glucose levels following glucagon injection are presented in Figure 21.

The portal blood glucose levels following glucagon injection in glucocorticoid-treated normal and shock animals follows a similar pattern as that seen in the hepatic blood, however no significant increases are seen.

(ii) Epinephrine

The effect of glucocorticoid administration on the hepatic blood glucose response curve following in vivo injection of epinephrine is presented in Figure 22. Glucocorticoid administration produces

FIGURE 20

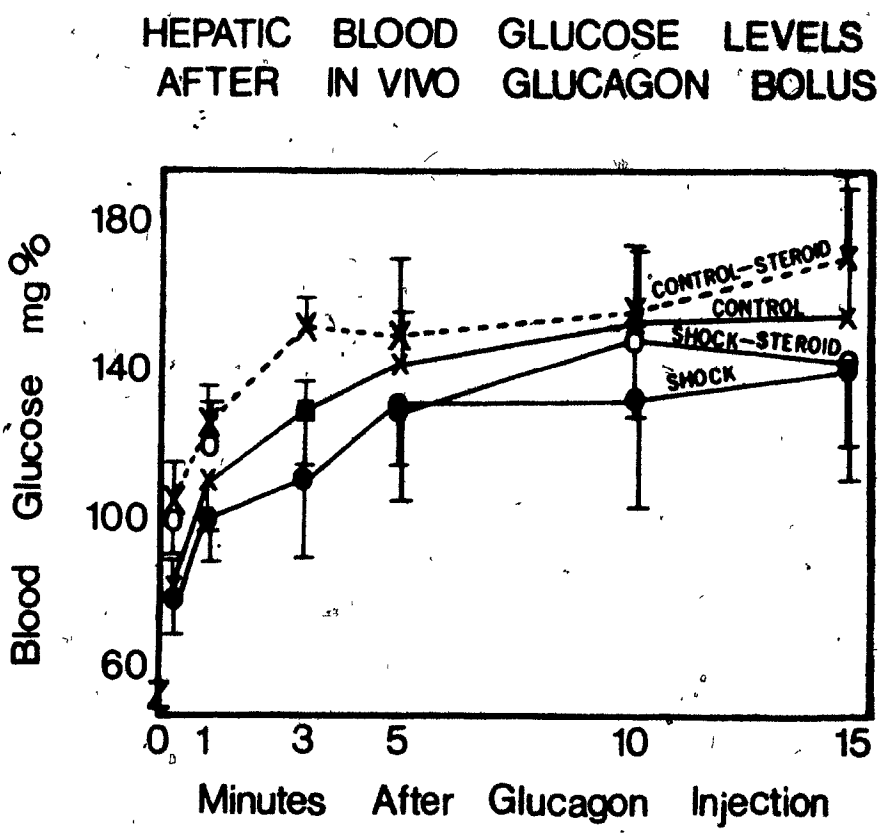

HEPATIC BLOOD GLUCOSE LEVELS
AFTER IN VIVO GLUCAGON BOLUS
Blood Glucose mg %
180
140
100
60
CONTROL-STEROID
CONTROL
SHOCK-STEROID
SHOCK
0
1
3
5
10
15
Minutes After Glucagon Injection

FIGURE 21

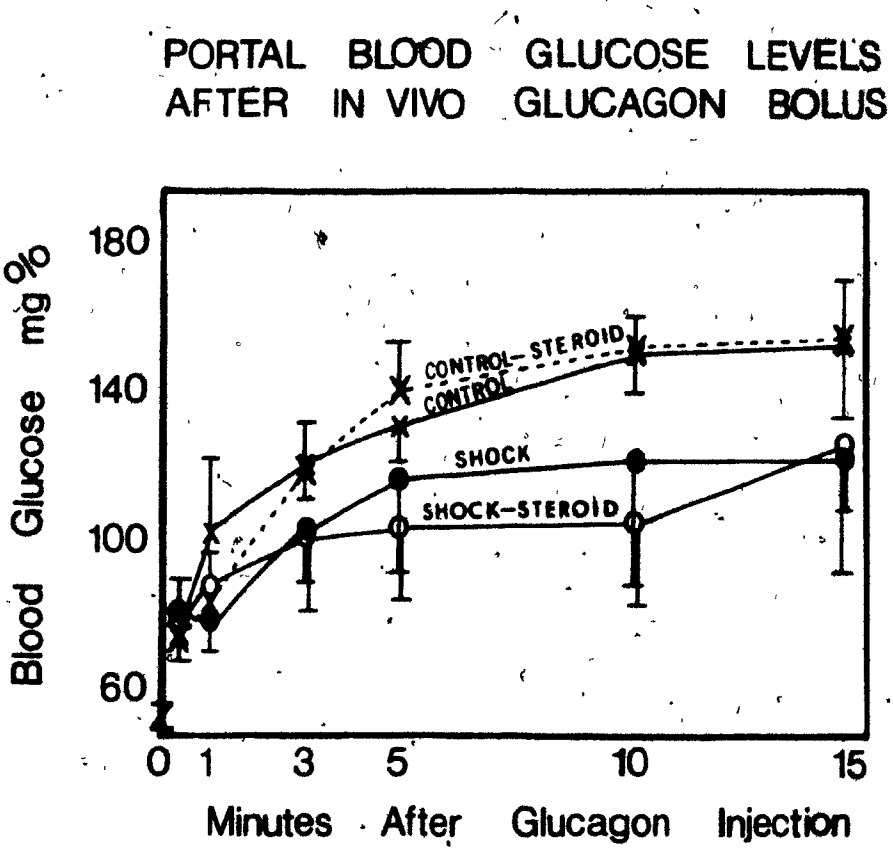

PORTAL BLOOD GLUCOSE LEVELS
AFTER IN VIVO GLUCAGON BOLUS
Blood Glucose mg %
180
140
100
60
CONTROL-STEROID
CONTROL
SHOCK
SHOCK-STEROID
0
1
3
5
10
15
Minutes After Glucagon Injection

FIGURE 22

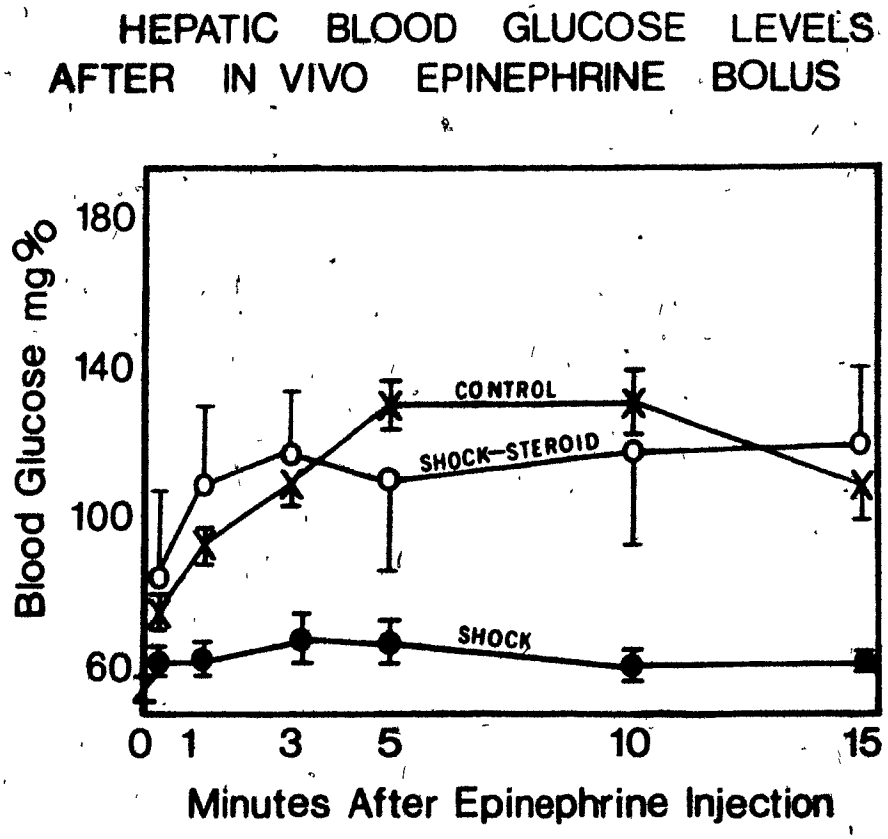
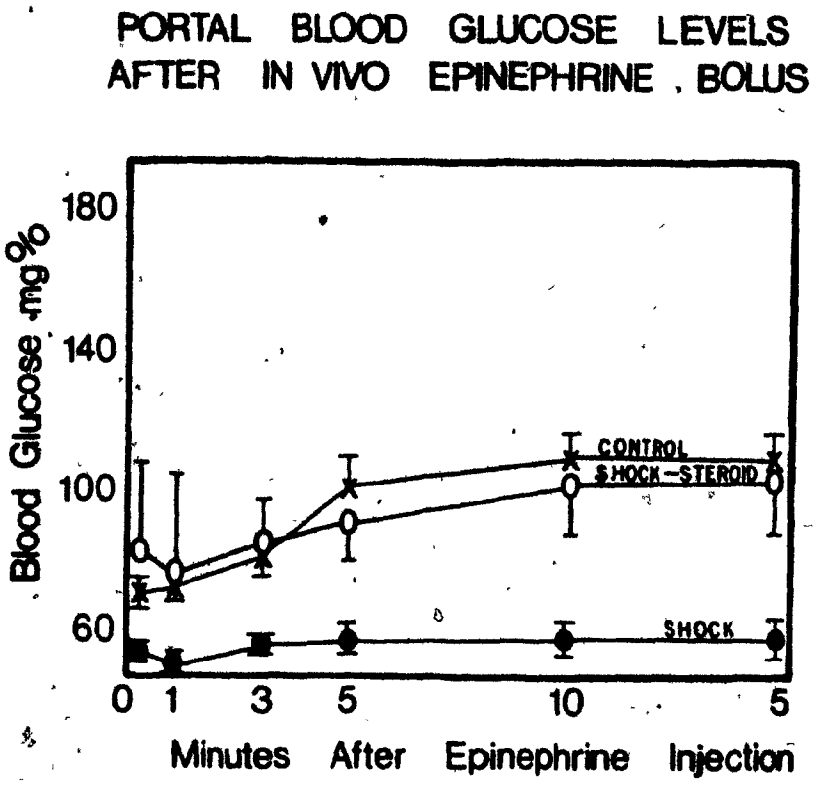

HEPATIC BLOOD GLUCOSE LEVELS
AFTER IN VIVO EPINEPHRINE BOLUS
Blood Glucose mg%
180
140
100
60
CONTROL
SHOCK-STEROID
SHOCK
0
1
3
5
10
15
Minutes After Epinephrine Injection

FIGURE 23


PORTAL BLOOD GLUCOSE LEVELS
AFTER IN VIVO EPINEPHRINE . BOLUS
Blood Glucose -mg%
180
140
100
60
CONTROL
SHOCK-STEROID
SHOCK
0 1 3 5 10 5
Minutes After Epinephrine Injection

a nonsignificant 42% increase in the basal resting blood sugar level over the slightly depressed shock level. Furthermore, it can be seen that the blood glucose response curve in the shock animals treated with glucocorticoids has been restored to the control pattern. Thus, is significantly elevated from the respective shock levels at 3 ($p < .02$), 5 ($p < .05$), 10 ($p < .01$) and 15 ($p < .001$) minutes following epinephrine.

A similar restoration of response is reflected in the portal blood glucose levels.(Figure 23).

Note that portal blood glucose levels are lower than their respective hepatic levels by 8 to 29%.

Glucocorticoids and Adenyl Cyclase Response:

(i) Glucagon

The effect of glucocorticoid administration on hepatic adenyl cyclase response to in vivo glucagon injection is presented in Figure 24.

It can be seen in Figure 24 that glucocorticoid administration to normal animals produces a 68% increase in basal ACA over untreated control basal ACA. There is no significant difference in AC response to glucagon injection between controls and steroid treated controls.

There is a significant ($p < .05$) depression in AC response at 2 minutes following glucagon injection in the untreated shock animals compared to the control-steroid animals. However, in the steroid treated shock animals the AC response at 2 minutes following glucagon injection is slightly reduced from the respective control value (not significant).

The effect of glucocorticoid administration on the intestinal AC response to in vivo glucagon injection is presented in Figure 25.

It can be seen that glucocorticoid treatment produces no significant changes in either control or shock intestinal AC response to in vivo glucagon injection.

(ii) Epinephrine

The effect of glucocorticoid administration in shock upon hepatic adenyl cyclase response to in vivo epinephrine injection is presented in Figures 26 and 27.

It can be seen in Figure 26 that steroid administration in shock animals produces small decreases in basal hepatic ACA ($p < .05$). However, the response at 2 and 15 minutes has been restored to normal.

In Figure 27 the results are presented as percent change in basal ACA 2 minutes following in vivo epinephrine injection. It can be seen that although the response to epinephrine is abolished in shock, steroid treatment in shock restores the response to levels that exceed the control response.

The effect of glucocorticoid administration in shock on intestinal AC response to in vivo injection of epinephrine is presented in Figures 27 and 28.

In Figure 28, it can be seen that steroid administration is associated with a significant ($p < .01$) depression in basal ACA. However, the ability of the intestinal adenyl cyclase enzyme to

FIGURE 24

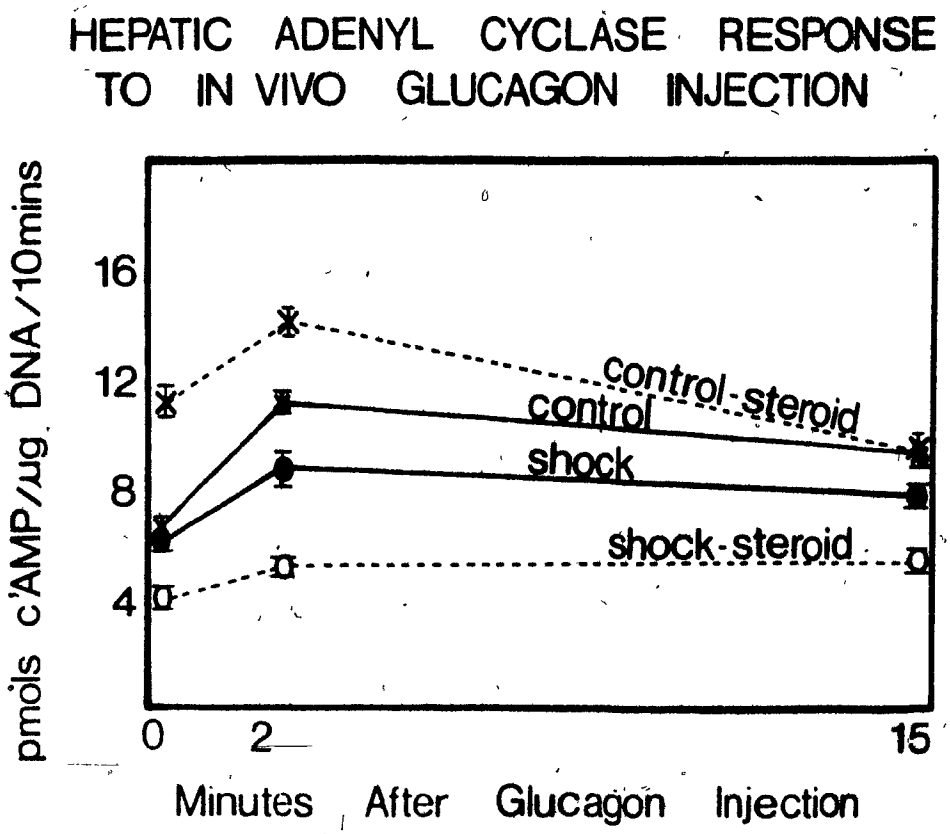

HEPATIC ADENYL CYCLASE RESPONSE
TO IN VIVO GLUCAGON INJECTION
pmols c'AMP/ug DNA/10mins
16
12
8
4
0
2
15
control-steroid
control
shock
shock-steroid
Minutes After Glucagon Injection

FIGURE 25

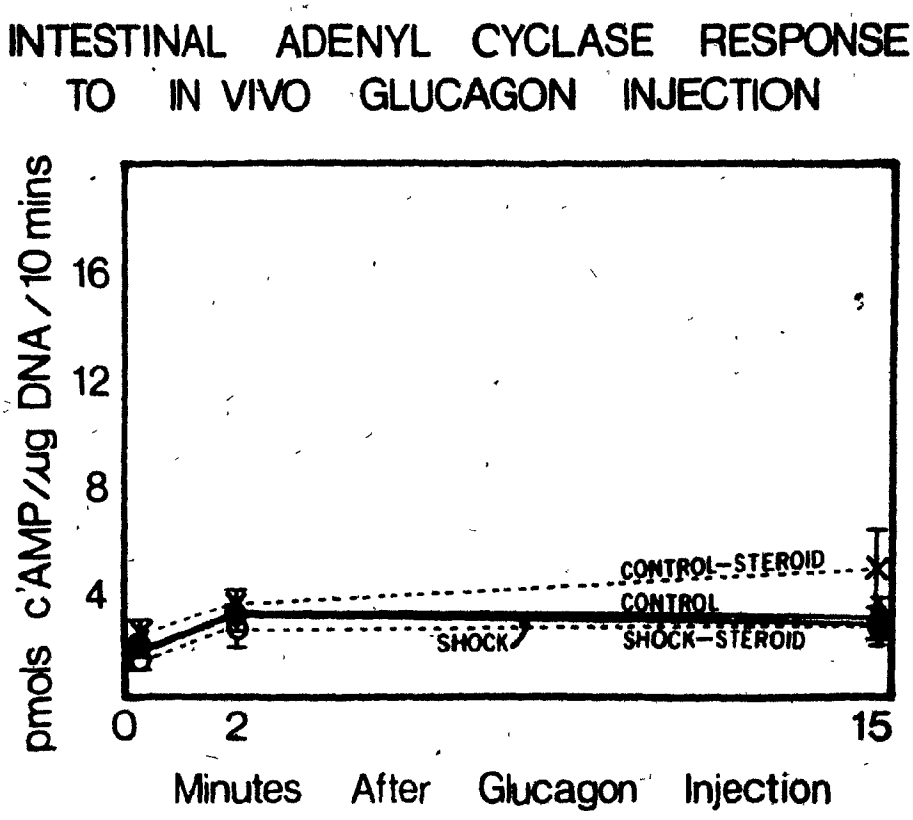

INTESTINAL ADENYL CYCLASE RESPONSE
TO IN VIVO GLUCAGON INJECTION
pmols c'AMP/ug DNA / 10 mins
16
12
8
4
0
2
15
CONTROL-STEROID
CONTROL
SHOCK
SHOCK-STEROID
Minutes After Glucagon Injection

FIGURE 26

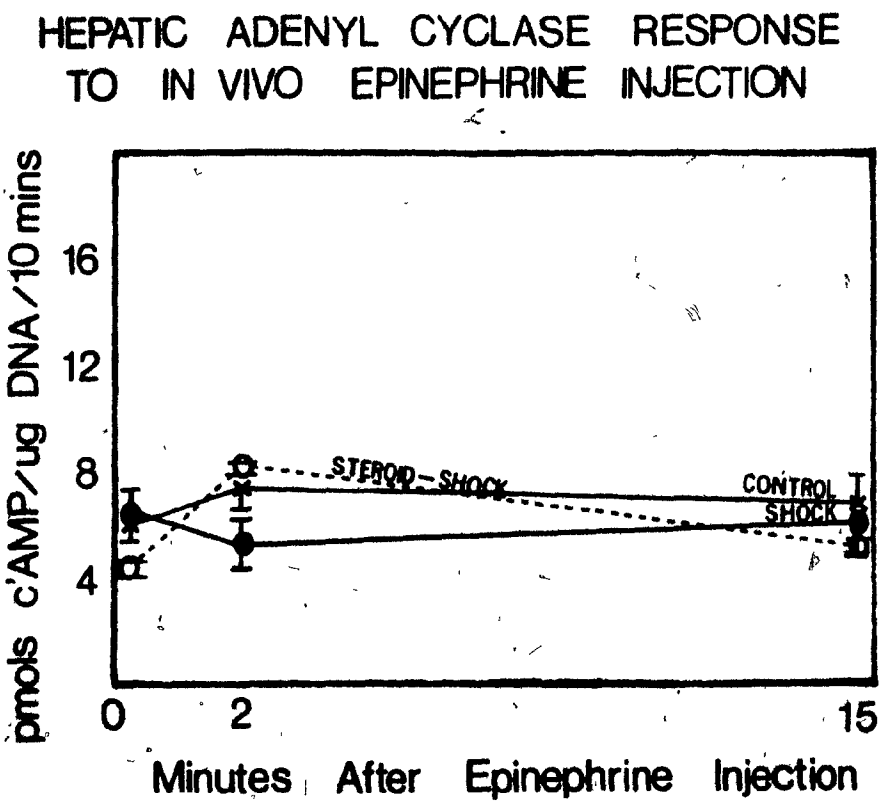

HEPATIC ADENYL CYCLASE RESPONSE
TO IN VIVO EPINEPHRINE INJECTION
pmols c'AMP/ug DNA/10 mins
16
12
8
4
0
2
15
STEROID-SHOCK
CONTROL
SHOCK
Minutes After Epinephrine Injection

