Insulin and Host Resistance to Endotoxemia

Bernard J. Buchanan
Loyola University Chicago

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INSULIN AND HOST RESISTANCE TO ENDOTOXEMIA

by

Bernard J. Buchanan

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

February

1975

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CHAPTER I
INTRODUCTION

The lipopolysaccharide components of the outer wall of Gram negative bacteria -- commonly referred to as endotoxins, have long been known to possess a wide range of biological activities when introduced into either experimental animals or man (27, 346, 352, 379, 488, 489, 530). Prominent among these activities is the capacity to induce a lethal shock syndrome -- generally referred to as endotoxic shock. Although the cardiovascular actions of endotoxin which result in shock have long been studied in some detail (209, 253, 476), only more recently have the metabolic consequences of endotoxemia and their intimate relationship to the pathogenesis of lethal endotoxin shock received a comparable degree of emphasis (50, 489).

Foremost among the metabolic sequela of endotoxemia are profound effects on caloric homeostasis. In particular endotoxemic alterations in hepatic function which lead to deranged carbohydrate homeostasis have been stressed; thus Berry (49, 50), Agarwal (5, 6) and others (204, 434, 458) have implicated depressed hepatic gluconeogenesis as a critical determinant in the evolution of a potentially lethal endotoxemia.

While carbohydrate homeostasis is regulated by a host of physiologic mechanisms, insulin is generally recognized as the dominant physiological regulatory influence (169, 170, 400, 429, 635, 669)
via its profound influences on both hepatic gluconeogenesis (151, 173, 191, 192, 196, 418, 463, 609, 669) and the rate of glucose utilization (169, 368, 400, 429, 508, 543, 635, 669).

The present study evaluated the proposition that insulin is involved in the hypoglycemic and carbohydrate depleting action of endotoxin and by influencing the manifestations of endotoxin toxicity it plays a critical role in the perpetuation of the shock syndrome. Since the reticuloendothelial system plays a critical role in the immunophysiologic resistance to potentially lethal shock (65, 132, 209, 536, 694) and deranged carbohydrate metabolism is a hallmark of endotoxemia, (50, 605) the influence of deranged carbohydrate metabolism on the host defense functions of the reticuloendothelial system was also investigated.
CHAPTER II

REVIEW OF LITERATURE, PURPOSE, AND OBJECTIVES

A. Deranged Carbohydrate Metabolism in Endotoxemia and the Occurrence of Hypoglycemia

In 1923 Menten and Manning (444) first described the characteristic hyperglycemic response appearing soon after injection of endotoxin; followed by a severe hypoglycemia in animals given a lethal dose of endotoxin. An iv injection of a lethal dose of heat-killed organisms of the Enteritidis-Paratyphoid B group was found to cause an almost immediate increase in blood sugar to 250 mg per dl in rabbits. The hyperglycemic peak was followed by a progressive hypoglycemia until the ultimate death of the animals. The authors felt it difficult to estimate the role of any specific organ in the alterations in carbohydrate metabolism since pathological changes appeared coincidentally in the liver, pancreas, and kidneys. Zeckwer and Goodell (692, 693) also investigated the changes in carbohydrate metabolism in rabbits which were challenged with heat-killed bacteria; they found a biphasic response of blood glucose levels. Two possible explanations for the rapid hyperglycemia produced by heat-killed bacteria were considered: (i) a failure of the utilization of glucose with its accumulation in the blood, or (ii) the sudden mobilization of glycogen and hence an increase in the amount of glucose entering the blood stream. Zeckwer and Goodell (693) dismissed the suggestion of Menten and Manning (444) that the initial hyperglycemia was due to injury of the pancreatic
islets and the failure of the tissues of the body to utilize glucose. The rapid reversal of the initial hyperglycemia within a matter of minutes was taken as evidence that the pancreatic islets had not been seriously damaged. Available evidence, including microchemical determinations of liver glycogen was interpreted as indicating that the initial hyperglycemia was secondary to the activation of glycogenolysis and it was thought that the sympathetic nervous system mediated this effect. In support of the idea that hypoglycemia was associated with lethal endotoxin shock, Zeckwer and Goodell (693) did not observe a hypoglycemic response in animals given less than a lethal dose of heat-killed bacteria even though the early hyperglycemic reaction still occurred.

The study of the mechanism by which endotoxin influences carbohydrate metabolism in experimental animals was further pursued by Kun and Miller (374) and Kun (372, 373). In the first series of experiments Kun and Miller (374) measured the changes in concentrations of glucose and glucose derived metabolites in blood and tissues of rabbits given a lethal dose of crude meningococcal or Salmonella endotoxin which caused death in 1.5 to 3 hrs. Despite the animals having been fasted 24 hrs, a transient hyperglycemia was observed with peak values averaging twice control at 30 minutes. By 90 minutes the hyperglycemia had subsided to control values; by 120 minutes, all animals were hypoglycemic with individual values of blood glucose as low as 10 mg per 100 ml. Miller and Kun (374) also reported glycogen depletion, accumulation of lactate and inorganic phosphorous and depression of pyruvate and glucose levels and suggested either a state of anoxia in
tissues or an inhibition of oxygen utilization was present. To pursue the latter possibility, muscle and liver were assayed for cytochrome oxidase and succinic dehydrogenase activities. Cytochrome oxidase activity was not affected by endotoxin treatment; however, endotoxin preparations injected in vivo caused a marked depression of succinic dehydrogenase activity when assayed in whole tissue homogenates of animals sacrificed as soon as only a few minutes after endotoxin injection. Kun and Miller (374) concluded that the inhibition of succinic dehydrogenase activity produced by endotoxin injection played an important role in the mechanism responsible for the changes in carbohydrate metabolites found in the blood and tissues of endotoxin treated rabbits. Kun (372) also reported experiments demonstrating an inhibition of the phosphorylation of glucose by endotoxin. It was found that the addition of endotoxin to rat muscle extracts inhibited their utilization of glucose in vitro. This inhibition of glucose utilization observed in the presence of endotoxin did not occur if Zn-free insulin was added to the reaction mixture. Since under the conditions employed the rate of glucose utilization was believed to be a measure of hexokinase activity, it was concluded that endotoxin may inhibit this enzyme. Furthermore, the endotoxin inhibition was reversible by insulin treatment. In a subsequent publication, Kun (373) reported the results of experiments on the effect of bacterial endotoxin on glycogen synthesis both in vivo and in vitro. Rats treated with endotoxin 1 hr before a glucose load showed no increase in hepatic glycogen while control rats increased their liver glycogen. In in vitro studies using rabbit liver slices, the addition of as little as 0.1 mg endotoxin
per ml to the incubation media depressed glycogen formation from glucose by over 50% and similarly depressed glyconeogenesis from pyruvate. Kun concluded that the inhibition of glycogen formation by endotoxin may be related to the inhibition of succinic dehydrogenase observed in a previous study (374). Since it was known that insulin increases the uptake of glucose by rat diaphragm muscle \textit{in vitro}, Kun (373) investigated the possibility of whether the action of insulin was affected by endotoxin or \textit{vice versa}. It was found that insulin still exerted its stimulating influence on glucose utilization in the presence of \textit{Salmonella} endotoxin as assessed using the diaphragm assay.

B. Mechanisms of the Carbohydrate Depletion in Endotoxemia

The effect of endotoxin on carbohydrate metabolism has been studied in some detail over the past 15 years by Berry and coworkers. Berry, Smythe and Young (57) reported the carbohydrate depleting action of endotoxin in relation to the protective role of cortisone in mice. A large dose of cortisone--5 mg per mouse--reduced lethality from about 90% to about 30%; a smaller dose of 1 mg of cortisone had no effect on endotoxin lethality. In mice which were not protected by cortisone and then sacrificed just before the first deaths would be expected, chemical analysis showed hypoglycemia, depletion of liver and muscle glycogen, and a reduction in total body carbohydrate to one fifth of control values. Treatment with a protective dose of cortisone maintained all of these parameters near control values. Berry et al. (57) interpreted this increase in carbohydrate stores to be largely the result of stimulated glyconeogenesis from body protein stores. However, they commented that it was not known whether the greater amount
of carbohydrate was due to greater synthesis, to diminished loss, or to both. Since dibenzyline treatment did not protect against lethal endotoxin shock and did not prevent the depletion of liver glycogen or alter the hypoglycemic response to endotoxin, it was concluded that any interaction between the adrenal medulla and bacterial endotoxin was of secondary importance and epinephrine was not responsible for the glycogen depleting action of endotoxin. To determine if endotoxin impaired the ability of the body to store carbohydrates, seventeen hour fasted mice were given a 50 mg glucose load following either saline or endotoxin pretreatment. Normal mice became hyperglycemic and showed a three-fold increase in liver glycogen with no change in muscle glycogen. In contrast, endotoxemic mice were hypoglycemic despite the glucose load and showed no change in hepatic glycogen with a small increase in muscle glycogen. In vitro endotoxin treatment of liver mitochondria increased the rate of ATP hydrolysis to an extent slightly greater than $10^{-4}$ molar dinitrophenol. This suggested to the authors that an interference with high energy phosphate metabolism offered a possible explanation for many of the metabolic changes observed after endotoxin poisoning. Berry and Smythe (52) studied the protein-carbohydrate balance following cortisone treatment of endotoxin poisoned mice as well as the response to ACTH and gastrointestinal absorption in endotoxemia. A 5 mg dose of cortisone given at the beginning of the overnight fast increased urinary nitrogen by an amount representing 52 and 57 mg protein in control and endotoxin poisoned mice respectively. A corresponding amount of carbohydrate could either be spared or synthetized. In normal mice cortisone caused the accumulation of
an amount of carbohydrate which agreed within 8% of the calculated amount of protein catabolized. However, endotoxin treated mice showed a deficiency in carbohydrate which indicated either (i) an increased utilization of glucose or (ii) an impairment in glucose synthesis from non-carbohydrate precursors. Berry and Smythe (52) stated that there existed no evidence for the former and they did not further consider this possible explanation. However, in a previous study (57) they had shown evidence of defects in carbohydrate metabolism in endotoxin treated mice and this was assumed to be the mechanism of the failure of endotoxin treated mice to exhibit a normal rise in carbohydrate stores following cortisone treatment. Investigations of gastrointestinal function in endotoxin treated mice showed that either the absorption or utilization of intra-gastric injections of tryptone broth was completely blocked. Furthermore, when endotoxin treated mice were given a test meal it remained in their stomach and proximal one third of the small intestine; in contrast it had traversed to the large intestine in control mice. It was concluded that endotoxin treatment caused the gastrointestinal tract to become almost completely paralysed. Since cortisone had been shown to protect mice from lethal endotoxemia (57), Berry and Smythe (52) studied the effect of ACTH and found that a dose of 8 units unexpectedly decreased survival from 75% in the control group to 10% in the treated animals. Further studies showed that endotoxemia blocked the effect of ACTH on metabolism as was observed in the control animals.

