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THE ROLE OF HYPOGLYCEMIA IN THE DEVELOPMENT OF CARDIOVASCULAR FAILURE FOLLOWING *ESCHERICHIA COLI* ENDOTOXIN ADMINISTRATION IN THE DOG

by

Philip M. Kober

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May

1982

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To my father who molded the person that I am and who taught me to persevere, to do everything to the best of my ability, and to live life intensely. He would be proud of my accomplishment if he were here.

ACKNOWLEDGEMENTS

I wish to thank Dr. James P. Filkins, my mentor, who not only provided guidance in the completion of my dissertation research project and taught me about the physiology of shock, but provided me with an excellent role model of a scientist and a teacher.

Special thanks must be given to Dr. John X. Thomas without whom the kind of research that is included in this dissertation -- relying on two of the strengths of the Department of Physiology, shock and metabolic physiology and cardiovascular physiology -- could never have been undertaken. Dr. Thomas, in an unofficial role as second advisor, has been instrumental in helping me set up the cardiovascular aspects of this study and has taken personal interest in my progress and in innumerable ways helped in the completion of this project. As he was also instrumental in my obtaining a postdoctoral position in San Diego with Dr. James Covell, I am doubly grateful for his efforts.

It is also appropriate to give special thanks to Dr. Mohammed M. Sayeed who through many discussions and his interest in my dissertation project has provided invaluable intellectual input.

I also wish to thank the other three members of my committee -- Drs. Robert Phillips, Richard Raymond, and

John Moran. Their assistance and thoughts during the final preparation of this dissertation have helped to make this a stronger dissertation and to make it a learning experience.

Dr. Walter C. Randall, who by allowing me to participate in Cardiovascular Journal Club and Advanced Physiology of the Heart and through many discussions, has been a key figure in my learning the cardiovascular physiology of shock and in keeping me abreast of the latest advances in cardiovascular physiology, also is gratefully acknowledged.

Thanks are also due to Dr. Michael Yelich who through many discussions and his help in setting up the insulin assay also provided invaluable assistance.

Several individuals must be acknowledged for their technical assistance. At the top of the list are two individuals without whom this dissertation project may never have been finished -- Ken Ellis who, though he worked with me for only one summer, was extremely helpful in getting the often tedious work of the dog experiments done and Ana Hernandez who must be given extra special thanks for her assistance in running assays for serum insulin and lactates, especially over the last few weeks in finishing up the remaining samples. Ana's patience and understanding during this time can never be repaid. The assistance of Ellen Newman in running the insulin assays during the early stages of this project was also indispensable. Janet Comer and Anne Alex must be acknowledged for their assistance

with the RES project. I have to say that quite often the technicians seem to be the real stars in research, but that they often do not receive the recognition which they truly deserve -- remaining instead unsung heroes. This dissertation, however could not have been accomplished without the assistance of these unsung heroes and their efforts are appreciated beyond measure.

Lastly, I must thank my mother who not only gave me her love and emotional support, but who in spite of her own busy schedule took time to type the final copies of this dissertation.

VITA

Philip M. Kober was born on June 20, 1952 to Philip J. Kober and Margaret M. Kober in Chicago, Illinois. He received his elementary and high school education on the North Shore and graduated from Evanston Township High School in June of 1970.

In August of 1970, Philip began his undergraduate work at the University of Tulsa, Oklahoma. In the fall of 1972, he transferred to DePaul University in Chicago where he graduated with honor with a B.S. in Chemistry in 1975.

He began his graduate work on the Master's degree level receiving an M.S. in Biology from Northwestern University in June of 1977. The author began his doctoral work in the Department of Physiology at Loyola University of Chicago in July of the same year. He has worked under the direction of Dr. James P. Filkins from June of 1978 through May of 1982. In July of 1980 he was awarded an Arthur J. Schmitt Dissertation Fellowship. Philip has taken a postdoctoral research position with Dr. James Covell in the Department of Medicine, University of California, San Diego, following the completion of the doctoral requirements. He currently has five publications.

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CHAPTER I

INTRODUCTION

Ever since the first clinical case of Gram-negative bacteremia was described by Brill and Libman in 1899 (19). septic shock and its research laboratory equivalent, endotoxic shock, have become an increasingly important clinical as well as experimental problem. Based on 1974 population studies. McCabe estimated that there would be 330,000 reported cases of bacteremia with 132,000 deaths within the United States per year (144). The mortality rate has remained extremely high, while the incidence of sepsis -- and presumably endotoxemia -- has increased over the years since 1974 (85). Using currently available statistics (206) and McCabe's estimates of incidence and mortality (144), there will be 380,000 cases of sepsis and 150,000 deaths per year. In his book The Lives of a Cell, Thomas (202) states that the presence of bacterial endotoxin in the blood stream is interpreted as "the very worst of bad news." Endotoxin provides a signal to the host of the presence of overwhelming numbers of Gram-negative bacteria and the response is a fight to the death. Unfortunately the massive, "panic-driven" mobilization of the defense only leads to the host's demise. As Thomas (202) concludes: "We are, in

effect, at the mercy of our own Pentagons ..." In spite of extensive experimental observations, the mechanisms which lead to the state of circulatory and metabolic collapse caused by Gram-negative bacterial endotoxins are nct clearly understood.

Recently, the state of metabolic failure which leads to severe hypoglycemia and which is accompanied by the state of circulatory failure has been a major focus. Siegel (192) and Duff (42) have proposed that the metabolic changes which occur during sepsis may underwrite the ensuing circulatory syndrome during clinical septic shock. In light of this concept they propose that extensive monitoring of the metabolic status along with the already accepted monitoring of circulatory status in septic patients be used as a guide to treatment and that, indeed, treatment should be aimed at correcting the disturbed metabolic state rather than merely supporting the cardiovascular system. While the profound, progressive hypoglycemia during endotoxin or septic shock has been well-documented in a number of species including dogs and man, the possible relationship between metabolic and cardiovascular failure has not been fully explored.

The research included in this dissertation examines the role of hypoglycemia in the development of cardiovascular failure during endotoxin shock in the dog in order to answer the following questions: Is hypoglycemia merely a parallel manifestation of the underlying endotoxic

syndrome, or does hypoglycemia play a causative role in the development of circulatory failure following endotoxin administration and if so, in what ways does hypoglycemia contribute to the pathogenesis of cardiovascular failure? Specifically, the purposes of this dissertation are fourfold: 1) to develop a canine model for the continuous monitoring of blood glucose levels for the induction of well-regulated alterations in the glycemic state -- i.e. a glucose clamp, and to assess cardiovascular performance; 2) to correlate hemodynamic events with changes in blood glucose levels following endotoxin administration; 3) to evaluate whether hypoglycemia <u>per se</u> induces changes in cardiovascular performance potentially leading to a state of circulatory shock and to compare the cardiovascular alterations of hypoglycemia with those of endotoxicosis; and 4) to evaluate the possible synergistic effects of hypoglycemia and endtoxin and conversely the potential beneficial effects of maintenance of blood glucose -- at either normoglycemic or hyperglycemic levels -- during shock.

CHAPTER II

LITERATURE REVIEW

Metabolic and Cardiovascular Failure during Circulatory Shock

Carbohydrate Dyshomeostasis during Endotoxin Shock: Alterations in Blood Glucose Levels

Observations on blood glucose levels during shock date back to Bernard (1877) who reported hyperglycemia following hemorrhage (214). While such hyperglycemia has been confirmed by a number of workers (see references 131, 157, 184, 214, 215 for a complete list of references with regard to studies of blood glucose levels during hemorrhage), it is evident that this is an early phenomenon and that hypoglycemia is seen following the transition from reversible to irreversible hemorrhagic shock (41, 214). The glycemic responses are variable: Some animals die in the hyperglycemic phase (131) while others show no initial hyperglycemia and exhibit a continuous fall in blood glucose from the onset of hemorrhage (36). Early hyperglycemia followed by hypoglycemia has also been described clinically during cardiogenic shock (174).

This biphasic pattern -- early hyperglycemia and late hypoglycemia -- is also seen during septic and endotoxic

shock and has been reported in several species (11, 51, 57, 88). Many of the early observations on blood glucose during endotoxin shock were made during experiments on rabbits (25, 122, 150, 151, 203, 223). Menten and Manning (150, 151) were the first to demonstrate early hyperglycemia followed by hypoglycemia in rabbits using dead bacteria, a crude endotoxin preparation. Penner and Klein (171) were the first to observe the blood glucose changes in dogs following endotoxin administration, but their results were anecdotal and they did not follow blood glucose through the late hypoglycemic stage. Berk and coauthors (9) reported finding two patterns in blood glucose levels in endotoxic dogs. One group showed hyperglycemia followed by hypoglycemia; the other proceeded immediately into hypoglycemia with a continuous fall in blood glucose from the onset of the syndrome. The results of Berk et al. (9) are similar to those of Cowley et al. (36) using a hemorrhagic model. However, neither group of investigators could be certain they did not miss a hyperglycemic phase with only periodic measurements of blood glucose. Hinshaw and coinvestigators also saw the second pattern of marked hypoglycemia with no early hyperglycemia in non-surviving endotoxic dogs (100). Dogs which survived endotoxemia, on the other hand, were better able to maintain their blood glucose levels. Wilson et al. reported a similar correlation between the ability to maintain glucose levels and survival in septic baboons (217).

Carbohydrate Dyshomeostasis: Mechanisms Leading to Hypoglycemia during Endotoxin Shock

The mechanism of shock-induced alterations in blood glucose is still a matter of debate, especially with regard to the development of hypoglycemia during endotoxin shock. Two mechanisms have been postulated to contribute to the profound hypoglycemia of endotoxin shock: 1) depression of hepatic gluconeogenesis -- along with glycogen depletion in fed animals -- (12, 53, 54, 57, 88, 147) and 2) increased peripheral glucose utilization (5, 53, 56, 57, 88, 152, 173, 218). Wolfe, Elahi, and Spitzer (218) have shown a failure to maintain an elevated level of gluconeogenesis in spite of the falling blood glucose level. Thus, while they conclude otherwise, gluconeogenesis was relatively depressed in their model since it was not elevated during developing hypoglycemia. The importance of increased peripheral glucose utilization versus decreased hepatic glucose production, probably depends on the stage of endotoxin shock: increased peripheral utilization making a greater contribution early with decreased hepatic glucose production playing an increasing role as the shock syndrome progresses (219). Kuttner and Spitzer (124) have shown that gluconeogenesis may, in fact, be elevated during early endotoxicosis.

The question remains how endotoxin causes these insulin-like effects. One possible mechanism is a direct effect of endotoxin on several tissues (notably adipose

tissue and skeletal muscle) to increase glucose oxidation (107, 110, 180, 197). While in some cases the direct effect has been difficult to establish (53, 110), it is generally accepted that endotoxin may have a direct insulin-like effect under at least some conditions. Since the direct effect has only been established in vitro or in isolated, perfused organ systems it is difficult, however, to assess whether these effects occur during the endotoxic syndrome in vivo.

The crux of the debate over the mechanism of endotoxin-induced hypoglycemia centers around the possible mediation by humoral factors: What substances are involved and what are the sources of these mediators? The stage of endotoxin shock is also an important consideration in discussing which mediators are involved since different factors may be released at different stages to initiate and maintain the metabolic events which lead to the progressive hypoglycemia. Buchanan and Filkins demonstrated a marked hyperinsulinemia in rats following endotoxin administration (23). Yelich and Filkins have shown with both perfused livers and pancreata (220) that this hyperinsulinemia is not due to an alteration in insulin clearance by the liver (a finding disputed by Cornell (35)), but to a hypersecretory state of the beta-cells of the endocrine pancreas. The levels of serum insulin decrease late in the endotoxic rat as the blood glucose level falls (52). This finding

of hyperinsulinemia is still disputed by Adeleye and co-workers (1). The role of insulin in the development of endotoxin hypoglycemia in the dog, however, is not well defined and even more confusing. Blackard and coinvestigators reported increased insulin secretion only when endotoxic dogs were given a glucose load (14). However, the results which they reported for untreated dogs are open to an alternative interpretation. Insulin levels were not increased above control in the untreated group; on the other hand, the levels do not decrease as would be expected in the profoundly hypoglycemic animal until the very late stages of shock immediately preceding death. This implies that there is at least a functional hyperinsulinemia since insulin secretion did not decrease normally with the drop in blood glucose. If insulin secretion was not properly braked in the face of extreme hypoglycemia, the pancreas may have been secreting more insulin than it should for the given blood glucose level -- thus, exacerbating the hypoglycemia. More recently, Spitzer and colleagues have described a brief increase in insulin release which occurs very early after endotoxin administration in the dog (199). After this early elevation, plasma insulin levels declined steadily for the remainder of the course of endotoxin shock in their model. Other groups have reported results which conflict with those of Blackard et al. even after a glucose load -- demonstrating

instead a hyporesponsive state of the endocrine pancreas (142). Thus, because it is hard to reconcile these facts, the possible role of insulin during canine endotoxin shock has never been clearly defined. Yet, the question of whether there is a hyperinsulinemia in the endotoxic dog is potentially important.

In addition to immunoreactive insulin, there may be one or more non-suppressible insulin-like substances involved in the pathogenesis of endotoxic hypoglycemia. Even in control animals non-suppressible insulin-like activity (NSILA) is 90% or more of the total biological insulin-like activity (222); therefore, it should not be surprising that NSILA is a factor in the development of endotoxic hypoglycemia. Filkins has recently demonstrated in the late stages of endotoxicosis in the rat that the NSILA is indeed elevated (52). In addition, Filkins has shown that at least a portion of this rise in NSILA may be due to the secretion of an insulin-like substance by macrophages (50).

Besides immunoreactive insulin and non-suppressible insulin like activity, other humoral substances may be involved in the development of endotoxic hypoglycemia. Berry and coworkers have found a substance produced by macrophages which antagonizes the gluconeogenic activity of glucocorticoids (10, 156). Pardini, Jones, and Filkins have shown that catecholamines are depleted from nerve terminals in the myocardium and spleen during endotoxin

shock in the rat (166). As this may apply to other organs such as the pancreas and liver, the catecholamine depletion may have several important effects on metabolism: First, the vasoconstriction caused by the excessive neural drive which leads to the depletion may cause an increased peripheral utilization of glucose because of the limitation on organ perfusion. Second, decreased flow of gluconeogenic substrate to the liver because of increased peripheral vasoconstriction may also help explain depressed gluconeogenesis. Third, if catecholamines are depleted from nerve terminals in the liver, such depletion would lead to loss of sympathetic support for gluconeogenesis. Fourth, if catecholamines are depleted in the endocrine pancreas, one of the normal brakes on insulin secretion would be released. While the catecholamine depletion has only been studied in heart and spleen, this mechanism -- while speculative -- may be important in the development of endotoxic hypoglycemia.

Another important factor which may mediate the effect of endotoxin on peripheral glucose utilization is tissue hypoxia. It is well-known that hypoxia and ischemia increase peripheral glucose utilization and it has been shown that this is a potential mechanism for increasing glucose use by skeletal muscle during endotoxin shock (181). However, Romanosky et al. (186) have shown that this is not a necessary condition for increased glucose utilization in this tissue.

Which tissues are utilizing glucose during endotoxin shock is another important question. Some tissues such as skeletal muscle may be using inordinate amounts of glucose at the expense of vital organs such as the brain and the heart. During endotoxin shock in the rat, Filkins and Figlewicz (56) have shown increased glucose oxidation by liver, hemidiaphragm, spleen and epididymal fat pad, but not in blood, lung, brain, heart, stomach, and kidney. Raymond and Emerson demonstrated depressed cerebral glucose utilization in the endotoxic dog (179). Spitzer and his colleagues (136, 198) have demonstrated a shift from free fatty acids to lactate in the myocardium. Liu, Long, and Spitzer (137) more recently have examined myocardial glucose utilization in vitro in response to endotoxin in addition to palmitate and lactate. Using isolated myocytes from normal dogs, they found that glucose oxidation was stimulated directly by increasing doses of endotoxin. While these in vitro data in large part parallel previous in vivo studies, the relationship between a direct in vitro effect and the in vivo effect is not clearly established since it is still possible that other mechanisms come into play in vivo. Kuttner, Apantaku, and Schumer (123) showed changes in glycolytic intermediates in endotoxic rat hearts which are consistent with increased glycolytic flux, but they did not measure glucose utilization. These results are also consistent with the findings of Marchetti et al. (143)

who demonstrated a slight increase in glucose utilization in endotoxic dog hearts in vivo. It has been demonstrated by several groups of investigators that the major increase in glucose utilization is seen in skeletal muscle which is more than 50% of the body mass (181, 186). While Hinshaw and coinvestigators (89) demonstrated increased glucose utilization in leukocytes from endotoxic dogs, it seems unlikely that blood cells can make a significant contribution to the increased peripheral glucose metabolism. Sayeed and Murthy (189) demonstrated an approximate doubling in glucose oxidation of endotoxic lung slices as compared to control slices. Overall, the consensus is that the major glucose utilizer during endotoxin shock is skeletal muscle with perhaps some minor contribution from fat, lungs, blood cells, and possibly the myocardium, thus depriving the brain of its necessary fuel. Although it has not been extensively studied, liver may also be an important utilizer of glucose following endotoxin administration (56).

Summary of Carbohydrate Dyshomeostasis during Endotoxin Shock

In summary, following the administration of endotoxin in a number of species -- including the dog -- a progressive, profound hypoglycemia develops which may or may not be preceeded by a brief period of hyperglycemia. This hypoglycemia is produced by a depression in hepatic

gluconeogenesis and increased peripheral utilization of glucose especially in skeletal muscle brought about by a direct insulin-like action of endotoxin and by the mediation of hypoxia and ischemia as well as several humoral factors such as insulin, non-suppressible insulin-like activity, and glucocorticoid antagonizing factor.

Circulatory Failure: The Endotoxic Syndrome

Classically shock is defined as a cardiovascular syndrome. This is no less true of endotoxin shock. The first systematic studies of the cardiovascular events following endotoxin administration in the dog were those of Weil and MacLean and their coworkers in 1956 (140, 209). Following the administration of endotoxin three hemodynamic phases have been described: an initial hypotensive episode within minutes after bolus injection, a recovery phase, and a subsequent more prolonged hypotensive phase culminating in death (30, 67, 157). The initial hypotensive episode is possibly caused by the release of histamines (98, 103, 207) as well as other mediators such as the endorphins (106, 183) and may well be a systemic anaphylaxis (140, 196, 209). The release of these mediators leads to dilation in the systemic vasculature, an increase in pulmonary resistance, and a decrease in venous return resulting in a decreased cardiac output. The inital hypotension leads to massive sympathoadrenal discharge.

(87) with peripheral vasoconstriction and an improvement in cardiac performance. Sympathetic outflow, thus, results in a partial recovery of venous return, cardiac output, and mean arterial pressure. There is, however, a second hypotensive phase characterized by a more gradual decline in blood pressure leading to irreversible shock and death (87). Early studies of this second phase demonstrated decreases in both peripheral resistance (61, 87, 95, 104, 209) and cardiac output (87, 95, 104, 209). In later studies increases (61, 78, 87) or no change (78, 87) in peripheral resistance were observed. The reasons for the discrepancy noted in peripheral resistance are not fully clear. Anesthesia, species differences, and the conditions of the experiment as well as when and where (i.e. which vascular beds) the measurements were made may very well make a difference in the response seen. Regardless of the response, both peripheral vascular and cardiac mechanisms are involved in the events which eventually lead to total circulatory collapse and death.

Cardiovascular Failure during Endotoxin Shock: Peripheral Vascular Mechanisms

Characteristics of the injuries resulting from endotoxin administration in the dog are the hemorrhagic intestinal lesions first noted by Weil and MacLean (140, 209). These lesions result from splanchnic vasoconstriction,

increased portal pressure, and hepatosplanchnic pooling and congestion (140, 209). Park, Baum, and Guntheroth (167) noted constriction of the portal vein in the dog. Active venoconstriction in the splanchnic bed following endotoxin administration was also seen in small mesenteric and intestinal veins by Meyer and Visscher (153) and Hinshaw and Nelson (99). While all of these groups obtained similar results on different veins, Park and colleagues disputed the concept of splanchnic pooling. The other groups felt that vasoconstriction was the mechanism responsible for the splanchnic pooling. This dispute can be reconciled because Park et al. considered pooling in the splanchnic circulation as a large vein phenomenon instead of considering the splanchnic circulation as a whole. Clearly, venoconstriction increases splanchnic venous pressures and decreases venous volume. However, venoconstriction also increases resistance to venous outflow and pooling will occur in the splanchnic microcirculation. The concept of splanchnic pooling is consistent with the finding of an increased splanchnic blood volume by Chien et al. (31). This conclusion is also consistent with the finding of increased hepatic wedge pressure by Tsargaris, Gani, and Lange (205). As a result of splanchnic pooling, venous return is decreased leading to a decrease in cardiac output and, thus, perpetuating the shock state. Splanchnic pooling along with an overall decrease in splanchnic flow

leading to severe splanchnic ischemia (77, 135, 165) can lead to the production and release of cardio- and vasotoxic materials from the ischemic splanchnic viscera (126, 127). Such toxic materials as the myocardial depressant factor (MDF) reported by Lefer and coinvestigators (126, 127) may be the primary mechanism leading to irreversible shock and death.

Decreases in renal blood flow have been reported following endotoxin administration in the dog in response to the systemic hypotension (102). The decrease in renal blood flow resulted in a decrease in GFR and urine output (102). More recently, using inert gas washout techniques, Neiberger and Passmore demonstrated that renal cortical flow was reduced to a greater extent than renal medullary flow in the endotoxic dog (160). These investigators also demonstrated that alpha-adrenergic vasoconstriction plays a major role in decreased renal cortical flow. While initially the decrease in renal blood flow is compensatory for the systemic hypotension, there are two important detrimental consequences which result from prolonged renal cortical ischemia: First, prolonged renal cortical ischemia and cortical necrosis would ensure that dysfunction is irreversible. Even if blood flow can be restored the kidneys would still fail. Second, relative maintenance of medullary flow would lead to a washout of the normal osmotic gradient for urine concentration. This latter

problem is particularly important since anuria does not always occur following endotoxin administration (102). Continued urine flow without the ability to properly concentrate the urine would further exacerbate the shock state.

Vasoconstriction has also been demonstrated in the skeletal muscle vascular bed following endotoxin administration (181). As mentioned previously this may contribute to the increase in peripheral glucose utilization and the end-product of glucose metabolism (e.g. lactate and hydrogen ions) in turn contribute to the vasoconstriction in this bed. Vasoconstriction is largely a compensatory mechanism, however, to help maintain systemic blood pressure and central blood volume.

Cerebral and coronary blood flow have also been measured during endotoxin shock. Myocardial ischemia has been demonstrated following the administration of endotoxin in the dog (15, 94, 115). Blood flow has been shown to be decreased in several different regions of the brain following endotoxin administration including several regions involved in cardiovascular and respiratory control (21, 168, 191). Altered autonomic control may be an important mechanism leading to decompensation and irreversible shock and a number of investigators (109, 191, 203) have postulated that depression of the central nervous system leading to failure of support of cardiac performance, peripheral vascular failure, and respiratory depression

is a major contributor to the cardiovascular and respiratory failure of endotoxicosis. The decrease in blood flow to the brain is largely due to the fall in blood pressure since Bryan and Emerson found either unchanged or decreased resistance in the seven regions which they studied (21). Parker and Emerson (168) also demonstrated that cerebral autoregulatory responses were maintained.

Besides the possibility of central nervous system involvement during endotoxin shock, Trank and Visscher (204) have shown that cardiovascular afferent systems may be altered following the administration of endotoxin in dogs. Using the isolated carotid sinus technique, these authors demonstrated higher carotid sinus nerve discharge frequencies at most pressures following the administration of endotoxin. Thus, the baroreceptors were found to be reset to a hypotensive level following endotoxin injection. This provides an explanation for the bradycardia and the variable response of total peripheral resistance during canine endotoxin shock. Now the cardiac and peripheral vascular responses will depend on the difference between the actual blood pressure and the new set point which is lower than the control set point. Paradoxically, then the baroreceptor reflex could operate to lower the blood pressure through decreases in heart rate and contractility and decreases in peripheral resistance as the blood pressure rises back <u>toward</u> control levels. Halinen (74) has

demonstrated that other cardiovascular afferent systems, particularly atrial receptors and the aortic arch baroreceptors, are also depressed during endotoxin shock. Thus, cardiovascular control may be upset following endotoxin administration for two reasons: 1) depression of the central nervous system and 2) resetting of baroreceptors and atrial volume receptors. In addition to these events in the systemic vascular beds, there is a maintained elevation in pulmonary vascular resistance throughout the course of endotoxin shock (87).

Along with the effects of endotoxin on systemic and pulmonary resistance and their neural control, endotoxin has been shown to cause an increase in capillary permeability in a number of vascular beds including the skeletal muscle, pulmonary, and splanchnic beds (32, 33, 194). Increased vascular permeability leads to a loss of fluid to the extravascular space and a consequent decrease in blood volume, thus, aggravating the shock syndrome. Endotoxin also triggers both classic and the alternate complement activation pathways as well as both the intrinsic and extrinsic coagulation pathways (158). These reactions lead to disseminated intravascular coagulation, increases in vascular permeability, and host defense reactions which cause tissue injury and decreased tissue perfusion.

Cardiovascular Failure during Endotoxin Shock: The Role of the Heart

The role of cardiac failure in the decompensatory phase of circulatory shock is often debated. Classically, it has been presumed that the function of the heart as well as the brain is preserved during circulatory shock or only secondarily fails late in the course of shock as a result of peripheral vascular failure (72, 86, 87, 95, 209). Early studies during endotoxin shock pointed to decreased venous return (101) as the major cause of decreased cardiac output during the initial phases of endotoxicosis and showed the heart to be resistant to the detrimental effects of endotoxin during the early stages (93, 97). The evidence of Solis and Downing (193), based on ventricular function curves in endotoxic cats, however, pointed to the potential for very early myocardial failure (within the first two hours). A large body of evidence in the endotoxin literature now points to the importance of early direct myocardial injury leading to cardiac failure. Recently Hess et al. (80) have attempted to systematically study the determinants of cardiac performance in endotoxic dogs: heart rate, preload, afterload, and myocardial contractility. They found increased afterload (as assessed by total peripheral resistance), decreased preload (as assessed by pulmonary capillary wedge pressure), and

decreased contractility (as assessed by stroke work along with no change in heart rate in their model). When animals were given a femoral artery-to-vein shunt, myocardial performance was improved along with preload and afterload (i.e. left ventricular filling pressure was increased while total peripheral resistance was decreased). While the methods may be somewhat crude, this study serves to point out that myocardial performance after endotoxin is affected both by changes in the extrinsic factors of preload and afterload -- i.e. peripheral vascular mechanisms -- and by an intrinsic depression of the ability of the myocardium to contract.