FIGURE 27

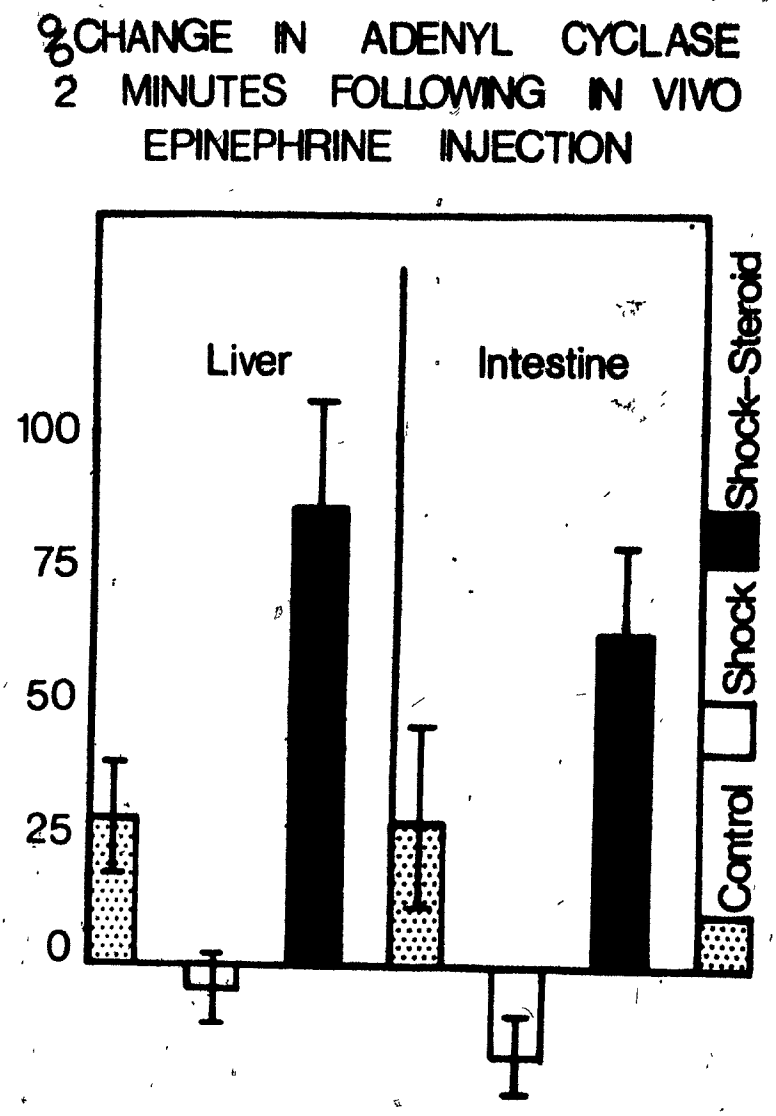
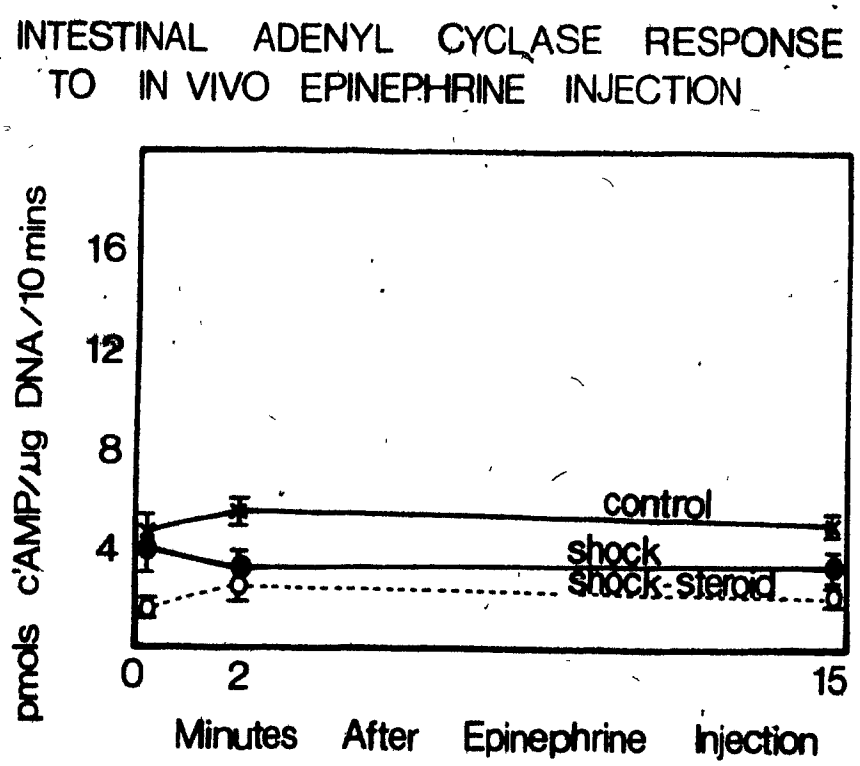

% CHANGE IN ADENYL CYCLASE
2 MINUTES FOLLOWING IN VIVO
EPINEPHRINE INJECTION
Liver
Intestine
100
75
50
25
0
Shock-Steroid
Shock
Control

FIGURE 28


INTESTINAL ADENYL CYCLASE RESPONSE TO IN VIVO EPINEPHRINE INJECTION
pmols c'AMP/µg DNA/10 mins
16
12
8
4
0
2
15
control
shock
shock-steroid
Minutes After Epinephrine Injection

respond to epinephrine has been restored, despite the fact that absolute values remain reduced from control ($p < .01$, $p < .001$).

In Figure 27 it can be seen that although the intestinal response to epinephrine is abolished in shock ($p < .01$), as seen in the liver, steroid treatment restores the response to levels that exceed the control response.

Note that hepatic basal ACA is almost always about 180% greater than basal intestinal ACA.

Tissue ATP Levels:

(i) Glucagon

Changes in hepatic and intestinal ATP levels following glucagon injection are presented in Tables III and IV.

Steroid administration in normal animals produces a non-significant 12% increase in hepatic ATP levels (Table III) compared to the untreated controls. There were no changes in ATP levels following glucagon injection in either steroid-treated or untreated controls. Steroid treated shock is associated with no change in baseline ATP levels, and the untreated shock ATP levels were also unchanged from control.

There were no changes in intestinal (Table IV) mucosal ATP levels in all groups of animals.

(ii) Epinephrine

Hepatic and intestinal ATP levels after epinephrine are shown in Tables V and VI.

TABLE II

Basal Adenyl Cyclase Response
To Shock and Steroid Therapy

	LIVER	INTESTINE
Control :	6.17 ± 0.62+ *	3.36 ± 0.64
Control - Steroid :	11.19 ± 2.92	2.39 ± 0.58
Shock :	5.77 ± 1.02*	2.66 ± 0.51
Shock - Steroid :	4.20 ± 0.57*	1.54 ± 0.18

+ All values represent pmols c'AMP/µg DNA/10 min. ± S.E.M.

* Significantly ($p = or < .05$) depressed from control-steroid (Student-T-Test)

TABLE III

Hepatic ATP Levels
Following In Vivo Glucagon Injection

TIME (Minutes)	0	2	15
Control :	1.333 ± .308[+]	1.662 ± .270[*]	1.504 ± .245
Control - Steroid :	1.497 ± .136	1.607 ± .198	1.423 ± .156
Shock :	1.628 ± .239	1.635 ± .220	1.685 ± .159
Shock - Steroid :	1.280 ± .177	1.628 ± .109	1.328 ± .042

[+] All values represent μmols/gm tissue ± S.E.M.
[*] Significantly increased over "0" time value (Paired-T-Test)

TABLE IV

Intestinal ATP Levels
Following In Vivo Glucagon Injection

TIME (Minutes)	0	2	15
Control :	1.798 ± .173[+]	1.716 ± .176	1.568 ± .102
Control - Steroid :	1.532 ± .201	1.512 ± .131	1.320 ± .177
Shock :	1.565 ± .102	1.770 ± .177	1.770 ± .177
Shock - Steroid :	1.184 ± .087[*]	1.464 ± .123	1.336 ± .089[**]

[+] All values represent μmols/gm tissue ± S.E.M.
[*] Significantly reduced from "0" control and shock values (Student-T-Test)
[**] Significantly reduced from "15" shock value (Student-T-Test)

TABLE V

Hepatic ATP Levels
Following In Vivo Epinephrine Injection

TIME (Minutes)	0	2	15
Control :	1.452 ± .176[+]	1.586 ± .137[*]	1.612 ± .176
Shock :	1.206 ± .197	1.342 ± .130	1.430 ± .270
Shock - Steroid :	1.424 ± .197	1.184 ± .130[**]	1.198 ± .158

[+] All values represent μmols/gm tissue ± S.E.M.
[*] Significantly greater than "0" value (Paired-T-Test)
[**] Significantly decreased from "2" control value (Student-T-Test)

TABLE VI

Intestinal ATP Levels
Following In Vivo Epinephrine Injection

TIME (Minutes)	0	2	15
Control :	1.824 ± .051[+]	1.890 ± .123	1.940 ± .101
Shock :	1.752 ± .217	1.590 ± .315	1.724 ± .273
Shock - Steroid :	1.788 ± .137	1.574 ± .250	1.820 ± .146

[+] All values represent μmols/gm tissue ± S.E.M.

There was a significant ($p < .05$) increase in hepatic tissue ATP (Table V) 15 minutes following epinephrine. The ATP level at 2 minutes following epinephrine injection in the shock-steroid treated group was significantly reduced from that seen in the control animals. Otherwise, there were no changes in ATP levels in treated or untreated shock compared to control, or each other.

No changes in intestinal ATP levels were observed following epinephrine injection in control, shock or steroid-treated shock animals (Table VI).

(iii) Norepinephrine

ATP levels in liver and intestine following norepinephrine injection are presented in Tables VII and VIII.

There was a significant increase ($p < .02$) in hepatic ATP levels (Table VII) in normal animals at 15 minutes following norepinephrine injection. No further changes in hepatic ATP levels were observed in control or shock animals.

There were no significant changes in intestinal ATP levels in control or shock animals following norepinephrine injection (Table VIII).

Glucocorticoids and Adenyl Cyclase Response to In Vitro Hormone Addition:

(i) Glucagon

The effect of glucocorticoid administration on the response of hepatic and intestinal homogenates to in vitro addition of 5

TABLE VII

Hepatic ATP Levels
Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	2	15
Control	1.524 ± .117+	1.808 ± .119	2.014 ± .133*
Shock	1.623 ± .285	1.723 ± .131	1.770 ± .183

+ All values represent μmols/gm tissue ± S.E.M.
* Significantly increased over "0" value (Paired-T-Test)

TABLE VIII

Intestinal ATP Levels
Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	2	15
Control	1.752 ± .192+	1.662 ± .131	1.844 ± .173
Shock	1.848 ± .226	1.980 ± .172	2.040 ± .130

+ All values represent μmols/gm tissue ± S.E.M.

nanomoles of glucagon is presented in Figure 29. The results are presented as percent increase over basal ACA.

It can be seen that steroid administration in normal dogs results in no change in the percent increase in basal hepatic ACA over that seen in the untreated controls following in vitro glucagon addition. It can also be seen that the depressed response in shock is partially restored by steroid therapy.

The normal intestine is associated with a reduction in basal ACA upon in vitro addition of glucagon. Steroid administration in normal animals results in an increase over basal enzyme activity following in vitro glucagon addition. A similar pattern is observed for the shock and steroid treated shock tissues.

(ii) Epinephrine

The effect of glucocorticoid administration in shock upon the ability of hepatic and intestinal adenyl cyclase to respond to in vitro addition of 5 nanomoles of epinephrine is presented in Figure 30.

The shock liver shows a significant reduction in the in vitro response to epinephrine. Steroid treatment restores the response to normal.

The shock intestine also shows a marked reduction in the response to in vitro stimulation which is restored in the steroid treated animals.

FIGURE 29

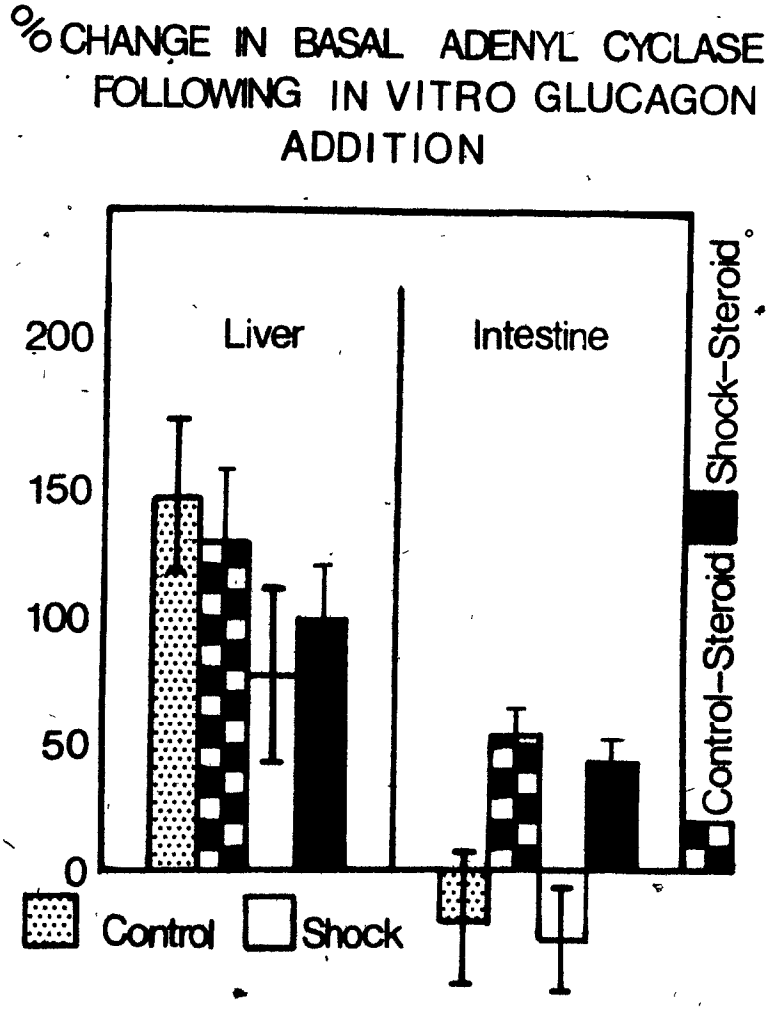
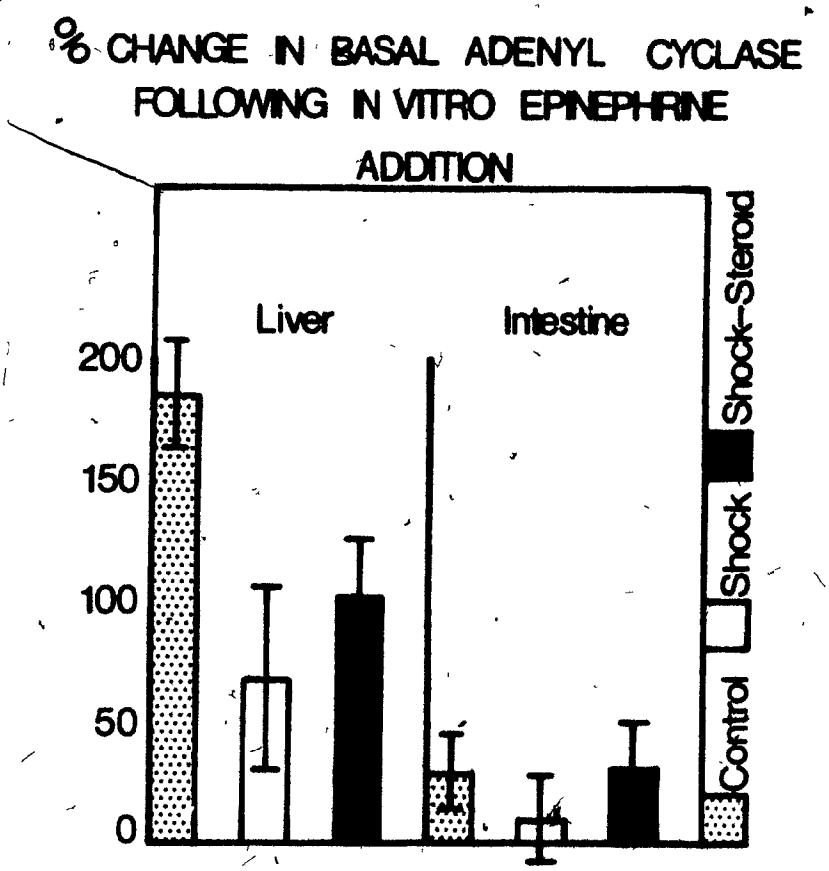

% CHANGE IN BASAL ADENYL CYCLASE FOLLOWING IN VITRO GLUCAGON ADDITION
200
150
100
50
0
Liver
Intestine
Shock-Steroid
Control-Steroid
Control
Shock

FIGURE 30

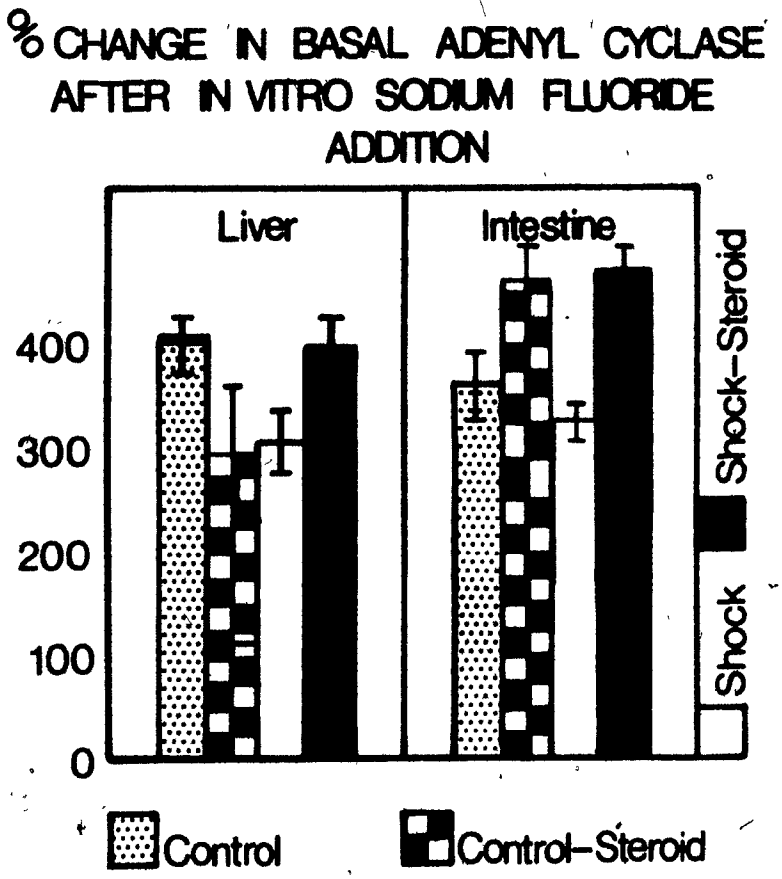

% CHANGE IN BASAL ADENYL CYCLASE FOLLOWING IN VITRO EPINEPHRINE ADDITION
Liver
Intestine
200
150
100
50
0
Control
Shock
Shock-Steroid

Glucocorticoids and the Adenyl Cyclase Response to In Vitro Addition of Sodium Fluoride:

The effect of glucocorticoid administration on NaF - stimulated adenyl cyclase activity in the liver and intestine is presented in Figure 31. The results are presented as a percent increase over basal ACA. It can be seen that in the normal liver 10 mM NaF produces a 410% increase over basal ACA. Steroid administration in normal animals results in a 68% increase in basal ACA and only a 23% increase in NaF - stimulated ACA over respective untreated control values. Therefore the percent increase over basal in the control steroid group appears slightly reduced. On the other hand, steroid administration in shock improves the ability of the tissues to respond to NaF, to a level not unlike that seen in the untreated controls.

Glucocorticoid administration in the normal intestine is associated with a slight increase in the NaF - stimulated enzyme activity from that seen in the untreated controls. Steroid treatment in shock shows an improvement in the ability of the tissue to respond when compared to the lower shock levels.

FIGURE 31


% CHANGE IN BASAL ADENYL CYCLASE AFTER IN VITRO SODIUM FLUORIDE ADDITION
Liver
Intestine
400
300
200
100
0
Shock-Steroid
Shock
Control
Control-Steroid

Introductory Discussion: Glucocorticoids

Glucocorticoids play a multifaceted role in cellular metabolism. The exact mechanism(s) of action of the steroids has been speculated upon, however there are biologic effects that cannot be accounted for by currently available information.

Glucocorticoids have been reported to play a permissive role or potentiate the effects of exogenous epinephrine and glucagon in carbohydrate metabolism in normal, (160, 161, 162, 163) and adrenalectomized (84, 296) animals. Pharmacological doses of steroids have been shown to effect several hemodynamic improvements in shock (74) by way of an alpha-adrenergic-like effect with a resultant preservation of the integrity of the peripheral capillary beds. Glenn and Lefer (121, 122) report that glucocorticoids stabilize cellular and lysosomal membranes in shock. They postulate that there is some involvement of the second messenger system in the lysosomal membrane stabilizing effects of the steroids (123). Furthermore, glucocorticoids have been shown to interact directly with the second messenger system at several levels (34, 206, 211). However, as yet, there is little agreement on the precise mechanism(s) involved in the aforementioned processes.

It was of interest therefore, to investigate whether the short-term administration of pharmacological doses of steroids to normal and shock animals alters the adenyl cyclase - phosphodiesterase responses to the hormones. In addition, it was of interest to see if

the steroids could afford protection from the ischemic damage incurred by the cell membrane in shock; especially with regard to the membrane-bound adenyl cyclase enzyme.

The results obtained by other investigators using steroid therapy in shock are contradictory. The "key" to estimation of the beneficial effects of steroid therapy in shock appears to be in the close regulation of the dosage and timing of administration (74). In addition, monitoring of fluid requirements is crucial. The same steroid therapy given at one time may have a completely different effect - even a detrimental effect - while given at another time - it proves beneficial. Therefore it was of interest to administer optimal doses (30 mg/kg) (7, 74, 200) of Methyl Prednisolone Sodium Succinate (MPSS) immediately following hypovolemia. Several studies have administered pharmacological doses of steroids before induction of shock. When translated into the clinical situation, pre-treatment of a "shock" patient is next to impossible - pre-treatment of the animals in this study was not attempted. The effects of administering the steroid immediately following the period of hypovolemia were studied.

The half-life of MPSS in normal animals is said to be about 3 to 4 hours (251, Melby, J.C., in 310). Therefore each dosage of MPSS was repeated every 3 hours until termination of the study in both normal and shock animals.

Blood Pressure Studies: Discussion

Blood pressure was monitored throughout all experiments. When MPSS is coupled with adequate volume replacement and a positive iontropic agent following shock, Dietzman and Castenada (74) have shown that survival rates are increased from 22 to 65% in experimental hemorrhagic shock in dogs.

There were no attempts to measure survival rates in this study. However, from previous experience in this laboratory, it is known that the particular modification of the Wiggers' procedure for hemorrhagic shock in dogs used in this study is associated with a mortality rate of 85%. Therefore, in this study, interest centered around the acute and lethal shock situation; no attempts at fluid replacement or use of iontropic agents was attempted.

Steroid treatment was beneficial, in this study, when administered soon after the hypovolemic episode. Reductions in blood pressure were minimized and myocardial response to the catecholamines was improved. In addition, despite the fact that many of the metabolic effects of epinephrine are potentiated by MPSS treatment, the tolerance to the extra insult of a 0.25 mg dose of epinephrine following three hours of hypovolemia, was increased.

Discussion:

Steroid-induced increases in hepatic glucose turnover and an enhanced rate of peripheral glycolysis has been shown to be associated

with a significant increase in resting hepatic blood glucose levels as seen here and elsewhere (103, 163, 250). When MPSS is administered acutely, and not over several days - the enhanced peripheral glycolysis is responsible for increased conversion of available lactate into glucose (103, 161). Lacticacidosis is a common feature of shock, and any substance that can actually put use to some of the excess lactate for production of endogenous glucose, must be of benefit. Glycolysis can proceed in hypoxic tissues and the fact that in the shock-steroid treated groups, the resting blood glucose is elevated from the respective untreated - shock values is evidence for this fact.