Berry and Smythe (53) later conducted experiments to discriminate between several possible explanations for the failure of endotoxin treated animals to respond to ACTH. Berry and Smythe (53) concluded
that the release of endogenously secreted adrenal corticoids in response to the stress of endotoxemia caused an increase in protein catabolism. However as judged by blood non-protein nitrogen changes and changes in urinary nitrogen excretion, excess urea formed from protein catabolism did not appear following ACTH treatment of endotoxin poisoned mice. It appeared that the kidney was unable to eliminate the additional quantities of nitrogenous wastes. In support of this possibility phenol red clearance, inulin clearance, and urea clearance were all found to be severely depressed in endotoxin poisoned animals.

In 1964 Berry (49) reviewed the literature from his own laboratory as well as others related to the metabolic effects of endotoxemia and the influence of endotoxemia on the activities of certain enzymes. The earlier work of Woods and associates demonstrating that lipopolysaccharides produce a primary metabolic alteration at the cellular and subcellular levels in in vitro experiments had suggested that endotoxin toxicity may be related to a metabolic derangement as will be further discussed below. Work in Berry's laboratory had recently suggested that endotoxin produced alterations in metabolism in vivo and that these alterations were modified antagonistically by cortisone treatment. Cortisone treatment appeared to cause an increase in key hepatic enzymes either wholly or at least in part by the synthesis of new enzyme protein. At this early time it was noted that tryptophan failed to protect against endotoxin even though this substrate might be expected to serve as a precursor of NAD or NADP synthesis which was thought to be one of the significant metabolic pathways suppressed by endotoxin. The conclusive evidence that tryptophan itself inhibited gluconeogenesis
had not yet appeared, although it had been reported that tryptophan had a hypoglycemic activity \textit{in vivo} \cite{454, 455} and that tryptophan prevented the degradation of insulin by liver homogenates when given \textit{in vivo} \cite{454}. Berry's hypothesis that cortisone protected against lethal endotoxemia by the induction of hepatic gluconeogenic enzymes was supported by experiments which demonstrated that both actinomycin D and ethionine which suppress RNA and protein synthesis respectively, prevented cortisone treatment from exerting its protective effects. Berry \cite{49} proposed as a generalization that agents which inhibit enzyme induction both sensitize endotoxin treated animals presumably by preventing the endogenous stimulation of enzyme synthesis by adrenal corticosteroids and also prevent cortisone from exerting its protective effect by the same mechanism. It also appeared that endotoxin prevented within 2 hr the \textit{de novo} synthesis of some enzymes stimulated by corticosteroids and by 4 hr cortisone no longer exerted any effect on hepatic enzyme synthesis. As would be expected if this hypothesis were true, actinomycin D given 4 hr after endotoxin should not affect lethality which was found to be the case in contrast to its potentiating effect on lethality when given concurrently with endotoxin.

To better define the applicability of their experimental findings in mice the effects of endotoxin and of cortisone on hepatic tryptophan pyrrolase activity was also studied in rats, rabbits, and guinea pigs \cite{49}. As in mouse experiments endotoxin depressed the activity of this enzyme in all species studied. Cortisone elevated the activity of this enzyme in rat and rabbit liver and protected these species from lethal endotoxin shock. In guinea pig liver the enzyme
activity was not increased by the dose of cortisone used and this was correlated with a failure of cortisone to protect guinea pigs from lethal endotoxemia. This was interpreted as support for the relationship between enzyme induction and protection from endotoxin shock (49).

Berry, Smythe, and Colwell (54) compared the inhibition of inducible liver enzymes in mice given either endotoxin or actinomycin D. It was shown that endotoxin at an LD-50 dose lowered the level of hepatic tryptophan pyrrolase in mice and prevented the induction of this enzyme by cortisone for up to 20 hrs. Actinomycin D had a similar effect. When either endotoxin or actinomycin D were given during the period of enzyme induction they both produced a rapid cessation of further enzyme activity increases and enzyme activity levels soon dropped to or below control values. However, not all enzymes were affected similarly by endotoxin treatment. Tyrosine-\( \alpha \)-ketoglutarate transaminase was induced by either endotoxin or cortisone in intact animals but was not induced by endotoxin in adrenalectomized animals, which suggested that endogenously secreted steroids might have been responsible for the endotoxin initiated rise in activity. Actinomycin D depressed the activity of this transaminase and prevented corticosteroid induction of the enzyme. It was concluded that the site of action of endotoxin and actinomycin D may appear to be similar for some enzymes but that endotoxin exhibits a specificity in the inhibition of the induction of hepatic enzymes which differentiates it from the generalized action of actinomycin D on RNA directed protein synthesis (54).

In a subsequent study Berry, Smythe, and Colwell (55) investigated the effect of endotoxin on the cortisone mediated induction
of other hepatic enzymes. In this paper it was stressed that events that occur in the first few hours after endotoxin injection are obviously important in determining the final response to endotoxin some hours later when the animal either succumbs to lethal endotoxin shock and dies or is able to resist the stress and survives. This was based on the supposition that endotoxin may prevent the synthesis of key enzymes with half-lives of only 2 to 3 hrs and thus rapidly limit the metabolic potential of the liver in maintaining metabolic homeostasis. In this study it was found that endotoxin inhibited the inductive activity of cortisone on the key gluconeogenic enzyme phosphoenolpyruvate carboxykinase. Endotoxin also prevented gluconeogenesis in the liver in response to cortisone stimulation. Induction of tryptophan oxygenase was also inhibited in intact animals and doses as low as one forty thousandth of an LD-50 or 0.01 µg significantly inhibited the induction of this enzyme following cortisone treatment of adrenalectomized mice thus demonstrating the potency of endotoxin in this biological activity. Although it had been indirectly inferred for a number of years that endotoxin prevented the synthesis of certain hepatic enzymes but not of other enzymes, the possibility of an inhibitor which depressed the activity of certain enzymes under assay conditions was only recently excluded. Rippe and Berry (542) quantitated hepatic tryptophan oxygenase in the livers of endotoxin treated and control mice using a radial immunodiffusion assay. It was consistently found that there was always less immunologically active enzyme protein after endotoxin treatment. The conclusion was drawn that endotoxin interfered with the synthetic process that resulted in enhanced levels of tryptophan oxygenase. This
conclusion was supported by a correlation between the diameter of immunoprecipitation ring and the catalytic activity of the enzyme in the liver homogenate from which it was taken. The effect of endotoxin on hepatic phosphoenolpyruvate carboxykinase was studied using a similar immunoprecipitation technique as well as an assay based on inhibition of this enzyme's activity by a specific anti-enzyme antibody (51). It was found that endotoxin inhibited the synthesis of phosphoenolpyruvate carboxykinase and thus reduced the level of assayable enzyme rather than inactivating or inhibiting the enzyme already present. Actinomycin D treatment resulted in levels of this enzyme not different from those observed with endotoxin treatment irrespective of possible differences in their mode of action. These experiments confirmed the previous conclusions of Berry, Smythe, and Colwell (55) that endotoxin can inhibit the induction of a key gluconeogenic enzyme to a stimulus which would be expected to result in its synthesis.

Snyder, Deters, and Ingle (598) studied the changes in the activity of hepatic pyruvate kinase in mice treated with endotoxin, cortisone, and saline. While endotoxin increased the activity of this enzyme, cortisone alone depressed it, and the combination of the 2 agents did not significantly change the activity of pyruvate kinase, the authors concluded that increased pyruvate kinase activity may limit gluconeogenesis in endotoxemia and thus lead to rapid carbohydrate depletion, where as cortisone may exert its protective effect at least in part by conserving phosphoenolpyruvate for subsequent gluconeogenesis. The demonstration by Pieroni and Levine (514) showing synergy between insulin, which induces this enzyme, and
endotoxin in producing lethal endotoxin shock was said to support this hypothesis. Elliott and Snyder (167) studied the levels of hepatic phosphoenolpyruvate carboxykinase in mice given endotoxin and suggested that the inhibition of this key gluconeogenic enzyme may in part account for the dramatic loss of body carbohydrate observed in endotoxemia. A decrease in the activity of this gluconeogenic enzyme would be expected to amplify the carbohydrate depleting effect of enhanced glycolysis promoted by increased pyruvate kinase activity demonstrated by these workers (598). In mice made tolerant to endotoxin, alterations in hepatic phosphoenolpyruvate carboxykinase activity did not occur following endotoxin challenge. McCallum and Berry (434) have recently investigated the physiological consequences of endotoxin treatment as they relate to gluconeogenesis and glycogen metabolism in mice in vivo.