Two aspects of cardiac performance are altered following endotoxin injection: 1) heart rate and rhythm and 2) contractility. Most studies have emphasized the depression in myocardial contractility, however, the heart rate and rhythm disturbances may be as important. Levy and Blattberg (133) describe some important effects of endotoxin on heart rate depending on anesthesia. In unanesthetized dogs they found a triphasic change in heart rate: a transient tachycardia, followed by bradycardia and then a secondary tachycardia. In dogs anesthetized with pentobarbital there was a similar initial tachycardia followed by a prolonged bradycardia, while in morphine-chloralose-urethane anesthetized dogs there was an initial tachycardia over a somewhat longer period than in the other

two groups followed by a return of heart rate to control levels and then a second acceleration in heart rate. They discussed the role of reflex activation of the sympathetic versus activation of cardiac vagal fibers in determining the heart rate under the various anesthetic conditions following endotoxin injection. Of particular interest is the paradoxical bradycardia seen during endotoxin shock. While the mechanism of this bradycardia is incompletely understood, it has drastic consequences for the maintenace of cardiac output following endotoxin administration. As mentioned before, the resetting of various cardiovascular receptors may play a role. More recent studies have shown that an unidentified serum factor may be involved in altering cardiac electrophysiology during endotoxin or septic shock (27, 28). Recently, Pellet et al. (169) have demonstrated increased effective refractory periods, increased conduction times (as measured with His bundle recordings) and bradycardia along with increased sino-atrial node recovery times after an extrastimulus in endotoxic dogs. These results imply depressed automaticity of the sinoatrial node and depressed conduction during canine endotoxicosis. The authors speculatively discuss the possible role of depressed responses to sympathetic stimulation and of myocardial depressant factor in producing these effects. However, they do not present data pertaining to these potential mechanisms.

Myocardial ischemia has been demonstrated during endotoxin shock (15, 94, 115). Thus, one would expect that all of the myriad effects of myocardial ischemia on myocardial performance -- including dysrhythmias and depressed contractility -- occur following endotoxin administration. While a number of investigators have demonstrated a coronary flow deficit during endotoxin shock there remains, surprisingly, some debate as to the effects of this flow deficit on myocardial function. While Bohs et al. (15) found a biphasic increase in coronary vascular resistance and a decrease in coronary flow they were unable to get an improvement in myocardial function after increasing coronary flow with dipyridamole. Thus, while demonstrating the presence of myocardial ischemia during endotoxin shock, these investigators provide some evidence that myocardial depression may not simply be due to the ischemic process. Other factors may be involved. On the other hand, dipyridamole has been shown to be ineffective during angina pectoris since it merely causes a redistribution of flow rather than improving flow to ischemic regions -- the so-called coronary steal phenomenon (40). The results of Bohs et al. (15) also conflict with those of Peyton et al. (172) who found significant improvement in myocardial function following treatment with sodium nitroprusside which lowers left ventricular afterload thus decreasing the left ventricular oxygen demand relative to

the supply (40). Hess and various coinvestigators (115, 118, 120) have shown dysfunction of sarcoplasmic reticulum calcium uptake and myofibrillar ATPase which is greater in the subendocardium than in the subepicardium. This is consistent with a global ischemic process and the greater subendocardial flow deficit which occurs under these conditions. These investigators also presented microscopic evidence of differential subendocardial injury. Other studies from this same laboratory (20, 81, 195) found that maintenance of venous return protected against myocardial failure following the administration of endotoxin. Hess and his colleagues concluded that the maintenance of venous return with a bypass pump or via a femoral artery-to-vein shunt resulted in a maintenance of coronary blood flow. Coronary blood flow, however, was not measured in these studies and other explanations for improved myocardial performance during maintained venous return experiments are possible. In studies with retrograde perfused canine hearts where myocardial blood flow was artificially maintained, Elkins et al. (44) also found improved myocardial function. Improved function was also associated with increased coronary flow in studies of the effects of glucose and insulin on myocardial performance in endotoxic dogs (4, 90). In these studies, however, the effect of the treatment could not conclusively be attributed to an improvement in blood flow.

Another major hypothesis for the deficit in myocardial performance following endotoxin injection is the toxic factor theory principally espoused by Lefer and associates (70, 126, 127). Lefer and colleagues have discovered a myocardial depressant factor (MDF) which is produced from proteins released into the blood stream from the ischemic pancreas during shock and acted upon by lysosomal hydrolases from the liver to form MDF (49, 128, 129). This serum factor has been shown to depress the contraction of isolated papillary muscle strips (126, 130) and to be transferable to non-shocked animals (185). This theory is consistent with the results reported by McCaig and Parratt (145) who found depressed inotropic responses to calcium administration in vivo but not in vitro. In another study, McCaig et al. (146) could find no in vitro physiological or ultrastructural evidence of myocardial depression in isolated atria or papillary muscle taken from cats 2.5 hours after endotoxin administration. This is again consistent with the idea that a serum factor causes the endotoxic myocardial dysfunction rather than intrinsic damage to the heart. Other investigators have not been able to demonstrate a myocardial depressant factor (96). In addition, shock still occurs following endotoxin injection in eviscerated dogs (173). Thus, while the evidence for a toxic factor is fairly strong, the possible role of MDF in the pathogenesis of endotoxic cardiac failure has been questioned.

In addition to a possible toxic factor other humoral agents may be involved in the pathogenesis of myocardial failure during endotoxicosis. Recently Reynolds et al. (183) have demonstrated improved myocardial function as assessed by maximum left ventricular dP/dt as well as increased survival following treatment of endotoxic dogs with naloxone. Thus, negative inotropic effects of endogenous opioids may play a role in the development of endotoxic cardiac failure. Another potential humoral mediator of endotoxic cardiac failure is histamine. Krause and Hess (119) demonstrated an improvement in the ability of myofibrillar ATPase to hydrolyze ATP following treatment with diphenhydramine, an antihistamine. Another study of the same laboratory showed a beneficial effect of diphenhydramine on the uptake of calcium by sarcoplasmic reticulum in endotoxic myocardium (79). While this evidence for histamine involvement is very indirect, and really inconclusive in the absence of data showing that histamine depresses myocardial function in either control or endotoxic hearts, it nevertheless points to a potential mechanism for endotoxic myocardial failure.

Pulmonary resistance is increased throughout the course of endotoxin shock (87). The afterload to right ventricular ejection is, thus, increased. This is a classical mechanism leading to right heart failure. High right ventricular afterload, thus, undoubtedly, contributes

to endotoxic cardiac failure.

Another aspect of endotoxic myocardial failure may invclve the response to positive inotropic stimuli. Archer and coinvestigators (6) found depressed responsiveness to infused epinephrine as assessed by maximum negative and positive left ventricular dP/dt, and myocardial power and efficiency. A depression in the coronary blood flow response to catecholamines was also demonstrated in this same study, thus, potentially exacerbating the myocardial ischemia. A similar depression in the response to norepinephrine has recently been demonstrated by Lust et al. (138). These decreases in responsiveness are in addition to the previously mentioned depletion of myocardial norepinephrine late in endotoxin shock demonstrated by Pardini and his coinvestigators (166).

Hinshaw (84) has also suggested that disturbances in myocardial calcium and potassium may contribute to the myocardial failure. Another contribution to the endotoxic cardiac failure may be the mitochondrial swelling, disruption of the contractile elements with intracellular edema, and the increase in extracellular fluid in the myocardial interstitium which have been reported in the endotoxic heart (34, 91, 92, 149).

Cardiovascular Failure during Endotoxin Shock: Summary

Both peripheral vascular collapse and cardiac failure have been shown to contribute to endotoxic circulatory collapse. An overall increase in total peripheral resistance is seen which may or may not be maintained over the entire time course of shock. Specific increases in resistance have been reported in the splanchnic circulation where pooling and congestion leading to hemorrhagic intestinal lesions which are pathognomic of endotoxin shock in the dog. Arteriolar constriction in the splanchnic bed may lead to the production of cardio- and vasotoxic materials which are released during an autoregulatory escape in the splanchnic vasculature. Resistance has also been shown to increase in the kidneys where renal cortical ischemia and an imbalance between cortical and medullary blood flow have detrimental consequences for survival, and in skeletal muscle where ischemia contributes to the increase in peripheral glucose utilization. Blood flow to the brain and heart have also been shown to be decreased following endotoxin administration. Cardiovascular reflexes are depressed as a result of central nervous system depression and by resetting of baroreceptors and atrial volume receptors. Several mechanisms contribute to endotoxic cardiac failure: Ischemia, myocardial depressant

factor, endogenous opioids, histamine, altered sympathetic responses, increased afterload to right ventricular ejection, myocardial edema, and altered myocardial calcium and potassium have all been postulated to contribute to this failure. None of these postulated mechanisms is mutually exclusive and several or all of them may contribute to the myocardial failure seen after endotoxin injection. The exact mechanism is still in dispute.

Early Studies of the Relationship of Metabolic and Circulatory Failure Following Endotoxin Administration

Several groups of investigators have examined the effects of glucose infusion during endotoxin or septic shock. In 1975, Weisul et al. (211) studied the beneficial effects of glucose, insulin, and potassium solutions in patients with sepsis and speculated on the potential role of hypoglycemia in the myocardial lesions of clinical septic shock. Berk et al. (9) found significant improvement in the survival of endotoxic dogs following glucose administration except at the higher doses of endotoxin studied. These authors also found differences in blood pressure based on whether the dogs went through an early hyperglycemic phase. Hinshaw and coworkers (100) demonstrated improved survival in the dog when glucose levels were maintained at preshock levels, and, in addition, found a positive correlation between the ability of an animal to

maintain glucose homeostasis and survival -- only three out of eleven animals surviving endotoxic administration without glucose treatment. Similar results have been obtained by Wilson et al. (217) in the baboon. Hinshaw's studies (100), however, in contrast to the results of Berk (9), demonstrated protection even at the relatively high dose of endotoxin used in this study (LD_{70}). Hinshaw et al. (90) and Archer et al. (4), however, were unable to demonstrate a protective effect of glucose on the endotoxic myocardium, but these results were obtained using a donor perfused dog heart model in which myocardial failure could not always be demonstrated following endotoxin administration (84). Spitzer, Wagner, and Blackard (200) also have published data which is consistent with a beneficial effect of glucose treatment -- showing improvement in mean arterial pressure, heart rate, and cardiac output in dogs receiving an LD_{70} dose of endotoxin and glucose treatment. These authors also found significant hyperinsulinemia following glucose infusion in endotoxic dogs. This explains both the lack of any increased 24 hr survival in this study since all infusions were stopped after 5 hrs and provides a potential explanation for the differences in the results of the Hinshaw experiments (100) and the Berk (9) studies -- at the higher doses of endotoxin in the Berk studies the animals may have become significantly hyperinsulinemic so that following glucose treatments the dogs became hypoglycemic

leading to the death of the animals.

The bulk of the data in these studies consists of survival data. Thus, it is not clear what the mechanism for increased survival is. It seems likely that maintenance of blood glucose levels during endotoxin shock will be beneficial whether or not glucose dyshomeostasis plays a causative role in the demise of the animal or whether it is merely symptomatic of the overall cardiovascular and metabolic failure. More importantly, the technology for continuous blood glucose monitoring and thus for maintenance of controlled alterations in the glycemic state was not available until recently. The glucose clamping experiments included in this dissertation are based on new techniques and are appropriate to provide more definitive evidence for the role of hypoglycemia in the pathogenesis of circulatory failure following endotoxin administration. While the idea that hypoglycemia plays an important role in the development of circulatory failure during shock is not new -- Carl Wiggers speculated on this point in his classic monograph (214) -- and while several studies have lent credence to this concept, none are wholly conclusive. Furthermore, the site of action of the protective effects of glucose and presumably the detrimental effects of hypoglycemia has not been clearly established.

CHAPTER III

EXPERIMENTAL DESIGN AND RATIONALE

Two sets of experiments were designed to study the role of glucose dyshomeostasis in the develpoment of cardiovascular failure during endotoxin shock. The first major set of experiments entailed the measurement of various cardiovascular parameters (see section on cardiovascular measurements in Chapter IV - Methods) along with glucose, insulin, and lactate without any interventions to alter blood glucose in order to correlate the changes in blood glucose with the depression of cardiovascular function during endotoxin shock. The second major set of experiments was an attempt to alter the pattern of the cardiovascular events of endotoxin shock by controlling blood glucose -- at hypoglycemic, normoglycemic, and hyperglycemic levels -- using a "glucose clamping" technique. If glucose dyshomeostasis plays a critical role in the development of circulatory failure, then 1) development of metabolic failure must precede development of circulatory failure (or at least the critical events of metabolic failure must precede the critical events in the development of cardiovascular failure) and 2) altering the pattern of metabolic failure by controlling the blood glucose levels should lead to a

detectable alteration in the development of cardiovascular failure.

To test the aforementioned hypothesis four types of experiments were performed: 1) unclamped, 2) hypoglycemic clamped, 3) hyperglycemic clamped, and 4) normoglycemic clamped. In each experiment one group of dogs received saline after the appropriate control period and the other group of dogs received endotoxin. The endotoxin injected dogs were followed until their death from endotoxin shock up to a maximum of 10 hrs. The saline injected dogs provided a time-matched series of control dogs which were followed for a period equal to or exceeding the maximum time of survival of the corresponding endotoxic dogs.

Relationship of Hemodynamic Events to Metabolic Events during Endotoxin Shock -- Unclamped Studies

The first major set of experiments in which no interventions were made allowed the assessment of the time relationships of the events of metabolic and cardiovascular failure during canine endotoxicosis. While all of the individual measurements made in this set of experiments have been made previously by others and published, without continuous glucose monitoring it has not been possible to make this type of correlation. Furthermore, the presence or absence of an early hyperglycemic phase and the relationship of such a phase to the development of circulatory

failure could not be conclusively demonstrated without continuous blood glucose monitoring. Thus, this first set of experiments is important for determining the sequence of metabolic and vascular events.

Cardiovascular Function in the Endotoxic Dog during Altered Glycemic States -- Glucose Clamping Studies

The primary purpose of the glucose clamping experiments was to alter blood glucose concentration in order to determine whether such an alteration changes the pattern of cardiovascular events in the endotoxic dog. If development of glucose dyshomeostasis plays a critical role in the development of circulatory shock (even if it is not the only factor), then clamping the glucose at different levels -- hypoglycemic, normoglycemic, and hyperglycemic -- should cause a change in the pattern of development of circulatory shock: glucose deprivation would be detrimental to the maintenance of circulatory function, while maintenance of glucose supplies would be beneficial. Thus, glucose clamping should lead to predictable alterations in cardiovascular performance and survival in the endotoxic dog.

A secondary purpose of glucose clamping was to study the insulin responsivity and glucose utilization rates of endotoxic dogs. The question of insulin responsivity in endotoxicosis is an important and much debated question. The technique of glucose clamping -- at hyperglycemic and

normoglycemic levels -- has been used by DeFronzo, Tobin, and Andres (38) to measure glucose utilization and insulin responsivity in humans. If this technique could be adapted to the endotoxic dog in combination with continuous glucose monitoring, it would help to answer this important question.

In the DeFronzo technique (38) the hyperglycemic clamp provides an index of the beta-cell response to glucose while the normoglycemic clamp provides an index of tissue insulin responsivity. The measured parameters in this technique are blood glucose, serum insulin, and the rates of glucose and insulin infusion. In the hyperglycemic clamp, the infusion rate of glucose reflects glucose utilized after correcting for the error between the desired clamp level and the actual blood glucose level -- i.e. the glucose utilized will be greater than or less than the glucose infused depending on whether the actual blood glucose is less than or greater than the desired glucose level. This error can be corrected for with knowledge of the glucose space and of the urinary excretion of glucose during the clamp. The hyperglycemic clamp can also be used to determine the tissue responsivity to endogenous insulin levels using the ratio of glucose utilized to the peripheral insulin level.

In the normoglycemic clamp, as developed by DeFronzo (38), insulin is infused at a constant rate sufficient to raise the serum insulin levels significantly above normal. Glucose is then infused at the rate needed to maintain

normoglycemia. Again the glucose infused is converted to glucose utilized in response to the insulin load. In normoglycemic clamps the glucose utilized reflects the tissue responsivity to exogenous insulin. In addition, the metabolic clearance rate of insulin can be determined by dividing the insulin infusion rate by the difference between the basal insulin concentration and the concentration during the infusion. The adaptability of this technique to the endotoxic dog is dependent on two factors: First, that endogenous glucose production is negligible during glucose infusions, and second, that the glucose space does not change significantly during endotoxin shock. It is possible that neither of these factors is negligible in the endotoxic animal, but it is nevertheless worthwhile attempting to adapt this technique to the endotoxic dog since it provides a simple way of studying tissue insulin responsivity, beta-cell glucose responsiveness, and peripheral glucose utilization in the intact animal.

CHAPTER IV

METHODS

Experimental Groups

A total of 46 dogs were included in these experiments. Another 28 dogs were excluded from the study because of difficulties in completing the protocol or in tolerating the instrumentation procedures. The 46 dogs included in these studies were divided into 8 groups: 1) unclamped saline-treated control dogs (N=5), 2) unclamped endotoxin-treated dogs (N=16), 3) saline-treated hypoglycemic-clamped dogs (N=5), 4) endotoxin-treated hypoglycemic-clamped dogs (N=6), 5) saline-treated hyperglycemic-clamped dogs (N=3), 6) endotoxin-treated hyperglycemic-clamped dogs (N=4), 7) saline-treated normoglycemic-clamped dogs (N=3), and 8) endotoxin-treated normoglycemic-clamped dogs (N=4). Specific protocols are discussed in the following section.

Endotoxin Preparation

Escherichia coli endotoxin (055:B5), prepared by Difco (Detroit, MI) using the Boivin method, was used in this study. The same batch of endotoxin (control number 6555468) was utilized in all experiments to provide for consistent

potency throughout the study. The endotoxin was suspended in 0.9% saline to a concentration of 5 mg endotoxin/ml. Every dog received the same weight-adjusted volume of endotoxin or vehicle. The endotoxin dose of 8 mg/kg was chosen to provide a very high lethality (100%) over a 10 hr period in untreated dogs. Using this dose of endotoxin (in a total of 53 dogs given endotoxin and undergoing at least part of one of the four protocols) one dog succumbed during the initial hypotensive phase and one additional dog died during the early hyperglycemic phase. Also two dogs died during the glucose clamping phase of the hypoglycemic clamp experiment and one during the clamping phase of the hyperglycemic clamp experiment. Thus, while 8 mg/kg is a high dose of endotoxin, most dogs went through the usual pattern of events -- the only real effect of this high dose being to shorten the time course so that the blood sampling needed for the various measurements could be made without compromising the ability of the animals to survive.

Canine Model

Mongrel dogs of either sex weighing 18.9±0.5 kg (X±S.E.M., range 14.5-26 kg, N=46) were used in this study. All dogs were kept in the animal facilities for at least one week prior to their experimental use. Dogs were fed (Purina Dog Chow) midmorning every day through the day prior to the acute experiment. Dogs were also free of any

obvious signs of infection such as mucous discharge from the nose or diarrhea prior to the acute experiment. When necessary, dogs were given a course of penicillin or other appropriate antibiotics to treat an existing infection and observed to be outwardly symptom-free prior to their experimental use. Thus, although the previous infectious history was highly variable and largely unknown, all dogs were in apparent good health at the time of experimentation. All dogs were routinely given a prophylactic course of tetracycline antibiotics and were treated for intestinal parasites. In addition all dogs were vaccinated against rabies, canine distemper, and parvo virus.

Initial Surgical Procedures

Food was withheld from all dogs for the 24 hrs prior to the acute experiment (i.e. they were last fed on the morning of the previous day). During this period of time all dogs continued to receive water ad libitum. Dogs were anesthetized intravenously via the cephalic vein of the forelimb with sodium pentobarbital (NEMBUTAL, 30 mg/kg). This dose was supplemented with 3-4 mg/kg every 1-1.5 hrs during the experiment to maintain a level of anesthesia (see (69) for dosage schedule). In order to minimize the cardiovascular depressant effects of pentobarbital -- especially in endotoxic dogs -- these supplemental doses were diluted to 10 ml with saline so that they could be

given slowly over a 3-5 min period. In spite of this precaution, twelve endotoxic dogs died during anesthetic supplementation partway through a protocol. Data from those dogs which died during anesthetic supplementation were excluded from analysis.

Following initial anesthetization a tracheotomy was performed. All dogs were then intubated with a glass or plastic Y-tube and allowed to breathe on room air spontaneously. Both femoral veins were cannulated to allow for intravenous infusions and for simultaneous venous sampling. The right femoral artery was cannulated for withdrawal of arterial samples for the measurement of arterial blood gases and pH. Arterial PO_2, PCO_2, pH and bicarbonate were measured in blood samples obtained every 15 min during control periods and every 30 min during experimental periods using a Corning 165/2 semi-automatic blood gas and pH analyzer. The blood gas analyzer was calibrated daily with standard buffers of pH 6.838 and 7.382 and with calibration gases containing 12% O_2, 5% CO_2, balance nitrogen and 0% O_2, 10% CO_2, balance nitrogen. The ambient barometric pressure was checked daily and the percentage compositions were converted to partial pressures assuming a body temperature of 37°C and saturation with water vapor (vapor pressure at 37°C, 47 mmHg). The calibration gases are bubbled through separate humidifier bottles to saturate the gases prior to entering the sample chamber. The calibration for both pH

and blood gases (oxygen and carbon dioxide) was checked periodically throughout the experiment and remained relatively stable (pH: ±0.005 units and oxygen and carbon dioxide: ±2 mmHg). If the calibration drifted outside these specified ranges the instrument was recalibrated. Rectal temperature was monitored in all dogs with a YSI thermistor probe. As body temperature can significantly affect metabolic variables, this temperature was maintained between 36 and 39°C with a heating pad and by covering the animal with surgical drapes during the experiment.

Cardiovascular Instrumentation

Electrocardiogram

All recordings were made on a Grass model 7 eight channel polygraph. A lead II electrocardiogram was routinely recorded using needle electrodes in the skin of both hindlimbs and the right forelimb. This lead was chosen to provide reasonably large upright QRS complexes. Heart rate was counted using fast traces of the electrocardiogram taken every fifteen minutes.

Left Ventricular Performance and Systemic Arterial Pressure

A Millar catheter-tip transducer (model PC 470) was introduced via the left femoral artery and positioned in the left ventricle for the measurement of left ventricular

pressure. The pressure wave form was used as the sole guide in proper placement of this transducer. Occasionally a ventricular dysrhythmia was induced during the placement of the Millar catheter. Such dysrhythmias could usually be suppressed by manipulating the catheter within the ventricle or in some cases by treating with lidocaine as necessary (1-2 mg/kg iv). In any case no animal was used for experimentation in which the presence of a dysrhythmia or the treatment necessary to suppress it compromised ventricular performance as assessed by left ventricular pressure, dP/dt, cardiac output, and systemic blood pressure. Two dogs died of ventricular fibrillation after placement of this catheter and three additional dogs could not be used due to the presence of ventricular tachycardia which persisted in spite of treatment even after the catheter was removed from the ventricle.

This transducer provides a good frequency response (0 to 20 KHz) and avoids the problems of fluid-filled catheter systems. There is less than 1 mmHg/hr drift; thus, over the course of a 10 hr experiment there is less than 10 mmHg drift. The catheter could be placed during an acute experiment without opening the chest and without requiring prior chronic implantation as with some other intravascular transducers.

The signal from this transducer was electronically differentiated and recorded on an adjacent channel. The

differential could then be used as a marker of end-diastole on fast traces so that end-diastolic pressure could be measured. In some cases a Millar catheter with a second transducer placed 4 cm behind the tip (Model PC 770) was used allowing the simultaneous recording of systemic blood pressure from the root of the aorta. This method of measurement of systemic blood pressure was useful since a check on valve performance was provided. However, this particular catheter could not be used in every case since blind placement using only the pressure as a guide is more difficult due to the smaller diameter of the distal catheter segment and the greater flexibility of the material that this segment is made of. In most cases the single transducer catheter was used and systemic blood pressure was measured from the catheter in the right femoral artery using a Statham P23ID transducer.

Left ventricular stroke work was calculated from the peak left ventricular pressure, the cardiac output (see the following section), and the heart rate using the following formula:

$$\text{Left Ventricular Stroke Work} = \text{LVP} \times (\text{C.O.}/\text{HR}) \qquad (1)$$

where, LVP = peak systolic pressure
C.O. = cardiac output
HR = heart rate

Because of the frequency response characteristic of the Grass model 7 recorder dP/dt cannot accurately be measured.

In the present study dP/dt was used only for timing of cardiac events because of this limitation of the recorder. Thus, stroke work was used instead to quantify cardiac performance. Both dP/dt and stroke work, however, suffer from the problem of being sensitive to preload, afterload, and heart rate as well as myocardial contractility. Thus, with either index it is difficult to separate peripheral vascular and cardiac events. Both indices have been used to assess cardiac performance during endotoxin shock. However, caution must be used in concluding that a cardiac mechanism is involved.

The Measurement of Cardiac Output and Mean Pulmonary Artery Pressure

A number seven French Swan-Ganz balloon catheter (110 cm in length) for the measurement of mean pulmonary artery pressure and cardiac output was introduced into the right external jugular vein and guided through the right side of the heart and into the pulmonary artery using the pressure wave form. The catheter was inserted until it was wedged with the balloon fully inflated; after which the balloon was deflated and the catheter was secured in place. Visual inspection on post-mortem examination has demonstrated that the tip was then in one of the major branches of the pulmonary artery. Numerous studies by Edwards Laboratories have shown that the trans-cardiac loop of

catheter can either shorten or lengthen depending on changes in cardiac performance and warming of the body of the catheter to blood temperature -- thus, either wedging the tip of the catheter or allowing it to fall back into the right ventricle. Thus, it was necessary to periodically readjust the position of the catheter when mean pulmonary pressure could no longer be measured at the tip (either because it was wedged or because the tip had moved back into the right ventricle as determined by the pressure record). In some dogs a few ventricular premature beats were seen during the passage of the catheter through the right heart. Inflation of the balloon whenever the catheter was in the right ventricle minimized dysrhythmias since floating the catheter tip tended to keep the catheter away from the ventricular wall. The soft material with which this catheter is made and the brief excursion through the right side of the heart also tended to minimize the occurrence of dysrhythmias.