Issekutz and Borkow (163) also studied hepatic blood glucose response curves following exogenous glucagon administration in MPSS - treated normal dogs. However, unlike our findings, they observed a spectacular potentiation of the normal hepatic blood glucose response curve in the MPSS - treated animals. This was explained in several ways: Glucagon was infused (0.06 µg/kg/min) over a period of 2 hours. In addition, the MPSS was administered over a three day period prior to the glucagon administration, allowing for the glucocorticoid - induced "de novo" synthesis of several gluconeogenic enzymes (350) and an increase in hepatic glycogen stores, with a resultant supranormal response of the hepatic target cell to glucagon. Furthermore, in vivo, the rate of formation of glucose from lactate depends on the plasma lactate levels and on the turnover rate of lactate (103, 161). Normally, glucagon does not increase

plasma lactate levels (5, 163), however, when MPSS was administered over 3 days, lactate levels were significantly increased (163). These factors probably account for the greater potentiation of response following glucagon seen in this other study (163).

De novo synthesis of hepatic gluconeogenic enzymes in the shock state is very unlikely, especially following short-term MPSS treatment. However, glucocorticoid-stimulated gluconeogenesis and glycogen production from lactate (74, 252, 302) in the presence of increased endogenous lactate may contribute to the restoration of the hepatic blood glucose response to epinephrine in shock.

Steroid treatment in shock is associated with a dramatic restoration in hepatic blood glucose response following epinephrine bolus. It was of interest to see if any changes in adenyl cyclase response might play a role in this improvement.

Very little data is available on the direct effects of steroids upon the AC enzyme. Most reports deal with the effects of steroids upon increased "de novo" synthesis of enzymes and the resultant potentiation of the particular physiologic response. For example, the enhanced response to ACTH in fat cell ghosts that have been incubated for several days (34), or the enhanced gluconeogenesis observed in normal and adrenalectomized animals treated with steroids over several weeks (84, 296, 297).

However, there is one report by Logsdon et al (206) that suggests a direct effect of steroids upon AC activity. They found

a rapid (within 5 minutes) 25 - 36% increase in basal ACA when 10^{-6} M cortisol was added <u>in vitro</u> into a leukocyte incubation mixture.

MPSS treatment in normal animals for a total of 4 hours prior to intervention was associated with a significant 68.5% increase in basal ACA over untreated controls. However, there was no significant difference in response between the two groups following glucagon injection. This is not due to the lag in glucagon action in the liver, as a later increase in ACA would most likely be reflected in further significant increases in hepatic blood glucose levels - which did not occur. Recently, it was shown that insulin is a very potent inhibitor of the glucagon stimulated release of cyclic AMP from the perfused rat liver (85, 86). When cyclic AMP levels are increased, i.e., in the presence of glucagon, the cyclic AMP - lowering effect of insulin can readily be demonstrated (195). The mechanism by which insulin produces this effect is uncertain. Furthermore, inhibition by insulin of hepatic glucose release has also been observed (235). Treatment with MPSS significantly increases insulin levels (185) and following glucagon injection, insulin levels were found to peak at levels almost 5 fold greater than basal levels (Tables XIV and XV). Therefore it is possible that such excessively high insulin levels may in part be responsible for the lack of significant potentiation of AC and glucose responses in the control steroid treated animals, compared to the untreated controls.

Steroid treatment in shock is associated with reductions in basal and glucagon responsive ACA in the liver. However, the percent response at two minutes following glucagon injection (Figure 24) is the same in the steroid-treated controls, and shock animals and the untreated shock animals - despite lowered absolute values. All these extreme changes in AC response to glucagon are associated with very little alteration in the primary physiological response - hepatic blood glucose output.

The insulin levels measured in the steroid treated and untreated shock animals (Tables XIV and XV) were significantly elevated from the untreated controls. The steroid treated shock group more so than the untreated shock group - which might account for the greater reductions in shock basal ACA if, in fact, insulin does decrease ACA (271). However, insulin levels following glucagon injection in shock were only slightly higher than in the controls - probably not elevated enough to explain the lower AC responses in steroid - treated and untreated shock. The question still arises as to how there can be an unchanged glucose response curve in the face of a depressed AC response!

The second messenger system involved in the hepatic glucagon-glucose output interaction may be saturated. In otherwords, the degree of AC activation observed in the control animals far exceeds that required to produce the normal blood glucose response curve. Furthermore, Robison states (284, Chapt. 7 p. 272) that, "glucagon is capable

of increasing the concentrations of cyclic AMP in the liver to levels much higher than are necessary to produce a maximal stimulation of glucose output". The remaining cyclic AMP is put to other uses within the cell, is broken down by PDE or appears in the extracellular fluid. There is great adaptive value to this potential. Sixty percent of the glucagon - AC function may be destroyed, as seen in this study, however the physiologic response remains intact. Thus, the levels of cyclic AMP are still high enough that no effect upon glucose output can be seen. Glucagon appears to be an efficient glucose - supplying hormone of stress.

Steroid administration in normal or shock animals has little effect upon intestinal ACA. There is a slight increase in basal ACA in the steroid treated controls (Figure 25) and a slight decrease in the steroid treated shock intestine. There appears to be a slight response to glucagon in the steroid treated shock group at 2 minutes and the steroid treated control group at 15 minutes (Figure 25). This may be due to the effect of MPSS on intestinal PDE activity, which will be discussed.

Bitensky et al (22) has presented substantial evidence for the existence of two separate and distinct receptors in the liver for glucagon and epinephrine. This becomes even more evident when one observes the totally different manner in which response to these hormones is altered in shock.

Steroid treatment in shock completely restores the hepatic

blood glucose response to epinephrine which was abolished in shock. This is associated with a complete restoration of adenyl cyclase response. In fact, the percent response in both the liver and intestine is supranormal in the steroid treated shock animals (Figure 27).

The insulin levels in the epinephrine animals, (Tables XVI and XVII) control, shock and steroid shock, are not significantly different from those seen in the respective animals at 5 minutes following glucagon injection (Tables XIV and XV). However, the fact that epinephrine inhibits insulin release (262) and is associated with a significant depression in portal insulin levels by 1 minute (Table XVII), may help to augment the AC response at 2 minutes following epinephrine injection by exerting little or no inhibitory influence.

The supranormal response to epinephrine in the steroid-treated shock animals agrees with data by others (144, 211, 269) in unrelated studies, that indicate glucocorticoids enhance the sensitivity of β-adrenergic receptors to stimulation by catecholamines. This action of glucocorticoids in shock appears to be very effective and at a time when the receptors are devoid of sensitivity to hormonal stimulation.

The beneficial effect of steroid therapy may be directly related to an improvement in blood flow to the affected organs. This, in turn, may provide an increased availability of the hormone in question. Dietzman and Castenada (74) have reported that MPSS in doses of 30 mg/kg acts as an effective peripheral vasodilatory agent

in clinical and experimental shock. Tissue perfusion is enhanced as reflected in an increased oxygen consumption (74) and tissue ATP levels. The positive effect upon peripheral circulatory status may in turn help to reduce the severity of ischemic damage to cellular components within the tissues.

Steroid administration in normal animals produces a 12% increase in hepatic tissue ATP levels. This is similar to results obtained by Feigelson and Feigelson (92). McArdle et al (224) obtained a similar increase in hepatic ATP levels following administration of an α-adrenergic blocking agent, acepromazine maleate. On the other hand, Young (361) states that hydrocortisone may cause a decrease in the level of intracellular ATP, which is what was found in the normal intestine in the steroid-treated control animals (Table IV).

Shock, in both steroid-treated and untreated animals, was associated with no significant alterations in tissue ATP levels. Numerous investigators (50, 51, 158, 288) have reported significant reductions in tissue ATP levels during oligemia. However, by 2 hours following re-infusion of blood, ATP levels may recover to within 30 to 40 percent of normal (152, 176, 224). The tissue biopsies in this study were obtained 4 hours following re-infusion of blood and were not significantly different from control values. Steroid therapy in shock, therefore, provided no improvement in tissue ATP levels. There was no correlation between altered adenyl cyclase function and changes in tissue ATP levels.

Goldfarb and Glenn (123) state that steroids may maintain lysosomal stability in ischemic tissues by restoration of cyclic AMP levels. It is interesting that they did not state, that because cellular and lysosomal membranes are stabilized by the steroid, the cyclic AMP system is therefore restored. Ignarro et al (155) report that liver lysosomal fractions contain β receptor sites which when stimulated by catecholamines, inhibit lysosomal enzyme release by a cyclic AMP - mediated process. The β response to epinephrine is abolished in shock as seen in this study. Therefore, by restorating the β receptor response to catecholamine stimulation, the steroids may also restore the effectiveness of the cyclic AMP - mediated lysosomal stabilizing effect.

The question arises as to whether the improvement in adenyl cyclase function is associated with improved delivery of the hormone or actual enhancement of receptor and/or catalytic function. Since the response to epinephrine in steroid treated shock is supranormal, there must be improved enzyme function in addition to improved peripheral blood flow.

Steroid treatment in normal livers is associated with no increase in response to in vitro glucagon addition (Figure 29). However, there is a stimulation of basal ACA in the intestine of steroid - controls where normally there is none in response to in vitro addition of glucagon (Figure 29). This effect of the steroids is most probably related to the α adrenergic blocking properties

(74) of MPSS. In blocking any α or inhibitory influences upon intestinal ACA, basal and hormonal activities become sensitized, and to some degree stimulated - even in shock.

Steroid therapy in shock is associated with significant improvements in hepatic response to in vitro addition of epinephrine and glucagon (Figures 29, 30). This effect is more prevalent with epinephrine than with glucagon.

Steroid therapy in shock is probably associated with a stabilization of the cell membranes with a resultant improvement in the receptor - subunit function of the membrane-bound AC.

The NaF - stimulated ACA is not significantly different between the steroid-treated and untreated control groups in either the liver or intestine (Table IX). However, since MPSS administration caused an increase in basal ACA in the normal liver (Figure 24), hepatic NaF - stimulated ACA in Figure 31 appears reduced from the untreated control value. It may thus be concluded that short-term administration of pharmacological doses of MPSS does not induce the synthesis of new AC enzyme. Furthermore, there appears to be no significant inhibition or interference in the ability of NaF to interact with and stimulate ACA in the presence of MPSS. However, the ACA measured in the presence of NaF is significantly reduced from control in shock and in steroid-treated shock in both the liver and intestine, when all the data is grouped (Table IX). As mentioned previously, shock and ischemia in the splanchnic tissues is associated with a

TABLE IX

Changes in Sodium Fluoride Stimulated Adenyl Cyclase Activity Following Steroid Administration in Normal and Shock Animals

	LIVER	INTESTINE
Control :	29.69 ± 2.52[+]	13.60 ± 1.45
Control - Steroid :	32.46 ± 7.04	11.15 ± 1.30
Shock :	21.80 ± 2.85[*]	9.44 ± 1.69
Shock - Steroid :	17.07 ± 2.45[*]	7.08 ± 0.72[*]

[+] All values represent pmols c'AMP/µg DNA/10 min. ± S.E.M.

[*] Significantly ($p = or < .05$) reduced from control (Student-T-Test)

loss in the total amount of AC enzyme - as represented by reductions in NaF - ACA. Steroid therapy in shock does not induce synthesis of new adenyl cyclase enzyme, however, the ability of the remaining AC to respond to NaF (Figure 31) has been improved from that seen in shock. The steroids do not create new enzyme, nor do they restore all the lost enzyme. They do, perhaps through a membrane stabilizing effect, enhance the function of both receptor and catalytic subunits in the remaining AC enzyme.

Throughout this study, it has become increasingly evident that absolute ACA in the liver exceeds the same in the intestine. Basal, NaF - stimulated, in vitro epinephrine and in vivo epinephrine stimulated ACA in the liver ranges from 150 - 200% greater than the respective enzyme activities in the intestine. The reason for this phenomenon, has been touched on before, Rodbell et al (285a) have shown that the liver binds 20 times more glucagon than do fat cells (which are specific for epinephrine) where glucagon - sensitive AC is 25 fold less than hepatic AC. This principle applies also in the intestine, where there is a lack of glucagon - sensitive AC (312) and a presence of epinephrine sensitive AC. The fact that the absolute values for intestinal NaF - stimulated ACA are so much lower than those in the liver probably indicates there is less adenyl cyclase/mg intestinal tissue than per mg hepatic tissue. The reason for this discrepancy is most likely centered around the physiological need for the AC system. Beta adrenergic stimulation is of limited physiological significance in the intestine as compared with the liver (318).

Glucocorticoids and Phosphodiesterase:

(i) Glucagon

Changes in hepatic phosphodiesterase (PDE) activity following in vivo glucagon injection are presented in Figure 32.

Control hepatic PDE activity in this series of animals was 154.49 ± 18.31 pmols cyclic AMP destroyed/µg DNA/5 minutes, and did not change following glucagon injection. In shock, the basal PDE activity is decreased by 23% (not significant) and gradually increases to control levels by 15 minutes following glucagon injection. Steroid treatment produces a 44% ($p < .05$) decrease from control basal PDE and a 27.10% decrease from the respective shock value. The steroid treated PDE activity does not change from basal levels after glucagon administration.

Changes in intestinal PDE activity following glucagon injection are presented in Figure 33.

Control basal PDE in the intestine is 14.67 ± 1.45 pmols cyclic AMP destroyed/µg DNA/5 minutes, and does not change following glucagon injection. In shock there is a significant ($p < .025$) 25% decrease in basal PDE, which remains the same after glucagon administration. Steroid treatment restores PDE activity toward control levels.

(ii) Epinephrine

Hepatic PDE activity following epinephrine pulse is presented in Figure 34. Control hepatic PDE is 75.67 ± 15.13 pmols

FIGURE 32

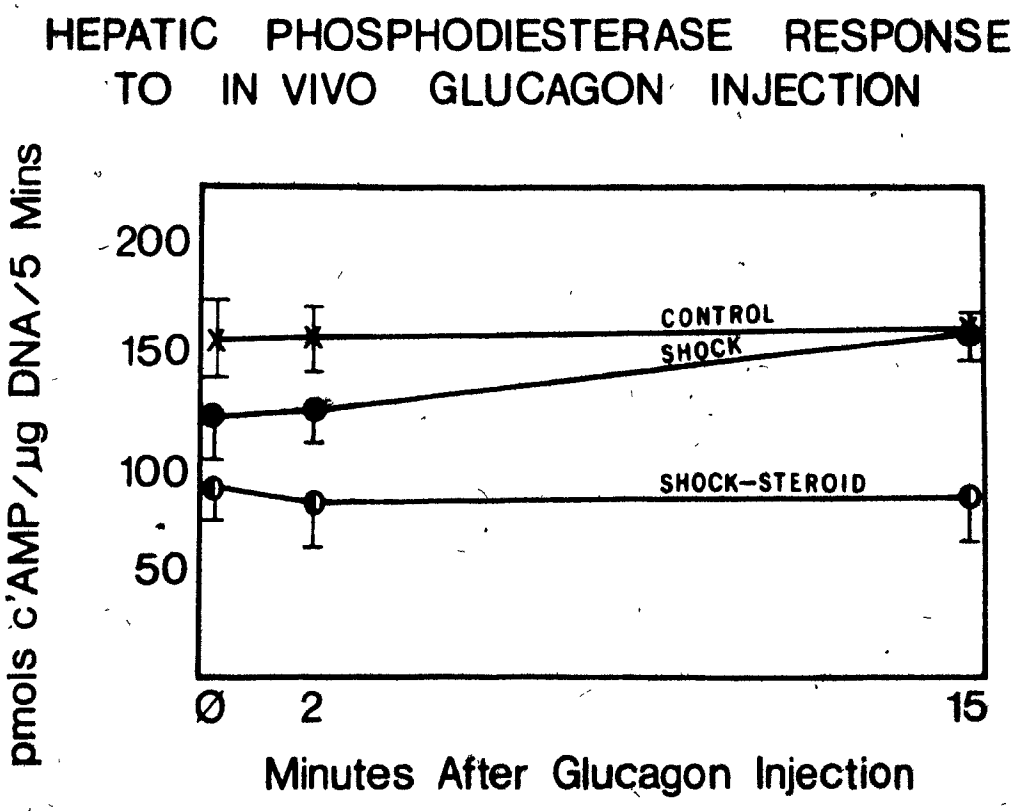
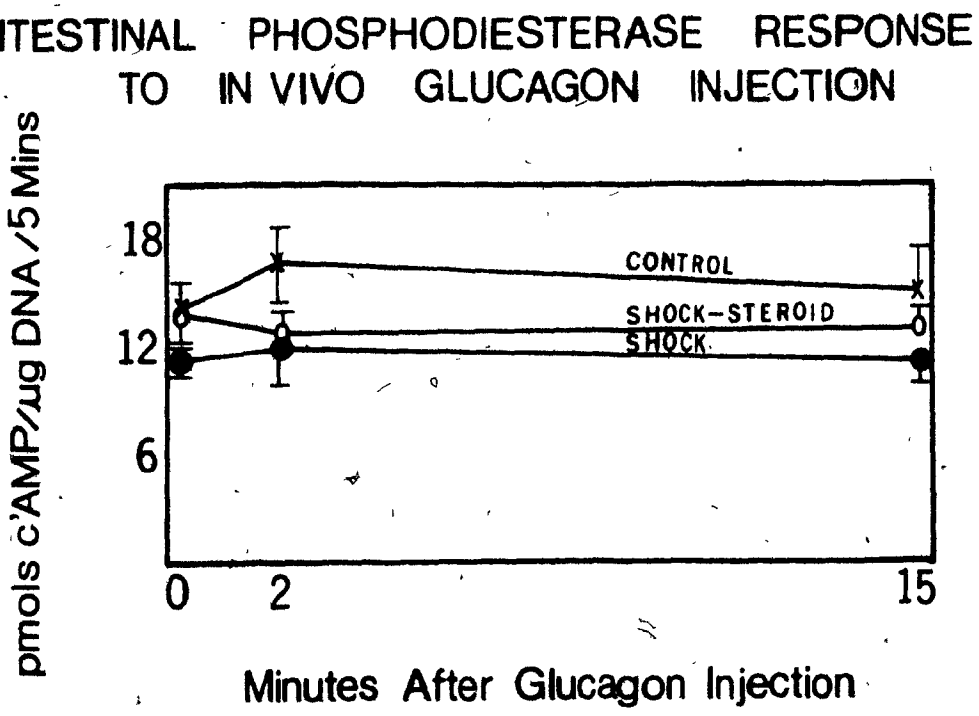

HEPATIC PHOSPHODIESTERASE RESPONSE TO IN VIVO GLUCAGON INJECTION
pmols c'AMP/µg DNA/5 Mins
200
150
100
50
CONTROL
SHOCK
SHOCK-STEROID
Ø
2
15
Minutes After Glucagon Injection

FIGURE 33


INTESTINAL PHOSPHODIESTERASE RESPONSE TO IN VIVO GLUCAGON INJECTION
pmols c'AMP/µg DNA/5 Mins
18
12
6
0
2
15
CONTROL
SHOCK-STEROID
SHOCK
Minutes After Glucagon Injection

destroyed/μg DNA/5 minutes. This value does not increase significantly after epinephrine bolus. In shock, basal PDE is significantly increased ($p < .025$) before and at 2 ($p < .025$) and 15 minutes ($p < .0025$) after epinephrine injection. Steroid treatment is associated with a significant ($p < .05$) 27% decrease in PDE from shock levels which remains unchanged.

Intestinal PDE activity following epinephrine pulse is seen in Figure 35. Control intestinal PDE activity is 21.25 ± 3.35 pmols cyclic AMP destroyed/μg DNA/5 minutes. This value does not change after epinephrine pulse. In shock the PDE activity is unchanged from the control activity and remains the same following epinephrine injection. Steroid treatment in shock is associated with a significant ($p < .05$) 20% reduction in PDE from shock levels at 15 minutes following epinephrine bolus.

(iii) Norepinephrine

Changes in hepatic PDE activity following norepinephrine injection are presented in Figure 36. A slight decrease (nonsignificant) from basal levels is seen following norepinephrine bolus in normal animals. In shock, basal PDE is only slightly decreased (nonsignificant) from control and this value rises only slightly by 15 minutes.