Studies were conducted during the period between 12 and 24 hrs of fasting during which time control mice exhibited active glycogen synthesis. Endotoxin treatment severely depressed liver glycogen levels and prevented the secondary rise in liver glycogen levels and hepatic glycogen synthase activity during fasting which was presumed to be due to inhibition of enzyme induction. The incorporation of C-14-label from alanine-U-C-14, pyruvate-2-C-14, or glucose-U-C-14 into blood glucose and liver glycogen was substantially impaired in endotoxin treated mice with decreases observed as early as 4 hr after endotoxin. The administration of a glucose or pyruvate load to endotoxin treated mice did not restore their hypoglycemic blood glucose levels, gluconeogenesis, glycogen synthesis, or liver glycogen synthase activity to normal levels. The data indicated that the incorporation of glucose directly into liver glycogen
was not as severely depressed following endotoxin as was the conversion of alanine or pyruvate to hepatic glycogen. Further experiments led McCallum and Berry (434) to conclude that their results clearly established that gluconeogenesis was more severely impaired than was glycogen synthesis following endotoxin treatment.

Aside from the above mentioned reports from the laboratory of Berry as well as others which have stressed alterations in hepatic enzyme induction as key events leading to the carbohydrate depletion and profound hypoglycemia of endotoxin shock, a number of other investigators have documented other derangements in carbohydrate homeostasis in animals either given endotoxin or in septic shock. Sanford and coworkers (559) studied the mechanism of the glycogenolytic action of bacterial endotoxin in rats. As a result of their findings using adrenalectomized animals maintained on replacement doses of corticosteroid and dietary manipulations they concluded that liver glycogen may either rise or fall following endotoxin injection and that derangements in carbohydrate metabolism observed following sublethal quantities of endotoxin represent not a direct hepatotoxic effect of endotoxin, but are rather a consequence of alterations in the secretions from endocrine glands. Hamosh and Shapiro (292) pursued the cellular mechanism by which endotoxin treatment resulted in hepatic glycogenolysis and found that glycogen phosphorylase was near completely converted to the active form following the injection of a purified endotoxin. Similar changes were observed with liver homogenates and slices incubated in vitro with endotoxin. Hamosh and Shapiro (292) concluded that the adrenal gland played no important part in the phosphorylase activation
since similar changes were observed in adrenalectomized animals. Fukuda (227) studied the influence of antipyretics on endotoxin shock in intact and adrenalectomized rabbits. It was shown that although antipyretics could inhibit symptoms such as dyspnea and diarrhea and survival could be improved, the metabolic disturbances leading to hypoglycemia were unaltered and this remained the main cause of death in adrenalectomized animals. These findings lead to the postulation of a dual mechanism of endotoxin intoxication, one relating to the central fever mechanism mediated by autonomic pathways and the other resulting from the peripheral metabolic disturbances which lead to carbohydrate depletion and are responsible for the late death syndrome. It was further found that vagotonia leading to depressor responses was a prominent component of endotoxicosis. Fukuda and Akiyama (228) investigated the metabolic basis of endotoxin tolerance in rabbits. Tolerance to the lethal action of endotoxin was found to be characterized by a diminution of the liver glycogen depleting response to massive doses of endotoxin. A decreased response to thyroid extract in the tolerant state suggested that the change involved an intrinsic stability of hepatic metabolism. In experiments on dogs Fukuda and coworkers (231) studied the mechanism by which glucocorticoids protect from endotoxin shock. Glucocorticoid treatment prevented the near total depletion of hepatic glycogen characteristic of endotoxin shock. It was stressed that glucocorticoids must be given early to prevent the release of an endogenous "shock-inducing factor" as well as the carbohydrate depletion which both follow as a result of endotoxin treatment. Fukuda and Fukuda (229) investigated a suggestion of previous workers that purine
nucleotides might be involved in the stress resistance afforded by glucocorticoids. Pretreatment with 3' (2')-GMP or uridine prevented the acute deaths however, concurrent corticosteroid treatment was required for survival to be insured. In GMP treated dogs hypoglycemia could be easily corrected by glucose infusion which was never the case in control dogs. In rats pretreated with GMP, treatment with glucose prolonged survival. Fukuda and Hata (230) studied the anti-endotoxic effects of endotoxin conditioning and of glucocorticoid treatment in rats and stressed that in contrast to the hypoghesis of Berry and coworkers (57), corticosteroid protection did not relate with liver glycogen storing activity nor did it prevent the accumulation of lactate in hepatic tissue. Fukuda and Hata found that bilateral vagotomy prevented the appearance of the preterminal phase of shock which usually followed the hypotensive phase and suggested that the terminal events may be the result of progressive central nervous system effects presumably mediated by a vagal discharge. Fine and coworkers (208, 209, 518) have also stressed the role of the central nervous system in mediating endotoxin toxicity and have recently (127) implicated a corticosteroid increase in calcium conductance as a potential mechanism of the protective effect on the disturbance of excitable membranes caused by endotoxin. In conjunction with the neural mediation of endotoxin toxicity Palmerio et al. (502) reported denervation of the abdominal viscera to be an effective means of treatment of experimental shock induced by either iv or intraventricular injection of endotoxin.

The initial response to endotoxemia or acute gram negative sepsis has long been considered to be an early hyperglycemia in
association with the vasodilating and hypotensive action of endotoxin. This hyperglycemia has long been attributed to an activation of the sympathoadrenal system with concomitant hepatic glycogenolysis (131, 282, 302, 374, 444, 476, 559, 693) and an inhibition of insulin release (136-139, 189, 285, 605) as well as a reflex increase in glucagon secretion mediated by the autonomic nervous system (67, 544, 560). However, not all investigators have observed this pattern of events, especially when less than massive acutely lethal doses of endotoxin were administered. Berk et al. (45) administered doses of 0.25 to 2.0 mg per kg endotoxin to dogs and monitored metabolic as well as hemodynamic parameters. In slightly over one half of the animals a limited hyperglycemia during the first hour was observed. In these animals after the modest hyperglycemic response and in all other dogs a steady fall in blood glucose to hypoglycemic levels was observed. The data were interpreted as indicating that endotoxin produced a metabolic effect which increased glucose utilization above the rate of glucose production and thus lead to hypoglycemia. It was suggested that hypoglycemia was a factor influencing survival at all endotoxin doses studied but with the larger doses glucose treatment was ineffective due to the activation of additional lethal factors. Burrows (89) studied endotoxemia in the conscious pony given an iv dose of only 50 to 200 micrograms of endotoxin per kg. An early hyperglycemia was observed with blood glucose levels reaching twice control at 1 or 2 hrs after endotoxin. Hypoglycemia rapidly appeared with blood glucose levels less than 40 mg per 100 ml attained by 4 to 6 hrs after endotoxin. Surviving animals showed a slow return of blood glucose toward normal
by 24 hrs. Holper, DiLuzio, and coworkers (318) also studied the effects of lethal doses of endotoxin alone in conjunction with their studies of the sensitizing effect of lead acetate treatment on endotoxin shock in baboons. They noted an initial hyperglycemia at 3 hrs after endotoxin followed by a hypoglycemic state at 24 hrs after endotoxin which was said to be dose dependent. Blood lactate showed a dose related increase which reached higher levels at each sampling time until a peak of over 8 times control was attained. Blood pyruvate levels showed no significant change thus producing over a 6 1/2 fold increase in the lactate to pyruvate ratio which accompanied the onset of severe acidosis. Spitzer, Hinshaw and coworkers (605) studied the effects of endotoxin shock on the metabolism of the non-hepatic splanchnic area in baboons. In samples drawn at greater than 1 hr after endotoxin injection a depression of serum free fatty acids was observed in conjunction with a hypoglycemia. This was attributed to a depletion of liver glycogen coupled with a failure of gluconeogenesis to keep pace with the progressively increasing carbohydrate utilization. Decreases in the arterial levels of acetoacetate and in the ratio of acetoacetate to beta hydroxybutyrate suggested to the authors that the tissues deprived of adequate glucose and free fatty acids increased their utilization of ketone bodies. The increase in arterial lactate observed both in early and late endotoxin shock was attributed in part to a failure of the liver to clear lactate or in severe cases to the production of lactate by the liver. It was concluded that although there were interesting differences in the mesenteric vascular response in the baboon compared to the canine model, the systemic metabolic hallmarks
of endotoxin shock, hypoglycemia and lactacidemia, were still present. Tissue levels of metabolites in lethal hemorrhagic and endotoxin shock were compared by Blackwood and associates (63) using splenectomized dogs. The results suggested that tissue changes occur soon after endotoxin which produce a rising level of lactate despite a relatively normal concentration of ATP in liver, small bowel, and muscle. In the ensuing discussion W. Schumer stressed the failure of gluconeogenesis in the conditions of both endotoxic and hypovolemic shock and related this to a mitochondrial defect produced by endotoxemia which might explain the ATP depression and lactic acidosis observed by Blackwood et al. (63). Tissue substrate fluxes in endotoxemia and hemorrhage were studied by Printen et al. (526) who devoted special attention to the role of sympathetic activation evoked by a progressive hypotension. Divergent patterns of free fatty acids and glucose were seen with the former being more elevated in endotoxin shock and with glucose rising in the hemorrhaged animals but a relative hypoglycemia observed in the endotoxin shocked dogs. This lead to the conclusion that metabolic responses to endotoxin are more complex than the mass sympathetic response to hypotension.