Cardiac output was measured every fifteen minutes throughout the experiment using the thermodilution technique (see (132) for a good recent review of the thermodilution technique). Cardiac output was converted to cardiac index by dividing cardiac output by the surface area of the dog which was calculated on the basis of weight with the formula appropriate for the weight range of dogs used in this experiment: $S.A. = 0.114 \times W^{2/3}$ (13, 75: note that the constant 0.114 is the average of the two values given in the

references). The appropriate mean arterial pressure is divided by the cardiac index to determine the systemic and pulmonary vascular resistance (the venous pressure for both beds is assumed to be 0 for this calculation). The average of 3-5 serial measurements of cardiac output made at the end of each fifteen minute period is reported. With each measurement 3 ml of ice-cold 0.9% saline were injected as rapidly as possible into the proximal port (nominal volume 0.92 ml) of the Swan-Ganz catheter. This port is located 30 cm behind the tip of the catheter and when the catheter is properly placed it lies in the superior vena cava proximal to the right atrium. This positioning allows for adequate mixing before reaching the pulmonary artery. Care was always taken to inject during the same part of the respiratory cycle (end-inspiration) -- this is especially important in the dog because of the large azygous vein drainage in this species which is intermittent during the respiratory cycle. Care was also taken to minimize syringe handling prior to injection in order to avoid significant warming of the injectate. The temperature change is detected by a thermistor embedded in the catheter 4 cm behind the distal port at the tip. This thermistor is connected to an Edwards model 9520A cardiac output computer which performs the necessary calculations to obtain cardiac output in liters per minute. A second thermistor used to monitor the temperature of the injectate bath is also connected

to the cardiac output computer. Both bath temperature and blood temperature can also be displayed by the cardiac output computer.

The thermodilution technique is essentially an indicator-dilution technique in which temperature rather than concentration is used to determine the distribution of the indicator. The temperature detected by the thermistor in the pulmonary artery is dependent on the properties of heat exchange between the ice-cold saline injectate and the blood and surrounding tissues. These properties in turn are dependent on the heat capacity of saline and blood and the rate of blood flow past the thermistor. Since the heat capacities are a constant and known quantity, the temperature will vary according to the rate of flow past this thermistor -- i.e. at high rates of flow heat exchange is more rapid and the temperature change will be smaller while at low flow rates the "cold" injectate is carried away less rapidly and the temperature change is consequently larger and more prolonged. The mathematical derivation of this property is the following equation:

$$\text{flow} = \frac{\rho_I \, C_{P_I} \, 60 \, C_T \, V_I \, (T_B - T_I)}{\rho_B \, C_{P_B} \int \Delta T_B(t)\,dt} \qquad (2)$$

where, ρ_I = density of injectate

C_{P_I} = heat capacity of injectate

60 = seconds/minute

C_T = correction for temperature change prior to injection

V_I = injected volume (corrected for residual volume of catheter

T_B = blood temperature

T_I = injectate temperature

ρ_B = density of blood

C_{P_B} = heat capacity of blood

$\int \Delta T_B(t)dt$ = integral of thermodilution curve

The terms which do not include temperature are all constant and are combined to form a calibration constant which is dialed into the cardiac output computer prior to use. For 3 ml of ice-cold saline the calibration constant calculated by Edwards Laboratories after numerous trials is 0.132 for the model 9520A cardiac output computer. This constant is reasonably accurate for injectate temperatures in the range 0-5°C. Blood and injectate temperatures are continuously stored prior to injection and the cardiac output computer uses the values stored just prior to starting the integration in the calculation of the cardiac output. The Edwards

computer calculates an analog integration of the thermodilution curve which is automatically terminated at the point where the curve returns to 30% of its peak. This cuts-off approximately 22% of the area which is compensated for mathematically in the calculation of cardiac output by electronically multiplying the integral by 1.22. This cutoff point was chosen experimentally by Edwards Laboratories as being the point of minimum variability in the downslope of the curve. Beyond this point there is a release of heat from the heart and blood vessel walls which distorts the tail end of the curve. This is not, however, true recirculation which does not occur in the thermodilution technique.

Several factors influence the reproducibility and accuracy of the measurement of cardiac output by the thermodilution method. First, the volume and temperature of the injectate are important variables (16, 48). The greater the volume and the lower the temperature of the injectate (i.e. the greater the difference in temperature between blood and the injectate) the greater is the signal-to-noise ratio. Thus, high volume, low temperature injectates have the best signal-to-noise ratios: However, the cold temperature and the high volume can also affect cardiac output by altering heart rate and contractile force. Therefore, it was necessary to compromise in these experiments by using the smallest allowable volume (3 ml) of ice-cold saline minimizing the problem of the volume of injection

(which may be a very important consideration under the low cardiac output, high pulmonary resistance state of endotoxin shock) while giving a reasonable signal-to-noise ratio. This does not, however, mitigate the problem of the rapid injection of ice-cold saline into the right atrium on the SA node or on AV nodal conduction. The continuous monitoring of the ECG and of hemodynamic parameters during injection provided some check on this problem.

A second factor is the speed of injection (45, 60, 62). All injections should be made smoothly and rapidly (Edwards Laboratories requires a speed no slower than 4 ml/sec (26)). Injections which are too slow or not made at a uniform speed will not give true cardiac output readings.

Reasonable care in making injections and attention to the factors which may affect cardiac output measurement by this technique results in measurements which correlate very well with those made by the Fick principle (16) or with electromagnetic flow probes (169). Using either cannulating electromagnetic probes or periaortic probes, Pelletier and associates (169) obtained correlation coefficients of 0.98 and 0.96, respectively, with the thermodilution technique using ice-cold injectates in dogs ($P<0.001$) in 100 or more determinations. All of these determinations were made in control dogs under steady-state conditions. This raises one other consideration: During circulatory shock the cardiac output is no longer in a steady-state.

There may be wide variations over a short period of time resulting in differences between serial measurements of greater than 5% and occasionally greater than 10%. While this may make individual measurements less accurate it is likely that trends over long periods of time will still be accurately reflected and that differences in the pattern between groups will still be faithfully recorded. Thus, the technique is still a useful tool even with the loss of accuracy under non-steady state conditions and has been found to be useful in critically ill patients under those conditions (45). Thus, thermodilution cardiac outputs are used to provide an index of flow in these experiments without having to open the chest either acutely or during prior surgery to implant an electromagnetic flow meter.

Summary

To summarize, the following cardiovascular parameters are measured or calculated: Lead II electrocardiogram, heart rate, left ventricular pressure, left ventricular dP/dt, left ventricular stroke work, cardiac output, cardiac index, pulmonary artery pressure, systemic arterial pressure, and systemic and pulmonary vascular resistance (calculated from the mean systemic and pulmonary arterial pressures and the cardiac index).

Metabolic Measurements

Metabolic Measurements: Glucose

Glucose was measured using the YSI model 26 Continuous Glucose Monitor. This instrument combines the electronics for the measurement of blood glucose by the glucose oxidase method with a peristaltic pump for the continuous withdrawal of the sample. Blood is withdrawn via a double lumen catheter which adds a small amount of heparin and sodium fluoride (both of which have been shown by Yellow Springs Instruments not to interfere with the glucose oxidase measurement (221)) via one lumen while the blood is being withdrawn via the other lumen at the rate of 2, 5, or 10 ml/hr. The cannula for blood glucose sampling is placed by venipuncture in the left external jugular vein. Using this method of withdrawal there is no systemic anticoagulation of the animal and there is only a slight dilution of the sample from this source which is controlled for by calibration using the same catheter system. Throughout most of the experiment the pump is on the slowest rate of withdrawal so that only minimal volumes of blood are withdrawn for the measurement of glucose. During glucose clamping the 10 ml/hr speed is used to provide for better resolution of any changes in glucose level with time. The pump also introduces an appropriate amount of buffer into the sample

chamber to dilute the sample and to maintain pH for the glucose oxidase reaction. Glucose oxidase is immobilized on a glutaraldehyde resin membrane. This layer is sandwiched between a polycarbonate membrane on the outside facing the sample chamber with a pore size large enough to readily pass glucose, oxygen, and hydrogen peroxide, water, and salts and a cellulose acetate membrane on the inside facing the electrode with a pore size that excludes glucose, ascorbic acid, and most interfering substances but which permits the passage of hydrogen peroxide, water, and salts. The buffer also contains excess amounts of catalase to destroy any back-diffusing hydrogen peroxide. During the glucose oxidase reaction glucose combines with oxygen to form gluconic acid and hydrogen peroxide. The hydrogen peroxide then can diffuse across the inside of the membrane to the electrode. At the electrode the hydrogen peroxide is oxidized creating a current which is proportional to the amount of hydrogen peroxide present in solution. Since hydrogen peroxide is produced in the glucose oxidase reaction in stoichiometric proportion to glucose consumed, the current is also related to the amount of glucose in the sample. Thus, the monitor can -- using appropriate calibration (buffer -- i.e. 0 mg/dl -- and a 200 mg/dl standard were used for calibration and a nominal 500 mg/dl standard for a linearity check) -- then translate this current into glucose concentration in mg/dl. This method correlates well

with other methods of measuring blood glucose (108).

Metabolic Measurements: Insulin

Using the radioimmunoassay technique, insulin was measured in serum samples taken every 15 min throughout the experiment from a catheter in the right femoral vein. Pharmacia Diagnostics Phadebas Insulin kits were used for this measurement. In this assay anti-insulin antibodies are immobilized on Sephadex beads. The radioimmunoassay technique employs the displacement of iodine-125 insulin from the antibodies by non-radioactive insulin from the sample. This displacement occurs in a stoichiometric manner such that the greater the insulin concentration in the sample the greater the amount of radiolabelled insulin which is displaced. The antibodies and any bound insulin are immobilized on Sephadex beads which can be separated from the supernatant solution. The remaining bound radioactivity is quantified using a gamma counter. The counts obtained can then be related to the amount of insulin in the sample using appropriate standards to form a standard curve. For these experiments the standard curve and the samples are analyzed using a computer program developed by Kober and Yelich (see Appendix B) for the digital PDP 12 computer utilizing a log-logit type analysis. Data is entered into the computer from the gamma counter using a paper type reader. The log-logit analysis was shown to

give values which correlate very well with those obtained by reading off of the standard curve by hand. Correlation coefficients for the log-logit plots made by the computer were usually greater than 0.98 demonstrating the appropriateness of the log-logit linearization technique. Standard points which were off of the standard curve could be removed from the fit using an error correcting routine within the program. When the 0 μU/ml standard (H_o) was less than 15% of the total activity the kits no longer gave good results and the results were not used. The H_o standard for brand new kits was usually about 25% of the total activity. This assay method has been shown by this laboratory to give reasonably good results with rat insulin (221). In addition, Pharmacia reports within and between assay coefficients of variation of approximately 5 to 10 per cent using human control sera. Initially the results with dog serum samples were found to be reasonable as well. However, during the course of this study it has been discovered that under certain conditions spuriously high results may be obtained (fasting control samples in the range of 100's to 1000's of μU/ml). Since others have had a similar problem (personal communication with Nancy Manson), this does not seem to be an isolated instance and may be a factor in the wide variability in the literature of the reported insulin levels in the endotoxic dog (14, 142, 198). The interpretation of the insulin results included in this dissertation

must, therefore, be made with a great deal of caution. This problem will be discussed further, where appropriate in the results and discussion sections.

Metabolic Measurements: Lactate

Deproteinized whole blood lactate samples obtained every fifteen minutes (from the right femoral vein) were precipitated in 0.8% perchloric acid (0.5 ml of sample in 1 ml of perchloric acid). The samples were then centrifuged to obtain the supernatant and lactate was determined with the Calbiochem-Behring Rapid Lactate kits using an enzymatic method. Samples were pooled into control (prior to endotoxin or saline injection), early (first half of time course) and late shock (second half of time course) samples for unclamped endotoxic dogs, and into postinjection clamp (15-120 min) and post clamp (135 min-termination of the experiment) samples for glucose clamped dogs. The assay method uses lactate dehydrogenase isolated from beef heart to convert the lactate to pyruvate while NAD is reduced to NADH. The reaction is driven to completion by adding glutamate and glutamate-pyruvate transaminase to the reaction mixture. Thus, the pyruvate which is produced from lactate is converted to l-alanine in the transamination reaction. The NADH absorbs light in the ultraviolet range at 340 nm. The NADH produced and consequently the lactate consumed can thus be determined by measuring the UV absorbance of the

samples after the reaction is driven to completion (60 min at 30°C). The reaction is buffered in pH 9.5 TRIS buffer. All samples were run in duplicate and an average of the two absorbance readings was taken as the reported values. The lactate concentration in the diluted samples is calculated from the linear regression of a standard curve using the following standards (run singly with each assay): 1) 0.44 mM, 2) 1.10 mM, 3) 2.20 mM, 4) 3.30 mM, and 5) 4.40 mM. The linear regression and the calculation of sample concentrations including corrections of the concentration for the dilution of the original sample were performed on an HP97 calculator using a curve fitting program obtained from the Hewlett-Packard statistics package for the HP67/97.

Summary

To summarize the metabolic variables: Whole blood glucose, serum insulin, and deproteinized whole blood lactate were measured as indices of the carbohydrate status. Arterial blood gases and pH were also measured in samples taken from the right femoral artery.

Protocols

Relationship of Hemodynamic Events to Metabolic Events during Endotoxin Shock -- Unclamped Studies

Following a 30 min control period in which measurements were taken every 15 min (i.e. three control measurements were taken) the animals were given either saline or endotoxin. All dogs were then monitored for 10 hrs or until death in the endotoxin group with measurements being made at the previously stated intervals. No other interventions were made in this set of unclamped experiments.

Cardiovascular Function in the Endotoxic Dog during Altered Glycemic States -- Glucose Clamping Studies: The Hypoglycemic Clamp

After 30 min control period all animals were given an intravenous bolus dose of insulin consisting of the following components: 80 U/kg of regular insulin, 18 U/kg LENTE insulin, and 9 U/kg ULTRALENTE insulin. An infusion via the left femoral vein was also started at this time at 2 ml/min of 5 U/ml of regular insulin. This infusion was maintained at this rate until the desired level of blood glucose was reached. After 45 min the blood glucose had dropped to 27±2 mg/dl. At this time dogs were given either saline or 8 mg/kg endotoxin. The blood glucose was then maintained at a mean level between 10 and 30 mg/dl (19.7

±0.8 mg/dl) for a period of 2 hrs with intermittent infusions of 5 U/ml regular insulin at 2 ml/min or 5% glucose (glucose was given on an empirical basis -- usually 10-20 ml were given over a period of about a minute) as needed. These doses and infusion rates of insulin and glucose were chosen by trial-and-error in order to reach and maintain the desired level of blood glucose. Because the hypoglycemic clamp places considerable stress on the animal and consequently leads to powerful reflexes to return the blood glucose back to normal, even these doses of insulin proved inadequate in some animals to maintain the clamp -- particularly over the first hour of the clamp in endotoxic animals. Dogs (N=5) in which the clamp could not be maintained for 2 hrs were excluded from the study. After the 2 hr period all animals were monitored for an additional 3 hrs or until death without further infusions.

Cardiovascular Function in the Endotoxic Dog during Altered Glycemic States -- Glucose Clamping Studies: The Normoglycemic Clamp

After a 30 min control period an infusion of 5 U/ml regular insulin was started at a rate of 1 ml/min along with an infusion of 50% glucose at a rate of 1 ml/min (both infusions were given through a catheter in the left femoral vein). These infusions were then periodically adjusted by trial-and-error to maintain blood glucose at a mean level between 75 and 95 mg/dl (85±1 mg/dl). After 45 min the

dose of saline or endotoxin was administered as described previously. The clamp was maintained for 2 hrs postenotoxin or saline injection after which all infusions were stopped. All dogs were then monitored for another 6 hrs after ending the clamp or until death in the endotoxic group.

Cardiovascular Function in the Endotoxic Dog during Altered Glycemic States -- Glucose Clamping Studies: The Hyperglycemic Clamp

Following a 30 min control period animals were given a bolus dose of 300 mg/kg glucose (20% glucose in water). At the same time an infusion via the left femoral vein of 50% glucose was started at 1 ml/min. The infusion rate was adjusted periodically to maintain blood glucose at a mean level between 145 and 165 mg/dl (156±3 mg/dl); these adjustments were made by trial-and-error to achieve the desired level of blood glucose. Infusions were maintained over the same period of time as for the hypoglycemic clamp. Forty-five minutes after giving the bolus dose of glucose, animals were given either saline or 8 mg/kg endotoxin. The clamp was maintained for 2 hrs after the injection of saline or endotoxin and then all infusions were stopped and the dogs were monitored for an additional 8 hrs or until death.

Data Analysis

In the unclamped experiments data were divided into a control and a post injection period. Because of the variable time course of endotoxin shock, the post-injection or post-saline injection time course was normalized as a percentage of the time to death or the end of the experiment in the unclamped groups. This normalization device allowed the comparison of animals in what is assumed to be similar pathophysiological states. The data points obtained from the normalization procedure were then used for statistical comparisons. Statistical comparison between groups in the unclamped experiments was made using a factorial analysis of variance. Significant differences between groups were identified using a modified t-test at the various points.

In the glucose clamping experiments data were grouped into a control (-75 to -45 -- i.e. prior to any experimental manipulations), a preinjection clamp (0 min -- i.e. after the glucose clamp is established but prior to saline or endotoxin injection), a postinjection clamp (15 to 120 min -- i.e. during glucose clamping after the injection of saline or endotoxin), and a postclamp period (>120 min -- i.e. after the infusions are stopped). Data from each dog were averaged over each period of time and the resultant means were taken as representative data points for each time period. Measurements taken just prior to the end

of the experiment were also compared (for some parameters such as systemic and pulmonary resistance this was not done since some or all of the animals in the endotoxin groups died during the course of the experiment). Statistical comparisons were made using a one-way analysis of variance to determine the effect of the three clamps in saline and endotoxin injected animals. Significant differences between groups were identified using a modified t-test.

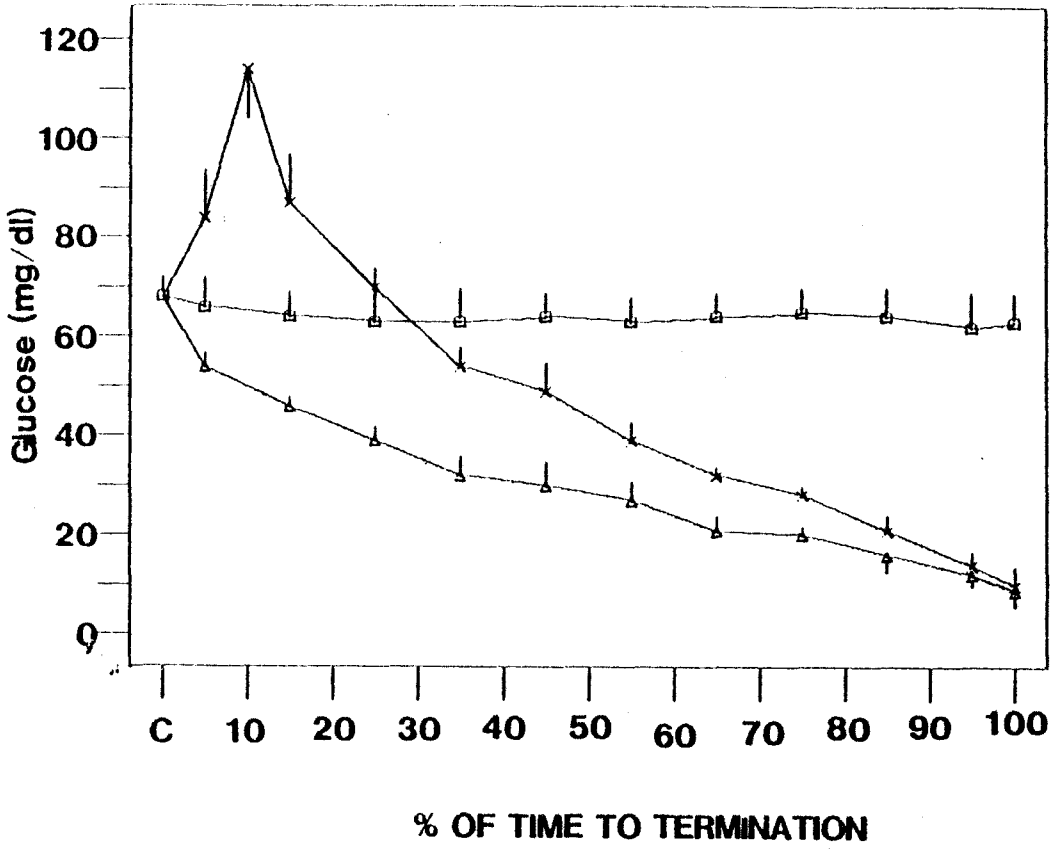
CHAPTER V

RESULTS

The Unclamped Experiments

A total of 21 dogs, 5 saline-injected and 16 endotoxin-injected, were used in this set of experiments. As can be seen from figure 1, the endotoxic group can be divided into a group of 6 dogs which showed an early hyperglycemic phase and a group of 10 dogs which showed no hyperglycemic phase during continuous blood glucose monitoring. While such an arbitrary division can perhaps be made using other criteria, glucose is the parameter of interest in this study and, thus, it is of interest to make the division in this case based on differences in the pattern of glucose dyshomeostasis following the administration of endotoxin. This difference in the pattern of changes in blood glucose is readily apparent in the records of the individual animals (see Appendis A, fig. 20) and it is therefore easy to make this kind of division of the endotoxic dogs into two groups. Statistical analysis clearly demonstrates that all three groups of dogs (control and the two endotoxic groups) had different patterns of blood glucose levels ($P<0.05$). The peak glucose level in the hyperglycemic group of 114±10 mg/dl occurred at 40±5 min following the administration of

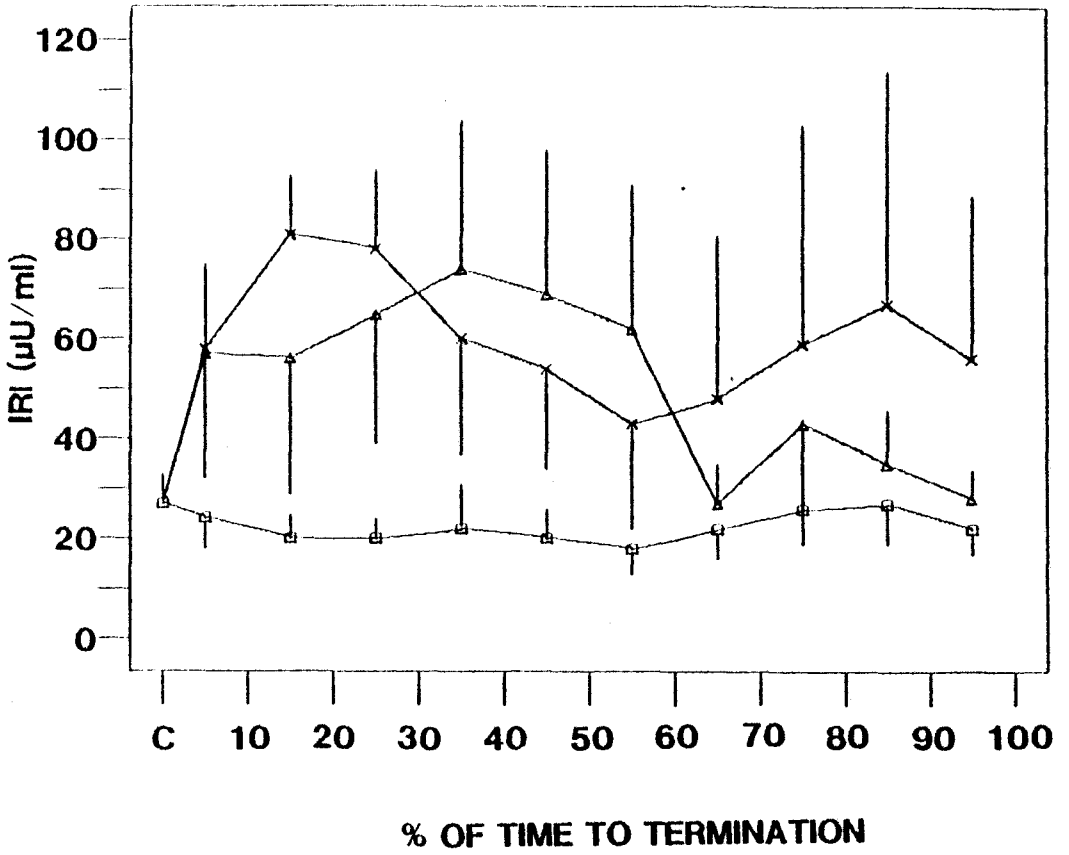
Fig. 1. Glucose -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=6), Endotoxin w/no hyperglycemia (Δ, N=10). All three groups exhibit different patterns of blood glucose ($P<0.05$).


120
100
80
60
40
20
0
Glucose (mg/dl)
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

endotoxin (at approximately 10 per cent of the average time course of 398±72 min in the hyperglycemic, endotoxin group of dogs). The mean time of survival of endotoxic dogs not showing any hyperglycemic stage was 276±47 min. Using an unpaired t-test this is not significantly different from the hyperglycemic group although the trend is toward shorter survival times ($t=1.3874$ with 14 degrees of freedom, $p=0.094$). This probably is a reflection of the highly lethal model of endotoxin shock used in these experiments. Near death, both groups of endotoxic dogs were profoundly hypoglycemic (10±4 mg/dl in dogs exhibiting an initial hyperglycemic phase and 9±3 mg/dl in dogs showing no hyperglycemia). In contrast glucose was fairly well maintained over a 10 hr period in dogs given saline.