Changes in intestinal mucosal PDE activity after norepinephrine bolus is presented in Figure 37. Control basal PDE activity does not change following norepinephrine injection. There are no

FIGURE 34

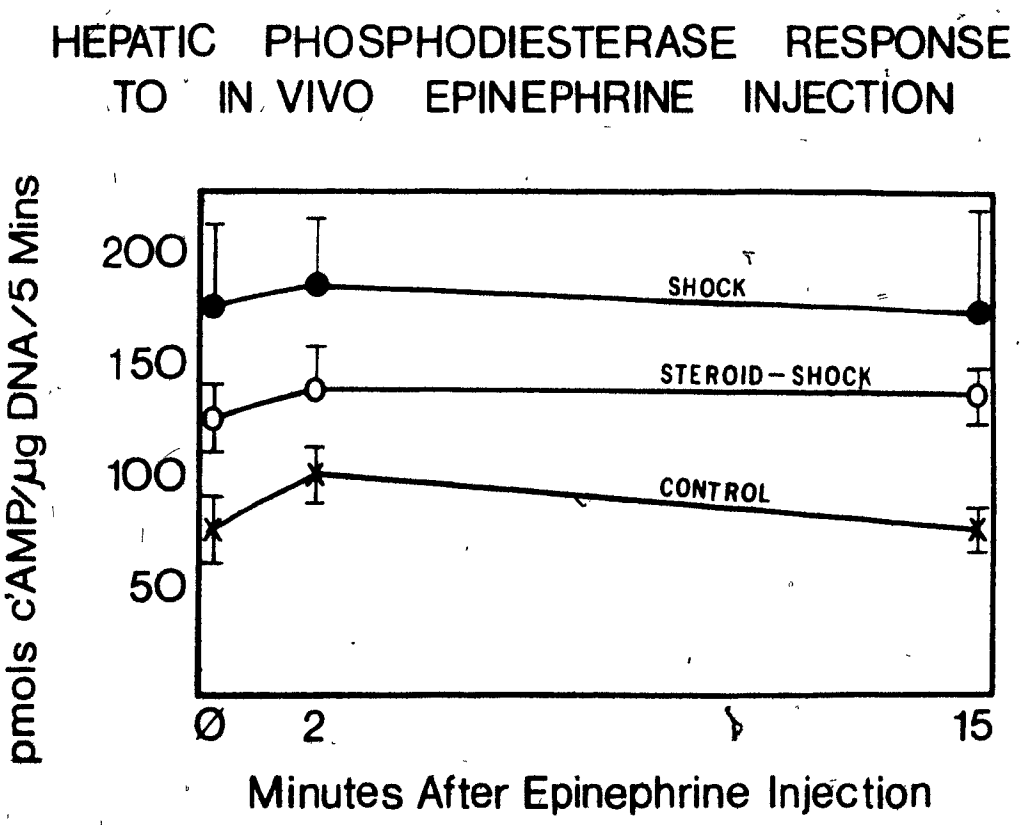

HEPATIC PHOSPHODIESTERASE RESPONSE TO IN VIVO EPINEPHRINE INJECTION
pmols c'AMP/µg DNA/5 Mins
200
150
100
50
SHOCK
STEROID – SHOCK
CONTROL
Ø
2
15
Minutes After Epinephrine Injection

FIGURE 35

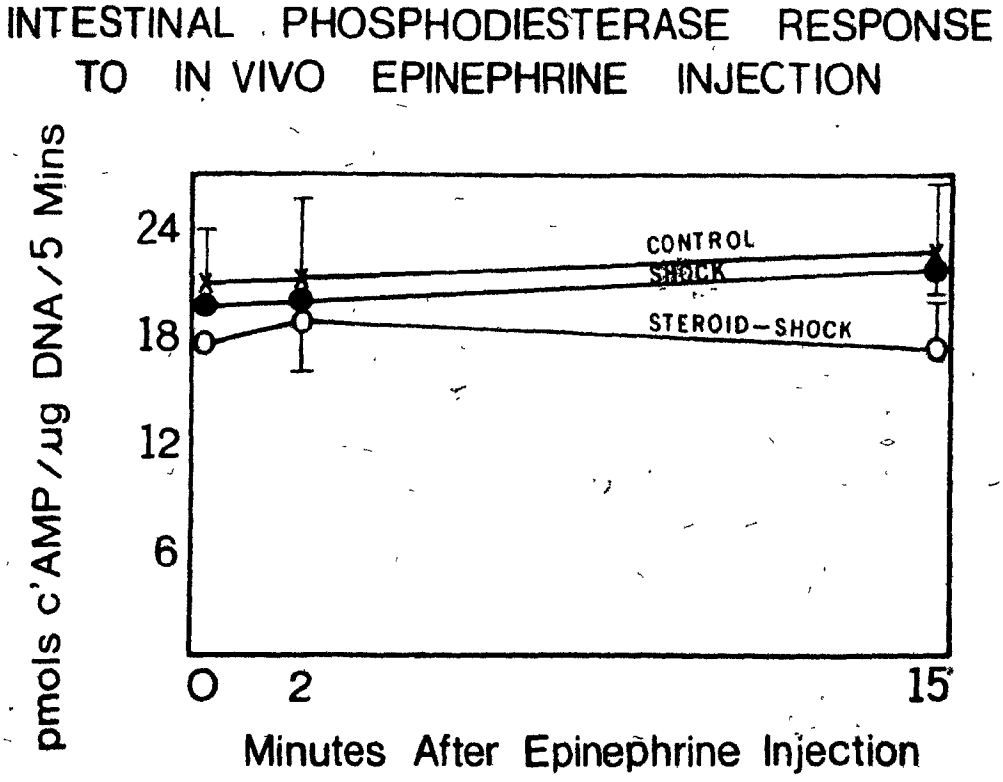

INTESTINAL PHOSPHODIESTERASE RESPONSE
TO IN VIVO EPINEPHRINE INJECTION
pmols c'AMP/µg DNA/5 Mins
24
18
12
6
CONTROL
SHOCK
STEROID–SHOCK
O
2
15
Minutes After Epinephrine Injection

FIGURE 36

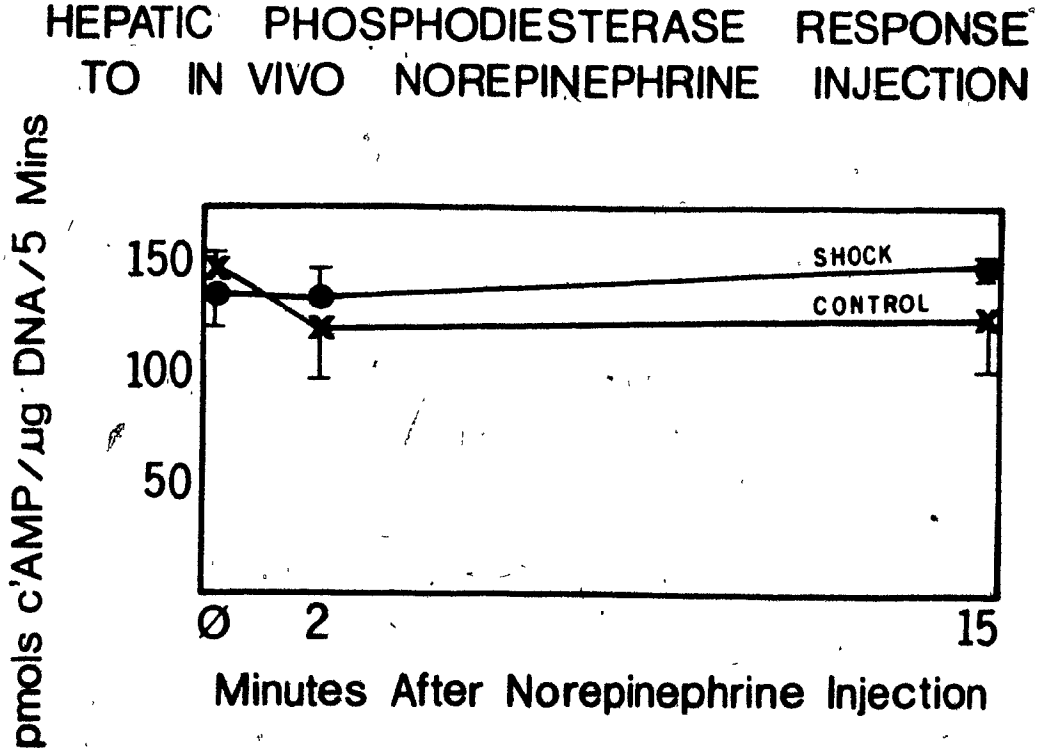
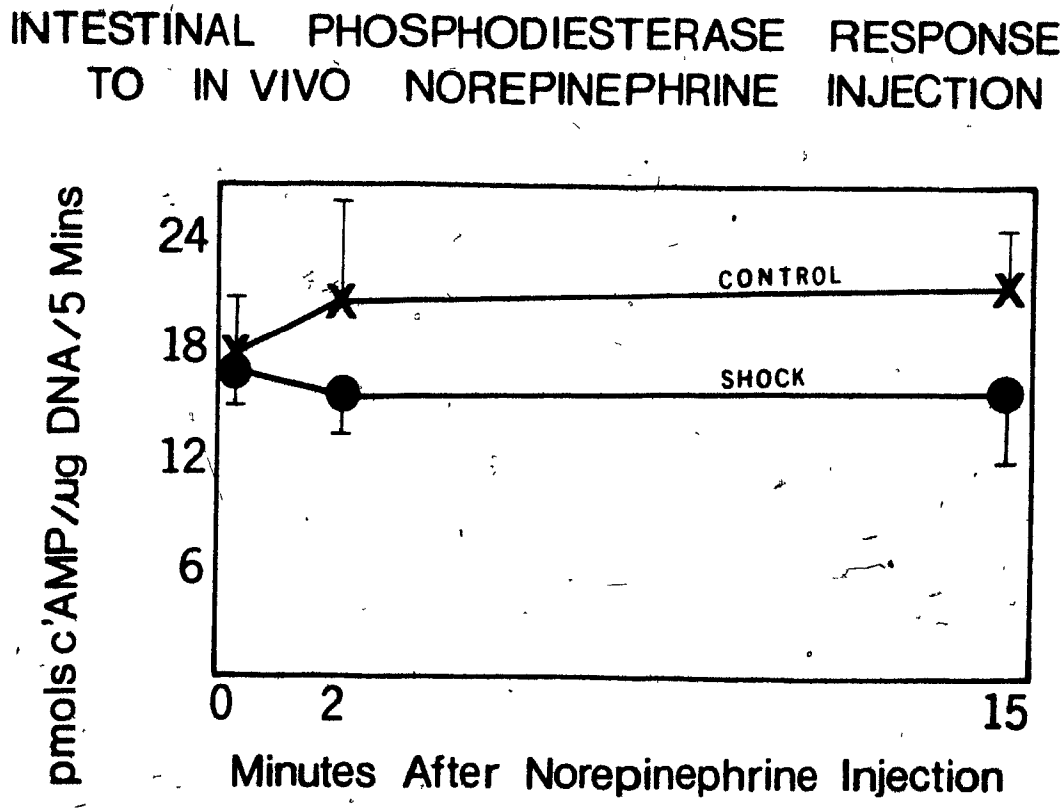

HEPATIC PHOSPHODIESTERASE RESPONSE TO IN VIVO NOREPINEPHRINE INJECTION
pmols c'AMP/µg DNA/5 Mins
150
100
50
SHOCK
CONTROL
Ø
2
15
Minutes After Norepinephrine Injection

FIGURE 37


INTESTINAL PHOSPHODIESTERASE RESPONSE
TO IN VIVO NOREPINEPHRINE INJECTION
pmols c'AMP/µg DNA/5 Mins
24
18
12
6
CONTROL
SHOCK
0
2
15
Minutes After Norepinephrine Injection

significant changes in intestinal PDE in shock, and the levels do not differ from control levels.

Hepatic PDE is generally 6 fold greater than intestinal PDE (Table X).

TABLE X

Baseline Phosphodiesterase Activity
in Shock and Steroid Therapy

	LIVER	INTESTINE
Control :	123.50 ± 12.80+	17.94 ± 2.75
Shock :	144.19 ± 22.02	16.49 ± 1.80
Shock - Steroid :	110.98 ± 4.50*	16.23 ± 1.28

+ All values represent pmols c'AMP/µg DNA/10 min. ± S.E.M.

* Significantly reduced ($p < .05$) from shock value (Student-T-Test)

Discussion:

The amount of cyclic AMP present at any one time is influenced by it's rate of synthesis, but also by it's rate of destruction. Therefore, it is of great importance to observe changes in phosphodiesterase (PDE) activity before comments can be made about tissue and plasma levels of cyclic AMP.

The AC response to epinephrine in the steroid-treated shock tissue is supranormal. This may indicate that related and compatible changes are occurring within the cell to potentiate the β-adrenergic response. For example, in the presence of a β-agonist, PDE activity may be decreased, and cyclic AMP breakdown reduced - thus potentiating the β response. Furthermore PDE activity has been shown to be altered by several hormones and drugs. Therefore it was of interest to observe the changes in hepatic and intestinal PDE activity following in vivo hormone injection in the normal, shock, and steroid-treated shock animals.

Numerous investigators (54, 202) have noted that most tissues contain both a low Km (0.2 - 3 μM) and high Km (15 - 250 μM) PDE enzymes. Others (239, 343) have shown up to 7 separate PDE enzymes in one tissue. Furthermore, Sutherland and Rall (335) have shown that PDE activity can vary considerably from tissue to tissue. Hepatic low Km PDE activity in this study is almost 6 fold greater than intestinal low Km PDE activity. Since most changes are said to occur in the low Km PDE fraction (213) - it was this enzyme which was

chosen for study.

Pawlson et al (256) and others (66, 212, 365) have shown that cyclic AMP, in addition to being the substrate, may also play a role in the acute regulation of PDE activity. PDE activity is increased 35 to 50% by in vitro addition of epinephrine or ACTH-agents which increase fat cell content of cyclic AMP (256). PDE activity reaches a maximum by 4 - 7 minutes, thus paralleling changes in cellular cyclic AMP production; then falls toward basal levels by 20 to 30 minutes. Similar changes were observed in this study following in vivo epinephrine injection (Figure 34). Control liver samples obtained at 2 minutes following epinephrine injection showed a 30% increase over basal PDE levels. Zinman and Hollenberg (366) also found a 30% increase in PDE activity in the presence of epinephrine. The increased PDE in the liver is associated with the increased ACA. Furthermore, a 15% fall in hepatic PDE was seen at 2 minutes following norepinephrine injection (Figure 36) which was associated with no increase in cyclic AMP production. Glucagon increased hepatic cyclic AMP production, but was associated with no change in PDE activity (Figure 32). This may represent further evidence for the specificity of hepatic AC response to epinephrine or glucagon. The cyclic AMP generated by either hormone may be immediately compartmentalized and directed toward specific physiologic responses. The cyclic AMP generated by glucagon may be reserved primarily for hepatic glucose metabolism while that generated by epinephrine may be

used in a more ubiquitous fashion and only a small portion directed toward glucose metabolism. The remaining portion may be directed to the vicinity of PDE in order to increase cyclic AMP destruction and limit the action of epinephrine.

No increase in PDE was seen in the intestine following epinephrine, glucagon or norepinephrine injections (Figures 33, 35, 37). Acute regulation of the cyclic AMP system may not be needed in the intestine to the degree that it may be required in the liver.

Little concrete data is available on the effects of epinephrine, glucagon, norepinephrine or shock upon intestinal low Km PDE. Most of the work done on PDE has been performed in the normal liver or adipose tissue. In addition, the few reports available on hepatic PDE changes in shock are conflicting. Glenn and Goldfarb (118) report that in shock, hepatic PDE activity increases by 50%. Furthermore, Cheung (53) states that citrate and ATP produce a 50 to 75% inhibition of PDE activity. It may be that in normal tissues, PDE is operating under the inhibitory influence of citrate and ATP, while in shock tissues which contain reduced amounts of citrate and ATP, PDE is released from this particular influence and may remain unchanged or increase.

Changes in the intracellular ion content may also affect PDE activity. Since magnesium ions are required for PDE activity (78, 335), and hypoxic cells tend to lose magnesium (231), then PDE activity might be expected to fall. Calcium ions are said to

inhibit PDE (9, 53, 138) and are known to accumulate within shock cells; then PDE might again be expected to decrease in vivo. On the otherhand, Rutenberg (unpublished data) states that there is no change in hepatic PDE in shock. The effects of shock upon basal hepatic and intestinal PDE in this study, were variable (Figures 32 to 37). There appears to be no relationship between increases or decreases in PDE activity and altered receptor and/or catalytic function of the AC enzyme. The epinephrine-induced increase in hepatic PDE is significantly reduced in shock (Figure 34). This may be due to the fact that AC response and cyclic AMP generation are abolished. The grouped data on basal PDE response in shock (Table X) shows a non-significant 16% increase over control basal PDE. PDE may be associated primarily with the soluble fraction of the cell and not the membrane. Therefore, ischemic damage to the cellular membrane is not necessarily associated with similar alterations in PDE function as seen with the AC enzyme.

Manganiello and Vaughan (213) and Senft (316) have shown that adrenalectomy is associated with increases in tissue PDE activity. Furthermore, numerous investigators (44, 124, 186, 206, 211, 316) have reported that 25 - 50% depressions in basal PDE activity occur in normal tissues when steroids are administered either in vivo or in vitro.

The effects of shock upon hepatic and intestinal PDE are variable, however the changes associated with steroid treatment are

not. Basal hepatic PDE in the steroid treated animals was significantly decreased from shock levels by 27% in both the glucagon and epinephrine series (Figures 32, 34). The inhibitory influence of steroids upon PDE activity in normal tissues is still evident in shock tissues. Thus, the mechanism by which MPSS alters PDE activity is not abolished in shock.

The 30% increase in PDE that occurred 2 minutes following epinephrine injection in normal animals (Figure 34) which was absent in the shock animals, was partially restored in the steroid-treated animals. Since the AC response to epinephrine was restored in the steroid treated - shock animals, it is not unlikely that cyclic AMP is again acutely regulating PDE activity as seen in the control tissues.

The influence of insulin upon PDE activity must be mentioned. Several investigators (153, 207, 213, 317, 366) have reported that exogenous administration of insulin increases homogenate PDE activity. Pawlson et al (256) studied this effect of insulin upon PDE activity and determined that the mechanism of insulin - induced PDE increases is different from the cyclic AMP - induced PDE increases. The results obtained in this study agree with Pawlson and furthermore indicate that probably the cyclic AMP influence upon PDE activity overrides the insulin influence.

Insulin levels in the control animals increased much more immediately following glucagon injection than following epinephrine

injection (Tables XV, XVII). However hepatic PDE did not increase following glucagon injection (Figure 32). The basal circulating insulin levels in shock were highest in the glucagon series, however shock basal hepatic and intestinal PDE in this series were lower than in the epinephrine series. The basal shock insulin levels in the norepinephrine series (Table XIX) were greater than the respective control values, but hepatic PDE activity was lower in shock. The basal insulin levels in the glucagon series (Table XV) of steroid treated shock were two fold greater than the respective value in the epinephrine group (Table XVII). However, again, hepatic and intestinal PDE activity were greatest in the epinephrine series (Figures 34, 35). Therefore, it appears that there is no relationship between changes in endogenous insulin levels and PDE activity. There are several possible explanations for this. The effect of insulin on PDE has been studied following exogenous in vivo or in vitro administration of insulin. The endogenous levels obtained in this study may not be great enough to exert a noticeable influence. Furthermore, it is not known whether insulin and cyclic AMP affect the same PDE enzyme.

The data so far indicate that steroid treatment in shock tends to restore both the AC and PDE responses to hormonal stimulation.

Glucocorticoids and Plasma Cyclic AMP Levels:

(i) Glucagon

The effect of glucocorticoids upon hepatic plasma cyclic AMP levels following *in vivo* glucagon injection in normal and shock animals is presented in Figure 38.

The control basal hepatic plasma cyclic AMP level is 41.04 ± 12.71 nmols/l. This value is significantly increased ($p < .05$) by 1 minute following glucagon pulse and continues to rise ($p < .025$) to peak at 10 minutes, then fall slightly by 15 minutes. Steroid administration in control animals is associated with no significant change in baseline plasma cyclic AMP levels, however the levels by 5 minutes following glucagon injection increase at a greater rate and peak at a higher level by 10 minutes than in the untreated controls. They also fall off slightly by 15 minutes. In shock, there are no significant differences in the basal and initial plasma cyclic AMP response to glucagon pulse, however after 5 minutes the levels drop off slightly from those in the control and steroid treated control animals at 10 and 15 minutes after glucagon. Steroid administration in shock is associated with a greater rate of hepatic plasma cyclic AMP appearance initially, however by 5 minutes the levels no longer increase and remain stable.

The effect of glucocorticoid administration upon portal plasma cyclic AMP levels following glucagon injection in normal and shock animals is presented in Figure 39.

FIGURE 38

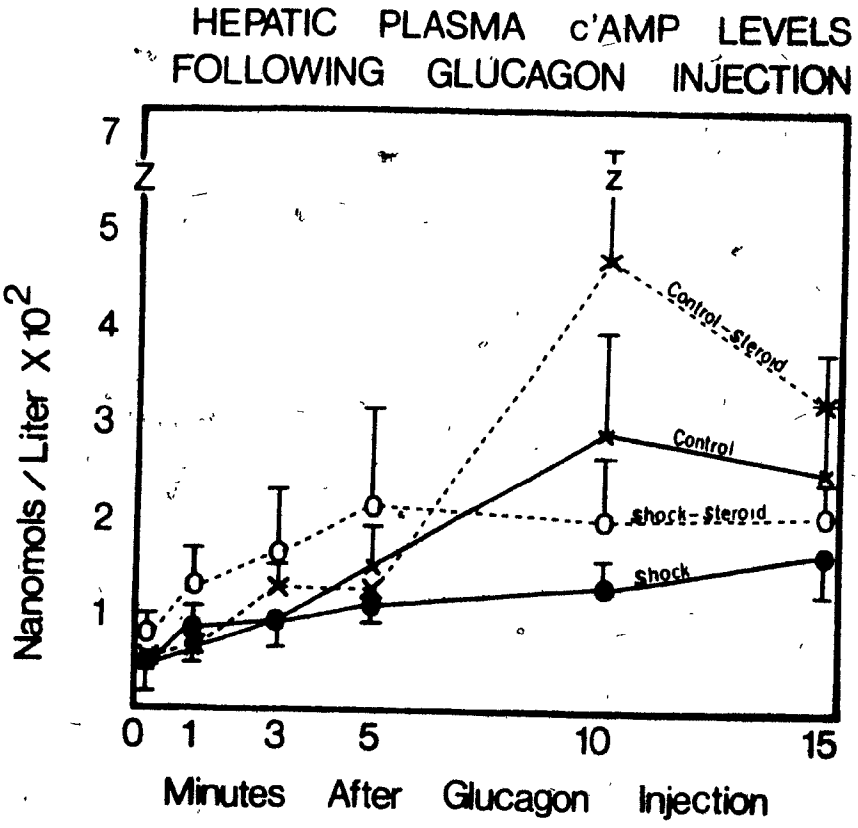
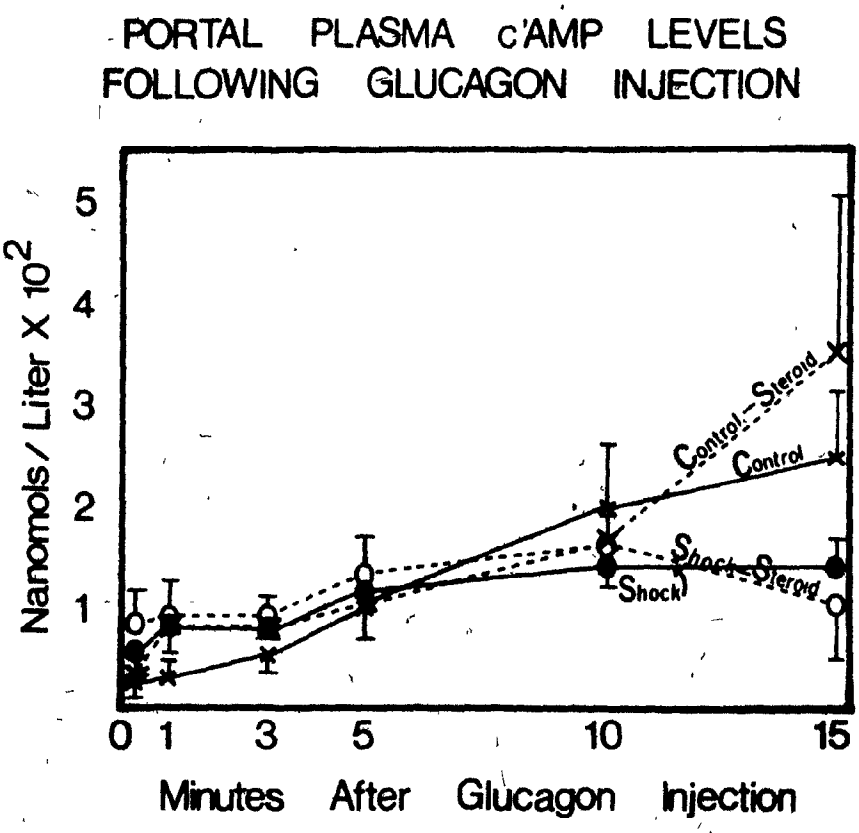

HEPATIC PLASMA c'AMP LEVELS
FOLLOWING GLUCAGON INJECTION
Nanomols / Liter X 10²
7
5
4
3
2
1
0
1
3
5
10
15
Minutes After Glucagon Injection
Control-Steroid
Control
Shock-Steroid
Shock

FIGURE 39


PORTAL PLASMA c'AMP LEVELS
FOLLOWING GLUCAGON INJECTION
Nanomols / Liter X 10^2
5
4
3
2
1
0
1
3
5
10
15
Minutes After Glucagon Injection
Control + Steroid
Control
Shock + Steroid
Shock

There is a smaller cyclic AMP response in the normal portal plasma than in the hepatic blood following glucagon injection. The increase in the normal animal becomes significant by 3 minutes and continues to rise gradually until 15 minutes following glucagon pulse.