LaNoue et al. (381) studied gluconeogenesis by liver slices of animals which had received a lethal dose of endotoxin. Endotoxin treatment in vivo severely depressed the ability of the liver slices to synthesize glucose from radiolabeled pyruvate and caused a net utilization of glucose from the incubation medium which suggested a mechanism for the hypoglycemia found in endotoxin treated rats in vivo. An increased rate of O₂ consumption by liver slices of endotoxin
treated rats demonstrated that the depression of gluconeogenesis was not due to a generalized decrease in cellular viability. A depression in the activity of glucose-6-phosphatase was found in endotoxin poisoned rats and it was suggested that a depression of this key gluconeogenic enzyme may contribute to the impaired gluconeogenic activity of liver slices from endotoxin treated rats. However, it was postulated that some factor other than the observed depression of gluconeogenic enzymes must be operative to account for the severe hypoglycemia in endotoxin treated rats. Williamson, Refino, and LaNoue (670) studied the effect of endotoxin on hepatic carbohydrate metabolism using the isolated perfused rat liver and analysis of tissue levels of gluconeogenic intermediates. The results demonstrated that the energy cost for gluconeogenesis was greater in livers of endotoxin treated rats since they consumed $O_2$ at an elevated rate and lactate at a normal rate but failed to produce glucose. The proposed mechanism of failure of gluconeogenesis was believed to be either a failure of fructose diphosphatase or a stimulation of phosphofructokinase resulting in an accumulation of fructose-1,6-diphosphate with a depletion of fructose-6-phosphate and glucose-6-phosphate. The fructose diphosphate would activate pyruvate kinase thus diminishing net phosphoenolpyruvate formation and hence net glucose formation while ATP utilization and $O_2$ consumption remain elevated. A mitochondrial defect was suggested to be the underlying cause of the elevated fructose diphosphate levels. Also notable was the observation that the infusion of a short chain free fatty acid, oleate, increased lactate uptake and doubled glucose production in the livers of normal rats but had no such effect in the
livers of endotoxin treated rats, further suggesting altered mitochondrial metabolism. A recent report from this laboratory (204) has stressed the transition from hepatic glycogenolysis to gluconeogenesis as a critical stage in the metabolic adaptation and resistance to lethal endotoxin shock. In both fed and fasted rats given an LD-100 dose of endotoxin terminal hypoglycemia and hepatic glycogen depletion were observed. When gluconeogenesis was stimulated by an overnight fast, rats were resistant to endotoxin which suggested that the induction of gluconeogenesis may be depressed in endotoxin poisoned rats. When gluconeogenesis was evaluated using the method of Cornell et al. (130) endotoxin poisoned rats had an impaired hyperglycemic response and a reduced incorporation of C-14-label from alanine-C-14 into blood glucose which suggested a defect in hepatic function. That the impaired gluconeogenesis involved an intrinsic hepatic metabolic lesion was demonstrated using hepatocytes isolated from rats given endotoxin in vivo.

C. Sub-Cellular and Insulin-Like Actions of Endotoxin

The mechanism by which shock and endotoxemia produce their characteristic derangements in cellular metabolism have been the subject of numerous investigations. Berry and coworkers (57) first reported a dinitrophenol like uncoupling action of endotoxin on isolated liver mitochondria. Mela and coworkers (441) studied rat liver mitochondrial function after hemorrhagic or endotoxic shock and found the abnormalities to be similar. State 4 activity was increased indicating loose coupling while state 3 activity was decreased which depressed respiratory control ratios to less than one third the control values. ADP utilization and ATPase activity were depressed suggesting a possible
defect in adenine nucleotide translocase. More recently Mela and coworkers (442) found about a 50% loss in mitochondrial Mg$^{++}$ following endotoxin shock which was accompanied by an almost complete loss in the capacity of the isolated mitochondria to transport Ca$^{++}$. Decreases in mitochondrial respiratory control ratios were also correlated with apparent rupture of hepatic lysosomes in vivo. When ruptured hepatic lysosomes were added to normal mitochondria in vitro the respiratory control ratio was found to drop exponentially due to an increase state of respiration indicating loose coupling. ATPase activity was likewise depressed by adding ruptured lysosomes in vitro. It was concluded that the experimental evidence suggested a role for lysosomal enzymes in the etiology of the mitochondrial lesions of shock.

In experiments in which endotoxin was added to isolated mitochondria in vitro, Mela (440) reported that some of the effects of endotoxemia in vivo were produced including alterations in the energy-dependent Ca$^{++}$ uptake which suggested that endotoxin directly affected mitochondrial membrane transport. Greer, Epps, and Vail (274) also reported inhibition of state 3 respiration and depressed respiratory control in rat liver mitochondria. Since these effects were observed in the presence of 12.5 molar Mg Cl$_2$ it was suggested that the loss of mitochondrial cations may be an important toxic action of endotoxin on rat liver mitochondria. Schumer and coworkers (565) extended the study of endotoxemic alterations in mitochondrial function to skeletal and cardiac muscle mitochondria since these tissues are responsible for a major fraction of basal oxygen consumption and abnormalities in cardiac function are known to occur in shock. State 3 respiration was
depressed in the mitochondria of both tissues and there was a marked loss of respiratory control. As had been observed in liver mitochondria, skeletal muscle mitochondria of endotoxin treated rats showed evidence of loose coupling. It was suggested that elevated levels of endogenous compounds such as free fatty acid anions or thyroxine may be responsible for the mitochondrial alterations observed.

Baue and coworkers (37) have studied ATP utilizing and ATP yielding processes in liver cell fractions isolated from animals in early and late shock and found a decrease in ATP yielding reactions as reflected by mitochondrial state 3 activity coupled with an increase in ATP dependent reactions as evaluated by Na⁺ - K⁺ activated ATPase activity. It was suggested that these changes may lead to alterations in membrane transport which may be an important etiologic factor in the initiation of cell injury in shock. In a subsequent study from the laboratory of Baue (109), the effect of low ATP levels on glucose uptake was studied in an effort to define the role of ATP as a feedback inhibitor of glucose uptake by skeletal muscle. It was found that anoxia alone was a sufficient stimulus to increase glucose uptake in intact rat soleus muscle which led to increased lactic acid production. It was considered relevant that Gould and Chaudry (275) had shown that insulin was not necessary for basal glucose uptake by soleus muscle, but insulin was required for anoxia stimulated glucose transport. This suggested to the authors that shock per se did not produce any alteration or modification in the function of endogenous insulin. Rutenberg et al. (552) studied the hepatic cyclic AMP response to glucagon in shocked rabbits. Basal cAMP levels were depressed in shocked rabbits below
control levels and the cAMP response to a supramaximal dose of glucagon in vivo was only one half that observed in control rabbits and not significantly greater than basal levels. In contrast, the in vitro response to non-specific adenylate cyclase stimulation by NaF was not different in the shocked and control liver preparations even though the response to a series of glucagon concentrations was severely impaired at all dose levels in the shocked livers. ATP levels in shocked livers were about ten-fold lower than in controls. It was concluded that the observed defect appeared to reside in the glucagon receptor without any intrinsic malfunction in adenylate cyclase or its catalytic properties; the ATP deficit was considered inconsequential to cAMP generation. The reported defect in glucagon stimulated adenylate cyclase activity in shock stands in contrast to the studies of Bitensky et al. (62) showing a selective stimulation of epinephrine sensitive adenylate cyclase in the membrane fraction of the livers of mice given endotoxin. An in vitro sensitization of the adenylate cyclase activity of the membrane fraction to epinephrine stimulation was also reported. The authors suggested that adenylate cyclase may be an initial locus of action of endotoxin and may explain the glycogenolytic response to endotoxin as well as the well known sensitivity to catecholamines observed in endotoxemia.

Gimpel and coworkers (254, 255) have also reported effects of endotoxin on hepatic adenylate cyclase activity in guinea pigs. In vivo endotoxin treatment significantly stimulated basal adenylate cyclase activity in liver biopsies taken 4 hrs after endotoxin injection, however a possible residual effect of hormones secreted in vivo was not excluded. Addition of endotoxin to liver homogenates in vitro produced a very limited but
statistically significant stimulation of adenylate cyclase activity which was only a small fraction of the stimulation observed with NaF. It was further shown that the increase in cAMP production could not be attributed to either an inhibition of phosphodiesterase activity or to the production of a dialysable factor which stimulated adenylate cyclase. The authors related their findings to the well recognized glycolytic stimulation, glycogenolytic activation, and glycogen synthase inhibition observed after endotoxin treatment which were suggested to be due to a direct interaction between endotoxin and membrane bound adenylate cyclase.