The serum immunoreactive insulin levels are shown in figure 2. There were no significant differences in insulin levels between any of the three groups at any point. There was a high degree of animal-to-animal variability in the endotoxic groups as demonstrated by the large standard errors in these two groups (see also fig. 21, Appendix A). This high degree of variability probably reflects the highly variable time course of changes in insulin levels seen in endotoxic animals. These results are indicative of at least a functional hyperinsulinemia in the endotoxic dog. These results, however, must be viewed with a certain amount of caution in light of the previously mentioned problem with

Fig. 2. Insulin -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=6), Endotoxin w/no hyperglycemia (Δ, N=10). No significant differences between groups.


120
100
80
60
40
20
0
IRI (μU/ml)
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

spuriously high insulin levels obtained in other experiments (see results of glucose clamping experiments). The fasting control level of 27±6 μU/ml obtained with this group of 21 dogs is certainly reasonable and while the elevations seen in some cases following the administration of endotoxin are dramatic (highest individual insulin measurement: 300 μU/ml) they are not unreasonable -- none of the measured control or experimental values in the unclamped series of dogs was higher than the highest standard concentration, in contrast to the results in the glucose clamping experiments. Because of the problem encountered in assaying the samples from the glucose clamping experiments, these results in unclamped animals cannot be taken as conclusive proof of hyperinsulinemia in the endotoxic dog.

Tables I, II, and III show the arterial blood gas and pH data for the unclamped experiments while the plasma lactate data is included in Table VIII along with the lactate data from the glucose clamping experiments. No consistent changes in either arterial PO_2 or PCO_2 were seen in either group of endotoxic dogs. Both groups of endotoxic dogs became acidotic, but there was no difference in the degree of acidosis seen in either group. Lactate was elevated in the 9 endotoxic dogs in this group in which these measurements were made. Insufficient samples, however, were taken from dogs showing hyperglycemia (N=2) to make a comparison of lactate levels in the two endotoxic groups. The similarity

Table I Arterial pH -- Unclamped Studies

	pH			
	Control	Early[1]	Mid[1]	Late[1]
Saline (5)[2]	7.37±0.02[3]	7.39±0.03	7.39±0.03	7.36±0.04
Endotoxin w/hyperglycemia (4)[2]		7.25±0.01[4]	7.15±0.06[4]	7.09±0.06[4]
Endotoxin no hyperglycemia (8)[2]		7.24±0.03[4]	7.18±0.05[4]	7.06±0.03[4]

1: Early = 0-25% of postinjection time course, Mid = 25-75%, Late = 75-100%

2: (N)

3: N=17 for Control

4: Significantly different from saline, $P<0.05$

Table II Arterial PO_2 -- Unclamped Experiments

	PO_2 (mmHg)			
	Control	Early[1]	Mid[1]	Late[1]
Saline (5)[2]	85±4[3]	77±2[4]	74±3[4]	71±4[4]
Endotoxin w/hyperglycemia (4)[2]		63±10[4]	57±11[4]	55±9[4]
Endotoxin no hyperglycemia (8)[2]		82±7[4]	82±10[4]	74±7[4]

1: Early = 0-25% of postinjection time course, Mid= 25-75%, Late = 75-100%

2: (N)

3: N=17 for control measurement

4: No significant differences

Table III Arterial PCO_2 -- Unclamped Experiments

	PCO_2 (mmHg)			
	Control	Early[1]	Mid[1]	Late[1]
Saline (5)[2]	33±2[3]	33±1[4]	30±2[4]	29±1[4]
Endotoxin w/hyperglycemia (4)[2]		39±4[4]	42±4[4]	47±5[4]
Endotoxin no hyperglycemia (8)[2]		27±2[4]	30±6[4]	36±4[4]

1: Early - 0-25% of postinjection time course, Mid = 25-75%, Late = 75-100%

2: (N)

3: N=17 for Control

4: No significant differences

of pH in these dogs, however, is an indication that these levels would probably not be different.

Cardiac output is plotted in figure 3. The cardiac output fell rapidly after the administration of endotoxin and remained below control levels throughout the time course of shock in both groups of endotoxic dogs. The cardiac output in endotoxic dogs going through an early hyperglycemic phase tended to be higher than that seen in endotoxic dogs with no early sign of hyperglycemia. However, both groups of endotoxic dogs gradually approached the same end-point.

Both peak left ventricular systolic and end-diastolic pressures are plotted in figure 4. While peak pressure was below control in both endotoxic groups throughout the time course of shock, there were no differences between the two groups of endotoxic dogs. However, end-diastolic pressure fell to a greater extent in animals which demonstrated an early hyperglycemic phase.

Stroke work is shown in figure 5. Stroke work was markedly depressed below control levels throughout the course of endotoxin shock in both groups of endotoxic dogs. Following a pattern similar to that for cardiac output, dogs which showed no hyperglycemia performed significantly less cardiac work than did dogs which went through an initial hyperglycemic phase.

Mean systemic blood pressure is shown in figure 6 and calculated total peripheral resistance is shown in

Fig. 3. Cardiac Output -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=5), Endotoxin w/no hyperglycemia (Δ, N=10). ☆ $P<0.05$ compared to control; ★ $P<0.05$ compared to control and endotox w/hyperglycemia; ◇ $P<0.05$ compared to control, $P<0.1$ compared endotoxin w/hyperglycemia.

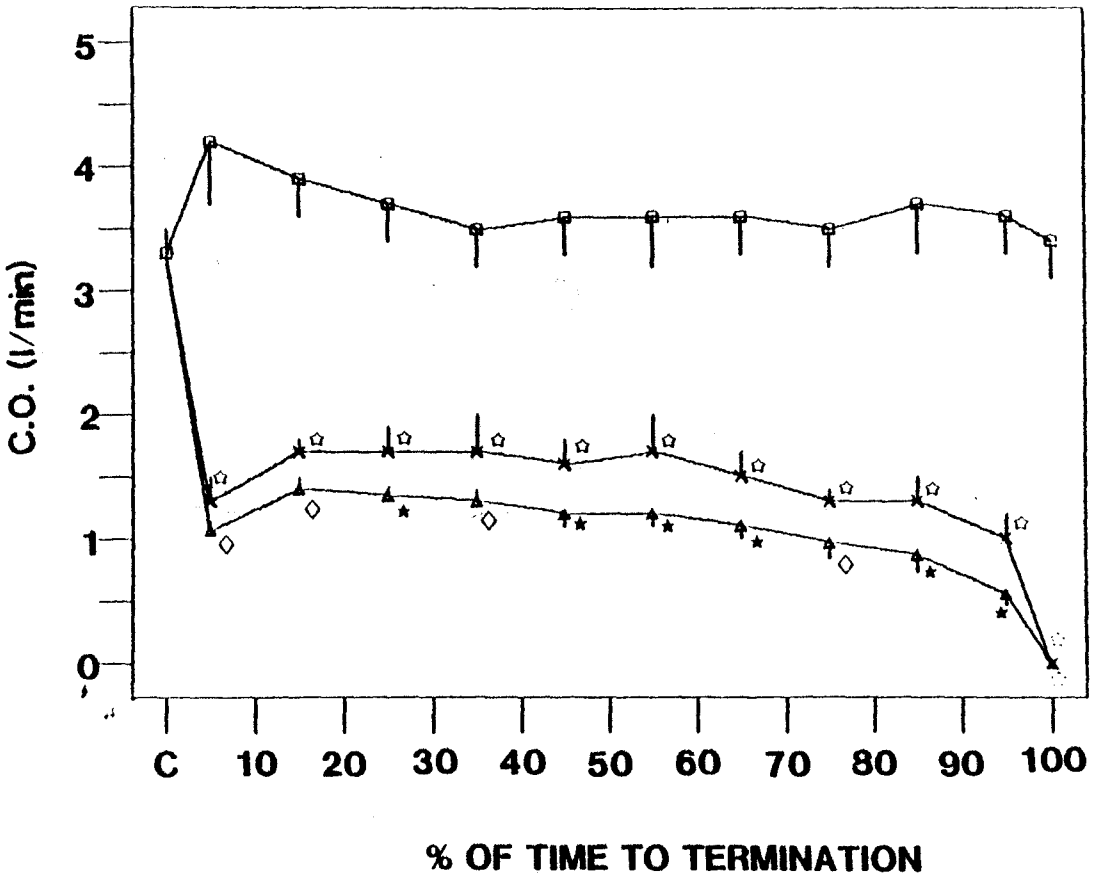

C.O. (l/min)
5
4
3
2
1
0
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

Fig. 4. Left Ventricular Peak Systolic and End-diastolic Pressures -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=6), Endotoxin w/no hyperglycemia (Δ, N=10). Peak systolic pressure -- endotoxin groups less than control, no differences between endotoxin groups. End-diastolic pressure -- endotoxin groups less than control, endotoxin w/hyperglycemia less than endotoxin w/no hyperglycemia.

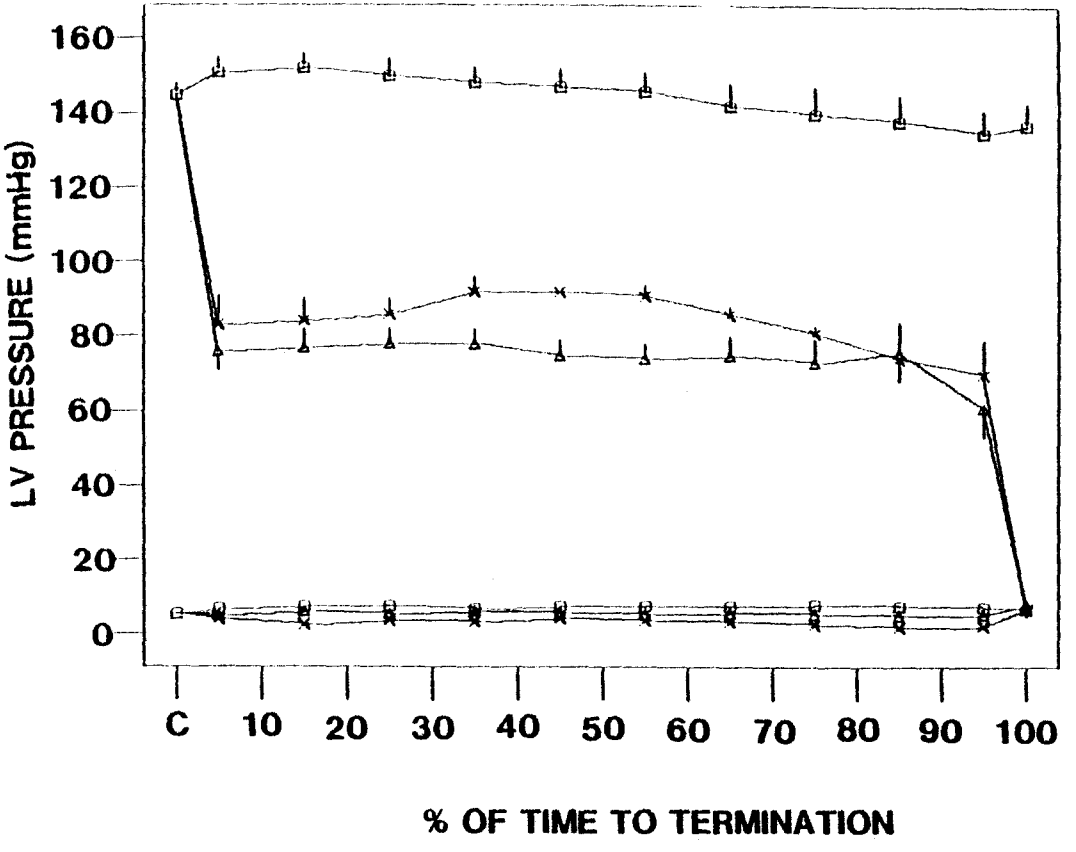

LV PRESSURE (mmHg)
160
140
120
100
80
60
40
20
0
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

Fig. 5. Left Ventricular Stroke Work -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=5), Endotoxin w/no hyperglycemia (Δ, N=10). ☆ $P<0.05$ compared to control; ★ $P<0.05$ compared to control and endotoxin w/hyperglycemia; ◇ $P<0.05$ compared to control, $P<0.1$ compared endotoxin w/hyperglycemia.

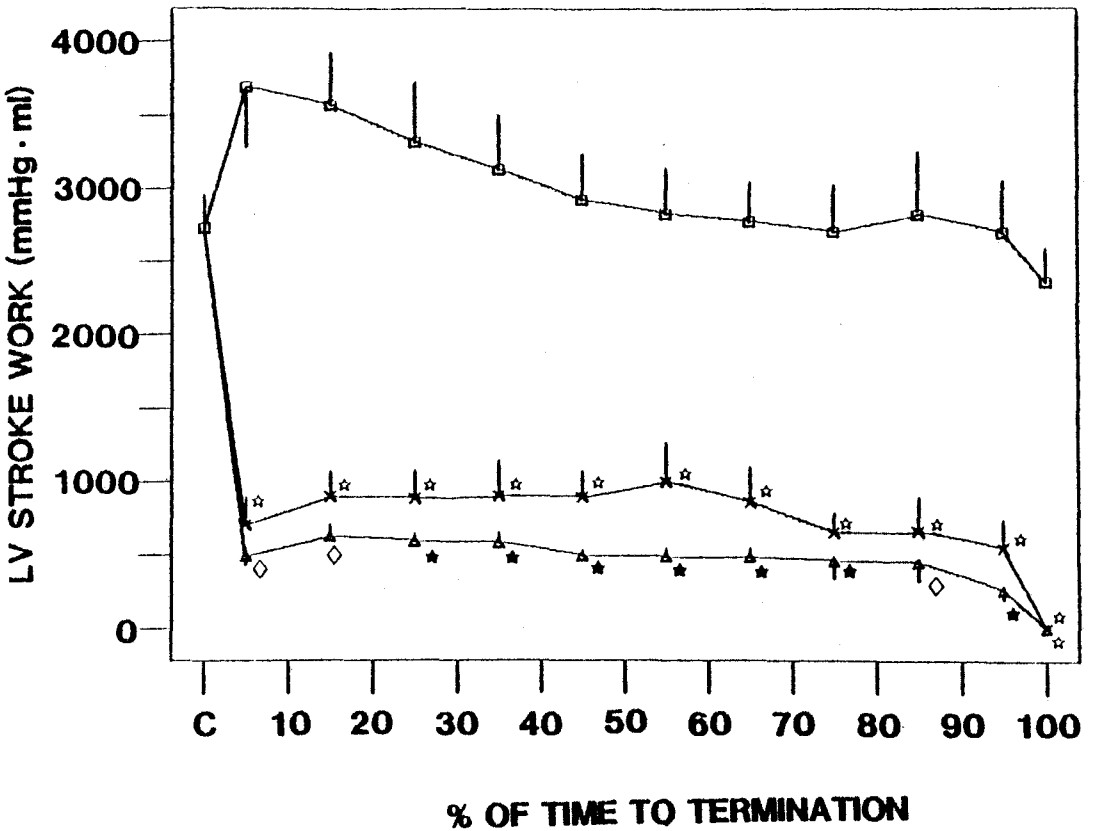

LV STROKE WORK (mmHg · ml)
4000
3000
2000
1000
0
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

Fig. 6. Mean Systemic Arterial Pressure -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=6), Endotoxin w/no hyperglycemia (Δ, N=10). ☆ $P<0.05$ compared to control; ★ $P<0.05$ compared to control and endotoxin w/hyperglycemia; ◇ $P<0.05$ compared to control, $P<0.1$ compared endotoxin w/hyperglycemia.

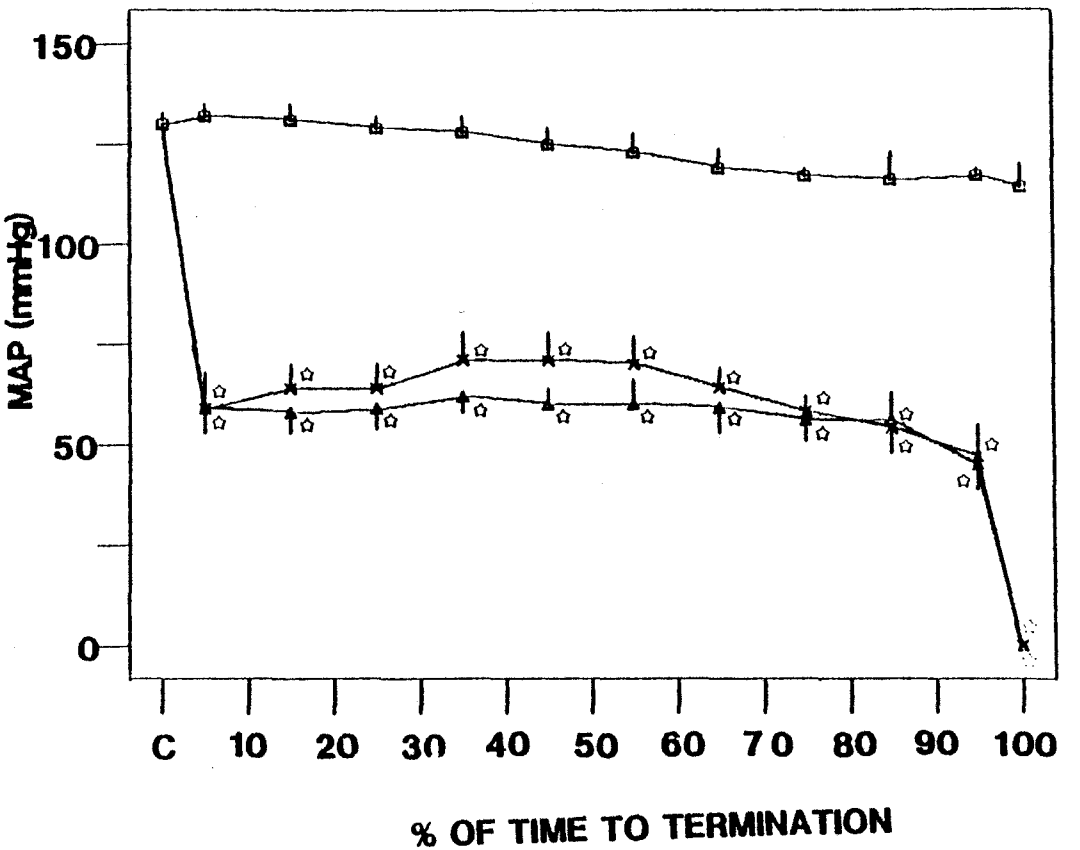

150
100
50
0
MAP (mmHg)
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

figure 7. Blood pressure fell to a similar extent in both groups of endotoxic dogs. As would be expected from the similar blood pressure but lower cardiac output seen in dogs which did not show an initial hyperglycemia, total peripheral resistance became progressively more elevated in this group than in either control dogs or endotoxic dogs exhibiting the hyperglycemic phase. These differences were particularly striking late during the course of endotoxin shock. A remarkably similar pattern was seen in the pulmonary circulation as shown in figures 8 and 9.

Glucose Clamping Experiments

A total of 25 dogs were included in these experiments: 5 hypoglycemic-clamped saline-injected dogs, 6 hypoglycemic-clamped endotoxin-injected dogs, 3 normoglycemic-clamped saline-injected dogs, 4 normoglycemic-clamped endotoxin-injected dogs, 3 hyperglycemic-clamped saline-injected dogs, and 4 hyperglycemic-clamped endotoxin-injected animals. In all of the figures the hypoglycemic clamp data is on the bottom, normoglycemic clamp in the middle, and hyperglycemic clamp data on the top. Dark bars represent data from endotoxin-injected dogs. Preinjection data includes all dogs in a particular clamp group. (11 hypoglycemic-clamped dogs, 7 normoglycemic-clamped dogs, and 7 hyperglycemic-clamped dogs.)

In the hypoglycemic clamp experiments the average

Fig. 7. Total Peripheral Resistance -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=5), Endotoxin w/no hyperglycemia (Δ, N=10). ☆ $P<0.05$ compared to control; ★ $P<0.05$ compared to control and endotoxin w/hyperglycemia; ◇ $P<0.05$ compared to control, $P<0.1$ compared endotoxin w/hyperglycemia.

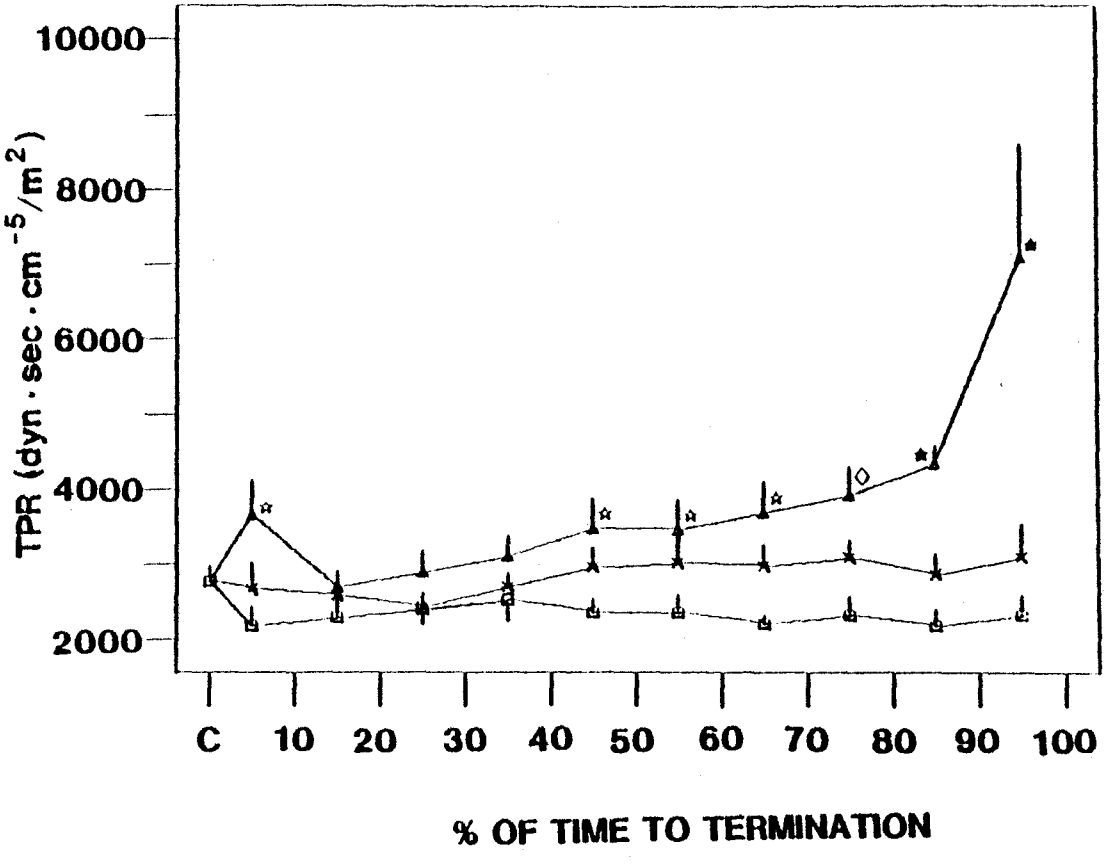

10000
8000
6000
4000
2000
TPR (dyn · sec · cm^{-5}/m^2)
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

Fig. 8. Mean Pulmonary Artery Pressure -- Unclamped Experiments: Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=4), Endotoxin w/no hyperglycemia (Δ, N=8). ☆ P<0.05 compared to control; ★ P<0.05 compared to control and endotoxin w/hyperglycemia; ◇ P<0.05 compared to control, P<0.1 compared endotoxin w/hyperglycemia.