Control steroid treatment is associated with a significant ($p<.05$) increase in portal plasma cyclic AMP at 1 minute following glucagon injection when compared to the respective untreated control value. The response remains similar to the untreated control until 15 minutes where it peaks at a slightly higher level. The increase in plasma cyclic AMP is significantly greater ($p<.05$) in shock, compared to the control at 1 minute following glucagon injection. However, the rise is very slow thereafter and the levels at 15 minutes are significantly ($p<.05$) depressed from the levels obtained in steroid treated and untreated controls. Steroid therapy in shock is associated with a slight elevation in basal portal plasma cyclic AMP level over the respective control value (not significant). In addition, the level at 1 minute following glucagon bolus is also slightly elevated over the respective control value. However, again the rise is slower and levels fall off by 10 minutes and are depressed from both steroid-treated and untreated controls by 15 minutes (not significant).

(ii) Epinephrine:

The effect of glucocorticoid administration on hepatic plasma cyclic AMP levels following in vivo epinephrine injection in

normal and shock animals is presented in Figure 40. Hepatic plasma cyclic AMP levels show a steep rise within 1 minute following epinephrine injection. They remain significantly elevated over basal levels at 3 ($p<.01$) and 5 ($p<.025$) minutes. There is a small decline at 10 minutes and then a peak again at 15 minutes. (Significantly ($p<.05$) elevated from basal level). The rise is more rapid in shock - the first peak is sustained from 1 to 3 minutes. The decline is more rapid and remains low from 5 to 10 minutes whereupon it rises again to a level similar to that seen at 15 minutes in the control situation. Steroid treatment in shock is associated with a small elevation in basal hepatic plasma cyclic AMP and a reduced but not significantly different response from that seen in the control animals.

The change in plasma cyclic AMP levels in the portal blood is presented in Figure 41. The response to epinephrine injection in the portal plasma is more rapid and remains consistently higher than that seen in the hepatic blood.

The levels became significantly increased over basal levels by 1 minute ($p<.025$); show a small decline at 3 minutes and peak again by 5, remaining constant until 15 minutes following epinephrine bolus. In shock, the basal level is significantly ($p<.05$) increased over the control level, however the response following epinephrine injection is significantly less than the control at 5 minutes after epinephrine pulse. Steroid treatment in shock is associated with a response

FIGURE 40

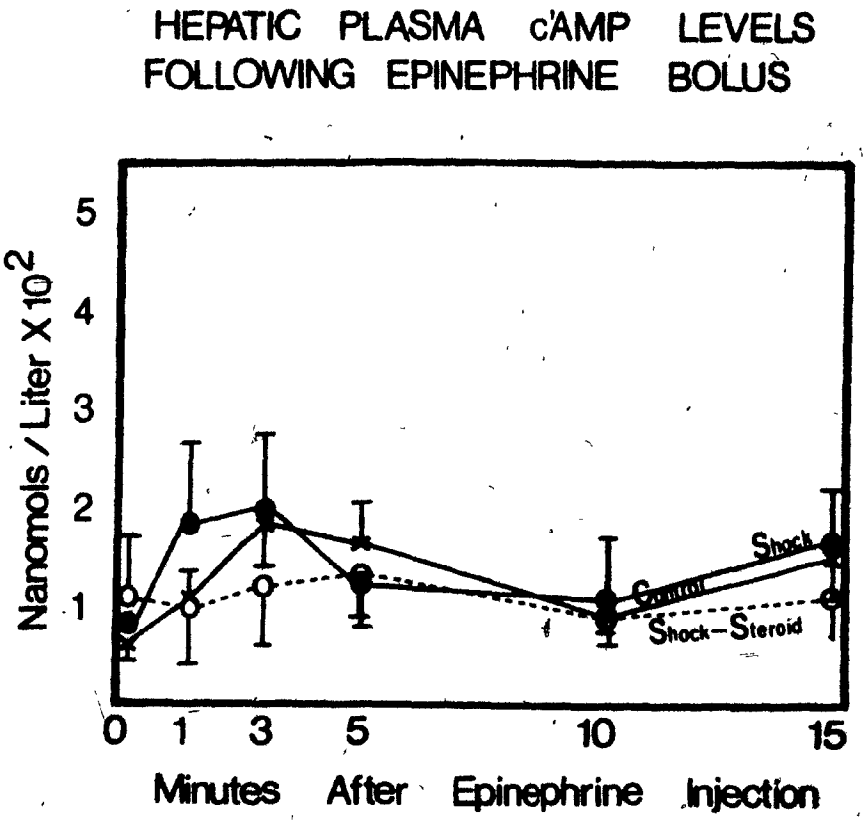

HEPATIC PLASMA cAMP LEVELS
FOLLOWING EPINEPHRINE BOLUS
Nanomols / Liter X 10²
5
4
3
2
1
0
1
3
5
10
15
Minutes After Epinephrine Injection
Shock
Control
Shock–Steroid

FIGURE 41

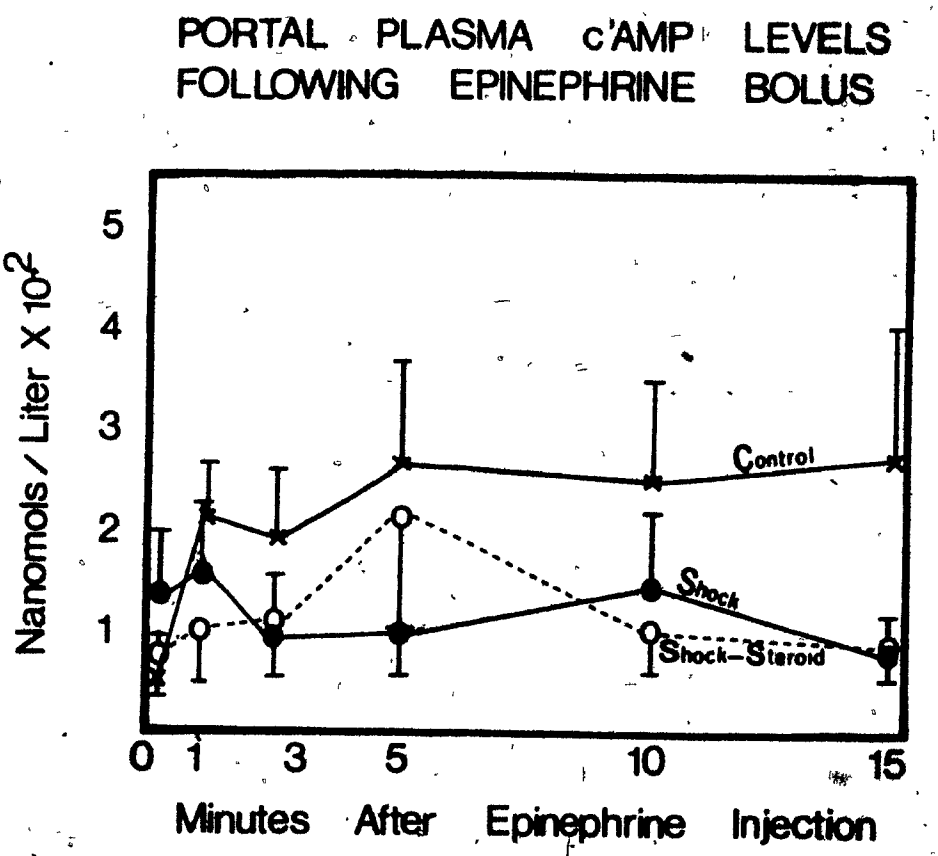

PORTAL PLASMA c'AMP LEVELS
FOLLOWING EPINEPHRINE BOLUS
Nanomols / Liter X 10^{-2}
5
4
3
2
1
Control
Shock
Shock–Steroid
0 1 3 5 10 15
Minutes After Epinephrine Injection

FIGURE 42

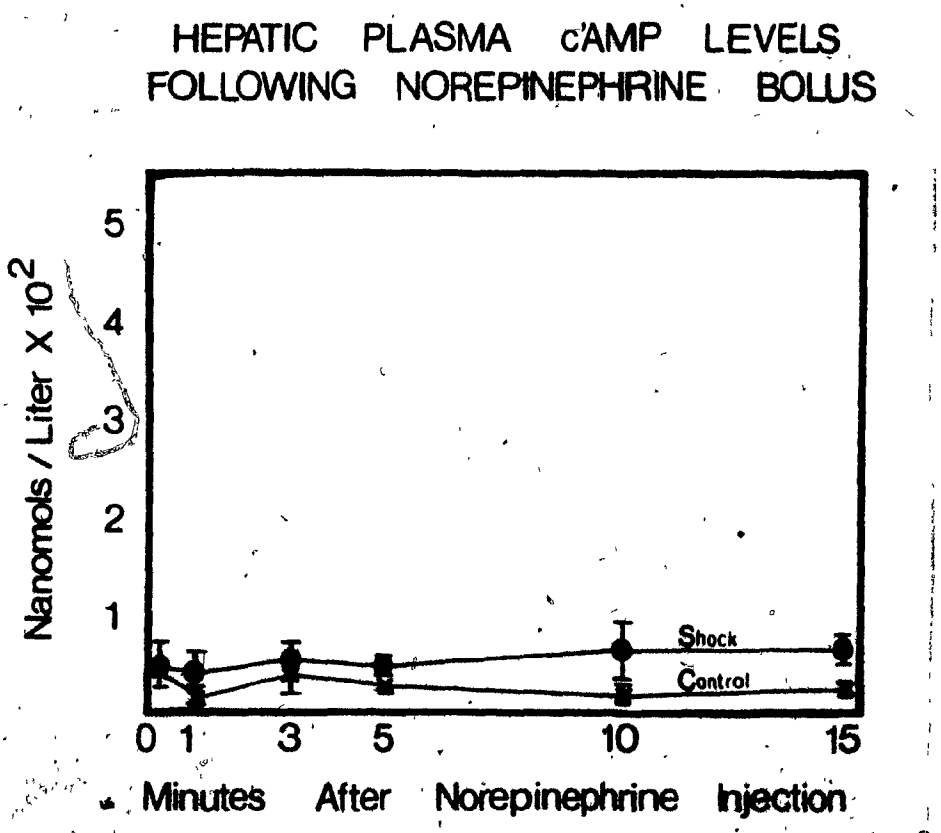

HEPATIC PLASMA c'AMP LEVELS
FOLLOWING NOREPINEPHRINE BOLUS
Nanomols / Liter X 10^2
5
4
3
2
1
Shock
Control
0 1 3 5 10 15
Minutes After Norepinephrine Injection

FIGURE 43

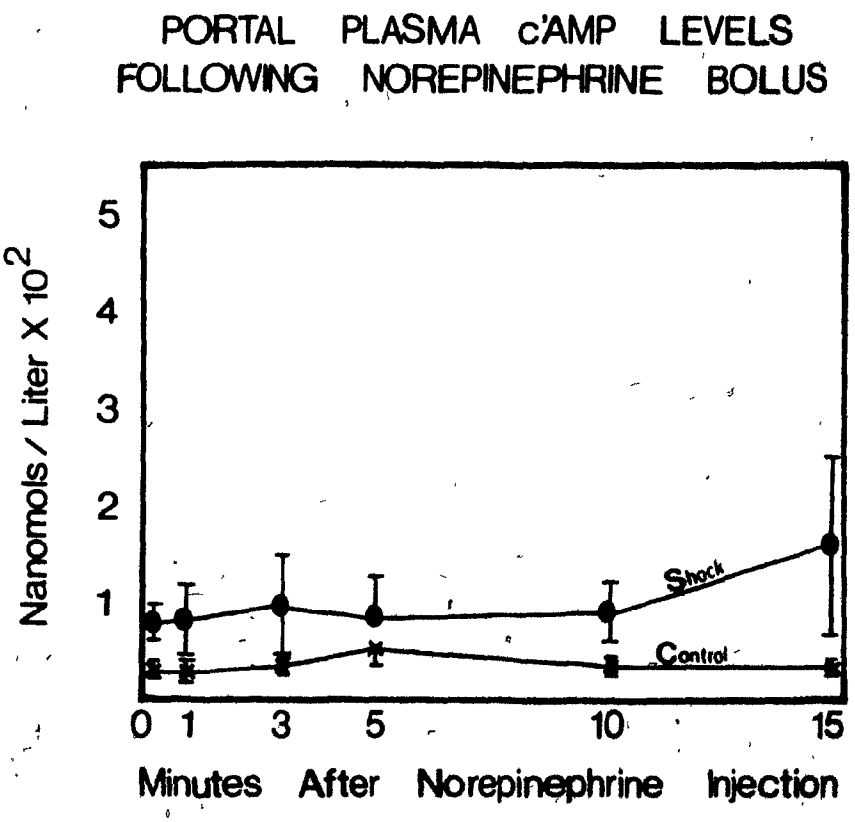

PORTAL PLASMA c'AMP LEVELS
FOLLOWING NOREPINEPHRINE BOLUS
Nanomols / Liter X 10^2
5
4
3
2
1
Shock
Control
0 1 3 5 10 15
Minutes After Norepinephrine Injection

TABLE XI

Baseline Plasma c'AMP Levels Following Steroid Administration in Normal and Shock Animals

	HEPATIC BLOOD	PORTAL BLOOD
Control :	49.42 ± 13.52[+]	35.83 ± 8.89[**]
Control - Steroid :	50.42 ± 14.06	33.75 ± 10.05[**]
Shock :	59.41 ± 26.11	93.69 ± 33.23[*]
Shock - Steroid :	88.45 ± 38.69	63.05 ± 23.33

[+] All values represent nmols c'AMP/L plasma ± S.E.M.
[*] Significantly ($p = or < .05$) elevated from control (Student-T-Test)
[**] Significantly ($p = or < .05$) decreased from shock levels (Student-T-Test)

TABLE XII

Baseline Tissue c'AMP Levels Following Steroid Administration in Normal and Shock Animals

	LIVER	INTESTINE
Control :	2.66 ± 0.53[+]	3.37 ± 0.45
Control - Steroid :	2.01 ± 0.43	2.04 ± 0.62[*]
Shock :	2.34 ± 0.40	3.24 ± 0.44
Shock - Steroid :	1.59 ± 0.21[*]	2.15 ± 0.33[*]

[+] All values represent pmols c'AMP/mg tissue ± S.E.M.
[*] Significantly ($p = or < .05$) reduced from control (Student-T-Test)

slightly lower than control which falls off significantly ($p < .025$) by 15 minutes.

(iii) Norepinephrine

The hepatic plasma cyclic AMP response to *in vivo* norepinephrine injection is presented in Figure 42. There are no significant changes in the hepatic plasma cyclic AMP levels following norepinephrine injection in the control animals. The response in shock is slightly elevated but is only significantly increased ($p < .01$) over control levels at 15 minutes.

The portal plasma cyclic AMP response to norepinephrine is presented in Figure 43. There is very little change in portal plasma cyclic AMP in normal animals following norepinephrine injection. The basal portal plasma cyclic AMP level is significantly elevated ($p < .025$) in shock and the response slightly elevated over control levels, significant ($p < .05$) at 10 minutes.

Hepatic plasma cyclic AMP levels exceed portal levels by an average of 40% (Table XI). However, in shock, this ratio is reversed and hepatic levels are 36% less than portal levels. Steroid administration in shock restores this ratio.

Glucocorticoids and Tissue Cyclic AMP Levels:

(i) Glucagon

Hepatic tissue cyclic AMP changes following *in vivo* glucagon injection in steroid-treated and untreated control and shock

animals is presented in Figure 44.

There is a significant ($p = .05$) 54% increase in hepatic cyclic AMP levels 2 minutes after glucagon injection. Tissue levels return to normal by 15 minutes. Steroid-treated controls show non-significant increases at 2 minutes and at 15 minutes. The response in shock is not different from that seen in the steroid controls. Steroid therapy in shock is associated with a slight depression in the basal cyclic AMP level and in the response to glucagon.

Changes in intestinal cyclic AMP levels following glucagon in steroid treated and untreated animals are presented in Figure 45.

There is a gradual decrease in intestinal cyclic AMP levels following glucagon pulse. This is significant ($p<.05$) at 15 minutes. Steroid treatment in control animals is associated with a non-significant decrease in basal tissue cyclic AMP levels. The levels remain low at 2 minutes and are signficantly ($p<.05$) reduced from controls at 15 minutes following glucagon pulse. Shock is associated with levels of intestinal cyclic AMP not significantly different from control values, or steroid-controls at 15 minutes. Steroid treatment in shock produces a significant depression in basal cyclic AMP levels when compared to untreated controls and shock. Tissue levels continue to fall and are not significantly reduced from control and shock levels at 15 minutes. There is no difference in intestinal cyclic AMP levels between the steroid-treated controls and the steroid treated shock animals.

FIGURE 44

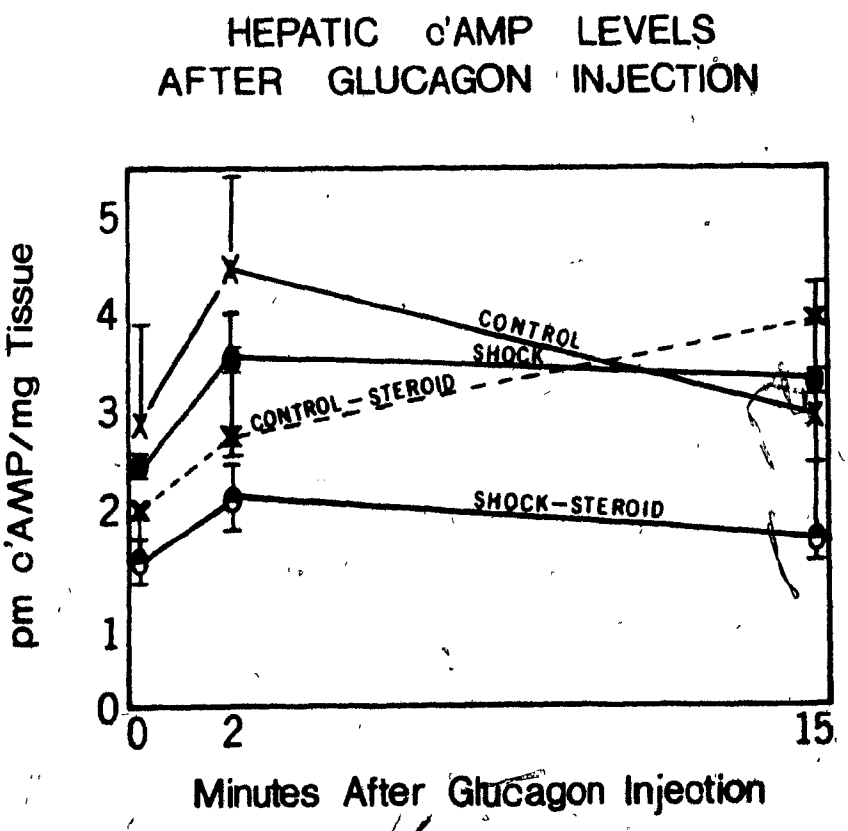
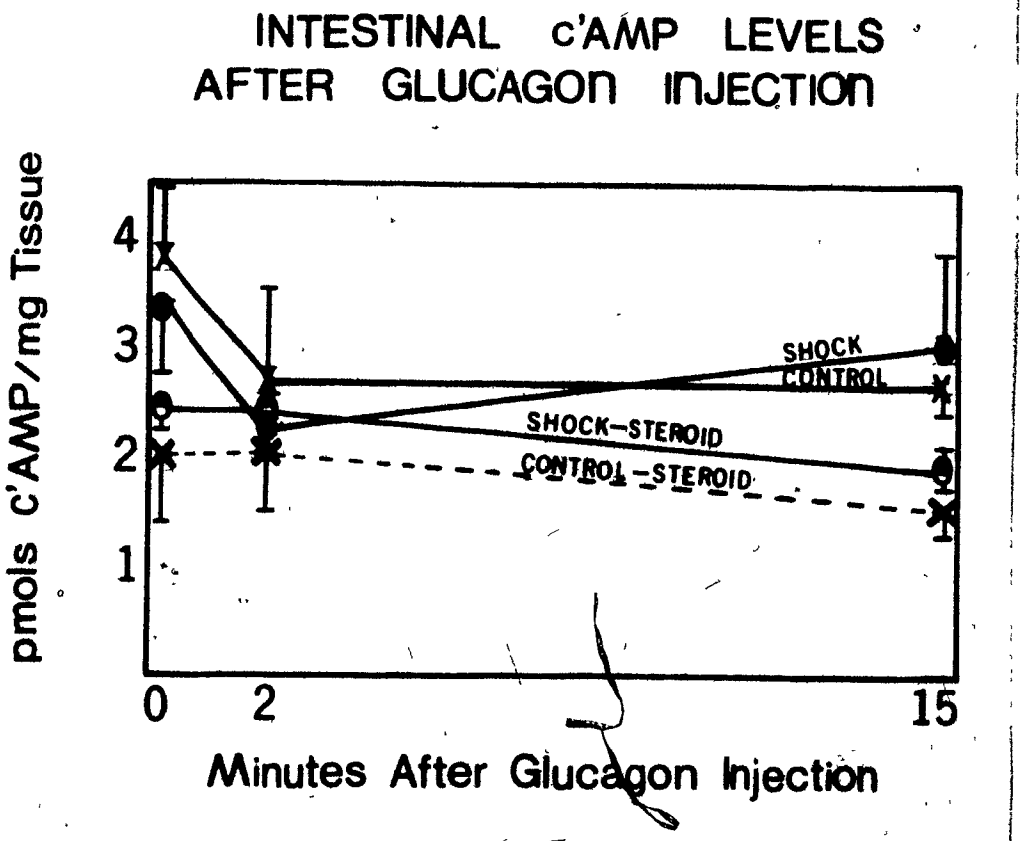

HEPATIC c'AMP LEVELS
AFTER GLUCAGON INJECTION
pm c'AMP/mg Tissue
0
1
2
3
4
5
0
2
15
Minutes After Glucagon Injection
CONTROL
SHOCK
CONTROL - STEROID
SHOCK - STEROID

FIGURE 45


INTESTINAL c'AMP LEVELS
AFTER GLUCAGON INJECTION
pmols c'AMP/mg Tissue
4
3
2
1
SHOCK
CONTROL
SHOCK–STEROID
CONTROL–STEROID
0
2
15
Minutes After Glucagon Injection

(ii) Epinephrine

Changes in hepatic cyclic AMP levels following epinephrine injection are presented in Figure 46.

There is a 6% and a significant ($p<.05$) 45% increase in the normal hepatic tissue cyclic AMP levels at 2 and 15 minutes respectively following epinephrine pulse. Shock is associated with a similar response; the cyclic AMP level at 15 minutes being significantly ($p<.025$) elevated above the basal level. Steroid treatment in shock produces a slight depression in hepatic cyclic AMP levels from control which is significant ($p<.05$) at 15 minutes.