Mitruka (456) has studied influence of gram negative sepsis on gluconeogenic and glycolytic enzyme patterns by assaying the levels of key enzymes in these pathways in tissues of rats infected with _S. typhimurium_. The glycolytic enzymes--glucokinase, phosphofructokinase, and pyruvate kinase--were all elevated 30 to 170% in kidney, spleen, and skeletal muscle of infected animals. _S. typhimurium_ treatment caused a 100 to 150% increase in key glycolytic enzymes in liver which was accompanied by a 20 to 80% decrease in key hepatic gluconeogenic enzymes. Isotopic tracer studies supported the inferences based on enzyme assays by showing that the metabolic flux in liver tissue of infected rats was increased toward the direction of glycolysis. It was noted that the effect of infection with _S. typhimurium_ was opposite that of starvation when enzyme profiles were compared, especially when the influence on hepatic gluconeogenic enzymes was compared. Smith and coworkers (405, 406, 596) have studied alterations in metabolites in mice given acutely lethal doses of non-gram negative bacteria. In experiments similar to
those of Berry et al. (57) homogenates were prepared from the carcasses of mice and analyzed for various metabolites and enzymes (405). A steady downward trend in carcass glucose was observed in mice given _S. aureus_ reaching 1/5 of control at 4 hrs and 1/12 of control at death. Further experiments showed the carbohydrate depletion could not be attributed to the bacteria per se but must be due to a host response. It was concluded that as in lethal endotoxemia, severe derangements in carbohydrate metabolism were part of the death mechanism in mice dying from staphylococcal infection. Smith (596) later reviewed this work and presented additional findings. In contrast to endotoxin poisoning which similarly depletes blood glucose and body carbohydrate stores, there was no increase in blood lactate in staphylococcal infected mice. This suggested that although blood pressure had been observed to be decreased, oxygenation of the tissues was still adequate. As in endotoxin shock, when the mice were pretreated with insulin a given dose of _Staphylococci_ produced a markedly more severe infection. When the oxidative activity of mouse liver was investigated _in vitro_ the tissue from infected mice showed a much higher rate of O2 uptake as well as evidence of uncoupling. In other experiments mice were given exogenous glucose and this produced a significant lengthening of survival time but no decrease in lethality. When given in massive doses testosterone and adrenal glucocorticoids were found effective in prolonging survival. Smith concluded that death from staphylococcal infection probably has its ultimate cause in changes in liver function and is intimately related to carbohydrate metabolism. Recently this group (466) has carefully examined lipid metabolism in mice with lethal _Staphylococcus septic_
shock. Increased plasma free fatty acids in infected mice were a most conspicuous feature with levels approaching ten times the normal fasted value. The authors suggested that the free fatty acids are probably not utilized because of the hypoglycemic state in infected animals. The rise in free fatty acids with a proportionately greater rise in saturated fatty acids was suggested to be the mechanism leading to mitochondrial damage and an uncoupling of oxidative phosphorylation.

In investigating the effects of endotoxin on metabolism at the cellular and sub-cellular levels, Woods and coworkers (677, 678) as well as others (252, 660) have shown striking similarities in the effects of endotoxin and those of insulin on carbohydrate metabolism. When tissues were either treated with endotoxin in vitro or obtained from animals poisoned with endotoxin in vivo, a marked stimulation of glycolysis was demonstrated. This glycolytic stimulation was observed only in tissues in which a similar response to insulin treatment was observed. It was suggested by Woods and coworkers (677) that endotoxin and insulin act as independent entities to stimulate glycolysis by competing for the same site of action which appeared to be at the level of glucose phosphorylation. These authors (677) concluded that the glycolytic stimulation effected by endotoxin like that of insulin, apparently involved an overcoming of the glycolytic restraint imposed by the action of a previously described (676) endogenous anti-insulin mechanism presumably involving endogenous steroid hormones, but which was accentuated by the addition of exogenous steroids. The capacity of various native endotoxin preparations as well as detoxified endotoxin to stimulate glycolysis was shown to parallel the in vivo activity of these preparations
in evoking characteristic host reactive responses. This evidence was interpreted as indicating that the features of the structure or composition of endotoxin responsible for the characteristic actions in vivo are the same as those involved in the influence on cellular glycolysis.

It was further suggested that the protective effects of cortisone in endotoxemia reported by Berry and coworkers (57) might be explicable at least in part by the ability of this steroid hormone at high levels to overcome the stimulation of glycolysis induced by endotoxin and thus play a role in the prevention of the severe depletion of carbohydrate reserves which occurs in endotoxin shock.

D. Hypoglycemic Activity of Endotoxin in Animals Sensitized to Lethal Endotoxin Shock

The hypoglycemic activity of endotoxin has been found to be accentuated in animals made sensitive to lethal endotoxemia by any of a number of experimental manipulations (50). The correlation was found to be so close that others (574) have suggested that hypoglycemia may be the common mechanism by which a number of experimental treatments sensitize animals to lethal endotoxin shock. Farrar and Watson (182) observed what they described as a progressive and profound hypoglycemia in carbon tetrachloride treated guinea pigs given a lethal dose of endotoxin. In control 12 hr fasted guinea pigs given a lethal dose of endotoxin a characteristic biphasic blood glucose response of hyperglycemia followed by a decline below control values was observed as the shock syndrome progressed. In guinea pigs treated with carbon-tetrachloride an immediate and progressive fall in blood glucose followed the endotoxin injection, reaching a mean value of 4 mg per 100 ml
at 4 hrs after endotoxin. The blood glucose response was not recognizably different in the carbon tetrachloride treated guinea pigs when the endotoxin dose was reduced to a level just sufficient to kill 90 to 95% of the sensitized animals. Glucose tolerance studies in carbon tetrachloride treated guinea pigs with and without endotoxin treatment showed no difference in rate of disappearance of glucose from the blood irrespective of endotoxin treatment which was interpreted as indicating that endotoxin treatment did not accelerate the rate of glucose removal at an early time after treatment with endotoxin. A failure in hepatic glucose production following endotoxin was felt to be the most likely explanation for the severe hypoglycemia in these animals with pre-existing liver damage. The impairment appeared to be in gluconeogenesis rather than a defect in glycogen storage which was elevated in carbon tetrachloride poisoned guinea pigs. Attempts were made to protect the animals from lethal hypoglycemia elicited by endotoxin by a constant infusion of 20% glucose at a rate of 50 mg glucose per 100 gm per hr. Glucose did not protect the animals and the small increase in mean survival time was not statistically significant. It was concluded that whereas hypoglycemia may be the cause of the early deaths following endotoxin in carbon tetrachloride sensitized guinea pigs, there appeared to be more complex changes which ultimately induced a lethal outcome even if exogenous glucose was supplied. Berry and coworkers (56) confirmed the original observations of Suter et al. (623) that mice infected with BCG were hyperreactive to bacterial endotoxin, and the time of maximal sensitivity to endotoxin the reticuloendothelial system was known to be activated (326). On this basis it was felt that BCG infection may be expected
to cause a resistance to endotoxin (56). However, it was found that the metabolic responses of BCG infected mice were abnormal and exhibited certain similarities in their metabolic responses to those previously found in endotoxin poisoned normal mice (53). Berry and coworkers (56) found that BCG infection depleted mice of liver and muscle glycogen and total body carbohydrate which were at a minimum when mice were most sensitive to endotoxin. It was suggested that the earlier approach of carbohydrate levels of endotoxin treated BCG infected mice to those found at death in normal animals given a lethal dose of endotoxin may explain why BCG infected mice die as early as 4 hrs after endotoxin where as normal survive usually about 18 hrs or more. Glucose injections were incapable of protecting BCG infected mice from the lethal effects of endotoxin. It was suggested that perhaps BCG infected mice have an impairment also in their ability to metabolize glucose. Finkelstein (210) studied the mechanism of the lethal effect of minute doses of endotoxin in chick embryos during their period of maximal sensitivity at 10 to 11 days incubation. In contrast to epinephrine, norepinephrine, histamine, serotonin, and acetylcholine, insulin in small amounts was very toxic to the embryos. A decrease in insulin sensitivity paralleled the increased resistance to endotoxin as chicks matured. Blood glucose studies showed that both insulin and endotoxin caused a severe hypoglycemia coincident with death. Finkelstein (210) observed that the chick embryo develops the capacity to handle exogenous insulin at the same time it becomes capable of coping with endotoxin and that the gross and microscopic pathology of embryos succumbing to insulin is similar to that observed in embryos given a lethal dose of endotoxin.
More recently, Shands and coworkers (574) have hypothesized and presented supporting evidence that it is the hypoglycemic activity of endotoxin which is responsible for the many fold sensitization to lethal endotoxemia produced by a variety of experimental manipulations known to markedly lower the resistance of animals to lethal endotoxin shock. This was supported by the observation of severe potentially lethal hypoglycemia in mice or rabbits made hyperreactive to endotoxin by BCG infection, zymosan treatment, or adrenalectomy. Detailed studies designed to delineate the mechanism of the hypoglycemic response to endotoxin in BCG infected mice were reported by Shands et al. (575). It was in these studies that the possibility of a role of insulin in the development of the lethal hypoglycemia induced by endotoxin was first given serious consideration. In addition the possibilities that endotoxin rapidly induces a hypermetabolic state or that it caused a block in gluconeogenesis in BCG infected mice were considered. However, Shands et al. (575) concluded from their studies in BCG infected mice that

The possibilities of an induced hypermetabolic state and an induced release of insulin in response to endotoxin as causes for the hypoglycemic responses were essentially ruled out. In addition, no clear-cut evidence of an insulin like action by endotoxin was found in the in vivo setting.