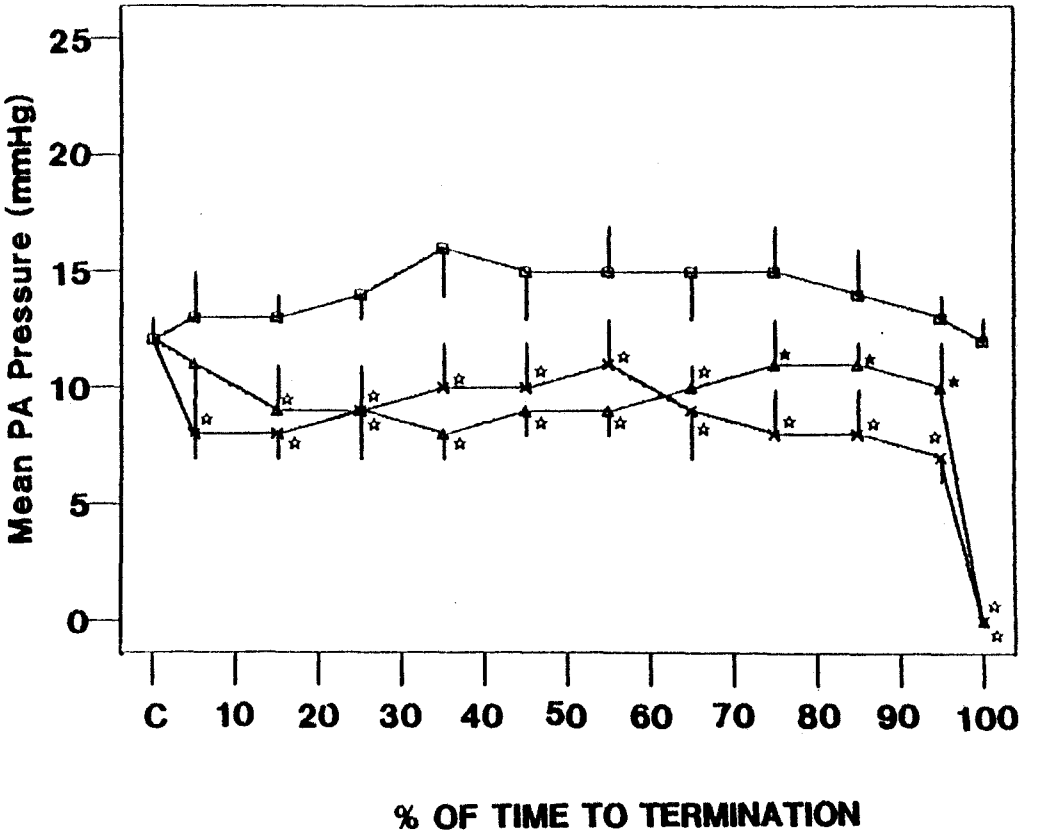
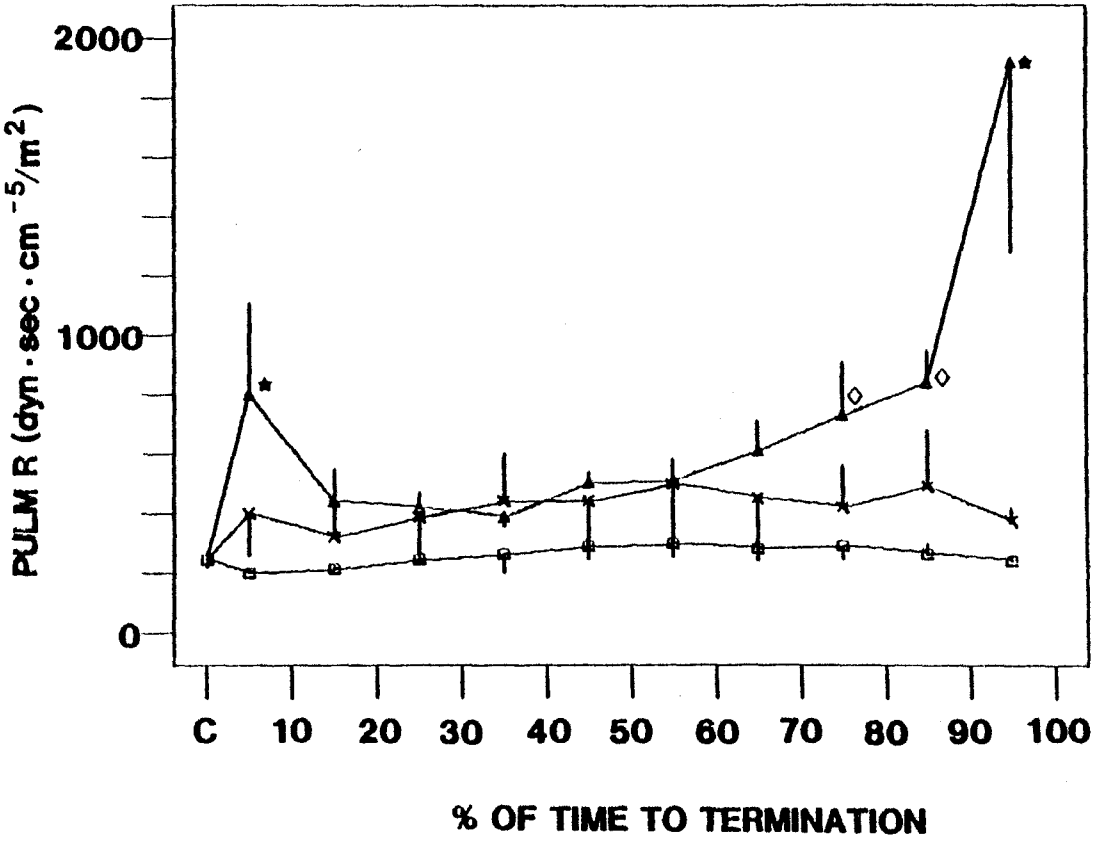

Mean PA Pressure (mmHg)
25
20
15
10
5
0
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

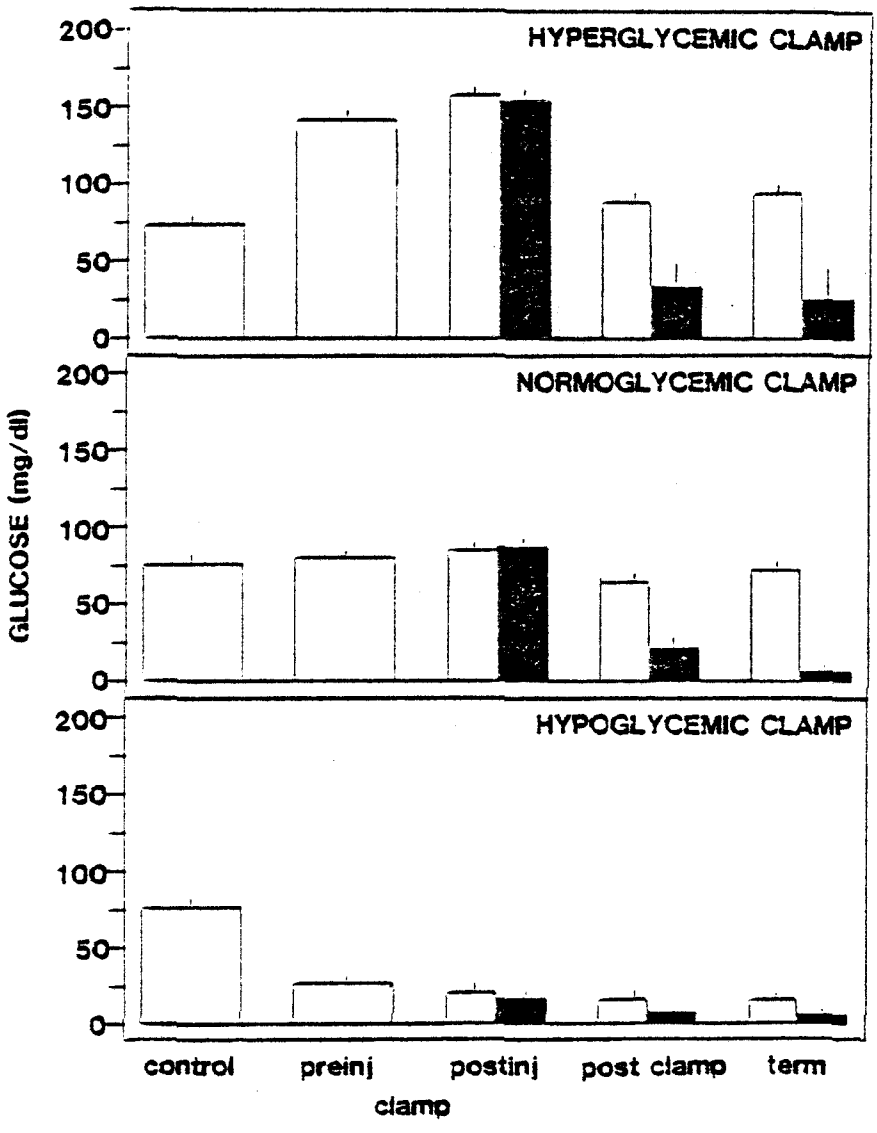
Fig. 9 Pulmonary Resistance -- Unclamped Experiments Saline (□ , N=5), Endotoxin w/hyperglycemia (X, N=4), Endotoxin w/no hyperglycemia (Δ, N=8), ☆ $P<0.05$ compared to control: ★ $P<0.05$ compared to control and endotoxin w/hyperglycemia; ◇ $P<0.05$ compared to control, $P<0.1$ compared endotoxin w/hyperglycemia.


2000
1000
0
PULM R (dyn · sec · cm $^{-5}$/m^2)
C
10
20
30
40
50
60
70
80
90
100
% OF TIME TO TERMINATION

time of survival for endotoxic dogs was 140±4 min following endotoxin administration. There were also 2 deaths in the hypoglycemic-clamped saline-injected group at 195 and 254 min. All normoglycemic-clamped saline-injected animals were alive at 480 min post-injection while the mean survival time in endotoxic normoglycemic-clamped dogs was 323±32 min. All hyperglycemic-clamped saline-injected dogs were also alive at the termination of the experiment (600 min) while there was one death in the endotoxic hyperglycemic-clamped group prior to 600 min (249 min post endotoxin). Thus, while hypoglycemia significantly shortened survival ($P<0.0001$) when compared to unclamped endotoxic animals (318±41 min), hyperglycemia markedly prolonged survival ($P=0.01$) and normoglycemia did not affect survival time ($P=0.48$).

Figure 10 shows the glucose levels in the three types of clamps (see also fig. 24, Appendix A). There were no significant differences in the glucose levels during the preclamp control period. All three groups were different during the preinjection clamp period ($P<<0.001$). The respective saline- and endotoxin-injected groups for each of the three clamps were maintained at similar levels during the postinjection clamp period ($P\geq0.2$) and the three clamp levels were all significantly different from each other ($P<<0.001$). Following the clamp the normoglycemic-clamped endotoxin-injected dogs dropped to blood glucose levels similar to the hypoglycemic-clamped dogs while three out

Fig. 10. Glucose -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemia (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparison of groups.


HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
GLUCOSE (mg/dl)
200
150
100
50
0
control
preinj
postinj
post clamp
term
clamp

of the four hyperglycemic-clamped endotoxic dogs also became hypoglycemic (one dog remaining normoglycemic). These trends continued to the end of the experiments. Normoglycemic-clamped saline-injected dogs fell to glucose levels slightly below control levels and then returned to control levels prior to the termination of the experiment. Hyperglycemic-clamped saline-injected dogs fell back to control levels or slightly higher and remained there until the end of the experiment.

Problems with the insulin assay prevent the reporting of reliable insulin data for the glucose clamping groups. Fasting control levels ranged from 5 to 1410 μU/ml (mean ±SD: 253.84±306.97 for 106 samples). This contrasts with control samples obtained during the unclamped experiments which averaged 27.27±21.97 for 261 control samples. A dog serum reference sample measured concurrently with the samples from the glucose clamping experiments ranged from 215 to 427 in 10 different assays giving a coefficient of variation of 24.6% in contrast to the Pharmacia interassay variation of 5-10% in human control sera as reported in their brochure. Assay-to-assay coefficients of variation ranging from 11.8% to 70% were also obtained on fasting control samples from the glucose clamping experiments when several repeat measurements were made. In two out of the three hyperglycemic clamp control dogs measured insulin levels remained elevated even 8 hr following cessation of glucose

infusion (100-600 μU/ml). Furthermore, despite the maintenance of normal glucose levels in normoglycemic-clamped saline-injected animals up to 6 hr after glucose and insulin infusions were stopped, levels were measured in the 1000's of μU/ml. Dilution of the dog reference serum sample in the manner of a standard curve resulted in a reasonable standard curve; therefore, it seems likely that the interfering factor is specific for insulin antibodies. The source of this interference is not completely known.

Glucose and insulin infusion rates for these experiments are shown in Table IV. There were no differences in glucose infusion rates between corresponding saline and endotoxin groups but the glucose infusion rates increased significantly with the clamp level. This lack of statistically significant differences between saline and endotoxic dogs, however, is somewhat misleading since only one out of five saline-injected dogs in this clamp group required any glucose to maintain the clamp level. In contrast all six of the endotoxic dogs in this group required varying amounts of glucose. While these infusion rates have not been corrected for errors from the "desired" level, lacking information as to the glucose space in the endotoxic dog such correction was not possible. In practice it became necessary to vary the insulin infusion in the normoglycemic clamp as well as the glucose infusion. The insulin infusion rates were significantly higher in the

Table IV Infusion Rates -- Glucose Clamping Experiments

	Glucose (mg/Kg/min)	Insulin (U/Kg/min)
Hyperglycemic Clamp		
Saline (3)[1]	6.72±1.38[2]	-----
Endotoxin (4)[1]	8.68±0.98[2]	-----
Normoglycemic Clamp		
Saline (3)[1]	4.72±1.30[3]	0.27±0.07
Endotoxin (4)[1]	4.11±0.53[3]	0.66±0.09[5]
Hypoglycemic Clamp		
Saline (5)[1]	0.23±0.23[4]	4.51±0.43
Endotoxin (6)[1]	0.99±0.33[4]	3.49 0.88

1: (N)

2, 3, 4: significant differences between clamps, $P<0.05$; no significant differences between endotoxin and corresponding saline group

5: significantly different from corresponding saline group, $P<0.05$

endotoxic normoglycemic-clamped dogs than in the corresponding controls. There were, however, no differences in the insulin infusion rates between the two groups of hypoglycemic-clamped dogs.

The arterial blood gas and pH data and the plasma lactate levels in the clamp groups are presented in Tables V through VIII. There was some tendency for the hypoglycemic-clamped groups to become hypoxic, although this was not significant. These groups also became significantly hypercapnic during the course of the experiment. No other consistent differences in arterial PO_2 and PCO_2 were found. All three endotoxic groups as well as hypoglycemic-clamped saline-injected dogs became acidotic -- hypoglycemic clamped endotoxin dogs to the greatest degree and the other three groups to about the same level. The plasma lactate levels are consistent with these results suggesting that the acidosis in all four groups was at least in part due to the lactacidemia.

Cardiac output is plotted in figure 11. There were no differences during the control or preinjection clamp periods. Following the injection of endotoxin all endotoxic dogs fell to similar levels while all three saline-injected groups remained at control levels. Following the clamp there was a further deterioration in the cardiac output in hypoglycemic-clamped endotoxin-injected dogs while all other groups essentially remained at the same

Table V Arterial pH -- Glucose Clamping Experiments

	pH			
	Control[1]	Preinj[1] Clamp	Postinj Clamp	Post Clamp
Hyperglycemic Clamp				
Saline (3)[2]	7.39±0.01	7.41±0.02	7.40±0.02	7.36±0.02
Endotoxin (4)[2]			7.22±0.03[3]	7.23±0.06[3]
Normoglycemic Clamp				
Saline (3)[2]	7.36±0.03	7.37±0.03	7.35±0.07	7.37±0.06
Endotoxin (4)[2]			7.19±0.05[3]	7.16±0.04[3]
Hypoglycemic Clamp				
Saline (5)[2]	7.34±0.02	7.31±0.03	7.19±0.05[3]	7.16±0.04[3]
Endotoxin (6)[2]			7.09±0.03[4]	6.99±0.04[4]

1: for control and preinjection clamp -- Hyperglycemic clamp. N=7; Normoglycemic clamp, N=7; Hypoglycemic clamp, N=11

2: (N)

3: significantly different from hyperglycemic and normoglycemic clamp saline, $P<0.05$

4: significantly different from all other groups, $P<0.05$

Table VI Arterial PO_2 -- Glucose Clamping Experiments

	PO_2 (mmHg)			
	Control[1]	Preinj[1] Clamp	Postinj Clamp	Post Clamp
Hyperglycemic Clamp				
Saline (3)[2]	85±3	95±4	95±5[3]	85±4
Endotoxin (4)[2]			73±4	73±8
Normoglycemic Clamp				
Saline (3)[2]	78±4	92±6	83±10	78±9
Endotoxin (4)[2]			82±4	77±4
Hypoglycemic Clamp				
Saline (5)[2]	93±2	96±4	74±4	66±5
Endotoxin (6)[2]			71±4	57±7

1: Hyperglycemic Clamp, N=7; Normoglycemic Clamp, N=7; Hypoglycemic Clamp, N=11

2: (N)

3: not significantly different from Normoglycemic saline (P=0.07), significantly different from all other groups (P<0.05); no other significant differences

Table VII Arterial PCO_2 -- Glucose Clamping Experiments

	PCO_2 (mmHg)			
	Control[1]	Preinj[1] Clamp	Postinj Clamp	Post Clamp
Hyperglycemic Clamp				
Saline (3)[2]	30±2	34±3	29±7	29±6
Endotoxin (4)[2]			35±1	42±6[5]
Normoglycemic Clamp				
Saline (3)[2]	33±3	28±2	33±4	26±2
Endotoxin (4)[2]			29±2	33±4
Hypoglycemic Clamp				
Saline (5)[2]	35±4	32±3	48±6[3]	57±4[3]
Endotoxin (6)[2]			43±4[4]	61±5[3]

1: Hyperglycemic Clamp, N=7; Normoglycemic Clamp, N=7; Hypoglycemic Clamp, N=11

2: (N)

3: significantly different from all other groups, $P<0.05$

4: significantly different from Hyperglycemic saline and Normoglycemic endotoxin, $P<0.02$

5: significantly different from Normoglycemic saline, $P=0.05$

Table VIII Plasma Lactate

Lactate (mmol/l)

		Unclamped	Glucose Clamping Postinj Clamp	Glucose Clamping Post Clamp
Control	(14)[1]	0.93±0.09		
Early Shock	(9)[1]	3.20±0.50[2]		
Late Shock	(9)[1]	4.20±0.70[2]		
Hyperglycemic Clamp				
Saline	(3)[1]		1.30±0.20	0.70±0.20
Endotoxin	(4)[1]		1.50±0.20[3]	2.20±0.10[3]
Normoglycemic Clamp				
Saline	(3)[1]		1.20±0.10	1.40±0.10[5]
Endotoxin	(4)[1]		2.00±0.20[3]	2.50±0.20[3]
Hypoglycemic Clamp				
Saline	(5)[1]		1.30±0.20	1.70±0.30[6]
Endotoxin	(6)[1]		2.90±0.20[4]	3.50±0.30[4]

1: (N)

2: early shock = 0-50% of postinjection time course; late shock = 50-100%; significantly different from control by unpaired t test, $P<0.001$

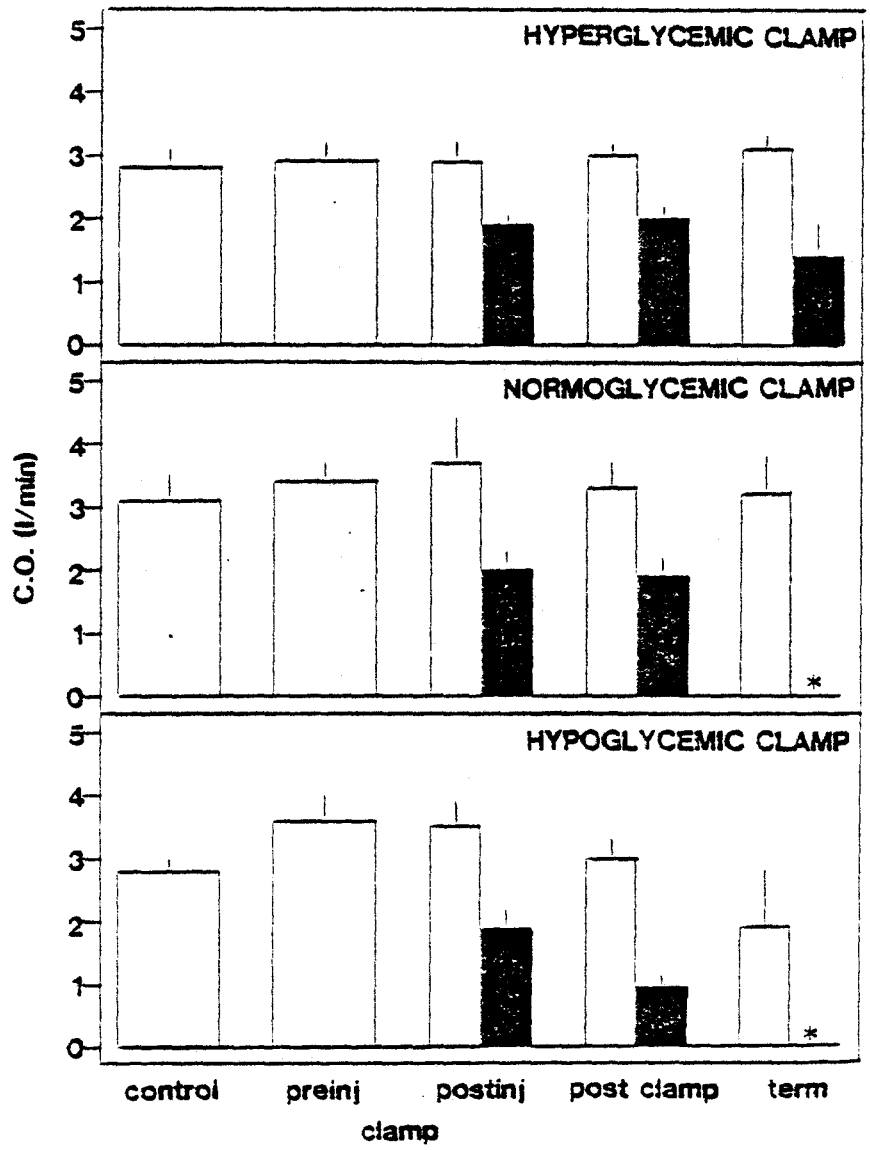
3: significantly different from saline-injected clamp groups and from control, $P<0.05$

4: significantly different from all other groups, $P<0.05$

5: significantly different from all groups except Hypoglycemic saline, $P<0.05$

6: significantly different from all other groups except Hyperglycemic endotoxin, $P<0.05$

Fig. 11. Cardiac Output -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection light bars=saline and dark bars= endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparison of groups. *All animals in these two groups were dead at the end of the experiment.


HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
C.O. (l/min)
0
1
2
3
4
5
*
*
control
preinj
postinj
post clamp
term
clamp

levels as during the clamp. Subsequently all of the hypoglycemic- and normoglycemic-clamped endotoxin-injected dogs died while the hypoglycemic-clamped saline-injected and the hyperglycemic-clamped endotoxin-injected dogs fell to similar levels. The normoglycemic-clamped and hyperglycemic-clamped saline-injected animals remained at control levels throughout the experiment.

Left ventricular peak systolic pressure (figure 12) and left ventricular end-diastolic pressure (figure 13) are shown in the next two figures. During the preinjection clamp period the hypoglycemic-clamped dogs began to fall to lower peak systolic pressures. With the injection of endotoxin left ventricular pressure fell to the same extent in all three endotoxin-injected groups. During the clamp the left ventricular peak systolic pressure continued to fall in the hypoglycemic-clamped saline-injected dogs but not to the same extent as any of the endotoxic groups. Following the clamp period left ventricular pressure continued to fall in the hypoglycemic-clamped endotoxic group but remained about the same in the other two endotoxic groups. The hypoglycemic-clamped saline group continued to decline but was still higher than any of the three endotoxic groups. Prior to the termination of the experiment left ventricular pressure was similar in the hypoglycemic-clamped saline-injected dogs and the hyperglycemic-clamped dogs while

Fig. 12. Left ventricular Peak Systolic Pressure -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11), Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparisons of groups. *All animals in these two groups were dead at the end of the experiment.

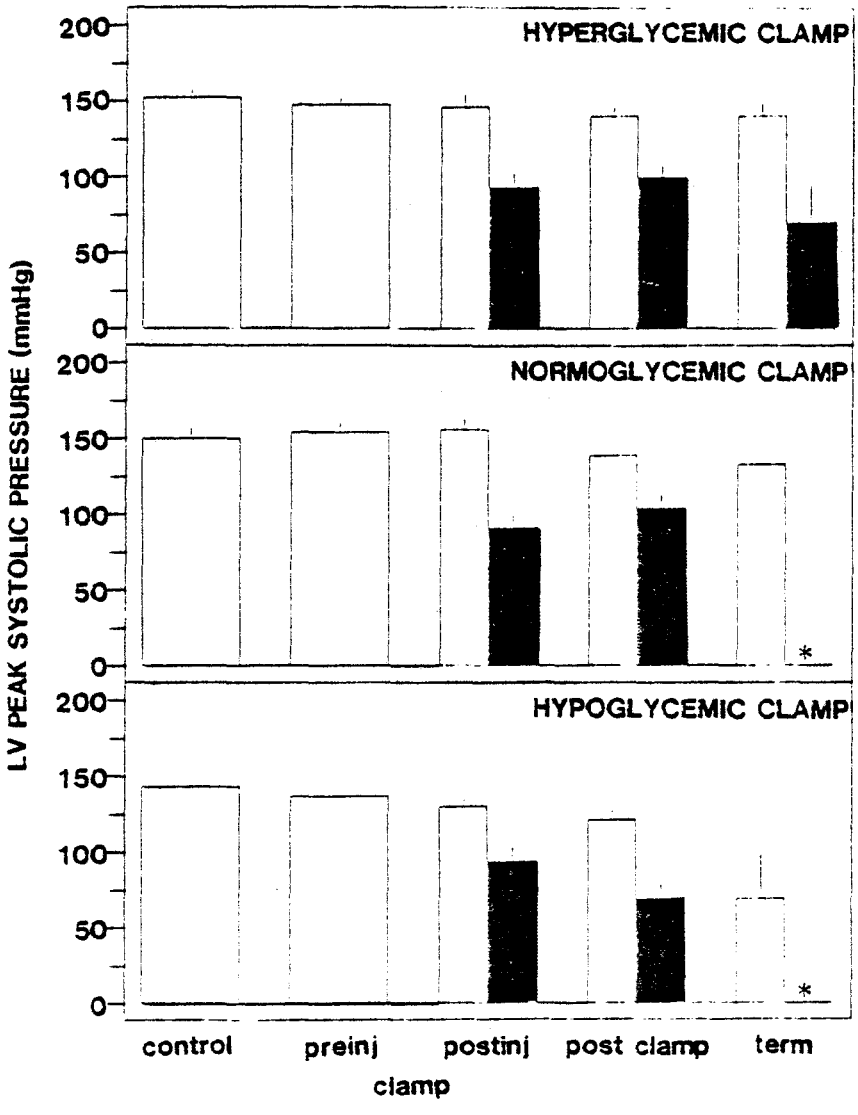
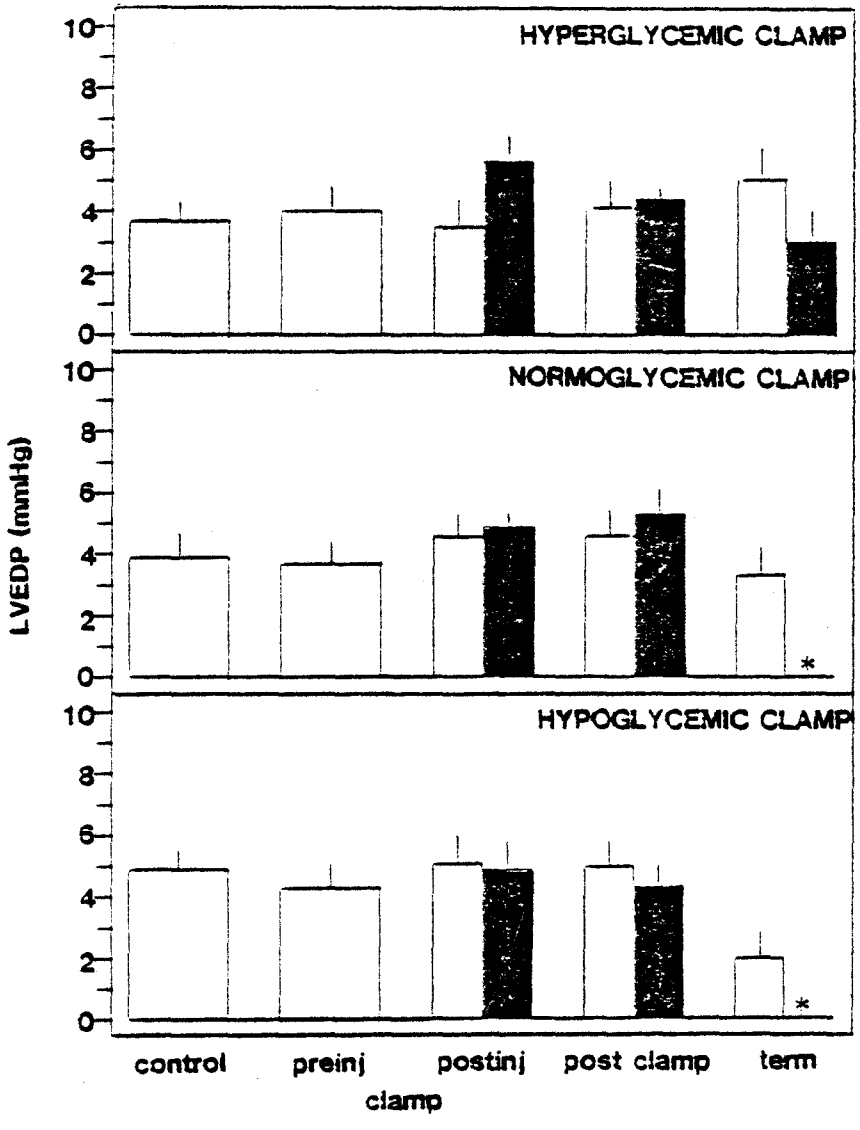

HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
LV PEAK SYSTOLIC PRESSURE (mmHg)
200
150
100
50
0
*
*
control
preinj
postinj
post clamp
term
clamp

Fig. 13. Left Ventricular End-diastolic Pressure -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to the text for comparisons of groups. *All animals in these two groups were dead at the end of the experiment.


HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
LVEDP (mmHg)
10
8
6
4
2
0
*
*
control
preinj
postinj
post clamp
term
clamp

the remaining endotoxic dogs all died. Left ventricular pressure remained fairly steady throughout the experiment in the normoglycemic- and hyperglycemic-clamped saline-injected control dogs. There were no differences in left ventricular end-diastolic pressures until just prior to the termination of the experiment in any of the groups. This is probably due to the quantity of fluid infused during the glucose clamps since end-diastolic pressure fell in unclamped dogs.

A similar pattern of events was also seen for stroke work, shown in figure 14, with the exception that hypoglycemic-clamped saline-injected dogs did not fall to the same extent as hyperglycemic-clamped endotoxic dogs. Otherwise, cardiac work deteriorated most rapidly in hypoglycemic-clamped endotoxin dogs, followed by normoglycemic-clamped and then hyperglycemic-clamped endotoxic dogs. Normoglycemic- and hyperglycemic-clamped saline-injected dogs remained at control levels throughout the experiment.

Figures 15 and 16 show the mean systolic blood pressure and the total peripheral resistance respectively. Systemic arterial pressure followed a similar pattern in all groups as for the cardiac performance parameters. While qualitatively the systemic resistance seems to follow the same pattern in hypoglycemic-clamped endotoxic dogs as for unclamped dogs not exhibiting a hyperglycemic =hase, there were no significant differences among any

Fig. 14. Left Ventricular Stroke Work -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to the text for comparisons of groups. *All animals in these two groups were dead at the end of the experiment.

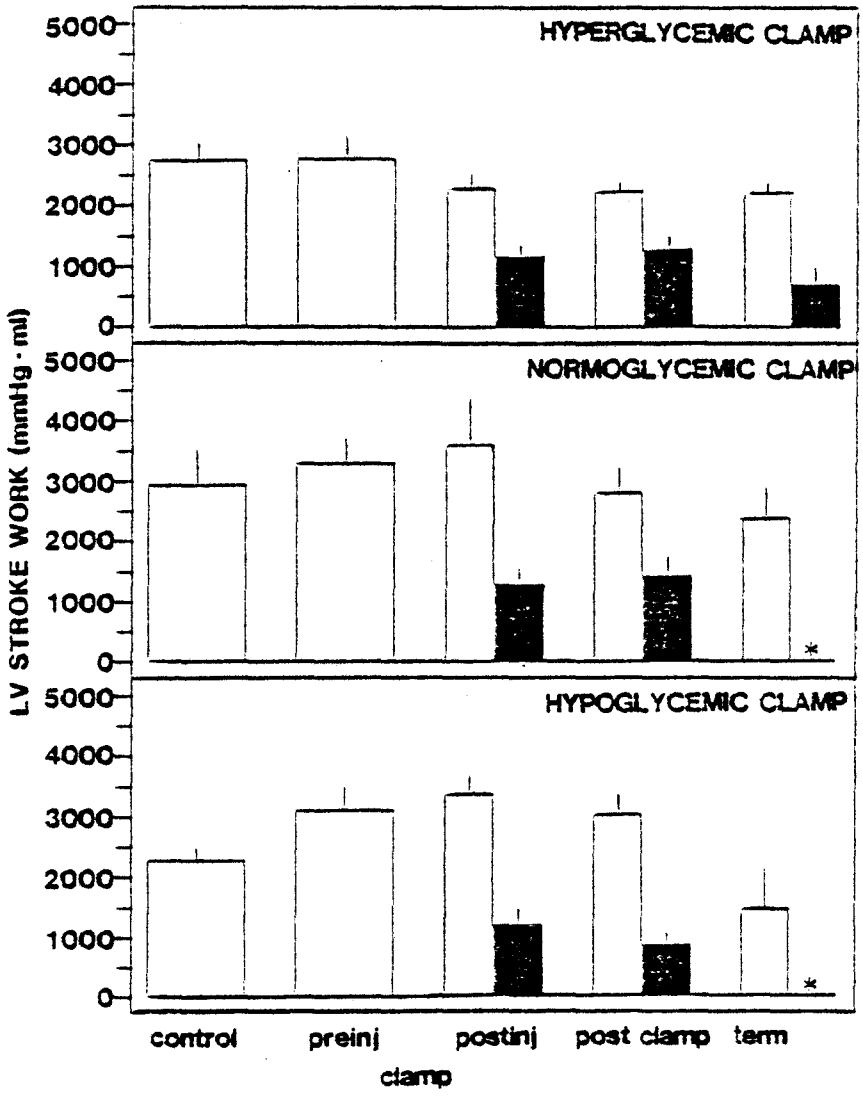

HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
LV STROKE WORK (mmHg · ml)
5000
4000
3000
2000
1000
0
*
*
control
preinj
postinj
post clamp
term
clamp

Fig. 15. Mean Systemic Arterial Pressure -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11), Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparisons of groups. *All animals in these two groups were dead at the end of the experiment.

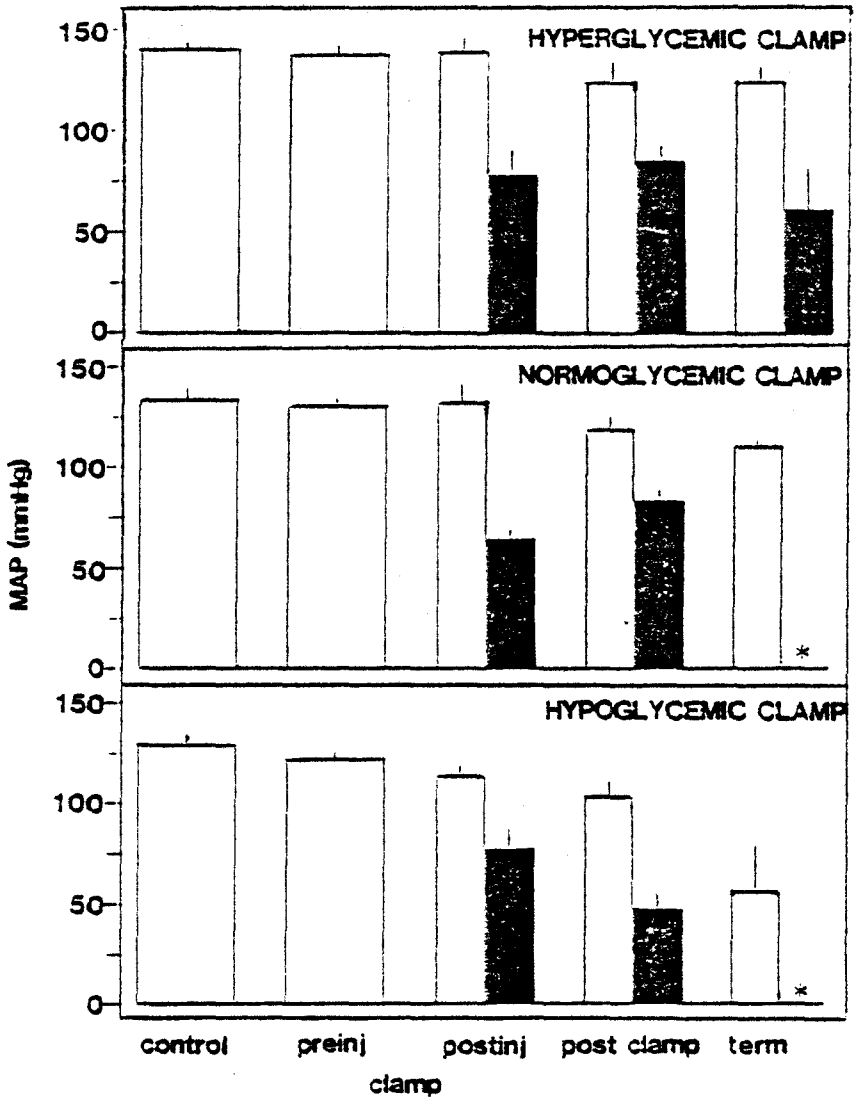
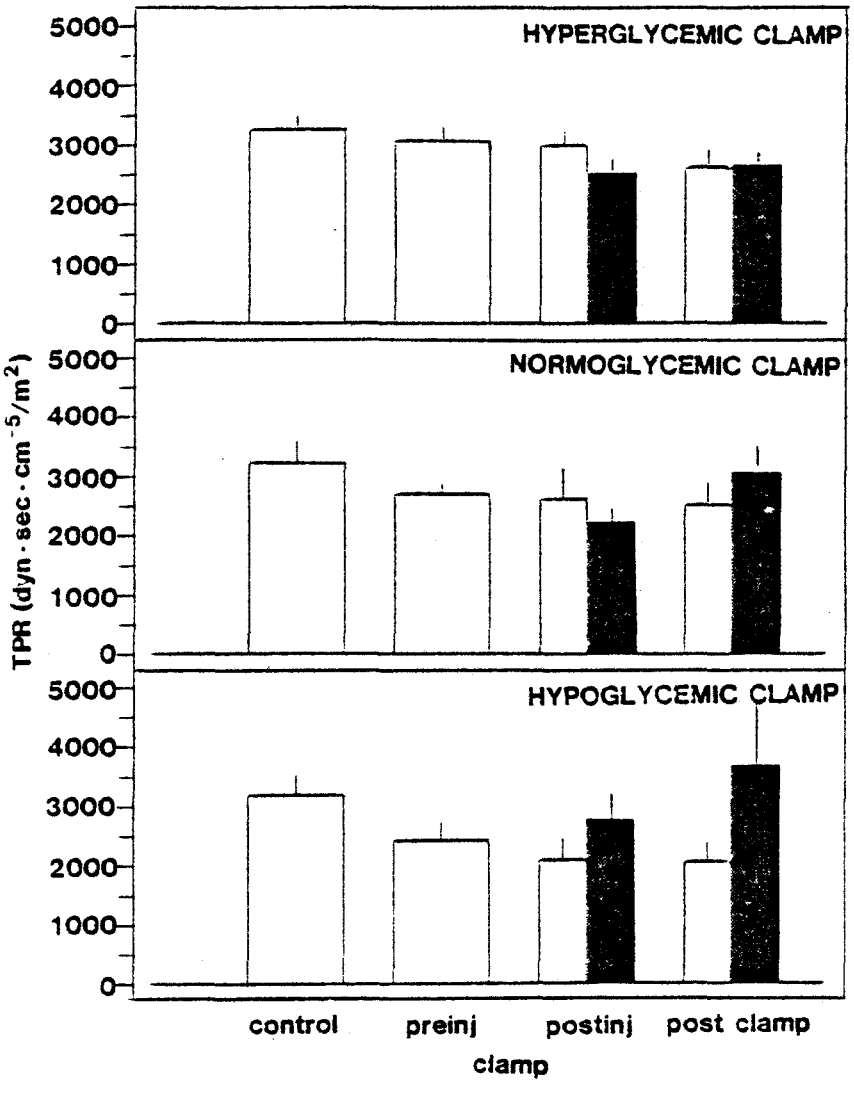

HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
MAP (mmHg)
150
100
50
0
*
*
control
preinj
postinj
post clamp
term
clamp

Fig. 16. Total Peripheral Resistance -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparisons of groups.


HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
5000
4000
3000
2000
1000
0
TPR (dyn · sec · cm⁻⁵/m²)
control
preinj
postinj
post clamp
clamp

of the groups at any of the time points. This is probably due to the high variability of the calculated resistance in general and the highly variable response of the other two endotoxic groups.

Figures 17 and 18 show the mean pulmonary artery pressures and the pulmonary resistance. There were no significant differences in mean pulmonary resistance. There were no significant differences in mean pulmonary artery pressure in any of the groups through the glucose clamping period. Following the clamp, however, pressure started to fall in all three endotoxic groups and the hypoglycemic-clamped saline-injected group. The pattern was similar at the termination of the experiment to that seen for all of the other cardiovascular parameters. Pulmonary artery resistance was significantly higher than any of the other groups during the postclamp period in hypoglycemic-clamped endotoxic dogs. Thus, pulmonary resistance in these animals followed a pattern reminiscent of the unclamped endotoxic dogs showing early hyperglycemia.

Summary of Results

To summarize the results: Two patterns of glucose changes were seen in unclamped endotoxic dogs. One group of dogs showed initial hyperglycemia while the other did not. Both patterns were associated with hyperinsulinemia.

Fig. 17. Mean Pulmonary Artery Pressure -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to text for comparisons of groups. *All animals in these two groups were dead at the end of the experiment.

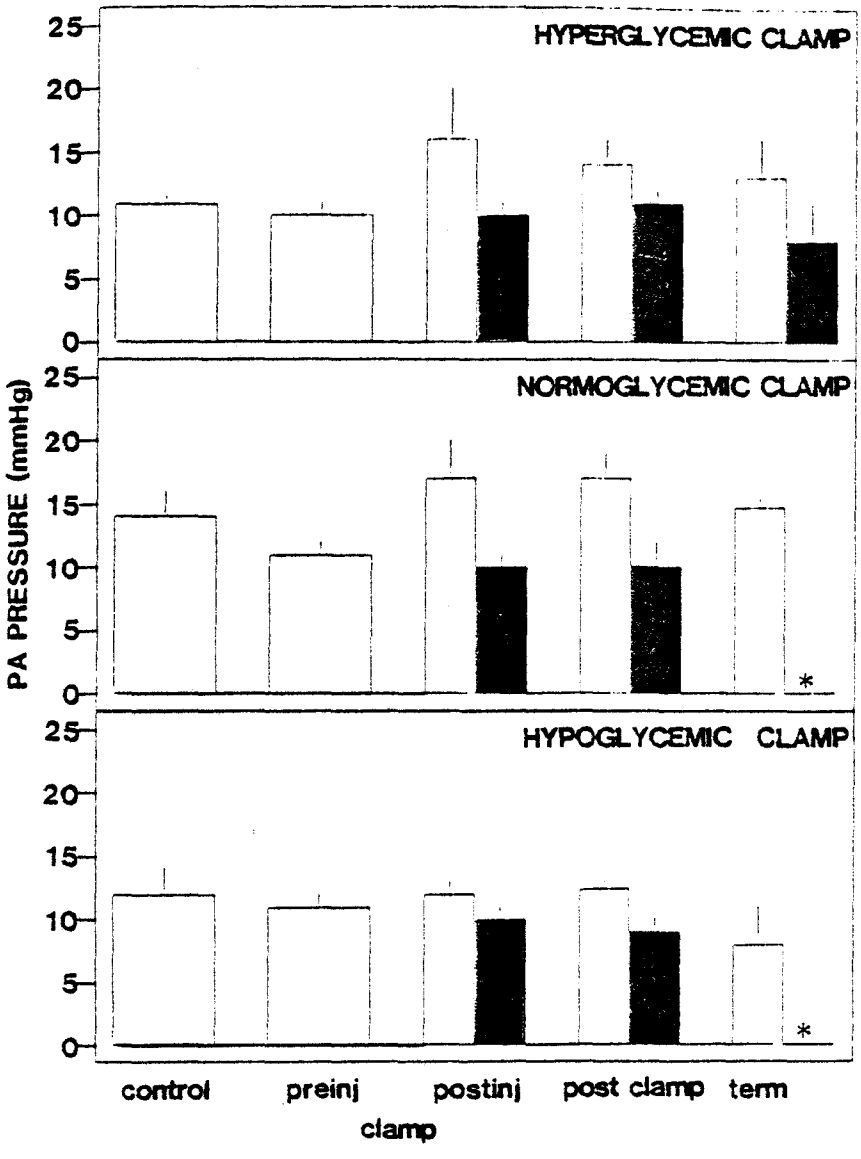
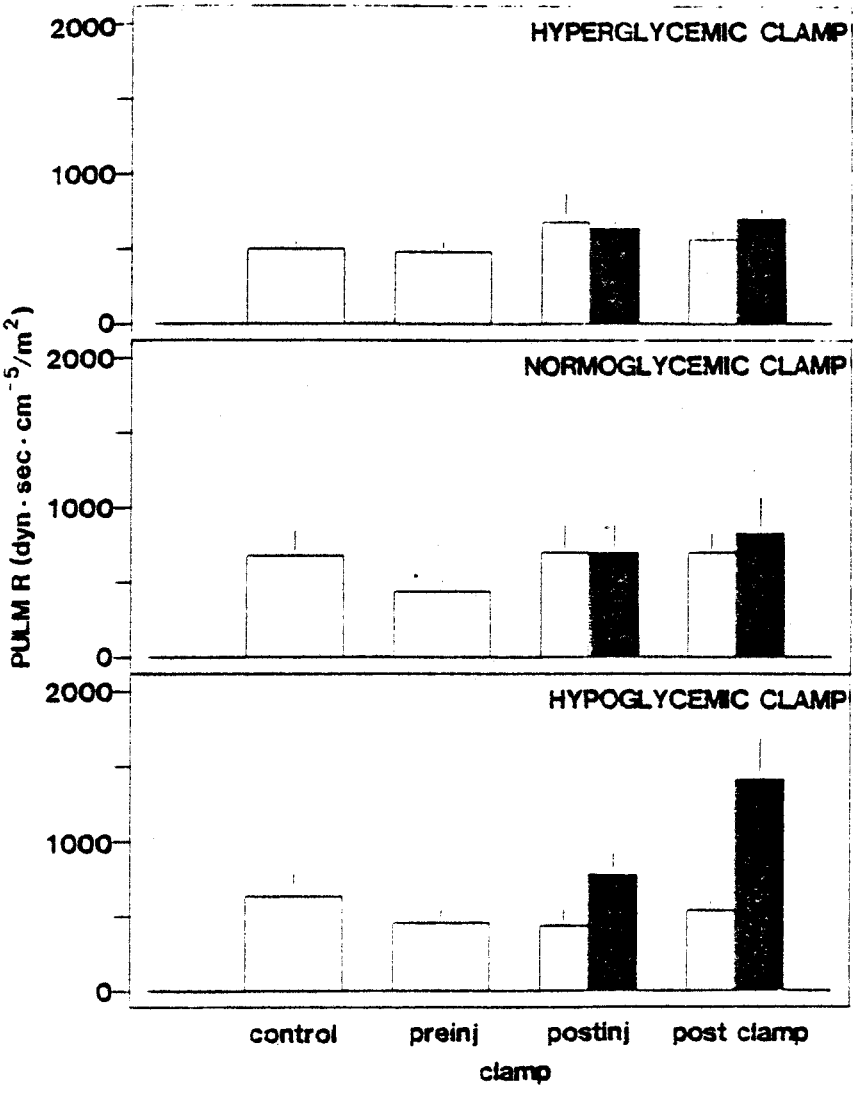

HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
PA PRESSURE (mmHg)
25
20
15
10
5
0
*
control
preinj
postinj
post clamp
term
clamp

Fig. 18. Pulmonary Resistance -- Glucose Clamping Experiments: Control and Preinjection Clamp -- Hyperglycemic (N=7), Normoglycemic (N=7), Hypoglycemic (N=11). Postinjection -- light bars=saline and dark bars=endotoxin. Hyperglycemic saline (N=3), Hyperglycemic endotoxin (N=4), Normoglycemic saline (N=3), Normoglycemic endotoxin (N=4), Hypoglycemic saline (N=5), Hypoglycemic endotoxin (N=6). Refer to the text for comparisons of groups.


HYPERGLYCEMIC CLAMP
NORMOGLYCEMIC CLAMP
HYPOGLYCEMIC CLAMP
2000
1000
0
PULM R (dyn · sec · cm⁻⁵/m²)
control
preinj
postinj
post clamp
clamp

Cardiac output and stroke work tended to be better in dogs exhibiting early hyperglycemia. While left ventricular pressure was similar in both groups of endotoxic dogs, animals showing the initial hyperglycemic phase had lower end-diastolic pressures. Mean systemic and pulmonary arterial pressures were also similar. This is due to higher resistance in both systemic and pulmonary circulations in dogs not experiencing the initial hyperglycemia. There were no consistent changes in arterial blood gases in either group of endotoxic dogs. Both groups of endotoxic dogs also developed lactacidemia leading to metabolic acidosis to about the same degree.

In glucose clamping experiments all endotoxic dogs had similar cardiovascular performance during the clamp. However, after terminating the clamp hypoglycemic-clamped endotoxic dogs rapidly deteriorated and succumbed to shock while hyperglycemic-clamped dogs were protected and normoglycemic clamped dogs followed a normal time course. The cardiovascular patterns reflect these differences in the ability to survive and hypoglycemic-clamped endotoxic dogs followed a pattern reminiscent of unclamped dogs which did not go through an early hyperglycemic phase. Cardiovascular performance was compromised in hypoglycemic-clamped dogs given saline to about the same extent as in hyperglycemic-clamped endotoxic dogs. All endotoxic dogs became acidotic

and lactacidemic -- hypoglycemic-clamped endotoxic dogs to the greatest extent. Hypoglycemic clamped dogs given saline also became acidotic. Both groups of hypoglycemic-clamped dogs also tended to become hypoxic and hypercapnic.

CHAPTER VI

DISCUSSION

Metabolic Effects of Endotoxin in the Canine Model

These results, using continuous glucose monitoring, conclusively demonstrate two patterns of glucose changes in endotoxic dogs: some dogs exhibit an initial hyperglycemic phase before becoming hypoglycemic, while other dogs proceed directly into the hypoglycemic phase. This confirms the earlier work of Berk et al. (9) and Hinshaw and colleagues (100) using discontinuous sampling of blood glucose. The mechanism behind these two patterns is unclear but may involve differences in glycogen reserves following an overnight fast, differences in nutritional status, or differences in the hormonal milieu. Further study is needed to determine the exact mechanism.

Hyperinsulinemia was also found to occur in the endotoxic dog analogous to the hyperinsulinemia which has already been demonstrated in the endotoxic rat by Filkins and colleagues (23, 220). At no time period did insulin fall below control in either group of endotoxic dogs. This result is in contradiction to several reports in the literature on endotoxin shock in the dog which either show depressed levels throughout or only early elevations in

association with a hyperglycemic phase (142, 199). There are several explanations for this discrepancy. First, in previous studies measurements were made at only a few time points. Since considerable fluctuation was seen in the present experiments in which measurements were made every 15 minutes (see Appendix A), it is possible that similar elevations of insulin levels were simply missed in earlier studies. Differences in the insulin response based on differences in lethality or endotoxin dose are also possible. Finally, the results of the present experiments cannot be taken as conclusive evidence of hyperinsulinemia in the dog since during glucose clamping experiments the possible presence of factors in dog serum which lead to spuriously high measurements in the radioimmunoassay for insulin was discovered. The nature of this interference is unknown. Since dilution of dog reference serum samples gave a reasonable standard curve, it is likely that the interfering factor is specific for insulin antibodies rather than interfering through non-specific binding. This is not an isolated problem since others have encountered the same problem of spuriously high insulin levels in dog serum (personal communication with N. Manson) and is worthy of further study since it may be the basis for the widely varying results obtained in studies of insulin levels in the endotoxic dog (14, 142, 199). The question of the role of pancreatic insulin in the development of hypoglycemia in the endotoxic

dog, thus, remains unanswered.

During glucose clamping no effect of endotoxin on glucose infusion rates was observed. This result is contrary to numerous other observations that glucose utilization is increased in endotoxic animals (5, 53, 56, 57, 88, 152, 173, 218). There are several explanations for this discrepancy. First, endogenous sources of glucose may be present which add to the glucose supplied exogenously by the infusion. Thus, the total glucose utilized was not accounted for by the infusion. Second, lacking specific information as to the glucose space in the endotoxic dog it is impossible to correct the glucose infusion rates to the actual glucose utilized. Since the glucose space may change following the administration of endotoxin and since different infusion rates might be due to wider variations of the actual glucose level from the desired level rather than differences in utilization, these corrections might be very important in the endotoxic dog model. Furthermore, since it became necessary to vary the insulin infusion rate as well as the glucose infusion rate to lower the glucose level in the normoglycemic-clamped dogs and lacking insulin data for the glucose clamping experiments, it is difficult to interpret the results in terms of tissue insulin sensitivity. In addition, in some animals it was necessary to give additional bolus injections of insulin or glucose in all three of the clamps in order to maintain the glucose level -- thus, adding

another complexity to the analysis and interpretation. While normoglycemic-clamped endotoxin dogs had higher insulin infusion rates but similar glucose infusion rates when compared to normoglycemic-clamped saline-injected dogs, possibly indicating insulin resistance, this conclusion is not supported by the results of the other two glucose clamps. There was a much greater increase in glucose utilization with hyperglycemia in endotoxin dogs than in saline-injected dogs (112% versus 43%). Furthermore, while there were no statistically significant differences in glucose infusion rates in hypoglycemic-clamped dogs, the statistics in this case are undoubtedly misleading since only one out of five dogs in this group given saline required any glucose to maintain the clamp level. In contrast, all six endotoxic, hypoglycemic-clamped dogs required varying amounts of glucose. Both of these results suggest insulin hypersensitivity in the endotoxic dog. Further refinements of the glucose clamping technique are clearly needed before these techniques can be used in the metabolic analysis of the endotoxic dog. In particular, it is necessary to anticipate the changes in blood glucose levels to a greater extent in order to minimize the deviations from the desired level as DeFronzo and his colleagues have done (38). While the manual control technique used in this study was useful in attaining the initial goal of developing a glucose clamping model in the endotoxic dog, there are certain

drawbacks to this technique. Pacini, Finegood, and Bergman (164) have recently pointed out that such human interaction entails a degree of bias and is also tedious. Because of these problems it is desirable to develop automatic means of feedback control of blood glucose for glucose clamping experiments. Thus, better results might be obtained if a more mathematical basis for the infusion was used rather than the trial-and-error method used in the present experiments. If the trial-and-error method, however, did not provide satisfactory control of glucose levels, even on a crude level, sophisticated mathematical modelling probably would not have helped. Fairly good control was obtained using the trial-and-error method (see Appendix A); this can, however, be improved upon with further refinements of the glucose clamping technique. These experiments thus provide a good foundation for the development of glucose clamping techniques in the endotoxic dog model.