Changes in intestinal cyclic AMP content following epinephrine injection are presented in Figure 47.

There is a small decline in tissue cyclic AMP levels following epinephrine injection which becomes significant ($p<.01$) at 15 minutes. Shock is associated with a significant increase ($p<.025$) in basal tissue levels, however levels fall off slightly following epinephrine pulse. Basal cyclic AMP levels are not significantly reduced from the respective shock levels in the steroid treated shock animals. There is a significant increase ($p<.01$) at 2 minutes which falls back to basal levels by 15 minutes.

(iii) Norepinephrine

Changes in hepatic cyclic AMP content following norepinephrine injection is presented in Figure 48.

There are no changes in hepatic cyclic AMP content follow-

FIGURE 46

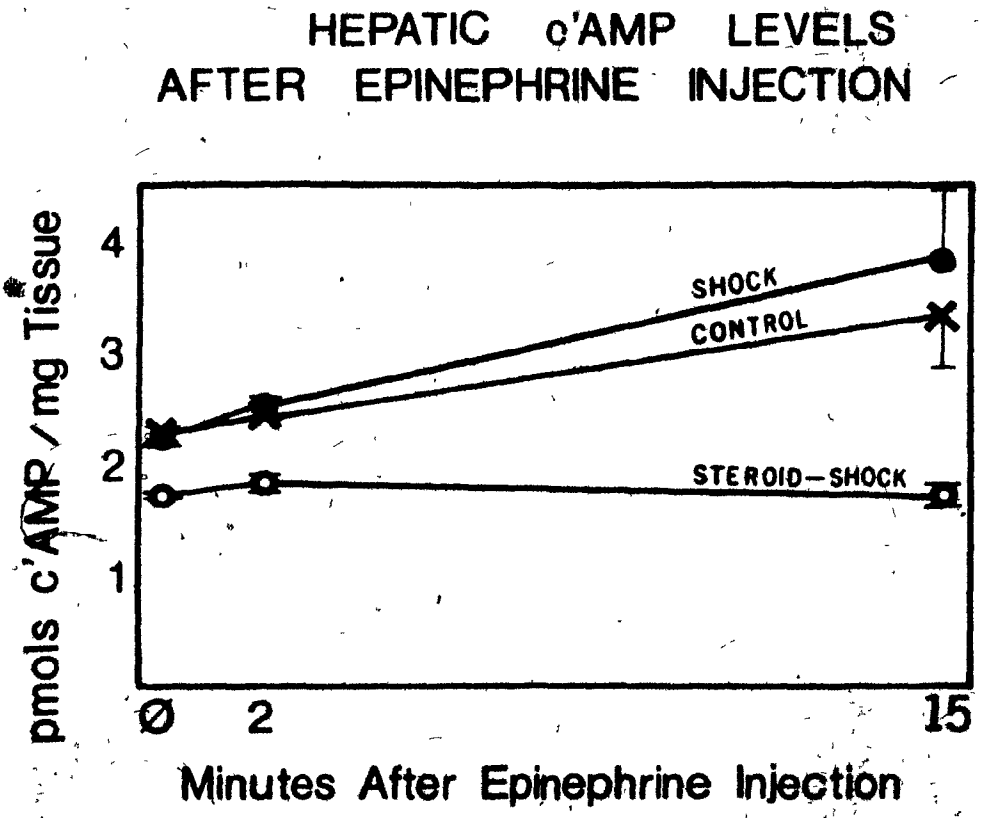

HEPATIC c'AMP LEVELS
AFTER EPINEPHRINE INJECTION
pmols c'AMP / mg Tissue
4
3
2
1
SHOCK
CONTROL
STEROID—SHOCK
Ø
2
15
Minutes After Epinephrine Injection

FIGURE 47

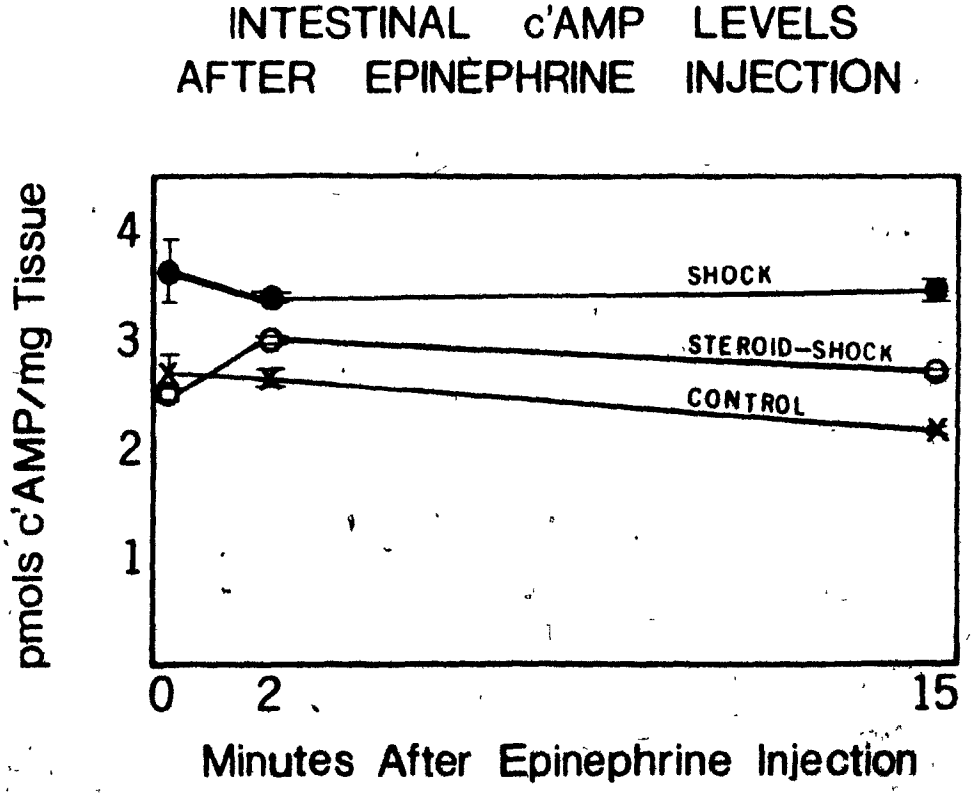

INTESTINAL c'AMP LEVELS
AFTER EPINEPHRINE INJECTION
pmols c'AMP/mg Tissue
4
3
2
1
SHOCK
STEROID-SHOCK
CONTROL
0
2
15
Minutes After Epinephrine Injection

FIGURE 48

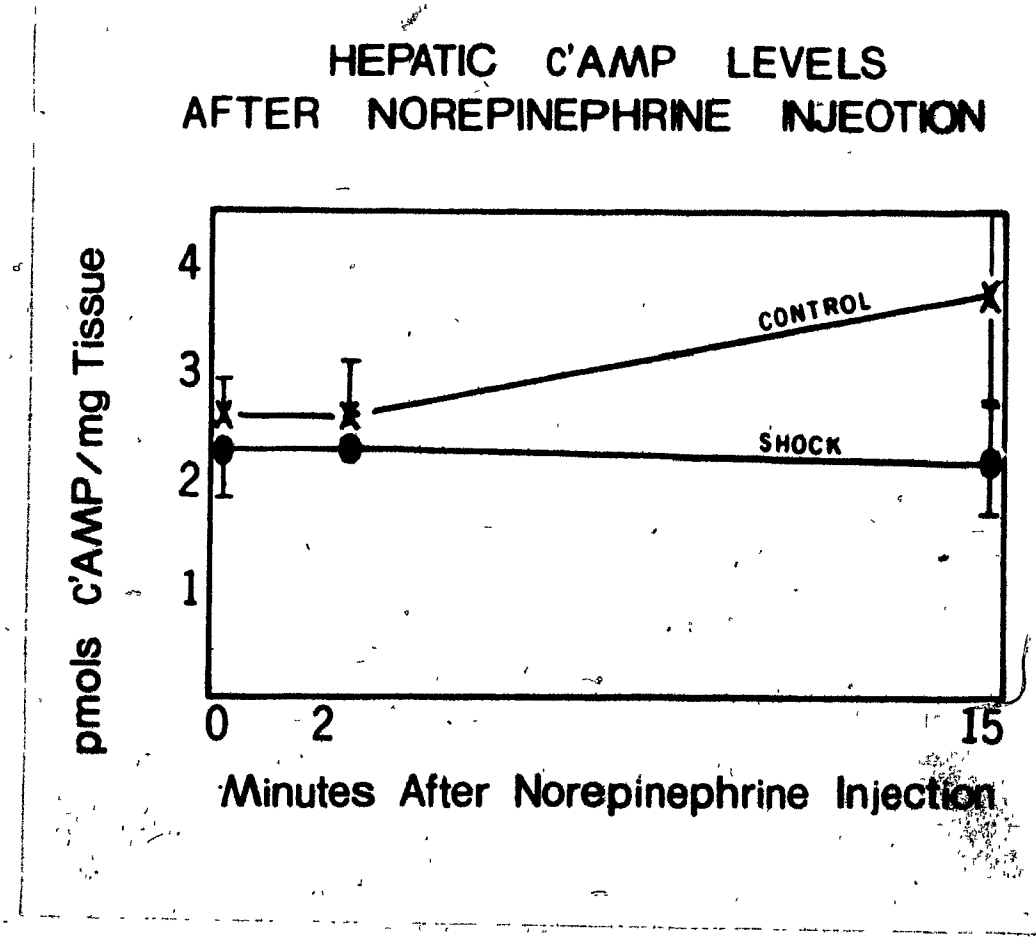
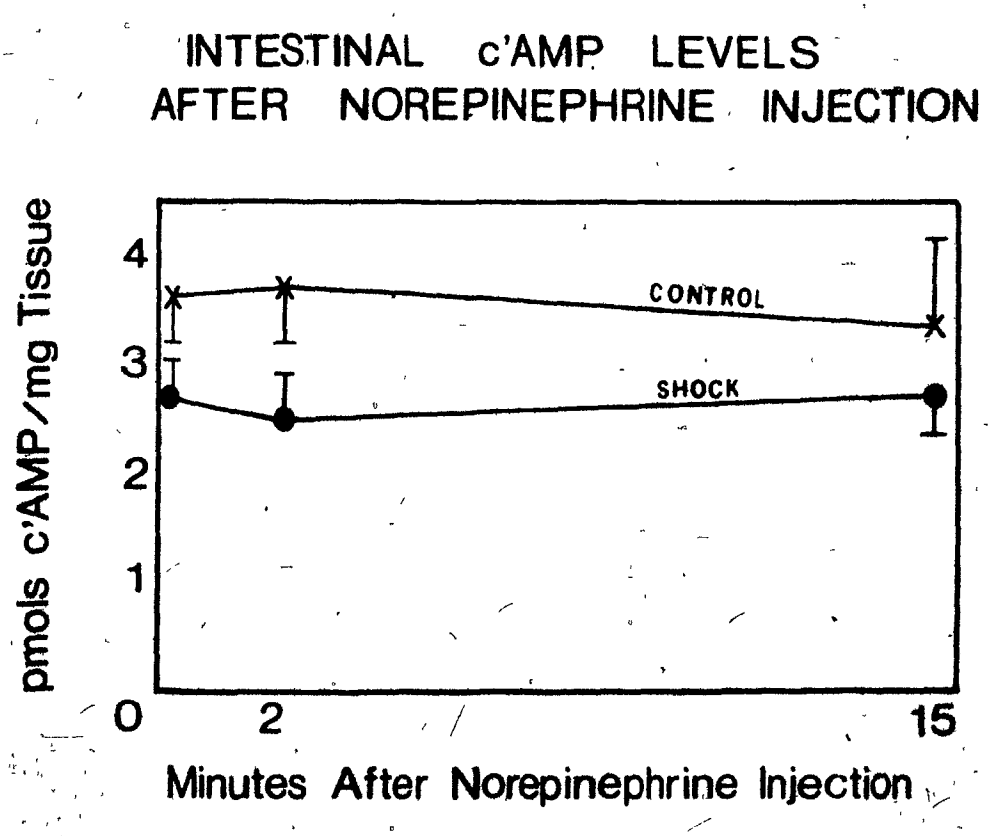

HEPATIC C'AMP LEVELS
AFTER NOREPINEPHRINE INJEOTION
pmols C'AMP/mg Tissue
4
3
2
1
CONTROL
SHOCK
0
2
15
Minutes After Norepinephrine Injection

FIGURE 49


INTESTINAL c'AMP LEVELS
AFTER NOREPINEPHRINE INJECTION
pmols c'AMP/mg Tissue
4
3
2
1
0
CONTROL
SHOCK
2
15
Minutes After Norepinephrine Injection

ing norepinephrine bolus, and in shock the values do not differ from the respective control values.

Changes in intestinal cyclic AMP content following norepinephrine injection are presented in Figure 49.

There is no change in cyclic AMP content in normal animals following norepinephrine pulse. Cyclic AMP content in the shock intestine is significantly reduced ($p < .05$) from control at 2 minutes after norepinephrine pulse.

The cyclic AMP content of intestinal tissue is generally 25% greater than the cyclic AMP content in the liver, however in the steroid treated controls, the cyclic AMP content is the same in the two tissues (Table XII).

Discussion:

Cyclic AMP is the product for AC and substrate for PDE. The data so far implicates changes in both these enzymes in shock. Glucocorticoid therapy in shock exerts a positive influence upon these enzymes tending to return their hormonal responses to normal. However, since tissue and plasma cyclic AMP are a net result of ACA, PDE activity, leakage and utilization and since the ultimate physiologic responses are mediated by cyclic AMP - it was of great import to study changes in both tissue and plasma cyclic AMP levels. The changes in cyclic AMP in hepatic and intestinal tissue and hepatic and portal plasma are discussed with respect to one another and to changes in ACA and PDE activity.

Measurement of total tissue cyclic AMP is not as easy to interpret as once was thought. Several investigators (49, 351) have reported that cyclic AMP and other adenine nucleotides are capable of crossing cellular membranes intact, to appear in the extracellular fluid. Exton et al (86) and others (12, 36) have shown that glucagon and epinephrine markedly increase net hepatic cyclic AMP generation, however the increase was more accurately reflected in the increasing extracellular fluid levels than in an intracellular accumulation. They (86, 185) found a better correlation between the glycogenolytic effect of these hormones and the rise in perfusate cyclic AMP rather than tissue levels, in the perfused liver. Furthermore, McArdle et al (224) have shown that epinephrine can stimulate cyclic AMP production

in the intestine *in vivo*, but the increased amounts of cyclic AMP leave the cells and enter the portal plasma resulting in no intracellular accumulation. Wehmann *et al* (351, 352) also came to the conclusion that the small intestine is an important source of plasma cyclic AMP.

A large portion of the intracellular baseline cyclic AMP content in the normal liver and intestine, may be in an inactive bound or compartmentalized form, whereas the active free cyclic AMP may leak into the extracellular space (85, 185). Furthermore, Anderson *et al* (8, 9) and Matsumoto and Uchida (219) report that no change in tissue cyclic AMP levels can occur when no significant alteration in the ratio between adenyl cyclase activity and phosphodiesterase activity takes place. Therefore, changes in the AC/PDE ratio may affect changes in intracellular and to a greater degree extracellular cyclic AMP levels.

The source of plasma cyclic AMP under basal conditions is rather uncertain. However, it is thought that organs release and take up cyclic AMP at the same time (351). The basal levels of plasma cyclic AMP obtained in this study range between 15 and 30 nanomoles per litre of plasma. Similar levels have been reported elsewhere in dogs (351, 352) and in man (115, 196, 265). Normal basal hepatic tissue cyclic AMP levels in this study ranged from 2.26 to 2.88 picomoles of cyclic AMP per milligram wet weight (pmols/mg) of tissue. This is within the range reported by Robison (284, p. 271, Chapt. 7). The baseline

levels of cyclic AMP in the intestinal mucosa ranged between 2.67 and 3.82 pmols/mg. This is in agreement with data reported by others (101, 224, 298, 299).

Glucagon is more potent in mobilizing hepatic glucose than is epinephrine. This was evident in the greater increases in ACA and tissue and plasma cyclic AMP levels following glucagon injection compared to epinephrine. These observations are in agreement with others (5, 88). Norepinephrine is an alpha-adrenergic agent and therefore produced little change in cyclic AMP production and glucose mobilization.

There is another essential difference between the glucagon and epinephrine responses. The pattern of tissue and plasma cyclic AMP response to epinephrine is biphasic in nature, while that following glucagon is not (88, 334). This finding was confirmed in the present study. The explanation for the biphasic response to epinephrine is not certain, however it has been proposed (334) that an increase in PDE, (which was seen in this study, Figure 34), might account for the decline seen following the peak in hepatic plasma cyclic AMP levels. This coupled with a massive initial release of cellular cyclic AMP might also account for the lack of tissue cyclic AMP accumulation. This theory is borne out by the fact that by 15 minutes PDE has returned to normal and cellular release has slowed down which allowed for a significant intracellular accumulation of cyclic AMP. Furthermore, in the glucagon group, PDE activity did not change and initially cellular release was slower than at the same time following epinephrine.

This allowed for a significant increase in hepatic cyclic AMP at 2 minutes which fell to normal by 15 minutes when cellular release was much greater than that seen after epinephrine.

Cyclic AMP, itself, appears to be partially responsible for terminating it's own actions. This is accomplished in two ways: by stimulating PDE activity to increase intracellular breakdown; and by escaping into the extracellular fluid. These two mechanisms may operate one at a time or in unison.

The pattern of cyclic AMP response to glucagon in the portal plasma probably reflects the changes in hepatic blood levels contributed by the liver (Figures 38, 39). Furthermore, there was a slight decline in intestinal mucosal cyclic AMP levels. This was not unexpected. Previously mentioned, was the fact that the intestine does not appear to contain significant amounts of AC sensitive to glucagon.

Field et al (95) stated that addition of epinephrine to an isolated strip of intestinal mucosa resulted in a decrease in tissue cyclic AMP levels and therefore concluded that epinephrine exerts an alpha-like effect upon the intestinal mucosal AC. There was also no intestinal accumulation of cyclic AMP following in vivo epinephrine pulse in this study (Figure 47). However, ACA was increased 30% by 2 minutes, and appearance of cyclic AMP into the portal circulation was rapid and sustained at a high level (Figures 11, 41). Furthermore, in vitro addition of epinephrine to intestinal homogenates produced a 30% increase in ACA (Figure 15). Although intestinal PDE did

not change *in vivo*, it appears that cyclic AMP leakage from the cells prevented a net intracellular accumulation following *in vivo* epinephrine as seen elsewhere (224). The discrepancy between these findings and those reported by Field *et al* (95), may be due to the fact that they did not take into account the fact that cyclic AMP may leave the tissues to appear in the external medium. However, epinephrine does stimulate both α and β receptors and the intestinal mucosa may contain both types of receptors. Alpha receptor stimulation and reduction in cyclic AMP production cannot be ignored as a possible contributing factor to fluctuations in tissue and plasma cyclic AMP following *in vivo* epinephrine injection.

Recently, changes in hepatic and portal vein cyclic AMP levels were studied (224) following a single bolus injection of 1 mg of epinephrine (4 times the does used in this study). The findings were similar to what was seen in this study, however there were some noteworthy differences. In the previous study, plasma cyclic AMP levels fell toward normal by 15 minutes following epinephrine injection - unlike this study where they remained elevated at 15 minutes. Noteworthy also, is the fact that the maximum levels of cyclic AMP reached in the portal blood following a 0.25 mg dose of epinephrine exceeded those seen following a 4 times larger dose of epinephrine (1 mg); while hepatic vein cyclic AMP levels were similar in both studies.

There is a distinct intestinal mucosal second messenger response to epinephrine and this response can equal or exceed that

of the liver following epinephrine with respect to plasma cyclic AMP increases. However, the pattern of the intestinal response appears to be dose dependant; when the epinephrine load becomes excessive (1 mg), the response is somewhat blunted and shorter in duration than that seen following a much smaller epinephrine load (.25 mg).

This may be a protective measure on the part of the second messenger system; when overwhelmed, it may "apply the brakes" in the form of a negative feedback system to reduce production of cyclic AMP. In addition, it may be that the vasoconstrictor effect of such a massive soe of epinephrine on the sensitive intestinal vasculature predominates and prevents full expression of the epinephrine effect upon the AC system.

There is little or no change in either hepatic or portal plasma and tissue cyclic AMP levels following norepinephrine pulse (Figures 42, 43, 48, 49). Ball et al (12) studied the response of plasma cyclic AMP in humans following norepinephrine and also found no changes in plasma cyclic AMP levels. This is not unexpected, as in low doses, norepinephrine is primarily an alpha agonist in the liver and intestine.

Basal hepatic vein cyclic AMP levels in shock were not different from their respective control values (Table XI). However, portal vein cyclic AMP levels were significantly elevated in shock (Table XI). Furthermore, the shock portal vein levels were slightly greater than the shock hepatic vein levels. Normally hepatic vein cyclic AMP levels are greater than portal vein levels under basal

conditions (351). This is consistent with the fact that the canine intestine is the well known "target organ" of shock with a tendency to develop necrotic lesions; and if an increased cellular release of cyclic AMP is a rough indication of tissue injury or stress, as shown by McArdle et al (224) in shock dogs, Prudhoe et al (264) in shock pigs, Rabinowitz et al (265, 266), Strange et al (332) and Vetter et al (346) for myocardial infarction, Sibbald et al (326) in clinical sepsis and Sehgal et al (314) in alcoholic pancreatitis; the increased portal levels may in fact be an index of the degree of severity of the insult as proposed by Sehgal et al (314).

Furthermore, Gill et al (115) have indicated that in surgery and trauma, the increases in plasma cyclic AMP preceded and appeared to be involved in the stimulation of cortisol release from the pituitary gland. They concluded that perhaps the extracellular fluid cyclic AMP increases seen in response to stress might be an additional form of physiologic compensation.

Cyclic AMP and other nucleotides have been shown to leak at increased rates into the extracellular fluid in vivo (99) and in vitro (183) from cells as a result of increased cellular membrane permeability which is known to occur in trauma and shock. This may also explain why the initial response of both shock liver and intestine to epinephrine or glucagon is greater than normal. Furthermore, such factors as renal insufficiency and reduced excretion of cyclic AMP may also contribute to the increased plasma cyclic AMP levels

or to maintenance of the increase in plasma levels. Despite the increased cell permeability, the response to epinephrine rapidly fell - no doubt due to the ablated AC and increased PDE activities. The rise in hepatic vein cyclic AMP in shock following glucagon continued at a rate slower than normal but did not fall off (Figure 38). Probably this reflects the continuing AC response coupled with a normal PDE activity. The altered pattern of cyclic AMP release into portal and hepatic blood appears to be a rough reflection of the defects present in the hepatic and intestinal second messenger system.