They interpreted their evidence as implicating an impairment of gluconeogenesis to be the mechanism by which BCG infection rendered mice hyperreactive to endotoxin. The persistence of a hypoglycemic activity of endotoxin in alloxan-diabetic BCG infected mice and the failure to observe a rise in mean blood glucose in mannoheptulose treated BCG infected mice following endotoxin poisoning were presented as evidence
that endogenous insulin secretion was not a requisite for endotoxin to exert its hypoglycemic activity in BCG infected mice. Based on the report of Pieroni and Levine (514) which demonstrated a marked sensitization to lethal endotoxin shock in normal mice by prior treatment with a sublethal dose of insulin, Shands and coworkers (577) re-examined the possible role of endogenous insulin secretion in the development of the profound hypoglycemia observed in BCG infected mice poisoned with endotoxin. The possibility was considered that although BCG infected mice per se were not markedly sensitive to insulin, perhaps endotoxin rendered such mice exquisitely sensitive to insulin. It was found that in combination with insulin a sub-lethal dose of endotoxin rapidly produced a severe convulsive hypoglycemia which was not produced by either agent alone when administered to BCG infected mice. Further studies were designed to examine the possibility that endogenous insulin secretion may have played a role in the previously reported (574, 575) unsuccessful attempts to protect endotoxin poisoned BCG infected mice from lethal endotoxemia by the parenteral administration of glucose. Since mannoheptulose prevented the abrupt fall in blood glucose to lethal levels following an intravenous dose of glucose it was suggested that endogenous insulin secretion played a role in this reactive hypoglycemia. The authors concluded that both the insulin sensitivity and the reactive hypoglycemia in response to exogenous glucose were attributable to an impairment of gluconeogenesis in endotoxin poisoned BCG infected mice and there were situations in vivo in which insulin may play a role in the development of a lethal hypoglycemia in endotoxin poisoned BCG infected mice. In search of the mechanism underlying the
endotoxin induced depression of gluconeogenesis and marked sensitivity to lethal shock in BCG infected mice, Shands and Senterfitt (570) studied the morphologic changes and the release of liver enzymes to serum in BCG infected mice given endotoxin. BCG infection alone caused an elevation in serum levels of hepatic enzymes which was maximal at the time the infected mice were most susceptible to endotoxin. Other experiments demonstrated that endotoxin treatment of BCG infected mice caused an acute elevation hepatic transaminases as well as lysosomal enzymes in the serum. Elevations in serum enzyme activities also occurred in normal animals given endotoxin. In morphological studies Shands and Senterfitt (576) found that BCG infection alone disturbed normal liver architecture by producing granulomas in the parenchymal tissue. After endotoxin treatment there was evidence of severe focal injury and circulatory stasis in the liver as well as glycogen depletion. Shands and Senterfitt concluded that their observations of morphological changes in BCG infected mice given endotoxin were comparable to what had been previously observed by others when endotoxin was given to non-sensitized animals but differed in that the changes occurred more rapidly and with a much smaller dose of endotoxin.

Work from our laboratory has sought to determine the mechanism by which lead acetate causes a 2,000 fold sensitization to the lethal effects of endotoxin in rats. After finding that lead acetate treatment did not affect the intravascular clearance of endotoxin nor did it impair the ability of liver homogenates to detoxify endotoxin, it was suggested that the mechanism of lead sensitization may relate to leads ability to alter metabolic responses during endotoxemia (202).
In a subsequent publication the metabolic alterations induced by endotoxin in lead sensitized rats were reported to be a profound hypoglycemia, lactacidemia, and depletion of hepatic glycogen (200). In studies with hepatocytes isolated from rats treated with lead acetate in vivo a depression of gluconeogenic capacity was found. It was suggested that the alterations in carbohydrate metabolism, in particular the depressed hepatic gluconeogenesis, were responsible for the sensitization to endotoxin produced by lead acetate. A preliminary report has also demonstrated a sensitization to insulin produced by lead acetate treatment (84) and this may play a role in the pathogenesis of lethal endotoxemia in lead sensitized animals. Endotoxin induced hypoglycemia has also been observed in subhuman primates sensitized to endotoxin. In a recent report from the laboratory of DiLuzio (318) the results of studies on the interaction between lead acetate and bacterial endotoxin were presented. The results clearly demonstrated that in presence of lead acetate, trace amounts of endotoxin can produce acute death. Although hypoglycemia and hyperlactic acidemia were observed in baboons given a lethal dose of endotoxin alone, only a more severe hypoglycemia with more nearly normal lactate levels were observed in lead sensitized animals. It was suggested that the finding of rapid onset of hypoglycemia and hypotension in the baboon contributed to the possibility of such a response in man.

E. The Response of the Endocrine Pancreas to Endotoxemia

The response of the endocrine pancreas to clinical as well as experimental hemorrhagic, traumatic, cardiogenic, and burn shock has been the topic of a number of published studies. However, few
Investigators have studied the pancreatic control of carbohydrate metabolism in endotoxemia and the related conditions of sepsis and infection. Bloom and associates (68) studied the influence of stress on the plasma levels of glucagon in baboons and rhesus monkeys. When conscious monkeys were given an iv injection of a small amount of endotoxin over a threefold elevation of plasma glucagon was observed in samples collected 2 hrs after endotoxin treatment. No change in blood glucose was observed and insulin levels showed only minimal deviation from the control values. The authors believed the increase in glucagon in response to endotoxemic stress was due to adrenergic stimulation of the pancreatic alpha cells mediated by the autonomic innervation known to richly supply the islets (680). Rocha and coworkers (544) studied glucagon secretion in clinical and experimental bacterial infections. Severe or moderate infections were found to be associated with striking hyperglucagonemia and a related increase in the insulin requirement to maintain normoglycemia. Glucagon was also believed to play a role in the catabolic response to infection as reflected by increased urinary nitrogen excretion and presumably an increased rate of gluconeogenesis. In the severely infected patients plasma insulin was more than doubled over control values but the greater relative increase in plasma glucagon caused the insulin to glucagon molar ratio to be depressed below normal values. This was thought to be the factor largely responsible for the negative nitrogen balance observed during infection. Lindsey et al. (406) studied the response of the pancreatic alpha cells in traumatized patients with and without accompanying shock. Hyperglucagonemia associated with hyperglycemia and an insignificant elevation of plasma insulin were
observed soon after the onset of shock. Patients undergoing surgical trauma who did not have associated hypotension had normal plasma glucagon levels. It was concluded that the injury induced modifications in pancreatic endocrine function, i.e., a relative hypoinsulinemia and an absolute hyperglucagonemia, must be regarded as protective physiological responses, and therapeutic efforts to correct the elevated glucagon to insulin ratio would be contra-indicated on theoretical grounds. Also noted were the risks of administering glucose to shocked patients with their relatively fixed insulin to glucagon ratio.