The Role of Hypoglycemia in the Development of Cardiovascular Failure in the Endotoxic Dog

Evidence from the Unclamped Studies

Associated with the two patterns of glucose changes in the unclamped experiments were differences in the pattern of circulatory failure. Dogs which did not show the early

hyperglycemia had poorer cardiac output and stroke work than dogs which did go through an early hyperglycemic phase. The finding of similar left ventricular pressures in the group which did not show hyperglycemia but at higher end-diastolic pressures is also consistent with the poorer cardiac function in this group. Since venous return and effective blood volume also fall during endotoxin shock (31, 101), endotoxic cardiac failure is complicated by changes in preload. This may explain the fall in end-diastolic pressure in both groups of endotoxic dogs. However, the lack of any differences in peak systolic pressure -- especially in light of a tendency toward greater afterload as assessed by total peripheral resistance in dogs with no hyperglycemic phase -- is harder to explain. Partly this may be the problem of finding statistically significant differences between experimental groups using an analysis of variance when there are relatively large differences between the control group and the experimental groups but only small differences between the experimental groups. Thus, the variance between the control groups and the experimental groups is large but between the experimental groups the variance is small and larger differences are needed to find a statistical significance. The high degree of lethality (100%) in both groups of endotoxic dogs also would tend to blur any real distinction in the cardiovascular performance of the two groups. In spite of

the poorer cardiac performance in dogs not showing early hyperglycemia, these dogs were able to maintain similar mean systemic arterial pressures at the tremendous expense of progressive increases in peripheral resistance leading to further tissue ischemia. Further experiments using a less potent model of endotoxin shock are needed to sort out the effects of the pattern of glucose dyshomeostasis on cardiovascular function and the importance of the ability of the animal to maintain blood glucose to survival and the maintenance of normal circulatory function.

Evidence from Glucose Clamping Studies

Hypoglycemia markedly curtailed survival while hyperglycemia even for only the initial two hours after endotoxin injection markedly prolonged survival. In contrast, maintenance of normoglycemia over the first two hours of endotoxicosis left the time course of endotoxin shock unaltered. Cardiovascular status paralleled these alterations in survival. In addition, hypoglycemia tended to depress cardiovascular function even without concomitant endotoxemia.

These effects of glucose clamping could be attributed to mechanisms other than control of blood glucose concentrations. First fluids given during the clamp may have provided some support to the cardiovascular system during shock. Volume overload, on the other hand, may be detrimental to an animal already in cardiac failure. The

lack of any differences in left ventricular end-diastolic pressure between any of the groups except preterminally makes it unlikely, however, that this is a factor. Second, insulin probably has its own effects on cardiovascular function (82, 117, 125, 161, 208, 211). Both Hiatt and coworkers (125, 161) and Downing and associates (82, 208) have shown that while insulin has a positive inotropic effect of its own, insulin also blocks the positive chronotropic and inotropic actions of catecholamines. Thus insulin or an insulin-like activity may in addition to causing the hypoglycemia of endotoxin shock, block the supportive effects of epinephrine and norepinephrine on the the heart. Furthermore, insulin and possibly insulin-like agents are known to have a number of effects on ionic fluxes -- especially potassium flux (117). Alterations in ion fluxes -- especially in the face of hypoglycemia -- may further disturb cardiac electrophysiology. It is possible that hyperinsulinism -- whether due to increased secretion of insulin by the pancreas or to insulin-like activities -- may further impair cardiac performance. Since most reports, however, ascribe positive inotropic and beneficial effects of insulin infusions under a variety of conditions (117, 211), it seems likely that the elevated insulin levels -- either because of an insulin infusion or in response to a glucose load -- in all three endotoxic clamp groups are beneficial and explain the similar levels

of cardiovascular variables during the clamp. It seems likely that insulin as well as the volume of infusions is supporting the cardiovascular system of endotoxic animals during these clamps. Following the cessation of all infusions the prevailing glucose level becomes the overriding factor in maintaining circulatory function and tissue perfusion. It is possible that the hyperinsulinemia which occurs following endotoxin administration might be a compensatory mechanism which together with hyperglycemia provides an endogenous glucose and insulin solution with the same beneficial effects as exogenously administered glucose-insulin-potassium solutions (117, 211). This mechanism -- in a similar manner to increases in peripheral resistance which help to maintain blood pressure at the expense of tissues which become ischemic -- becomes counterproductive and leads to the development of hypoglycemia when the hyperglycemia can no longer be maintained. The possibility that insulin blocks sympathetic support of the myocardium and, therefore, has detrimental effects, as postulated by Hiatt (82, 208) and Downing (125, 161) cannot, however, be eliminated. It seems likely, though, that the prevailing glucose levels are the controlling factor. Thus, one can alter the cardiovascular response to the administration of endotoxin and the ability of the dog to survive simply by altering the glycemic status. Dogs which are rendered hyperglycemic receive sufficient glucose to

more than keep up with metabolism even after the glucose infusion is stopped so that the needs of the cardiovascular system during the endotoxic stress continue to be supplied. Normoglycemic clamping, on the other hand, provides only sufficient glucose to supply the current needs and the hypoglycemic clamp removes the preexisting glucose reserve with detrimental consequences for cardiovascular functions. Hypoglycemia, therefore, plays a detrimental role during the course of shock while hyperglycemia appears to be a compensatory mechanism to maintain metabolic homeostasis and to prevent the ensuing cardiovascular failure.

Glucose Monitoring and Glucose Clamping and the Treatment of Endotoxin Shock

These experiments point to the need for metabolic as well as circulatory monitoring in septic patients as has been proposed by Siegel (192) and Duff (42). The results of the glucose clamping studies also indicate that glucose clamping may be an effective treatment for shock by helping to prevent the circulatory sequelae of sepsis. It is unknown whether there is a point where the effects of hypoglycemia become irreversible -- in analogy to the Wiggers' concept of irreversibility following hemmorhage (214). If glucose clamping is to be used as a treatment it is necessary to determine the best treatment regimen -- whether glucose alone or in conjunction with insulin is most effective.

Clearly it is important to have adequate assessment of metabolic status in order to appropriately administer treatment. In these experiments normoglycemic clamping was just as effective a treatment as hyperglycemic clamping early during the course of shock as assessed by the improvement in cardiovascular status, but only the hyperglycemic clamp was able to prolong survival following cessation of treatment. Furthermore, cardiovascular status deteriorated even in hyperglycemic-clamped endotoxic dogs. An assessment must be made as to when glucose clamping should be instituted and when it is safe to end that treatment. Further studies of the relationship of cardiovascular status to glucose homeostasis and the usefulness of glucose clamping as a treatment for endotoxic or septic shock are clearly needed.

Mechanism for the Role of Hypoglycemia in Cardiovascular Failure

Reticuloendothelial System Involvement

Buchanan and Filkins (22) demonstrated that hypoglycemia depresses phagocytosis of both colloidal carbon and endotoxin using an in vivo model of insulin-induced hypoglycemia and in vivo tests of phagocytic clearance. Thus, the hypoglycemia of endotoxin shock may contribute to the RES depression which occurs during endotoxin as well as other forms of circulatory shock (39, 59, 188). Since the RES is responsible for clearing cardio- and vasotoxic substances

as well as endotoxin from the blood, depression of this vital function may lead to cardiac and peripheral vascular failure following endotoxin administration by allowing such toxic substances to accumulate. In addition, there is evidence that RES depression alone can lead to a state of circulatory shock (7, 212, 213). Recently the results of Buchanan and Filkins have been confirmed using a slightly different model (116). There are, however, some striking differences between the quantitative results of this latter study and those of Buchanan and Filkins. These differences suggest that duration and the rate of induction of hypoglycemia may be important factors in the development of RES dysfunction as well as the plasma glucose level. Using an in situ perfused liver model, Kober and Filkins (116) also demonstrated that the hypoglycemic depression of RES phagocytosis is probably due to a direct metabolic effect of glucose deprivation on the Kupffer cells of the liver. Other mechanisms such as humoral mediators or maldistribution of blood flow in the liver cannot be eliminated and may operate in conjunction with a direct effect of glucose deprivation. A direct metabolic effect is entirely consistent with the evidence for a glucose-based metabolism in macrophages (76, 113, 163, 178, 187). It is also of interest to note that the RES may play a role in the development of hypoglycemia through its exocytic functions (58). Thus, both the endocytic and exocytic functions

of the RES may play an extremely important role in the pathogenesis of endotoxin shock by providing a positive feedback link via hypoglycemia (58).

The effects of hypoglycemia on RES function in the dog have not been explored. However, since this is a potential site for positive feedback in the development of circulatory shock, it is in theory an important mechanism.

Pulmonary Hemodynamics and Fluid Balance

The development of pulmonary edema during hypoglycemic episodes has been described clinically (8). The presence of a fulminating edema was also noted in conjunction with the previously mentioned studies of RES depression during hypoglycemia (unpublished observations). Two potential mechanisms for the pathogenesis of this pulmonary edema during hypoglycemia have been suggested in the literature (8). The first is a neurogenic mechanism leading to pulmonary vasoconstriction and increased pulmonary vascular resistance (139). Such increases in pulmonary vascular resistance are also important since the high afterload to right ventricular ejection can potentially lead to right ventricular failure. The second mechanism is an effect of hypoglycemia to increase alveolocapillary permeability -- either directly or indirectly through humoral mediators -- leading to increased fluid flux out of the pulmonary vasculature (71). Thus, through effects on pulmonary

vascular resistance and pulmonary capillary fluid balance, hypoglycemia can potentially contritute to the myocardial failure, hypovolemia, and the systemic hypoxia of endotoxin shock.

Lung fluid balance was not studied in the present set of experiments. However, both hypoglycemic-clamped endotoxic dogs and unclamped endotoxic dogs without early hyperglycemia show increases in pulmonary resistance after the administration of endotoxin. The increased pulmonary resistance could have contributed to the poorer cardiovascular picture in these groups of dogs by contributing to the development of myocardial failure or through an alteration in lung fluid balance. The contribution of increased pulmonary resistance to cardiovascular failure after endotoxin injection and the mechanism of the role of hypoglycemia in producing increased pulmonary resistance, however, requires further study.

Metabolic Requirements of Vascular Smooth Muscle

Until recently it was believed that vascular smooth muscle metabolism was largely dependent on carbohydrate as a source of fuel. It has now been shown that other substrates can be utilized by vascular smooth muscle (29) and that there may be differences between vascular beds (37). This issue remains controversial. As for other tissues,

glucose becomes a major substrate for vascular smooth muscle during hypoxia. It may be that as hypoglycemia and hypoxia develop during endotoxin shock that vasoconstriction cannot be maintained in non-vital beds. This would lead to a fall in systemic arterial pressure and a release of cardio- and vasotoxic substances from these beds. Malik and McGiff (141), on the other hand, have demonstrated increased responsiveness to adrenergic influences in the splanchnic circulation following glucose deprivation. This could lead to increased vasoconstriction in the splanchnic bed resulting in ischemia and production of cardio- and vasotoxic materials from damaged splanchnic organs. Thus, potentially, there are two opposing effects of hypoglycemia on the peripheral vasculature: decreased substrate availability to vascular smooth muscle and increased responsiveness to sympathetic nervous discharge. The balance between these two effects will determine the actual response to the hypoglycemia of endotoxin shock in a given vascular bed. In addition, this balance may change throughout the course of shock.

In these experiments, total peripheral resistance was maintained at control or elevated levels up until the time of death in all groups of endotoxic dogs. Furthermore, peripheral resistance seemed to be a function of cardiac output (i.e. the lower the cardiac output the higher the peripheral resistance). This implies that the peripheral

vasculature is capable of responding to the normal reflexes to maintain blood pressure. Thus, an overall effect of glucose deprivation during endotoxin shock on the ability of vascular smooth muscle to contract seems not to be present in spite of the very low levels of blood glucose. Effects on individual beds, however, cannot be ruled out. These data, on the other hand, are consistent with an enhancement of adrenergic vasoconstrictor mechanisms, since peripheral resistance was elevated in unclamped dogs not exhibiting early hyperglycemia and in hypoglycemic-clamped endotoxic dogs (although this did not reach statistical significance because of the high variability in the response of normoglycemic-clamped endotoxic dogs). This hypothesis requires more direct study of the vascular response to adrenergic stimulation after endotoxin and during hypoglycemia.

Hypoglycemia and Cardiac Function during Endotoxicosis

Evidence from the unclamped experiments suggests that the pattern of glucose dyshomeostasis affects primarily cardiac function (cardiac output and stroke work are affected by the pattern of glucose dyshomeostasis while the response of the peripheral vasculature follows reflexly to support systemic arterial pressure even in late shock. The results of the glucose clamping experiments are also consistent with this hypothesis since the same pattern of

deteriorating cardiac function with increasing systemic and pulmonary resistance is seen in hypoglycemic-clamped endotoxic dogs. This is also consistent with the current concept that the heart is the major site of circulatory failure during endotoxicosis rather than the peripheral vasculature (78). However, most of the studies of cardiac function following endotoxin injection -- including the present study -- have measured global circulatory parameters such as systemic and pulmonary arterial pressures, cardiac output, and left ventricular pressure which are affected by other factors as well as the contractile status of the heart. Further investigations utilizing more sophisticated means to assess cardiac performance and reserve -- such as wall thickness and regional shortening as well as methods of probing cardiac function -- are needed to define the role of the heart in endotoxic shock in general and more specifically the effect of the progressive hypoglycemia of endotoxin shock on cardiac function. Several mechanisms may lead to cardiac failure during endotoxin shock. The role of RES, pulmonary resistance, and lung fluid balance have already been discussed.

Some of the earliest clues to the potential effects of hypoglycemia on cardiovascular performance come from studies during the era of insulin shock treatments. Ernstene and Altschule (47) in 1931 discussed the dangers of diminished myocardial reserve during hypoglycemic episodes.

In the same year Middleton and Oatway (154) also noted the potential for myocardial injury following hypoglycemia. Additional evidence for this potential has been provided more recently by Libby, Maroko, and Braunwald (134). However, their 1975 study provided no information as to the mechanism and merely demonstrated the increase in myocardial injury following coronary artery ligation during hypoglycemia as assessed by S-T segment changes and myocardial creatine phosphokinase levels. Edwards and Page (43) as far back as 1924 emphasized the decreased functional capacity of the heart during hypoglycemic episodes and made the statement that the changes in cardiovascular function which they observed during hypoglycemia "may present the essential features of circulatory failure shock." In addition to these observations, the beneficial effects of insulin and glucose on the ischemic myocardium and also on the shock heart are well-known and have been extensively reviewed (117).

Bricknell and Opie (17, 18) have recently advanced the idea, supported by the earlier work of Gercken (66) and Gudbjarnason and coworkers (73), that the maintenance of membrane function in the heart is dependent on glycolytically produced ATP, and, thus, on a source of glucose. This concept is also supported by the work of Girardier (67) and of McDonald, Hunter, and McLeod (148) showing that glucose is necessary for the maintenance of normal action

potentials in the heart. As might be expected from this concept nodal tissue as well as tissue of the conducting system seems to be more dependent on carbohydrate metabolism (114, 121). These cardiac tissues are also more susceptible to the effects of glucose deprivation in the face of hypoxia (190). Hypoglycemia -- even without concomitant ischemia and hypoxia -- could, thus, lead to altered cardiac electrophysiology, dysrhythmias, depressed contractility, and altered responsivity to cholinergic and adrenergic influences resulting in autonomic imbalance via a depression of myocardial membrane function. Although the hypothesis of compartmentalization of ATP is certainly not a proven theory, it seems clear that while the major substrate of the heart under most circumstances is free fatty acids, the approximately 30% which is glucose and other carbohydrates (117) is just as important in maintaining normal cardiac function. During myocardial ischemia which has been demonstrated during endotoxin shock (15, 94, 115), glucose supply becomes even more critical since ATP can only be produced from free fatty acids oxidatively (117). Glycolysis, on the other hand, requires no oxygen. Furthermore, oxidative metabolism from glucose requires less oxygen than fatty acid metabolism (117). Thus, glucose becomes the preferred substrate during ischemia and hypoxia when oxygen supply is limited. Numerous studies have shown that myocardial function during ischemia and hypoxia is, in

fact, improved when glucose is supplied as the substrate as opposed to free fatty acids (117). In experiments in which high levels of free fatty acids were infused in normal dogs -- thus, turning off glucose utilization -- ischemic-like changes were produced in the electrocardiogram (105). The importance of glucose to the maintenance of cardiac performance is also suggested by clinical evidence that there is a relationship between the degree of glucose intolerance following myocardial infarction (so-called stress diabetes) and the degree of left ventricular dysfunction (182, 216). There is some similarity between the metabolic problems created by such glucose intolerance and those created by the hypoglycemia of endotoxin shock. During stress-induced glucose intolerance the supply of glucose is intact but transport into and utilization by the cells is impaired, whereas during endotoxic hypoglycemia the central supply of glucose is limited. The net result is effectively the same: The myocardial cells do not receive sufficient substrate to maintain adequate ventricular performance. It may very well be that the supply of glucose is a critical factor in several pathophysiological states effecting myocardial performance. Maintenance of normal glucose homeostasis may therefore be important in the maintenance of normal cardiovascular function. The implication is that the supply management system and the delivery system are highly integrated functions and that neither one works well by itself. This

concept is certainly worthy of further study.

In this set of experiments some support is provided for another potential mechanism of the effect of hypoglycemia on the heart. Increases in plasma lactate and decreases in pH -- both metabolic and respiratory in origin -- were seen in hypoglycemic-clamped dogs even in the saline-injected group. Furthermore, hypoglycemia exaggerated the development of endotoxic lactacidemia and acidosis (metabolic and respiratory). It has been shown that hydrogen ions adversely affect cardiac performance (162, 201). However, since there was no difference in the pH between the two unclamped endotoxic groups the impact of acidosis on cardiac function and the contribution of hypoglycemia to that acidosis during endotoxin shock requires further investigation.

Central Nervous System Depression

Since glucose is the major substrate of neural tissue (46), regulation of cardiovascular and respiratory function is deranged during hypoglycemic episodes. The areas of the brain which are most sensitive to glucose deprivation are the neocortex and the centers involved in cardiovascular and respiratory functions (46). Himwich (83) describes a progressive failure of the various regions of the brain during hypoglycemia, starting from the neocortex and progressing through the brain stem. These stages are dependent on the duration and degree of hypoglycemia. During each

stage there are different signs and symptoms which depend on the level of consciousness and the prevailing autonomic tone. Gellhorn pointed to the autonomic imbalance produced by the effects of hypoglycemia on the central nervous system -- especially in conjunction with respiratory depression leading to hypoxia and hypercapnia (63, 64, 65). Randall and coworkers (175, 176, 177, 210) have demonstrated the role of autonomic imbalance in the development of cardiac dysrhythmias using the technique of intrapericardial denervation. It is also noteworthy that Gellhorn observed periodic breathing (Cheyne-Stokes respiration) in response to hypoxia and hypoglycemia, but not in response to hypoglycemia alone (64). Recently Raymond and Emerson (179) studied the effects of hypoglycemia and diminished blood flow on cerebral metabolism during endotoxin shock in dogs. They concluded that the resulting depression of brainstem cardiovascular and respiratory centers may be critical to the pathogenesis of the shock state.

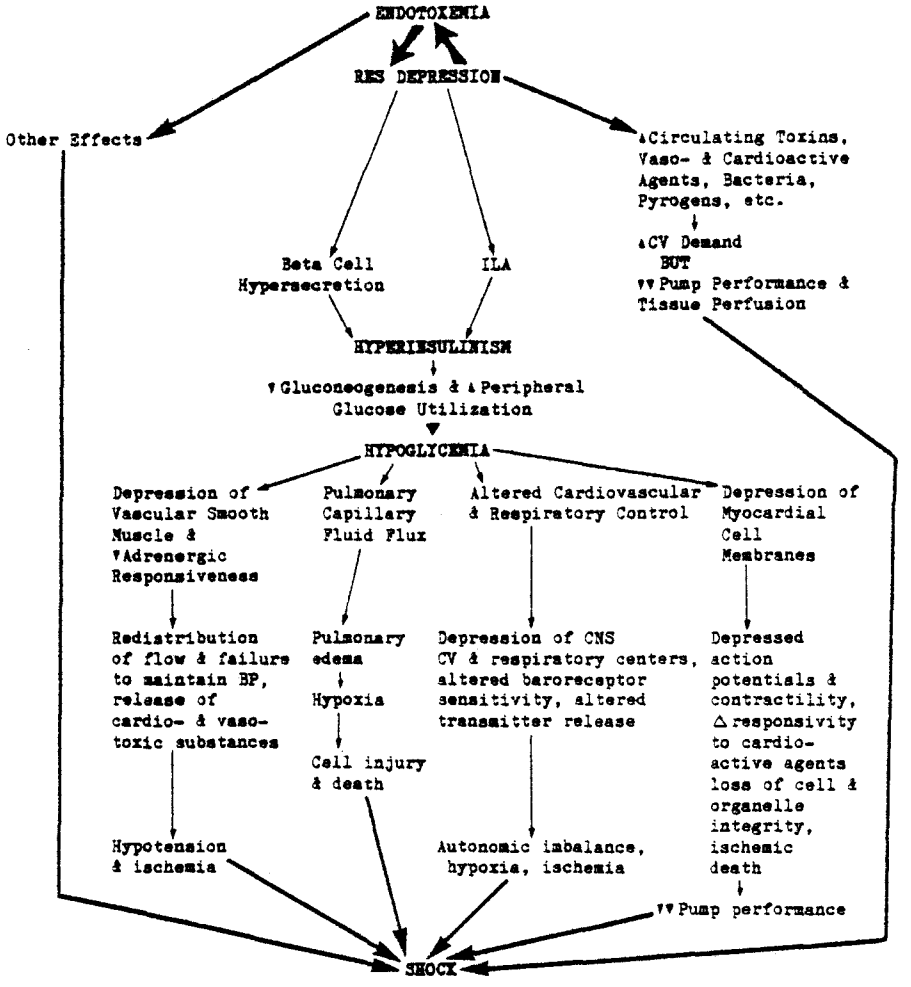
In addition to these effects on the central nervous system it is also possible that hypoglycemia affects peripheral autonomic nerve function and cardiovascular reflex mechanisms. Miles and Hayter (155) discussed the role of the baroreceptor reflex in the abnormal response to tilting during insulin-induced hypoglycemia sometimes seen in diabetics. While diabetic autonomic neuropathies may also be a contributory factor in the failure of these patients to

maintain blood pressure on a tilt table after induction of mild hypoglycemia, it is possible that under the more severe hypoglycemia of endotoxin shock that the baroreceptor reflex will nevertheless be attenuated by this mechanism. Appenzeller has suggested that the sensitivity of the baroreceptors may be altered by insulin or glucose (2, 3, 24). In addition Järhult and associates have demonstrated effects of carotid baroreceptors on the function of pancreatic islets as well as their more classical function in the cardiovascular system (111, 112). It is conceivable that insulin or glucose may feedback on the baroreceptors. While the evidence is largely speculative, hypoglycemia or hyperinsulinism might play a role in the resetting of the baroreceptors during endotoxic hypotension.

Summary

There are, thus, several mechanisms by which hypoglycemia can lead to cardiovascular dyshomeostasis and shock: 1) reticuloendothelial system depression, 2) altered pulmonary vascular fluid balance, 3) increased pulmonary vascular resistance, 4) altered vascular smooth muscle function, 5) synergism with ischemia to produce myocardial dysfunction, 6) acidosis leading to depression of cardiac performance, and 7) altered neural regulation of the cardiovascular and respiratory systems. These hypotheses are summarized in figure 19. None of these mechanisms is

Fig. 19. Hypothetical scheme of the role of hypoglycemia in endotoxin shock


ENDOTOXEMIA
RES DEPRESSION
Other Effects
▲Circulating Toxins, Vaso- & Cardioactive Agents, Bacteria, Pyrogens, etc.
▲CV Demand
BUT
▼▼Pump Performance & Tissue Perfusion
Beta Cell Hypersecretion
ILA
HYPERINSULINISM
▼Gluconeogenesis & ▲Peripheral Glucose Utilization
HYPOGLYCEMIA
Depression of Vascular Smooth Muscle & ▼Adrenergic Responsiveness
Pulmonary Capillary Fluid Flux
Altered Cardiovascular & Respiratory Control
Depression of Myocardial Cell Membranes
Redistribution of flow & failure to maintain BP, release of cardio- & vaso-toxic substances
Pulmonary edema
Hypoxia
Cell injury & death
Depression of CNS CV & respiratory centers, altered baroreceptor sensitivity, altered transmitter release
Depressed action potentials & contractility, Δ responsivity to cardio-active agents loss of cell & organelle integrity, ischemic death
Hypotension & ischemia
Autonomic imbalance, hypoxia, ischemia
▼▼Pump performance
SHOCK

mutually exclusive and it is possible -- even probable -- that more than one or even all of them are involved. The evidence presented in this study indicates that the major effect is most likely depression of myocardial performance -- either directly or indirectly -- rather than the peripheral vasculature. Further experiments are needed to look more closely at cardiac performance during endotoxin shock and to examine the different hypotheses for the mechanism of hypoglycemic depression of the heart. This is an important area since it opens up additional vistas linking metabolic homeostasis and cardiac function -- an area which has largely been ignored.