Shock basal hepatic and intestinal cyclic AMP levels were not significantly different from control. Robison, et al (283) also found that anoxic cardiac tissue cyclic AMP levels were unchanged from control. However, in contrast are the results of several investigators that found significant reductions in hepatic (228, 289) and intestinal (224) cyclic AMP levels in experimental hemorrhagic shock. Simultaneously measured ATP levels in these studies were also significantly reduced. There were few significant reductions in ATP levels in this study. The possibility of ATP being compartmentalized and that significant reductions in ATP could occur in the pool destined for AC cannot be ignored as a possible compromising factor in tissue cyclic AMP production.

There were some significant increases in shock tissue levels of cyclic AMP. These were associated with a normal or slightly increased ACA and a normal or slightly decreased PDE, and occurred

despite the increased cellular permeability, and could not be readily explained.

Simultaneous measurement of production, release and breakdown can account for most plasma and tissue cyclic AMP changes. However, certain discrepancies do arise and may be explained in several ways. For example, under appropriate conditions, concentrations of the catecholamines can be found which increase fat cell lipolysis (89, 90) or contractile force in cardiac muscle (254), without any detectable changes in total tissue cyclic AMP. Furthermore, the resting level of cyclic AMP in the liver, if evenly distributed intracellularly would, on the basis of in vitro studies, (284, p. 271 Chapt. 7) represent a concentration of the nucleotide sufficient to stimulate gluconeogenesis and glycogenolysis maximally. This would essentially eliminate glycogen synthesis. Since this is not the case, several investigators (10, 42, 100) have postulated that any total tissue pool of cyclic AMP is compartmentalized within the cells. Thus, the pool of cyclic AMP affecting any particular function may represent only a small fraction of the total tissue cyclic AMP. Therefore certain agents could double the size of it's specific "target" pool of cyclic AMP without its resulting in any measurable increase in the overall basal levels. On the otherhand, heterogenous tissues, such as the intestinal mucosa, may contain numerous large pools of cyclic AMP, which are not closely related to the particular function being studied. The intestine contains villus,

crypt and goblet cells. The extent to which each contribute to the absorptive and secretory functions produced by α-stimulation and β or cyclic AMP stimulation is not known. The ileal mucosa also contains many leukocytes (mostly lymphocytes) which could contribute to the cyclic AMP content of this tissue. Minute changes of only a few picomoles per mg of tissue conceivably are associated with maximal alterations in physiologic function. Changes of this magnitude might be undetectable when they occur in one compartment surrounded by other compartments with high basal cyclic AMP levels. Furthermore, relatively small decreases in one cell might be masked by high basal cyclic AMP levels in surrounding unaffected cells.

Issekutz (159) reported that Depo-Medrol treatment of 3.8 mg/kg x 3 days in normal dogs was associated with a 43% decrease in baseline plasma cyclic AMP levels. Short term administration of Solu-Medrol for 4 hours (30 mg/kg) was associated with no changes in baseline plasma cyclic AMP levels (Table XI). However in shock, steroid treatment tended to restore the reversed hepatic/portal plasma cyclic AMP ratio evident in untreated shock. This was accomplished by increasing hepatic vein cyclic AMP levels (49%) and decreasing (32%) portal vein cyclic AMP levels.

Initially, MPSS treatment in normal animals was associated with an increased ACA, decreased tissue cyclic AMP levels and decreased PDE activity. The excess cyclic AMP appeared in the plasma. However, steroid administration in normal animals for periods longer

than four hours may be associated with depressions in baseline ACA, coupled with decreased plasma levels.

Steroid therapy in shock appears to exert an inhibitory influence upon all levels of baseline function in the second messenger system as seen here and elsewhere (159). However, despite the depressed baseline values in shock and in control steroid treated animals (159) the responses of AC to hormonal stimulation were supranormal and cyclic AMP levels were restored in shock.

Recently, it has been shown that insulin is a very potent inhibitor of glucagon - stimulated release of cyclic AMP from the perfused rat liver (85, 86, 185). Methylprednisolone administration significantly increases plasma insulin levels in normal dogs (Figure 51), (45, 159) and in shock - steroid treated animals (Figures 51, 53). The increased circulating insulin levels in the shock and steroid-treated shock animals in this study could contribute to some of the decreases in plasma cyclic AMP response to glucagon stimulation.

The absolute values for baseline, basal and NaF - stimulated ACA are depressed from control and shock values in the steroid-treated shock animals. The decrease in NaF - stimulated activity probably indicates a reduction in the total amount of functional AC enzyme. However, the response of AC to hormonal stimulation is normal or supranormal in the steroid - shock group. Steroid administration in shock appears to potentiate the efficiency of the remaining enzyme to a point where the response to hormonal stimulation is greater than

normal. In conjunction with this positive effect upon AC, steroid therapy is associated with a decrease in PDE activity. The overall effect of steroids in shock appears to be aimed at preservation of AC response irrespective of baseline function.

Basal ACA does not appear to be of primary importance in determining the ultimate ability of the enzyme to respond to hormonal stimulation. Basal ACA in the steroid - treated controls was significantly elevated from untreated controls, while response to glucagon was not significantly altered from that of the untreated controls. The AC response to epinephrine in shock was abolished and was associated with a normal basal ACA. Basal ACA was reduced in the steroid-treated shock tissues, while AC response had been restored to normal. Therefore, changes in basal ACA do not necessarily represent a reliable prediction of the ability of the AC enzyme to function. However, when both basal and NaF - stimulated ACA are significantly reduced, the response of AC and the concomitant physiologic response are altered, as seen in shock following epinephrine pulse. This is no longer true in the presence of steroids where, even in shock, significant reductions in basl and NaF - stimulated ACA are associated with normal or supra-normal response.

Glucocorticoids and Serum Insulin:

(i) Glucagon

The effect of glucocorticoid administration upon hepatic serum insulin levels following glucagon injection in normal and shock animals is presented in Table XIV.

There are only small nonsignificant elevations in hepatic serum insulin levels following glucagon injection into control animals.

The changes in serum insulin levels in shock dogs following glucagon pulse are not significantly different from those seen in the normal animals.

Steroid administration in normal or shock animals is associated with slight elevations in basal circulating insulin levels. No significant alterations in the response curve following glucagon injection was noted when compared to the respective control or shock values.

Portal serum insulin changes following glucagon injection are presented in Table XV.

There is a slight elevation in serum insulin following glucagon injection in normal animals, however none of the increases are significant. Steroid administration in control animals is associated with a nonsignificant increase in basal insulin over the respective control value. The increases in insulin levels at 3, 5, 10 and 15 minutes following glucagon injection are also not significant. Within each group in which insulin levels were measured, there were

TABLE XIV

Changes in Hepatic Serum Insulin Levels Following In Vivo Glucagon Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	38.5 ± 17+	36.0 ± 11	58.2 ± 26	66.5 ± 38	59.5 ± 26	78.0 ± 30
Control - Steroid :	87.8 ± 36	123.8 ± 69	56.7 ± 18	128.8 ± 80	151.8 ± 63	171.0 ± 85
Shock :	23.0 ± 5	95.5 ± 40	55.7 ± 24	23.0 ± 9	28.7 ± 13	24.0 ± 12
Shock - Steroid :	57.3 ± 11	65.6 ± 15	104.4 ± 38	97.3 ± 41	92.2 ± 36	125.6 ± 34

+ All values represent μ units/ml ± S.E.M.

TABLE XV

Changes in Portal Serum Insulin Levels Following In Vivo Glucagon Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	22.2 ± 11+	41.7 ± 25	74.7 ± 52	73.9 ± 52	56.8 ± 30	61.3 ± 27
Control - Steroid :	66.3 ± 26	143.5 ± 65	200.5 ± 88	314.7 ± 110	285.8 ± 98	186.6 ± 56
Shock :	50.0 ± 21	190.0 ± 170	108.5 ± 71	143.3 ± 119	121.3 ± 101	121.5 ± 93
Shock - Steroid :	100.2 ± 39	186.8 ± 74	143.0 ± 86	107.8 ± 59	142.8 ± 76	84.2 ± 38

+ All values represent μ units/ml ± S.E.M.

always one or two animals that showed excessively high insulin levels following hormone injection; especially in the shock and steroid-treated animals. This was therefore associated with large standard errors and a lack of significance.

Steroid administration in both control and shock animals was associated with a trend toward greater elevations in portal insulin levels following glucagon injection. The levels attained following glucagon injection into steroid-treated controls tended to be greater than those seen in the steroid-treated shock animals.

(ii) Epinephrine

Changes in hepatic serum insulin levels following epinephrine injection are presented in Table XVI.

There is a significant ($p < .025$) decrease in serum insulin levels at 1 minute following epinephrine injection in normal animals. Levels rise slightly but are not significantly different than the basal levels. The response in shock is similar except the increases seen at 5 and 10 minutes are significantly ($p < 0.05$) increased from the shock basal level. There are no significant differences between the control and shock serum insulin levels following epinephrine bolus.

Steroid administration is associated with no significant changes in basal levels or response following epinephrine pulse.

Changes in portal serum insulin levels following epinephrine injection are presented in Table XVII.

Serum insulin levels remain low until after 1 minute following

TABLE XVI

Changes in Hepatic Serum Insulin Levels Following In Vivo Epinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	27.4 ± 5[+]	19.8 ± 3	20.8 ± 2	32.0 ± 7	54.4 ± 21	39.6 ± 16
Shock :	25.0 ± 9	21.3 ± 7	51.0 ± 21	53.8 ± 21	58.8 ± 24	46.8 ± 20
Shock - Steroid :	48.4 ± 12	33.2 ± 11	39.0 ± 14	49.0 ± 11	70.0 ± 21	46.5 ± 8

[+] All values represent μ units/ml ± S.E.M.

TABLE XVII

Changes in Portal Serum Insulin Levels Following In Vivo Epinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	22.6 ± 2[+]	25.4 ± 8	42.8 ± 16	81.1 ± 55	49.8 ± 17	92.0 ± 59
Shock :	12.7 ± 7	10.5 ± 5	18.0 ± 10	28.7 ± 11	30.4 ± 16	25.0 ± 9
Shock - Steroid :	75.6 ± 33	28.8 ± 3[*]	45.1 ± 10	137.6 ± 57	163.1 ± 61	127.0 ± 54

[+] All values represent μ units/ml ± S.E.M.

[*] Significantly greater than the respective shock value ($p < .05$)

epinephrine injection. There is a peak ($p < .05$) at 5 minutes, a trough at 10 and another peak at 15 minutes following epinephrine pulse. The response in shock is not significantly different from the control response.

Steroid administration in shock is associated with a non-significant increase in basal portal serum insulin levels. There is a slight (nonsignificant) decrease at 1 minute, which is significantly greater ($p < .05$) than the respective shock value. This is followed by a gradual increase which peaks at 10 minutes and falls off slightly by 15 minutes.

(iii) Norepinephrine

Changes in hepatic serum insulin levels following norepinephrine pulse are presented in Table XVIII.

There is a gradual and variable increase in serum insulin levels significant ($p < .025$) only, at 10 minutes following norepinephrine pulse. Shock is associated with a slight decrease in response which is significant ($p = .05$) at 10 minutes.

Portal serum insulin response to norepinephrine injection is presented in Table XVIII. The increase in control portal serum insulin levels becomes significant at 3 minutes ($p < .0125$) and remains so at 5 ($p = .05$) and 10 minutes ($p < .05$) following norepinephrine bolus. There is a significant increase in serum insulin levels at 1 and 5 minutes following norepinephrine injection in shock animals. The shock response is only significantly greater

TABLE XVIII

Changes in Hepatic Serum Insulin Levels Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	26.2 ± 8[+]	32.8 ± 8	53.8 ± 23	97.8 ± 56	84.0 ± 28[**]	67.2 ± 32
Shock :	36.5 ± 16	32.6 ± 9	37.4 ± 16	38.4 ± 13	31.0 ± 8 [*]	27.3 ± 8

[+] All values represent μ units/ml ± S.E.M.

[*] Significantly lower than the respective control value (p = .05) (Student-T-Test)

[**] Significantly elevated over baseline value (p <.05) (Paired-T-Test)

Changes in Portal Serum Insulin Levels Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	20.6 ± 4[+]	25.0 ± 11	48.4 ± 10[*]	108.8 ± 48[*]	76.4 ± 26[*]	67.2 ± 34
Shock :	84.7 ± 59	120.8 ± 75[*]	138.6 ± 83	166.3 ± 70[*]	76.0 ± 32	63.8 ± 20

[+] All values represent μ units/ml ± S.E.M.

[*] Significantly elevated over respective baseline values (p = or <.05) (Paired-T-Test)

than the control response at 1 minute after norepinephrine bolus.

Glucocorticoids and Hematocrit:

(i) Glucagon

Changes in hepatic blood hematocrit following glucagon injection are presented in Table XIX.

There is no change in hematocrit in the normal animal following glucagon injection. Steroid-treated shock or untreated shock are associated with a slight increase in hematocrit (nonsignificant) which remains unchanged after glucagon bolus.

A similar pattern in normal, shock and steroid-treated shock animals is seen in the portal blood (Table XX).

(ii) Epinephrine

Changes in hepatic blood hematocrit following epinephrine injection are seen in Table XXI.

There is a significant increase ($p < .0005$) in hematocrit 1 minute following epinephrine pulse, which remains elevated ($p < .0005$) until 15 minutes ($p < .0025$). There is no change in hematocrit at 1 minute following epinephrine injection in shock animals. This value is significantly ($p < .025$) reduced from the respective control value. The remaining values are not significantly different from the control values. Steroid therapy is associated with a slight increase in basal hematocrit which is not significant. There is a significant depression in hematocrit at 15 minutes in the steroid-treated group.

TABLE XIX

Changes in Hepatic Blood Hematocrit Following In Vivo Glucagon Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	45.8 ± 1.8+	45.8 ± 2.2	49.2 ± 2.0*	48.8 ± 2.0	47.6 ± 2.2	46.0 ± 2.7
Shock :	55.8 ± 6.5	54.5 ± 6.1	55.8 ± 5.8	55.5 ± 5.9	55.0 ± 5.9	54.3 ± 6.3
Shock - Steroid :	55.2 ± 3.7	53.2 ± 8.3	53.2 ± 8.3	53.3 ± 3.4	53.5 ± 3.2	51.3 ± 3.5

+ All values represent percent red blood cells ± S.E.M.

* Significantly ($p < .05$) elevated from baseline value (Paired-T-Test)

TABLE XX

Changes in Portal Blood Hematocrit Following In Vivo Glucagon Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	45.8 ± 1.4+	46.0 ± 2.1	48.6 ± 2.3*	48.8 ± 2.2*	47.6 ± 2.3	45.6 ± 2.6
Shock :	55.5 ± 6.5	56.5 ± 6.0	56.3 ± 5.9	56.0 ± 5.7	55.0 ± 5.9	53.5 ± 6.4
Shock - Steroid :	54.5 ± 3.5	51.6 ± 2.77	53.2 ± 4.0	52.8 ± 3.3	52.8 ± 3.3	49.4 ± 3.2

+ All values represent percent red blood cells ± S.E.M.

* Significantly ($p < .05$) elevated above baseline value (Paired-T-Test)

TABLE XXI

Changes in Hepatic Blood Hematocrit Following In Vivo Epinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	50.0 ± 4.9+	65.5 ± 3.1*	62.3 ± 3.1*	60.8 ± 3.5*	57.5 ± 3.8*	55.0 ± 4.1*
Shock :	53.8 ± 3.1**	56.2 ± 2.3	57.6 ± 2.4	57.0 ± 2.3	54.4 ± 2.6	53.2 ± 2.5
Shock - Steroid :	58.0 ± 1.1**	57.0 ± 2.2	56.8 ± 2.4	58.2 ± 1.7	56.0 ± 2.1	54.8 ± 1.9***

\+ All values represent percent red blood cells ± S.E.M.
* Significantly (p = or <.05) elevated from baseline value (Paired-T-Test)
** Significantly (p = or <.05) elevated from control value (Student-T-Test)
*** Significantly (p = or <.05) decreased from baseline value (Paired-T-Test)

TABLE XXII

Changes in Portal Blood Hematocrit Following In Vivo Epinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	45.4 ± 4.1+	55.5 ± 6.0*	57.0 ± 5.6*	56.0 ± 5.6*	55.0 ± 6.5*	50.4 ± 5.1*
Shock :	52.8 ± 2.6	57.2 ± 2.1	55.8 ± 2.0	54.6 ± 2.2	53.0 ± 2.4	51.6 ± 2.7
Shock - Steroid :	57.2 ± 2.2**	57.2 ± 1.4	58.2 ± 1.6	57.6 ± 1.6	55.4 ± 2.2***	54.2 ± 2.1***

\+ All values represent percent red blood cells ± S.E.M.
* Significantly (p = or <.05) elevated from baseline value (Paired-T-Test)
** Significantly (p = or <.05) elevated from control value (Student-T-Test)
*** Significantly (p = or <.05) decreased from baseline value (Paired-T-Test)

A similar pattern is observed for the portal blood hematocrit in Table XXII.

(iii) Norepinephrine

Changes in hepatic blood hematocrit following norepinephrine injection are presented in Table XXIII.

There is a significant ($p < .02$) increase in hematocrit at 1 minute following norepinephrine bolus. The hematocrit remains elevated at 3 ($p < .01$) and 5 minutes ($p < .05$) however falls to basal levels thereafter. There is a significant ($p < .025$) increase in basal hematocrit in the shock animals. There are no changes following norepinephrine pulse in shock.

Portal blood hematocrit (Table XXIV) shows a significant increase ($p < .001$) at 1 minute, which remains significantly elevated. There is a nonsignificant increase in the basal hematocrit of the shock animals and this does not change following norepinephrine bolus.

TABLE XXIII

Changes in Hepatic Blood Hematocrit Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	46.0 ± 2.3+	56.2 ± 2.7*	56.6 ± 2.2*	54.8 ± 2.4*	52.2 ± 2.5	50.8 ± 2.2
Shock :	55.8 ± 2.8**	54.3 ± 3.1	55.0 ± 3.8	54.5 ± 3.6	53.5 ± 3.3	53.3 ± 3.5

+ All values represent percent red blood cells ± S.E.M.

* Significantly (p = or <.05) elevated from baseline value (Paired-T-Test)

** Significantly (p = or <.05) elevated from respective control value (Student-T-Test)

TABLE XXIV

Changes in Portal Blood Hematocrit Following In Vivo Norepinephrine Injection

TIME (Minutes)	0	1	3	5	10	15
Control :	48.6 ± 1.3+	55.2 ± 1.6	55.2 ± 2.4	54.0 ± 2.5	52.0 ± 2.1	51.0 ± 2.2
Shock :	54.5 ± 3.2	55.0 ± 3.1	55.0 ± 3.1	54.3 ± 3.1	53.8 ± 3.4	53.8 ± 3.4

+ All values represent percent red blood cells ± S.E.M.

Discussion:

Insulin is capable of supressing the normal rise in tissue and plasma cyclic AMP in response to epinephrine or glucagon (283). Many of the hepatic effects of insulin are mediated by a decrease in cyclic AMP, either by reduction in adenyl cyclase activity or increase in phosphodiesterase activity or both. Since blood glucose, serum insulin and the second messenger system are intimately related in normal and pathological conditions, it was of interest to observe the insulin response to hormonal stimulation under normal conditions, in untreated shock and following steroid therapy.

The normal baseline levels of circulating immunoreactive insulin range between 20 to 30 micro units per millilitre of serum (μu/ml), values similar to those found in dogs by others (45, 159).

The maximal increase in portal blood insulin levels in normal dogs did not differ following epinephrine or glucagon (Tables XV, XVII), despite the fact that glucagon is a more powerful mobilizer of glucose, and also has been reported to stimulate insulin release in the absence of glucose (81). Glucose is the primary stimulus for increased insulin release. However, it appears that there is a limit beyond which subsequent increases in circulating glucose levels will produce further increases in insulin release. Thus, the maximal increases in insulin may be similar, however the patterns of insulin response following epinephrine or glucagon are different. Porte (262) and others (108, 146) report that epinephrine exerts a direct inhibi-

tory effect upon insulin release by stimulating the α-receptors of the pancreatic islet cells. However, the inhibitory influence exerted by epinephrine does not prevent insulin release completely. It is the glucose, and not amino acid (102) or free fatty acid (129, 214) induced elevations of serum insulin that are inhibited by epinephrine. Since epinephrine is known to mobilize fatty acids, then some increase in insulin release would be expected following epinephrine injection. The inhibitory effect of epinephrine probably accounts for the lag in insulin release seen following epinephrine pulse - which was not seen following glucagon, and which was associated with elevated glucose levels in both cases (Tables XV, XVII). Furthermore, the observed decline in insulin release at 10 minutes following epinephrine may represent the continuing controlling influence of epinephrine. Glucagon stimulates insulin release by directly stimulating the β receptors in the pancreatic islet cells (36, 63, 293). In addition, glucagon stimulates release of endogenous catecholamines (63, 71) which might account for the small decline in insulin release seen at 10 minutes after glucagon injection.

Cerasi et al (47) have shown that β-adrenergic blockade inhibits the rise in insulin during an infusion of glucose. Others (6, 162) have indicated that dibutyryl cyclic AMP infusion will increase insulin release. Still others (293) have indicated that increases in plasma cyclic AMP may directly stimulate insulin release which in itself may partially overcome the inhibitory influence of

epinephrine. Zawalich et al (362) have shown that there is a negative feedback relationship between plasma cyclic AMP and insulin. When an increased plasma cyclic AMP produces a increase in insulin release, the elevated levels of insulin tend to "feed back" upon the second messenger system to reduce cyclic AMP production. Furthermore, a highly significant ($p < .0005$) correlation between serum insulin and plasma cyclic AMP levels has been reported for hemorrhagic shock in pigs (264) and in acute myocardial infarction in humans (346). However, alterations in renal function were not examined in these studies and might contribute to such a close correlation. A positive correlation between serum insulin and plasma cyclic AMP levels was not seen in this study. The insulin responses were far more variable than the plasma cyclic AMP responses. In addition, Chiu et al (unpublished data) have also found no correlation between serum insulin levels and plasma cyclic AMP levels in patients undergoing minor or major (cardiopulmonary bypass) cardiac surgery.

Issekutz et al (162) have shown that despite the α-adrenergic nature of norepinephrine, insulin levels show a marked but transient increase upon norepinephrine infusion. The rapid rise in free fatty acids was thought to be the stimulus. Similarily, in this study, insulin levels showed a short-lived increase following norepinephrine injection (Table XIX). Since there was little change in blood glucose or plasma cyclic AMP levels, it may be that an increase in free fatty acids stimulated insulin release.

Blood glucose and insulin levels in shock are subject to tremendous variation. Differences in measured values depend on species, type of shock and sampling time. However, there is general agreement that hemorrhagic shock is like diabetes in that it is characterized by an insulin resistance and glucose intolerance (50, 290). Grouped data (N = 15; Table XIII) in this study indicate a 125% increase in portal levels of insulin in shock which is associated with an insignificant 7% decrease in blood glucose levels, both measured at 4 hours following hypovolemia. Following epinephrine injection, the blunted plasma cyclic AMP and insulin responses were associated with an abolition of the hyperglycemic response. The insulin response following glucagon injection in shock is greater than the control response. This is associated with a slightly reduced hyperglycemic response and probably is an indication of the insulin resistance.