Shambaugh and Beisel (573) were the first to report the insulin response to a glucose stimulus during acute infection of humans with Pasteurella tularemia. Within 8 to 24 hrs of the onset of clinical illness a significant decrease in glucose tolerance was observed in conjunction with a slight but statistically significant fasting hyperglycemia. By 2 weeks after antibiotic treatment glucose tolerance was found not to reflect the insulin response to the glycemic stimulus. The three hour insulin output following the glucose load was significantly greater during gram negative infection than during the control studies. Since the magnitude of the insulin response was inversely related to the rapidity of glucose disappearance during the infection as it was during control studies, the authors suggested that the effectiveness of insulin was dampened during sepsis by a peripheral inhibition of insulin action. From the observations of altered glucose tolerance and elevated insulin responses associated with an exaggerated rise in blood pyruvate following glucose, the authors attributed the changes to be due at least in part to a glucocorticoid excess. Also
suggested was a possible role of glucagon in the enhanced insulin response during sepsis. Beisel (40) reviewed the work from his laboratory and of others on the interrelated changes in host metabolism during generalized infections illness. He stressed that a number of models had been used to study acute infectious disease the metabolic response to these diverse diseases developed in a stereotyped manner. In all diseases plasma amino acids were depressed, and in some cases the principle gluconeogenic amino acids--especially alanine, were more severely depressed. The apparent increase in flow of amino acids from muscle and peripheral tissues to liver during infection was confirmed using the non-metabolized amino acid cycloleucine as a tracer in experiments with rats. Beisel (40) suggested that in early infection gluconeogenesis is increased at the expense of plasma amino acids. In terminal stages of overwhelming infection or endotoxemia it is Beisel's view that blood sugar values can not be maintained, tissue carbohydrate stores become exhausted, and plasma glucose precursor levels rise as hepatic gluconeogenic function fails. Felig and associates (193) have presented evidence that pattern is not limited to gram negative septicemia but may also be observed in acute viral disease. Rayfield et al. (533) recently studied plasma glucagon and insulin during a self-limiting viral infection in human volunteers. As had been previously reported there was a depression of glucose tolerance and a marked increase in the insulin response to an iv glucose load. Plasma glucagon was significantly elevated during the infection in the fasting state but was significantly depressed by the hyperglycemia following glucose loading as it had been in control studies. Growth hormone was tripled in the
fasting infected patients and exhibited a paradoxical increase following iv glucose in infected patients, whereas no change was observed in control studies. Urinary cortisol was increased 3 times over control values in the infected patients. Free fatty acids showed a two-fold rise during the infection. Hyperinsulinemia following glucose during the infection was considered compatible with the elevated plasma levels of glucagon, growth hormone, and free fatty acids. Rayfield and coworkers (532) recently reexamined the hypersecretion of growth hormone in response to iv glucose in conscious rhesus monkeys infected with Diplococcus pneumoniae or with Salmonella typhimurium. There were neither glucose intolerance or FFA abnormalities during the height of the illness. The paradoxical large increase in growth hormone following iv glucose was also observed in this model. Alpha adrenergic blockade by phentolamine did not block the growth hormone response, however an extended period of chlorpromazine treatment prevented it suggesting that it was mediated by a central nervous system mechanism. In a similar study this group (247) investigated the glucoregulatory hormonal responses during acute sepsis in rhesus monkeys. No differences in fasting growth hormone, fasting glucose, or the disappearance rate of glucose were found when control studies were compared with those following the induction of sepsis. However, during sepsis there was a relative hyperinsulinemia in response to glucose as well as a fasting hyperglucagonemia. Plasma amino acid levels were markedly lowered during sepsis. O'Donnell et al. (490) studied the relationships of hind limb energy fuel metabolism to the circulatory responses to sepsis in unanesthetized fasted pigs. On the basis of cardiovascular responses
3 days after cecal ligation the septic pigs were assigned to either a high flow group or a low flow group. Mean blood glucose was depressed in both septic groups compared to control values, but the depression was less severe in the low flow group. In the high flow septic group plasma insulin was significantly increased to over 4 times control levels. This elevation in plasma insulin was present despite a significant depression of blood glucose to 30 mg per 100 ml below control fasting levels. In the low flow septic group the insulin elevation was not statistically significant. Hindlimb glucose uptake was the same in all three groups suggesting a peripheral insulin resistance in the high flow septic group. Hind limb lactate production was strikingly elevated in the low flow septic group but not in the high flow septic animals. Arterial lactate levels reflected their rates of production. In both groups of septic pigs arterial free fatty acids were markedly depressed below the control fasting level. In sepsis the hind limb released fatty acids into the plasma in contrast to its net removal of circulating free fatty acids in control pigs. Glycerol release was depressed in high flow sepsis but was markedly elevated in low flow sepsis. The authors indicated that their study had demonstrated sepsis to be associated with 2 distinct patterns of substrate fuel utilization for energy production both of which differed significantly from the metabolic pattern of starvation. The metabolic pattern in sepsis appeared to be related to the circulatory status. The role of insulin as a key regulator of the flow and utilization of substrates was stressed by the authors. Insulin was viewed as playing a critical role in modulating fuel utilization in the high flow septic state, however
peripheral insulin resistance was also recognized. In the low flow septic state peripheral anaerobic glycolysis was the major fate of glucose in peripheral tissues. It was proposed that the factor responsible for the difference in the metabolic pattern in high and low flow sepsis was that catecholamine activity was enhanced in the low flow septic state but not elevated in high flow sepsis. Clowes et al. (120) extended the previous work of O'Donnell et al. (440) to include clinical observations of human sepsis. To examine the effect of glucose, potassium, and insulin on the high output and low output responses to septic peritonitis, experiments were carried out as previously described up through the baseline measurements in septic pigs. The pigs were then infused over a 10 min. period with 1 gm glucose per kg, 1.5 units of insulin per kg, and 10 mEq potassium per kg. At 15 min. and 2 hrs after the infusion circulatory and metabolic measurements were repeated. In response to the infusion of glucose, potassium, and insulin there was an increase in cardiac output in both low and high flow septic pigs. Both groups also demonstrated an increase in hind limb oxygen consumption and in glucose uptake. In the clinical study Clowes et al. (120) classified patients as high flow or low flow sepsis on the basis of if they were normotensive or hypotensive. Both groups were markedly hyperglycemic, however this parameter may have been influenced by previous iv treatment with glucose. In the high flow septic patients plasma insulin was elevated to over twice the control value. Ten patients were treated with glucose, insulin and potassium using the doses which had been used in the pigs. It was reported that the infusion caused a rise in both mean arterial blood pressure and in cardiac
index. The infusion caused a fall in plasma free fatty acids. A hypothesis was proposed that certain of the phenomena observed in the high output septic state may be protective mechanisms to prevent the hypoinsulinemia associated with cardiovascular insufficiency which characterizes the low output state. It was envisioned that reduced insulin may play a role in producing the low flow state. It was noted that in other studies these investigators found that the hypoinsulinemia and prolonged severe proteolysis could be averted or ameliorated by infusions of amino acids instead of glucose. Clowes et al. (120) proposed the possibility that low plasma insulin depressed cardiovascular function in septic patients, however stated that the mechanism underlying this relationship was not clear.

Griffiths, Groves, and Leung (280) reported a study designed to gain added insight into the relationship between plasma glucose, serum triglycerides, and hormones regulating the levels of these metabolites in dogs subjected to gram negative septicemia. In dogs which died within 12 hrs there were wide variations in the plasma insulin levels. Values as high as four times the control insulin level were observed at 4 hrs following bacteremia. In these dogs dying rapidly a fall in plasma glucose was associated with the increases in mean plasma insulin. In dogs surviving greater than 12 hrs a progressive hypoglycemia was associated with no change in plasma insulin. It was hypothesized that the rise in insulin observed in this study may have been antagonized by other hormones such as glucagon and growth hormone as well as the rise in cortisol observed in this study. The hypoglycemia observed in this study was attributed to a combination of
accelerated glucose utilization coupled with an impaired hepatic gluco-
neogenic function. Elevated insulin levels were suggested to be one
factor which impaired gluconeogenesis by limiting peripheral amino acid
release. Cryer, Herman, and Sode (137) initiated a study of carbohy-
drate metabolism in baboons subjected to gram-negative septicemia. Each
animal was given a dose of 10 ml per kg of a suspension of $10^{11}$ washed
E. coli per kg by iv injection. Animals surviving beyond the 2 hr point
were given 500 ml of 5% albumin in saline every 30 minutes until the 4
hr point was reached. Following E. coli injection significant hyper-
glycemia was observed and blood glucose remained elevated until the 120
min. point. By 240 min. significant hypoglycemia was observed. De-
spite the transient hyperglycemia plasma insulin dropped below the mean
level observed in saline treated controls but the difference was not
statistically significant until 2 hrs after E. coli. Regression analysis
showed that the change in plasma insulin was significantly correlated
to the changes in plasma glucose in septic baboons. The hyperglycemic
phase following E. coli injection was suggested to be due at least in
part to a lack of the expected insulin response. The suppression of
insulin release was thought to be due to catecholamine suppression of
the beta cell response to hyperglycemia and it was considered likely
that this effect was mediated by the islets' sympathetic innervation
rather than attributable to elevation of plasma epinephrine arising
from the adrenal medulla. It was further suggested that the hypo-
insulinemia could not be explained on the basis of either an increased
distribution space or an increase in the rate of degradation and that
the depressed plasma levels truly reflected a decreased pancreatic
insulin secretion. Cryer and coworkers (138) conducted experiments to further clarify the mechanism of the initial hyperglycemia and hypoinsulinemia in septic baboons. Intravenous glucose tolerance tests with 0.5 gm of glucose per kg at 20 min. after E. coli injection showed the insulin response to be suppressed compared to the insulin response in non-septic controls. During septicemia the half-time for glucose disappearance following the iv load was increased nearly two-fold from 41 to 75 min. In insulin tolerance tests 0.2 Units of regular insulin was given iv 20 min. after the induction of sepsis. In these experiments the insulin treated septic baboons all became severely hypotensive and usually died before the 2 hr point was reached. Both the control and septic baboons exhibited decreases in blood glucose following insulin. Although initially hyperglycemic the septic baboons exhibited a greater depression of blood glucose in response to insulin, thus demonstrating that they were not insulin resistant at this early time after sepsis. In septic baboons the half-time for iv insulin disappearance was increased over two-fold from a control value of 16 min. in the controls to 33 min. in the septic animals. It was concluded that insulin resistance was not a significant factor in the hyperglycemia observed after E. coli injection. It was further indicated that insulin could prevent the hyperglycemia, however the data showed that insulin treated animals died more rapidly and as noted by the authors exhibited a more rapid and profound hypotensive response. In a later study Cryer et al. (136) reported the influence of phentolamine induced alpha adrenergic blockade on septicemia in the baboon. When phentolamine was infused throughout the period of septicemia the depression in serum insulin
and hyperglycemia did not develop in the 2 1/2 hr observation period. The relationship between the degree of suppression of insulin secretion and survival time was studied using smaller doses of $3.0 \text{ to } 4.2 \times 10^{10}$ E. coli per kg and all animals were volume loaded with large quantities of albumin in saline. With this reduction in dose from $10^{11}$ E. coli per kg used in the previous studies only one half of the animals had depressed insulin levels. The duration of survival ranged from 2.1 to 24.3 hrs and was significantly correlated with the serum insulin levels expressed as a percent of the mean basal level at 30, 45, or 60 min. after E. coli. Five additional animals were given infusions of albumin containing insulin, glucose, and potassium. Despite evidence of a metabolic effect of the infused insulin as indicated by a 75% reduction in plasma free fatty acid concentration the mean overall survival of the treated baboons was 9.8 hrs compared to a mean survival time of 9.7 hrs for the controls given only albumin solution. In reviewing their work the authors noted that their evidence indicated that, "Stress-induced inhibition of insulin secretion appears to be a relatively high-threshold phenomenon. Definite suppression of insulin secretion occurred in only 3 of 6 septicemic baboons given a smaller but still lethal dose of E. coli." It was concluded that their observations did not support the pharmacologic use of insulin in the treatment of septic states. Cryer et al. (139) further considered the possibility that depressed insulin secretion may well be a component of a coordinated adaptive metabolic response to life threatening stress; however it was felt essential to examine the possibility that insulin lack may be detrimental to survival in baboons with severe sepsis. Baboons
were injected with 3.0 to 4.4 x 10^{10} E. coli per kg and treated with either 2.5% albumin in saline or 2.5% albumin containing glucose, insulin, and potassium. Mean arterial blood pressure showed little difference between the animals treated with glucose, insulin, and potassium and those given only the albumin. In septic baboons infused with glucose, insulin, and potassium a marked hyperinsulinemia and hyperglycemia were maintained. Blood lactate was severely elevated in all septic baboons and glucose, insulin, and potassium treatment had no effect on this metabolic parameter. Following the 30 min. sample the mean plasma pH of the insulin infused group was consistently lower than that of the septic controls at all sampling times. The overall duration of survival was not improved by glucose, insulin, and potassium infusion. It was claimed that it appeared that glucose, insulin, and potassium infusion prolonged the survival of a sub-group of animals which showed evidence of more severe initial stress of septicemia as reflected by a greater inhibition of insulin release. The meaning of this depression from control values is not readily interpreted since the authors conceded from experiments in which phentolamine was infused that their animals were already stressed to the point of inhibited insulin release and accompanying hyperglycemia in the control restrained state. More recently this group (129) has investigated changes in serum free fatty acids during E. coli septicemia using their previously described baboon model. The dose of E. coli was the lower yet still lethal dose of 5 x 10^{10} organisms per kg. In these experiments no significant hyperglycemia was observed, however hypoglycemia was evident by 90 min. after E. coli injection. Mean serum insulin was reduced early in
the course of septicemia, but by 90 min. after injection the septic animals were no longer hypoinsulinemic when compared to controls despite their reduced mean serum glucose levels. The controls showed no significant changes in serum free fatty acids. In the septic animals serum free fatty acids showed significant decreases to 72% of basal values at 60 and 90 min. after *E. coli* injection. The reduction in free fatty acids occurred despite the early depression of mean serum insulin levels. All septic baboons died within 2 hrs of *E. coli* injection. It was suggested that the depression of serum free fatty acid levels may have been due to the elevation of mean plasma lactate to values of about 80 and 130 mg per 100 ml at 60 and 90 min. after *E. coli* injection respectively. Again the authors explained that the stress the animals were subjected to prior to shock induction may have influenced the control values.