CHAPTER VII

SUMMARY AND CONCLUSIONS

A canine model for the continuous monitoring of blood glucose levels during endotoxic shock has been developed. This model has been used to develop the technique of glucose clamping at hypo-, normo-, and hyperglycemic levels in order to study glucose metabolism during endotoxin shock and the relationship of derangements in glucose homeostasis to the development of the collapse of the circulatory system.

Because of the use of continuous glucose monitoring, it has been conclusively demonstrated that some dogs go through an early hyperglycemic phase while others do not. Previously, with the availability of only discontinuous sampling, it was possible that the early hyperglycemic phase was simply missed by the sampling rather than being non-existent. The mechanism regulating whether a hyperglycemic phase will occur is not known. Regardless of whether an animal showed early hyperglycemia or not following the administration of endotoxin, a hyperinsulinemia with a highly variable time course was seen to occur during endotoxin shock. However, since in some cases spuriously high insulin levels could be obtained because of the presence of an interfering factor, the existence of hyperinsulinemia

in the endotoxic dog has not been conclusively demonstrated.

These results also have demonstrated that metabolic failure leading to profound hypoglycemia following endotoxin injection is an important -- even critical -- factor in the development of cardiovascular collapse while the initial hyperglycemia is a part of the compensatory mechanism to prevent the circulatory failure and its lethal consequences. The results are consistent with an effect of hypoglycemia to depress cardiac function in the endotoxic dog. Further experiments are needed to determine the exact mechanism of the effects of hypoglycemia on cardiac performance. The peripheral vasculature remains under neural control to maintain blood pressure but this is accomplished at the tremendous cost of reflex vasoconstriction leading to tissue ischemia. The results also suggest that glucose dyshomeostasis may also contribute to the increase in pulmonary resistance seen during endotoxin shock. Such increases in pulmonary resistance may contribute to the development of cardiac failure. Glucose clamping may be an effective treatment for the circulatory sequelae of endotoxin shock. Further refinements of the glucose clamping technique are needed before the technique can be used in a treatment regimen to prevent the hypoglycemia and the resulting circulatory failure following endotoxin administration.

Thus, in conclusion:

1) The ability to maintain and even elevate blood glucose levels is intimately associated with the ability to maintain cardiovascular function after endotoxin injection.

2) Conversely, the development of metabolic failure leading to profound, progressive hypoglycemia is critically involved in the circulatory collapse seen following endotoxin administration.

3) Hypoglycemia adversely affects cardiac performance during endotoxicosis.

4) Peripheral vascular mechanisms are still able to maintain blood pressure but at the cost of tremendous increases in resistance leading to tissue ischemia.

5) Glucose clamping to maintain a hyperglycemic state may be an effective treatment regimen to prevent the circulatory sequelae of endotoxin shock.

6) Further experimentation is needed to investigate the effect of the glucose dyshomeostasis of endotoxin shock on cardiac performance.

7) Further experimentation is also needed to refine the glucose clamping technique for further studies of endotoxic glucose metabolism in the dog and for the development of an effective treatment to prevent the progression to circulatory failure and shock.

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APPENDIX A

APPENDIX A

Additional Data Presentation

The following five figures show presentations of data for selected parameters for individual dogs in the unclamped groups (figures 20-23) and the average glucose levels during the clamps in the glucose clamping groups (figure 24). The 4 figures from the unclamped experiments demonstrate the wide variability in the time course of endotoxin shock in these sets of experiments and also show the two patterns of blood glucose changes seen (figue 20). Also seen in these figures is the highly variable pattern of serum insulin (figure 21). Figure 24 shows the average glucose levels during the glucose clamps. Statistical analysis (a factorial analysis of variance) demonstrates that all three clamps were different ($P<0.05$) with no differences between corresponding endotoxin and saline groups ($P>0.1$),

Fig. 20. Glucose: Individual Dogs -- Unclamped Experiments. Bottom=Endotoxin w/no hyperglycemia, Middle=Endotoxin w/hyperglycemia, Top=saline.

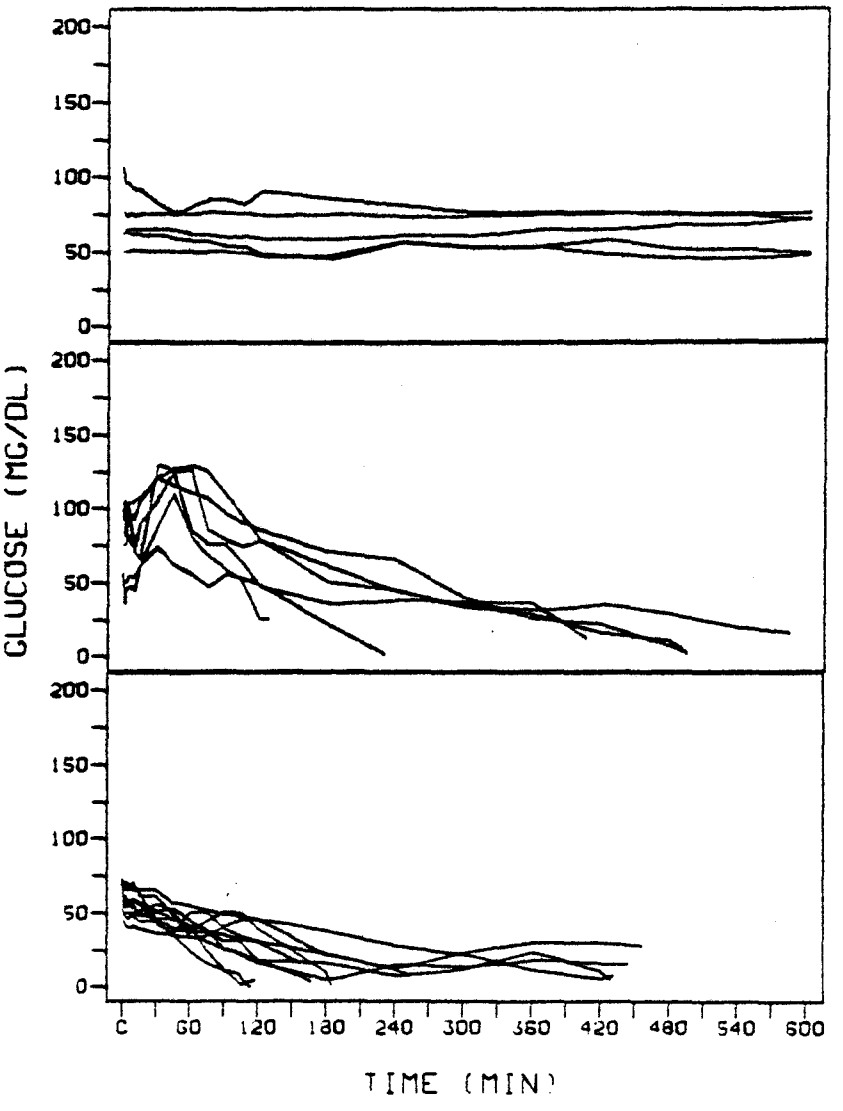

200
150
100
50
0
200
150
100
50
0
200
150
100
50
0
GLUCOSE (MG/DL)
0
60
120
180
240
300
360
420
480
540
600
TIME (MIN)

Fig. 21. Insulin: Individual Dogs -- Unclamped Experiments. Bottom=Endotoxin w/no hyperglycemia, Middle=Endotoxin w/hyperglycemia, Top=saline.

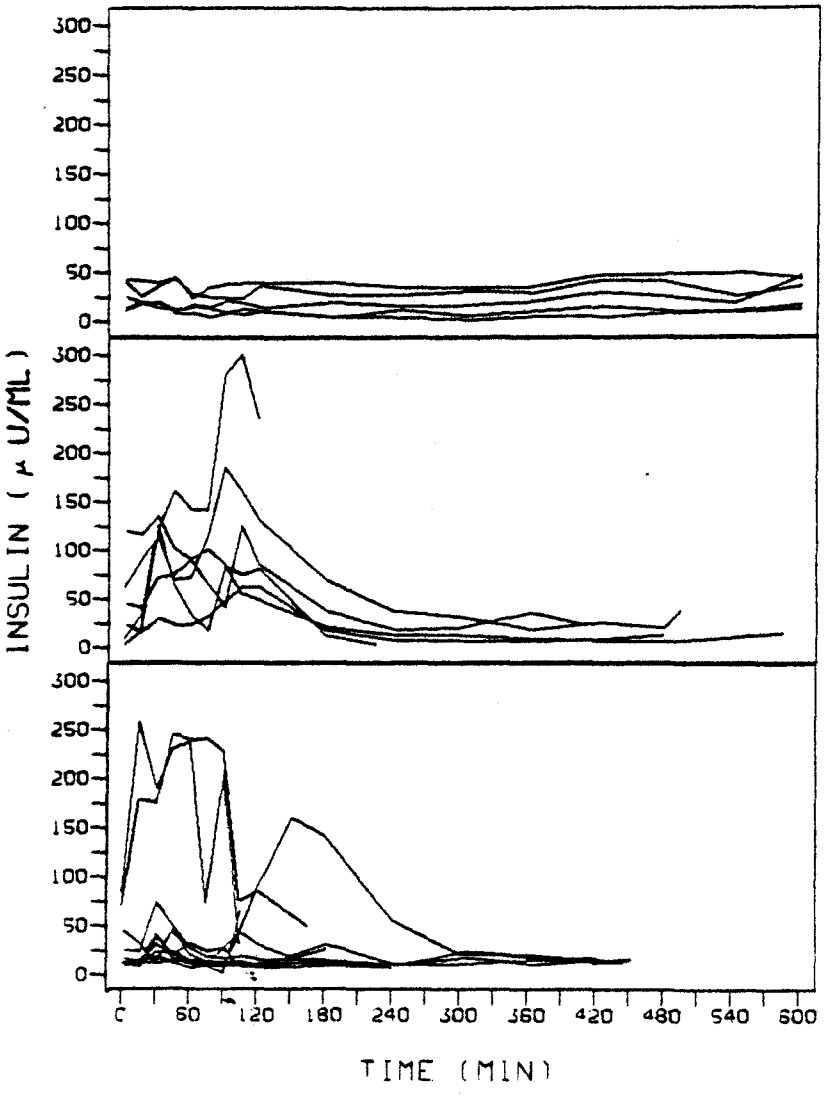

INSULIN (μU/ML)
300
250
200
150
100
50
0
0
60
120
180
240
300
360
420
480
540
600
TIME (MIN)

Fig. 22. Cardiac Output: Individual Dogs -- Unclamped Experiments. Bottom= Endotoxin w/no hyperglycemia, Middle=Endotoxin w/hyperglycemia, Top=saline.

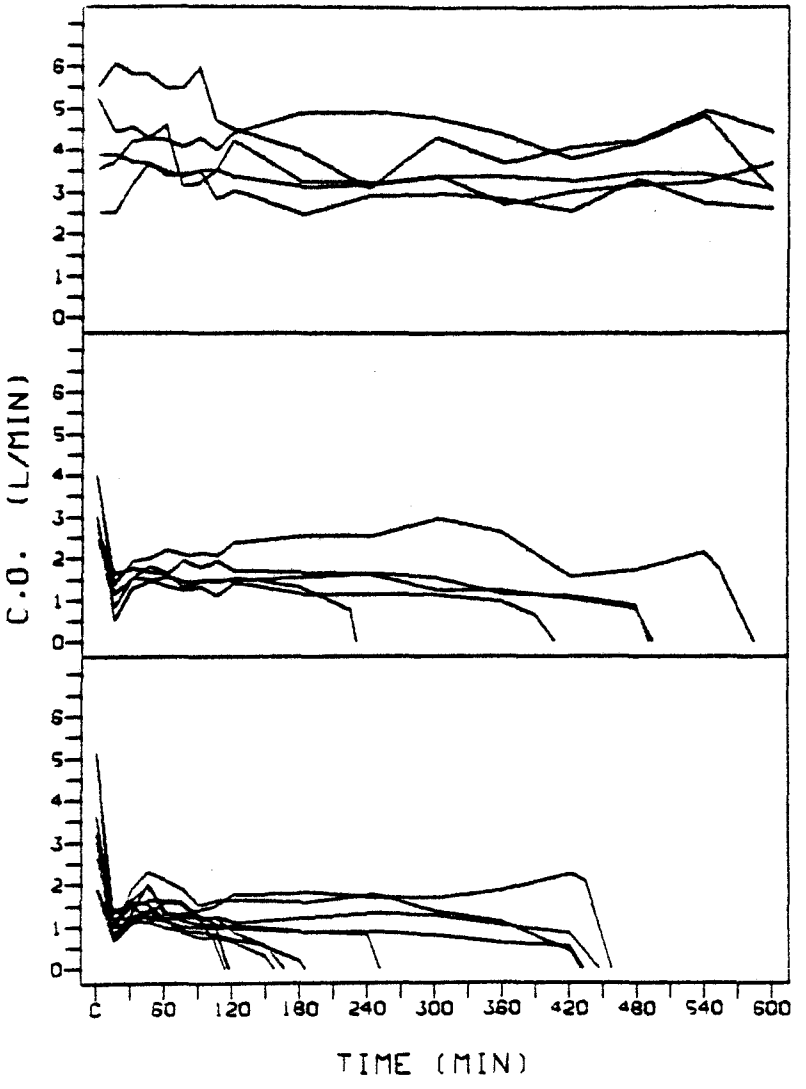

C.O. (L/MIN)
0
1
2
3
4
5
6
C
60
120
180
240
300
360
420
480
540
600
TIME (MIN)

Fig. 23. Mean Systemic Arterial Pressure: Individual Dogs -- Unclamped Experiments. Bottom=Endotoxin w/no hyperglycemia, Middle= Endotoxin w/hyperglycemia, Top=saline.

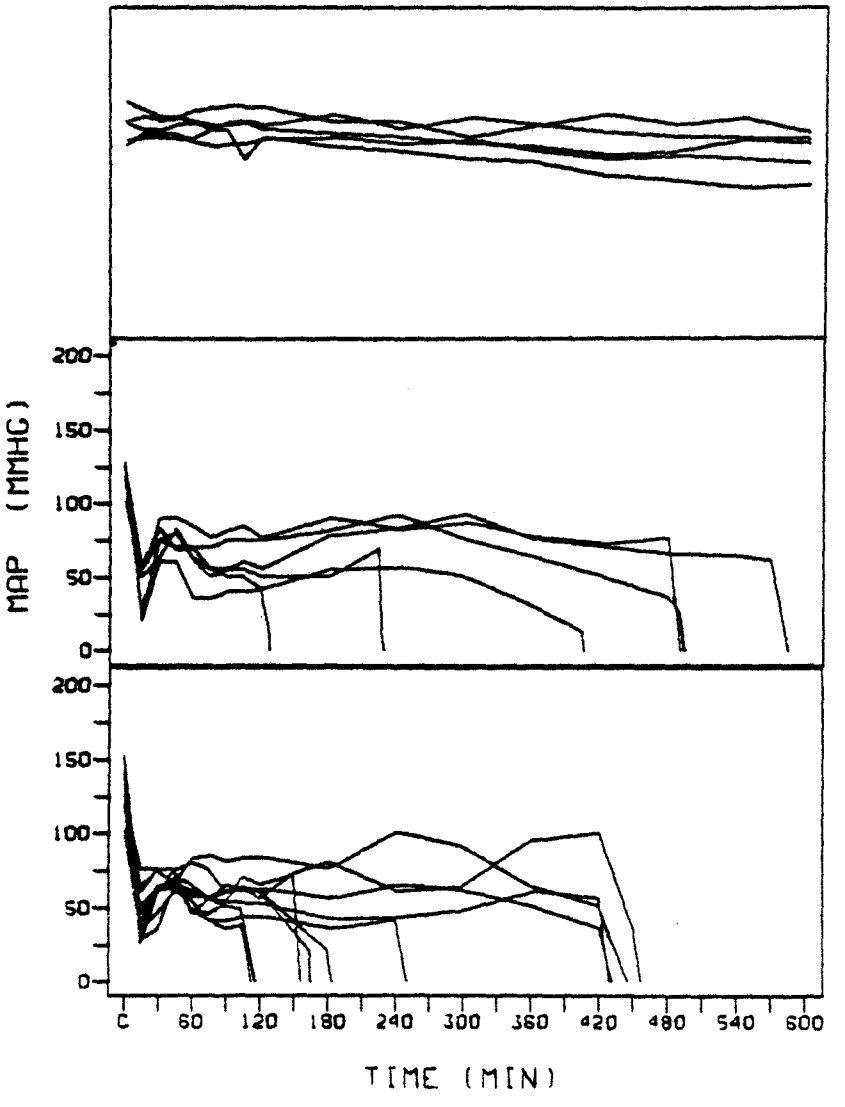
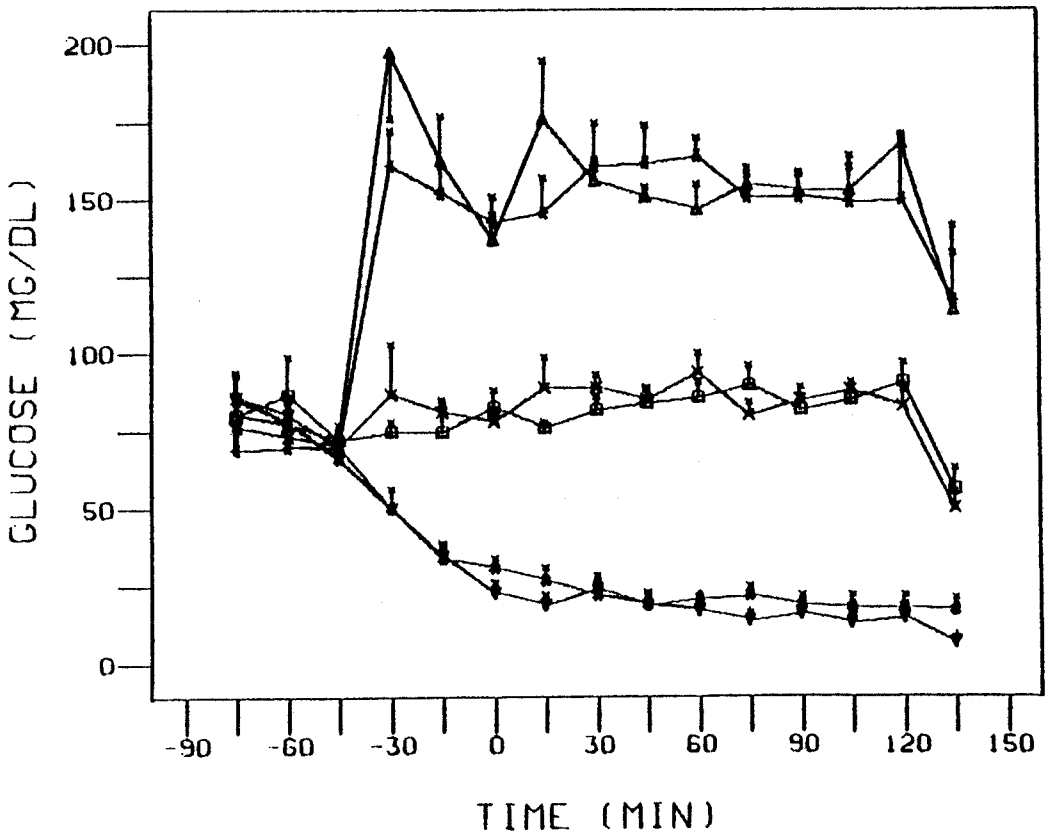

MAP (MMHG)
200
150
100
50
0
200
150
100
50
0
0
60
120
180
240
300
360
420
480
540
600
TIME (MIN)

Fig. 24. Glucose Clamps: ▽ Endotoxin, Hypoglycemic Clamp (N=6); # Saline, Hypoglycemic Clamp (N=5); X Endotoxin, Normoglycemic Clamp (N=4); □ Saline, Normoglycemic Clamp (N=3); * Endotoxin, Hyperglycemic Clamp (N=4); Δ Saline, Hyperglycemic Clamp (N=3).


GLUCOSE (MG/DL)
200
150
100
50
0
-90
-60
-30
0
30
60
90
120
150
TIME (MIN)

APPENDIX B

APPENDIX B

RIA PROGRAM

The following program, written in PFOCAL on PDP12 computer, was used to analyze the insulin data. Data from the gamma counter is read by the computer via paper tape. The program consists of two parts: 1) the main program and 2) the log-logit linear regression subroutine which is accessed by the main program.

Main RIA Program

```
01. 01 C RIA
01. 20 C CALCULATES RIA FROM DATA ENTERED INTO THE COMPUTER
01. 30 C VIA PAPER TAPE FROM GAMMA COUNTER
01. 40 C DEVELOPED BY P. M. KOBER AND M. R. YELICH
01. 50 C AUGUST 13, 1980
01. 60 A !!!"EXPERIMENT IDENTIFICATION #:"D
01. 70 A !!"NUMBER OF ASSAY TUBES?"N1
01. 80 D 8
01. 90 D 9

02. 05 S K=0
02. 10 T "WELL #  CPM(CH A)   B/BO   %B   AVG %B"I
       (K-1)2. 2,2. 15
02. 15 T :53"IRI   AVG IRI"
o2. 20 T !;D 6
02. 30 T !!!!!;S K=1;D 2. 1;T  !

04. 10 C IRI CALCULATIONS FOR SAMPLES
04. 20 O R I
04. 27 S K=);S S1=0;S I1=0
04. 30 F J=20,N1;D 5
04. 40 Q

05. 10 C SAMPLES
05. 20 A N, M, S, M, M, M
05. 30 S S2=S-BL;S S2=S2/H;S P(K)=S2
```

```
05. 40 I (K-1)5. 5,5. 9
05. 50 S S1=S1+S2;S K=1
05. 60 S I=FEXP(((FLOG(S2/(1-S2))-B)/Q)*FLOG(10));S I1=I1+I
05. 70 O I TTY
05. 75 T !,%4. 00,N, :9,%7.01,S, :23,%4. 03,S2, :31,%4. 01,
       100*52, :51%3. 00,I
05. 80 O R I; R
05. 90 S S1=(S1+S2)/2;S K=0
05. 92 S I=FEXP(((FLOG(S2/(1-S2))-B)/Q*FLOG(10));S I1=(I1+I)
       /2
05. 94 O I TTY:
05. 97 T ,:51,%3. 00, I, :59, I1; I (FABS(P(0)-P(1))-. 05)5.
       98,5. 98;T  :62"*"
05. 98 S I1=0;S S1=0
05. 99 O R I;R

06. 10 C STANDARD CURVE CALCULATION
06. 20 O I DF:RIA.FD
06. 30 F J=1,3;A M, M, M, M, M, M
06. 40 S K=0;S D1=0
06. 50 F J=4,19;D 7
06. 60 O I TTY:,ECHO
06 70 L G STCURV

07. 05 C STANDARDS
07. 10 A N, M, S, M, M, M
07. 20 S S=S-BL;S S(J)=S/H
07. 30 I (K-1)7. 4,7.8
07. 40 S S1=S1+S(J);S K=1
07. 50 O I TTY:
07. 60 T !,%4. 00,N, :9,%7. 01,S+BL, :23,%4. 03,S(J), :31,%4.
       01,100*S(J)
07. 70 O R I;R
07. 80 S S1=(S1+S(J))/2;S K=0
07. 90 O I TTY:
07. 92 T !,%4. 00,N, :9,%7. 01,S+BL, :23,%4. 03,S(J), :31,
       %4. 01,100*S(J), :40,S1*100
07. 94 S S1=0
07. 96 O R I;R

08. 10 C BLANK CALCULATION
08. 20 O I DF :RIA. FD
08. 30 F J=1,3;A M, M, X(J),M, M, M
08. 40 O I TTY:
08. 50 S BL=(X(2)+X(3))/2
08. 60 T !!!"CPM FOR BLANK+     "%6. 02,BL

09. 10 C STANDARD H CALCULATION (BO)
09. 20 S H=0
09. 30 O R I
09. 40 F J=4,19;D 10
```

```
09. 50 O C
09. 60 S H=H/2-BL
09. 70 T !"CPM FOR SAMPLE H (BO)="H, !!!!!

10. 05 C STANDARD H
10. 10 A M,M,H1,M,M,M;I (J-18)10. 2;S H=H+H1
10. 20 R
```

Log-Logit Linear Regression Subroutine

```
01. 10 C STCURV
01. 20 C LOG-LOGIT REGRESSION OF STANDARD CURVE FOR INSULIN
       RIA
01. 30 C USED AS SUBROUTINE FOR RIA PROGRAM
01. 40 C DEVELOPED BY P. KOBER AND M. R. YELICH
01. 50 C AUGUST 13, 1980
01. 60 S SX=0;S X2=0;S SY=);S Y2=0;S N2=14
01. 65 S D=193
01. 67 T !!!!!
01. 70 F J=4,2,16;T !"STD DOSE ";S D=FOUT(D);A "?"S1;S S1(J)
       =S1;S S1(J+1)=S1;S D
01. 80 F J=4,17;D 3

02. 10 S DX=X2-SX^2/N2;S DY=Y2-SY^2/N2
02. 20 S Q=(XY-SX*SY/N2)/DX;S B=(SY-SX*Q)/N2
02. 30 S R=Q*DX/FSQT(DX*DY);S S=DY-Q^2*DX;S E=FSQT(S/(N2-2))
02. 40 T !!!!!%4. 03"LOGIT Y=",Q, "*LOG X+"%5. 03,B, "+/-"E
02. 50 T !"R="R
02. 56 T !!
02. 60 A "GOOD STANDARD CURVE?D;T !!!!!
02. 70 I (D-OYES)4. 1;R

03. 10 S X=FLOG(S1(J))/FLOG(10);S Y=FLOG(S(J)/(1-S(J)))
03. 20 S SX=SX+X;S X2=X2+X^2;S SY=SY+Y;S Y2=Y2+Y^2; XY=XY+
       X*Y

04. 10 A "HOW MANY BAD POINTS?"D
04. 20 F G=1,D;D 5
04. 25 S N2=N2-D
04. 30 G 2. 1

05. 10 T "DOSE AND NUMBER OF BAD POINT"%3.00,G;A "?"S1,V
05. 20 S X=FLOG(S1)/FLOG(10);S Y=FLOG(S(V)/(!-S(V)))
05. 30 S SX=SX-X;S  X2=X2-X^2;S SY=SY-Y;S Y2=Y2-^2;S
       XY=XY-X*Y
*
```

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The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

9/25/82
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