Insulin has been reported to interact with the second messenger system resulting in a decrease in net cyclic AMP production (83, 258). The mechanism by which insulin affects the decrease is not certain. When endogenous insulin levels are decreased during starvation (86) or insulin deficiency (168), hepatic cyclic AMP levels are elevated. Robison et al (283) report that hepatic cyclic AMP levels are decreased in the presence of insulin. Ray et al (271) and others (157, 164, 184) state that insulin supresses tissue adenyl cyclase activity. Still others (260, 341) have not been able to show any change in adenyl cyclase activity (basal, epinephrine or glucagon

TABLE XIII

Baseline Hepatic and Portal Serum Insulin Levels
Following Glucocorticoid Administration

	HEPATIC BLOOD	PORTAL BLOOD
Control :	30.14 ± 10.20+	21.79 ± 5.64
Control - Steroid :	87.80 ± 36.00*	66.33 ± 25.49*
Shock :	28.17 ± 5.49	46.35 ± 28.95
Shock - Steroid :	52.87 ± 11.85	87.88 ± 36.10*

+ All values represent μ units/ml ± S.E.M.

* Significantly ($p = or < .05$) elevated from control (Student-T-Test)

stimulated) in the presence of insulin. On the otherhand, insulin has been shown to increase low Km hepatic phosphodiesterase activity *in vivo* (317) and *in vitro* (207, 213, 366). However, this could not be confirmed by Muller-Oerlinghausen *et al* (247) or Menahan *et al* (234).

Thus, it is possible that excessively high circulating levels of insulin may feed back upon the hepatic and intestinal second messenger system to produce a decrease in plasma cyclic AMP appearance. This may be what has occurred in the steroid treated group following glucagon injection. The circulating insulin levels are inappropriately high (probably due to steroid action upon gluconeogenic mechanisms and perhaps a direct effect upon insulin secretory rates, coupled with the insulin resistance of the shock state). Although AC response to the hormone is restored to normal, absolute levels and appearances of the cyclic AMP into the plasma is still reduced. There may, in fact, be a complicated feedback relationship between plasma cyclic AMP, glucose and insulin.

Hemorrhagic shock complicates this relationship with an added insulin resistance and further hormonal intervention with increased circulating levels of endogenous epinephrine, norepinephrine, glucagon and cortisol. Jefferson *et al* (168) state that in shock, the insulin control on cyclic AMP production is abolished. In addition, the reverse is also true: it appears that cyclic AMP control upon insulin release is also abolished in shock. In the shock - epinephrine

group (Figures 40, 41), the plasma cyclic AMP response is only slightly reduced, however both the hyperglycemic and insulin responses are abolished. In contrast, the insulin response in the shock - glucagon group (Tables XIV, XV), is greater in shock than in control and is associated with a slightly reduced hyperglycemic and plasma cyclic AMP response. Both insulin and cyclic AMP responses are elevated in shock following norepinephrine (Table XIX, Figures 42, 43).

Bauer and associates (17) report that dogs subjected to hemorrhagic shock develop a significant hyperglycemia accompanied by a corresponding increase in insulin levels. Other species such as sheep, Rhesus monkey and baboon, do not exhibit a significant insulin response to hemorrhagic shock despite a marked hyperglycemic response (136, 141, 240).

Several clinical studies have reported (46, 48, 97) low insulin levels in various types of shock. The baseline portal insulin levels in this study, (grouped data, Table XIII) agree with Bauer et al (17) in that there is a significant 112% increase in canine shock compared to control.

Steroid treatment in normal and shock animals provides further stimulus for insulin release. Along with epinephrine and glucagon, methylprednisolone is a powerful gluconeogenic agent. This is evident by the 200% increase in baseline portal insulin levels, over the untreated controls, in the steroid treated control animals (Table XIII, XV). Similarily, glucocorticoid therapy has been

shown to increase portal insulin levels in normal rabbits (45), by as much as 244% (156). Furthermore, insulin release following glucagon injection in the steroid-treated controls, although variable, is greater than that seen in the control, shock or steroid-treated shock groups. Baseline portal insulin levels were increased by 300% in the shock-steroid treated animals (Table XIII), and the response following either glucagon or epinephrine (Tables XV, XVII), was greater than the normal response, but only greater than the shock response in the epinephrine group. The insulin release following epinephrine in the shock steroid-treated group appears to have overcome the inhibitory influence of epinephrine as it is greater than in the control animals and is associated with a normal hyperglycemic response. However, the insulin responses in the shock steroid-treated group following glucagon injection appears to be inappropriately high for a subnormal hyperglycemic response.

It appears that in addition to providing extra endogenous glucose, the steroids sensitize the insulin responding system resulting in a greater outpouring of insulin in response to smaller changes in plasma cyclic AMP. Furthermore, the α-blockade (74) characteristics of MPSS may circumvent the epinephrine induced inhibition to a certain extent and allow for an increased sensitivity to β-adrenergic stimulation.

Epinephrine and norepinephrine are potent vasopressors which in the dog, and not in man, contract down the splenic capsule (216

Chapt. 24) to decrease the size of the spleen with a resultant increase in red blood cell release into the peripheral circulation. Thus, providing a physiologic mechanism for an increased amount of circulating hemoglobin during acute hypoxia, hemorrhage and other stresses that may increase catecholamine release. Furthermore, the catecholamines decrease circulating blood volume by increasing the loss of protein-free fluid into the tissues probably as a result of post capillary vasoconstriction. The resultant hemoconcentration is a rapid and significant response in this study (Tables XV, XVI). It becomes evident by one minute following either epinephrine or norepinephrine injection in normal animals. However, in shock, with or without steroid therapy, the baseline hematocrit is elevated from normal levels, but shows no further increase following catecholamine injection (Table XV). This is most likely due to a depletion in the splenic supply of red blood cells which accompanies the hemorrhage - induced disturbances in plasma volume.

Normally, a lower hematocrit is associated with a better survival rate in hemorrhagic shock. However, Manohar and Tyagi (215) have shown that in hydrocortisone pre-treated animals, that were subjected to superior mesenteric artery occlusion shock, the hematocrit was markedly elevated, but there were excellent survival rates (to 100%). Furthermore, Marks et al (216), have shown that adrenalectomy decreases plasma volume and is generally associated with poorer survival rates following shock than when the steroids are replaced.

They have indicated that increased cortisol levels are part of the physiologic requirement for restitution of blood volume following hemorrhage (216). Gann and Pirkle (112) have shown that the increased hematocrit, plasma protein concentration and osmolality associated with high cortisol and steroid levels is not due to elevated glucose levels alone; but due to an influx of fluid and electrolytes. Cortisol and it's synthetic analogues have been shown (338) to increase plasma volume secondary to an active transport of sodium out of the cells. The steroids appear to reverse the effect of shock upon the intracellular accumulation of excess sodium and water (242, 323). The endogenous steroids coupled with exogenous administration of steroids help the interstitial fluid to protect the plasma volume in shock.

GENERAL DISCUSSION

Homeostasis is affected by the response of the tissue cells to the hormones. The role of the second messenger, cyclic AMP, in mediating cellular responses to hormones is widely recognized. Evidence is rapidly accumulating to support the suggestion that defects in cyclic AMP metabolism may account for abnormalities in homeostatic function in various clinical situations. Changes in adenyl cyclase function and tissue and plasma cyclic AMP levels that have been measured in hemorrhagic shock (124, 224, 288), indicate a fundamental defect in the cellular machinery. Inadequate response of the second messenger system could result in the decreased ability of the tissues to respond to homeostatic and therapeutic controls; and therefore may contribute to irreversibility in shock.

Steroids have been shown to interact with and potentiate the response of the second messenger system to hormonal stimulation.

The purpose of the present investigation was to study the normal response of the second messenger system in splanchnic tissues following hormonal stimulation; and to observe the degree to which the hormonal response is altered in hemorrhagic shock; and to investigate whether the induced alterations could be made to respond to therapeutic administration of glucocorticoids.

The results obtained by the few investigators that have done any work in this field have been confirmed in this study. However, the defect in the second messenger response has been defined more specifically,

and a means of partially reversing the alteration has been accomplished.

Glucagon is a more potent hepatic glycogenolytic and gluconeogenic hormone than is epinephrine. The maximum hepatic blood glucose levels reached in this study following in vivo glucagon injection in normal animals was 33 percent greater than those reached following epinephrine injection (Figures 2, 4). Therefore, since cyclic AMP is said to mediate the effects of these hormones upon hepatic glucose output, it might be expected that glucagon produces greater increases in hepatic adenyl cyclase activity than does epinephrine. Indeed, this is what was seen. The maximal increase in adenyl cyclase activity following glucagon injection was almost 50 percent greater than that seen following epinephrine injection in normal animals (Figures 8, 10). Furthermore, the ability of the hepatic adenyl cyclase enzyme to respond to hormonal stimulation was decreased following glucagon and abolished following epinephrine injection (Figures 8, 10) in shock animals. Thus, the reduction and abolition of the hyperglycemic responses to glucagon and epinephrine respectively, are also accompanied by parallel changes in the adenyl cyclase response to these hormones.

The reports by others (88, 161, 165) indicating that small doses of norepinephrine have virtually no effect upon hepatic carbohydrate metabolism in normal dogs was confirmed in this study. Norepinephrine injection into normal animals was associated with no changes in tissue adenyl cyclase activity or hepatic blood glucose levels. Furthermore, no significant changes were noted when norepinephrine was

injected into shock animals (Figures 12, 13).

Basal adenyl cyclase activity was excluded as a reliable prediction of a tissue's ability to respond to hormonal stimulation in shock. The adenyl cyclase response to in vivo epinephrine injection was abolished in shock and was associated with a "normal-looking" basal ACA in both the liver and intestine (Figures 10, 11). Hepatic adenyl cyclase response to in vivo glucagon injection was only slightly reduced and was also associated with a normal basal enzyme activity (Figure 8).

The circulation to and within the splanchnic organs is severely compromised in shock. The question arises as to whether the defects observed are a direct result of a decreased delivery of hormone to the cells of the target tissues, or perhaps due to severe ischemic damage to the cellular membrane-bound enzyme.

The decreased perfusion in shock tissues may contribute to a depressed hormonal response by limiting the accessibility of the target tissue hormone receptors to the circulating hormone. In vitro addition of hormone to tissue homogenates obtained before and after in vivo hormone injection assures contact between the hormone and tissue receptors in question. These studies indicated that even if the hormones are able to reach the target tissue receptors in a normal fashion, the ability of the adenyl cyclase system in these shock tissues to respond is reduced by 40 percent following in vitro epinephrine addition (Figure 15) and 30 percent following in vitro glucagon introduction (Figure 14). Therefore, the reductions in both in vivo and in vitro responses to the hormones in

question are not totally a result of a reduction in the amount of hormone delivered to the target tissues in shock.

Sodium fluoride stimulates adenyl cyclase activity maximally and represents the intrinsic or catalytic capabilities of the adenyl cyclase enzyme - free from receptor subunit influence. Significant decreases in NaF - stimulated ACA were noted only in shock liver and intestine following in vivo epinephrine injection where the adenyl cyclase response was totally abolished; and in the shock liver and intestine in the norepinephrine group where basal adenyl cyclase activity was significantly reduced. Thus, slight decreases in AC response appears to be related to a receptor subunit defect, while catalytic subunit function remains relatively intact. However, when response to in vivo hormone injection is totally abolished, catalytic function of adenyl cyclase is also altered. It would appear that only when adenyl cyclase function is severely disrupted, is the intrinsic or catalytic activity of the enzyme disturbed.

The altered hormonal responses may reflect damage incurred by the cellular membrane structure induced by the ischemia of shock. Damage to structure and function of cell membranes is well documented in shock (31, 68). Since adenyl cyclase is a membrane-bound enzyme and therefore is dependent upon the structural integrity of the membrane for optimal enzyme function, then architectural changes in the membrane structure induced by ischemia, swelling and altered permeability could, therefore, induce abnormalities in adenyl cyclase function. Since absolute

NaF - stimulated adenyl cyclase activity represents the total amount of functional adenyl cyclase present, then the observed decreases in the NaF - stimulated adenyl cyclase activity in shock (Figures 17 to 19), probably represent a loss of functional adenyl cyclase enzyme.

ATP is the substrate for the adenyl cyclase reaction. It is possible that the well documented decreases in tissue ATP levels in shock might account for the observed reductions in adenyl cyclase response. However, the ATP levels in this study showed only minimal changes in shock (Tables III to VIII) and, in addition, the particular in vitro assay for adenyl cyclase employed in this study has been shown (21) to maintain the incubation mixture ATP concentration at a sufficient level to result in no compromise of maximal adenyl cyclase activity.

Glucocorticoids have been reported to potentiate the effects of exogenous glucagon and epinephrine on carbohydrate metabolism (160, 161, 162, 163); and to effect several hemodynamic improvements in shock (74); in addition to their membrane stabilizing effects (121, 122). Furthermore, glucocorticoids have been reported to interact with both the adenyl cyclase (206) and phosphodiesterase (211) enzymes.

Pharmacological doses of Methylprednisolone 4 hours prior to hormone injection in normal animals was associated with a significant increase in baseline hepatic adenyl cyclase activity (Figure 24). A similar effect upon basal adenyl cyclase activity of leukocytes incubated in cortisol was observed by Logsdon et al (206). However, the increase in basal adenyl cyclase activity in this study was not accompanied by a

significant potentiation of adenyl cyclase response or blood glucose output (Figures 20, 24) following glucagon injection when compared to the respective untreated control animals. It was concluded that the short-term administration of Methylprednisolone does not allow sufficient time for the glucocorticoid-induced "de novo" synthesis of several gluconeogenic enzymes (350) and the increased hepatic glycogen stores which in turn allows for a greater potentiation of the normal hepatic blood glucose output in response to exogenous glucagon. Furthermore, the excessively high insulin levels observed in the control steroid-treated animals (Table XIV), which have been shown to inhibit hepatic glucose release (235), may in part be responsible for the lack of potentiation of the adenyl cyclase and glucose responses.

Steroid therapy in shock was associated with a restored and furthermore supranormal adenyl cyclase response following in vivo epinephrine injection in both the liver and intestine (Figure 27). This was reflected by the return of a normal blood glucose response curve following epinephrine injection.

Despite the supranormal adenyl cyclase response following epinephrine in the steroid-treated shock animals, the absolute values for basal and Sodium fluoride stimulated adenyl cyclase did not exceed those values seen in the control animals.

No consistent effect of shock and ischemia upon tissue phosphodiesterase activity was observed. However, steroid administration produced consistent and significant 20 to 45 percent decreases in both

hepatic and intestinal phosphodiesterase activities. This effect of steroids upon tissue phosphodiesterase may have contributed to the restored and potentiated adenyl cyclase responses seen following epinephrine injection in steroid-treated shock by allowing for a greater intracellular accumulation of cyclic AMP. Thus, despite a lower basal adenyl cyclase activity, a response may be potentiated or become supranormal when there is a decreased rate of intracellular degradation.

The initial release of cyclic AMP into the plasma following in vivo hormone injection was greater in shock than in control or steroid-treated shock (Figures 38 to 41). This is probably indicative of an alteration in cellular permeability and a rough reflection of the extent of ischemic damage to the tissues.

The damage to the epinephrine - responsive adenyl cyclase system was far more extensive than that to the glucagon sensitive adenyl cyclase system in shock. The discrepancy may be related to the phenomenon of desensitization. Several investigators (22, 69, 150, 274, 340) have reported that exposure to high levels of a β-adrenergic drug renders a tissue less responsive to the same stimulator when introduced several hours later. Furthermore, reductions in hepatic cyclic AMP production (259, 363) and hormone-sensitive adenyl cyclase activity (69, 236) have also been reported following repeated and subsequent challenge with the appropriate hormone. Reports indicate up to 70 percent reductions in epinephrine responsive adenyl cyclase activity (236) and 30 to 40 percent reductions in glucagon responsive adenyl cyclase activity (69).

Throughout the hypovolemic period and then again in late refractory shock when the blood pressure is again falling, the tissue epinephrine receptors are bombarded with up to 100 fold increases in the circulating levels of epinephrine. Glucagon receptors, however, are not exposed to such excessive amounts of glucagon. Since epinephrine and glucagon have distinct receptors in the liver, and only epinephrine receptors in the intestine, it is conceivable that greater reductions in sensitivity to further epinephrine rather than glucagon challenge might occur in tissues obtained from shock animals.

Glucagon is a very powerful mobilizer of hepatic glucose. The present results as well as results obtained by others seem to indicate that the specific glucagon-sensitive adenyl cyclase system is easily saturated and produces far more cyclic AMP than is required for maximal hepatic glucose output. There is great adaptive value to this potential. Sixty percent of the adenyl cyclase response to glucagon may be lost in shock, however the physiologic response remains relatively intact. That is, the liver is still capable of a normal glucose producing response to glucagon stimulation in shock. Glucagon appears to be an efficient glucose-supplying hormone of stress.

Absolute levels of intracellular cyclic AMP were not consistently reduced in shock, whereas in steroid-treated shock they were reduced. The reliability of measuring tissue cyclic AMP levels has been questioned. Simultaneous measurement of production, release and breakdown of cyclic AMP can account for most of the tissue and plasma cyclic

AMP changes, however, certain discrepancies do arise and may be explained in several ways.

Exton et al (86) and others (12, 36) have shown that glucagon and epinephrine markedly increase net hepatic cyclic AMP generation, however, the increase was more accurately reflected in the increasing extracellular fluid levels than in the intracellular accumulation. Similar findings were observed by McArdle et al (224) in the intestine following in vivo epinephrine injection.

Under appropriate conditions, concentrations of the catecholamines can be found which increase fat cell lipolysis (89, 90) or contractile force in cardiac muscle (254), without any detectable changes in total tissue cyclic AMP. Furthermore, resting levels of cyclic AMP in the liver, if evenly distributed intracellularly, would, on the basis of in vitro studies, (284 Chapt. 7 p. 271) represent a concentration of the nucleotide sufficient to stimulate gluconeogenesis and glycogenolysis maximally. Since this is not the case, it has been postulated (10, 42, 100) that any total tissue pool of cyclic AMP is compartmentalized within the cells, and the specific pool of cyclic AMP affecting a particular function may represent only a small fraction of the total tissue cyclic AMP. Therefore, an agent could double the size of it's specific "target" pool of cyclic AMP without its resulting in any measurable increase in the overall basal levels. On the otherhand, heterogenous tissues, such as the intestinal mucosa, may contain numerous large pools of cyclic AMP including lymphocytes which could contribute to the cyclic

AMP content of this tissue. Minute increases or decreases of only a few picomoles per milligram of tissue may be associated with maximal alterations in function, while remaining undetectable when they occur in one compartment surrounded by other compartments with high basal cyclic AMP levels.

Steroid administration in normal and shock animals was associated with greater increases in serum insulin levels following in vivo hormone injection than were seen in animals not given steroids. Since steroids enhance and potentiate β responses to hormonal stimulation and are associated with elevations in blood glucose levels, it is not unlikely that the greater increases in insulin are in part a result of the direct steroid effects upon the liver.

The restoration of hepatic and intestinal response to epinephrine in shock suggests that the action of glucocorticoids is not restricted to de novo synthesis of gluconeogenic enzymes. The fact that this action of the steroids occurs within 4 hours in animals in shock also suggests a direct effect upon cyclic AMP metabolism. This may involve a re-integration or stabilization of the damaged cellular membrane which in turn restores receptor and/or catalytic subunit function of the adenyl cyclase enzyme. The effects of Methylprednisolone in shock appears to be aimed at preservation of adenyl cyclase response irrespective of baseline function. In addition, the results indicate the adenyl cyclase response is augmented by a supression of phosphodiesterase activity.

Steroid administration in shock enhances the ability of the tissues to respond to hormonal stimulation by restoring the adenyl cyclase response at a time when the tissue is devoid of β adrenergic sensitivity. Furthermore, the restoration of adenyl cyclase response is augmented by reductions in tissue phosphodiesterase activity.

CONTRIBUTIONS TO ORIGINAL RESEARCH

The following factors, which have not been clarified in the existing literature, have been found to influence the canine hepatic and intestinal second messenger system.

1. Control basal adenyl cyclase activity was increased by 68 percent in liver obtained from normal animals 4 hours following *in vivo* administration of 30 mg/kg Methylprednisolone Sodium Succinate (control-steroid treated animals).

2. The hepatic adenyl cyclase response following *in vivo* glucagon injection in the control-steroid treated animals was not significantly different from that seen in the untreated control liver.

3. The normal increase in hepatic adenyl cyclase activity seen following *in vivo* epinephrine injection was abolished in dogs subjected to hemorrhagic shock.

4. The intestinal adenyl cyclase response following *in vivo* epinephrine injection in control animals was also abolished in shock.

5. Basal adenyl cyclase activity in liver obtained from shock animals treated with Methylprednisolone Sodium Succinate was significantly reduced by 40 percent from the respective control value; however was associated with a supranormal response following *in vivo* epinephrine injection.

6. Baseline intestinal adenyl cyclase activity was decreased by 50 percent in the shock-steroid treated animals when compared to the

respective control value, however the adenyl cyclase response following epinephrine pulse was restored to normal.

7. The absolute levels of adenyl cyclase activity following glucagon injection into the shock-steroid treated animals were decreased from respective control values by 40 percent. However, this adenyl cyclase response was associated with a normal hepatic blood glucose response curve.

8. In vitro addition of 5 nanomoles of glucagon into hepatic homogenates obtained from control animals pretreated with glucocorticoids resulted in a final adenyl cyclase activity which was 55 percent greater than that obtained following in vitro addition of 5 nanomoles glucagon into hepatic tissue obtained from normal animals.

9. The final adenyl cyclase activity obtained after in vitro addition of 5 nanomoles of glucagon into intestinal homogenates obtained from steroid-treated control animals was 85 percent greater than that seen in intestinal tissue obtained from normal animals.

10. The final adenyl cyclase activity obtained following in vitro addition of 5 nanomoles of glucagon into hepatic tissue obtained from steroid-treated shock animals was increased by 22 percent over the respective value seen in liver obtained from shock animals.

11. The final adenyl cyclase activity obtained following in vitro addition of 5 nanomoles of epinephrine to hepatic tissue of steroid-treated shock dogs was 35 percent greater than the respective value in the shock liver.

12. The intestinal adenyl cyclase response to *in vitro* addition of 5 nanomoles of epinephrine was restored to normal in the shock animals treated with glucocorticoids.

13. Addition of 10 mM Sodium fluoride into hepatic or intestinal tissue homogenates obtained from control animals treated with glucocorticoids was associated with no significant change in the final adenyl cyclase activity from that observed in the untreated controls.

14. Baseline intestinal mucosal cyclic AMP levels in the steroid-treated control animals were decreased by 46 percent from the respective value in the normal intestine.

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