The reports of Cryer et al. (136, 137, 139) of severe hyperglycemia accompanied by a hypoinsulinemia in acute gram-negative septicemia in the baboon are contrasted by recent studies of clinical sepsis. Gump and coworkers (285) studied the iv glucose tolerance in patients with indwelling hepatic vein catheters which permitted measurement of hepatic blood flow by a dye infusion technique. In the septic patients in the basal state a slightly elevated arterial glucose level (94 ± 14 mg per 100 ml) was associated with an elevation of basal serum insulin to 150% of control values, an elevated hepatic glucose production, and an increase in forearm glucose consumption. Following the infusion of 0.5 gm glucose per kg there was a reversal of hepatic glucose production to glucose uptake in control subjects which was accompanied by an increase in forearm glucose utilization. Following glucose
infusion peripheral glucose uptake was also evident in the septic patients, however hepatic glucose production was not suppressed. Serum insulin determinations from hepatic as well as peripheral blood samples showed an exaggerated insulin response in the septic patients. The authors concluded that failure of insulin secretion does not appear to be a factor in the failure of glucose infusion to reverse hepatic glucose production in septic patients since splanchnic insulin release was significantly greater in the septic patients. It was concluded that the failure of insulin to shut down hepatic glucose production was a key factor in the altered carbohydrate metabolism observed in clinical sepsis.

F. Purpose and Objectives

The overall objective of this study was to evaluate the relationships between deranged homeostatic control of carbohydrate metabolism in endotoxemia, endogenous insulin secretion in endotoxemia, and host defense to lethal endotoxin shock. Special emphasis was directed toward answering the question of whether endogenously secreted insulin might play a role in the development of the profound hypoglycemia and glycogen depletion known to accompany endotoxin shock. In addition, the possible role of altered carbohydrate homeostasis in the perpetuation of the shock syndrome and ultimate demise of the challenged host via influences on the reticuloendothelial system was explored.

Specifically, the objectives of the present study were:

1) to evaluate and establish whether a significant relationship between the parameters of glycemic state, insulin secretory state, and the susceptibility to the induction of lethal endotoxin shock in
normal fed and fasted rats,

(2) to investigate the gluconeogenic capacity and glycogenic functions of the liver in vivo in endotoxin-treated rats and to determine the influence of prior glucose treatment on these parameters,

(3) to determine the temporal responses of both blood glucose and serum insulin in rats challenged with a potentially lethal dose of endotoxin and to relate these dynamic responses to resistance to the lethal effects of endotoxin,

(4) to evaluate the glucose tolerance and insulin secretory response to a glycemic stimulus in rats previously treated with endotoxin,

(5) to determine if endotoxin treatment induces a hypermetabolic state with respect to carbohydrate oxidation and to evaluate if this response is influenced by treatment with exogenous glucose,

(6) to determine the role of insulin in the control of aerobic glycolysis in endotoxin poisoned rats by manipulations which are known to influence insulin secretion,

(7) to determine the influence of insulin as well as insulin induced hypoglycemia on the phagocytic activity of the reticuloendothelial system and to relate this to resistance to experimental shock induction, and

(8) to determine the rate of disappearance of biologically active endotoxin from the vascular compartment of normal rats and to investigate how this is influenced by procedures known to sensitize rats to the lethal effects of endotoxin; lastly, to consider the possibility that the hypoglycemia of shock may play a role in the patho-
genesis and perpetuation of the shock syndrome at the level of reticuloendothelial host defense.
A. Animals and Care

Adult male rats of the Holtzman strain were obtained directly from the Holtzman Company (Madison, Wisconsin). All rats—except those made diabetic with streptozotocin—weighed 300 ± 20 grams when used in the fed state and approximately 15 grams less when used after an overnight fast. All rats were received at least 7 to 10 days prior to use and thus they were allowed to acclimate to our laboratory animal quarters. Throughout this period and during the experimental periods—except when overnight fasting is designated—Purina Laboratory Chow (Ralston Purina, St. Louis, Missouri) and fresh tap water were available ad libitum. Water bottles were not removed during overnight fasting or during experimental periods—except in the experiments dealing with the clearance of endotoxin from the vascular compartment; with these rats access to water terminated at the time of iv endotoxin injection.

Overnight fasting consisted of removing the food from the rat cages at approximately 6 PM on the evening immediately preceding the experiment.

Temperature in the animal room was maintained at 27 ± 1°C throughout these experiments and was monitored with a recording thermometer (Tempscribe, Bacharach, Pittsburgh, Pennsylvania). A lighting
A schedule of alternating 12 hr light-dark periods was employed with lights on from 7:00 AM to 7:00 PM. The rats were routinely kept in hanging stainless steel wire mesh cages with 5 animals per cage (7x10x17 inches).

B. Endotoxin Preparation

The endotoxin used was the Boivin lipopolysaccharide prepared from *Salmonella enteritidis* and purchased from Difco Laboratories (Detroit, Michigan). A single batch of this commercial preparation was used in all lethality and metabolic studies. This batch was distributed under the supplier's control numbers #275421 and #503046. In the experiments concerning the clearance of endotoxin *in vivo* and *in vitro*, another batch was used which had been assigned the control number #216338. In all cases the endotoxin was dispersed in sterile pyrogen-free 0.9% saline (Baxter, Travenol Laboratories Inc., Deerfield, Illinois) prior to either *in vivo* injection or addition to *in vitro* perfusion media. The endotoxin was weighed on a Cahn electrobeam balance (Model G, Cahn Division of Ventron Corporation, Paramount, California). In all lethality experiments and metabolic studies the endotoxin was prepared in saline on the day of the experiment such that a 1 ml volume contained the desired dose. In *in vivo* clearance studies the endotoxin was similarly prepared each day at a concentration of 500 micrograms per ml of saline and a volume to achieve the specified dose was administered.

In the *in vitro* clearance studies a stock solution of 200 µg endotoxin per ml saline was prepared and stored frozen in glass tubes. On the day of the experiment one tube was thawed and dilutions of this stock solution were prepared as known endotoxin standards; a 1.5 ml aliquot was also added to the 150 ml volume of perfusion media. There was no
evidence of deterioration of the lethal properties of endotoxin stored
frozen in saline for the three month period during which these studies
were in progress (Tables 18, 19, and 20).

C. Lethality Experiments

The specific protocols of the lethality experiments are given
in the "Results" section. In general rats were given known doses of
endotoxin suspended in 1 ml of saline by intravenous injection under
light ether (Anaesthesia Grade, Mallinckrodt, St. Louis, Missouri)
anæsthesia. All intravenous injections were made via the dorsal vein
of the penis as employed in this laboratory for a number of years. This
route of injection has been recently evaluated by Nightingale and
Mouravief (486) and was found to give the same pharmacokinetic data as
intracardiac injection. These investigators found no evidence of
shunting of blood from this site to the hepatic portal system prior to
entering the systemic circulation. Control groups received either no
further treatment or were treated with saline or the diluent of the
agent used in experimental groups. Agents given subcutaneously were
injected under the skin overlying the scapular region. Intraperitoneal
injections were made at midabdominal level on the right side of the mid-
line with care to avoid damage to vital organs. In all cases lethality
was tabulated at 48 hrs following injection of endotoxin as in previous
studies from this laboratory (198, 202). The vast majority of the shock
deaths occurred at 12 to 24 hrs after endotoxin injection as was found
in previous studies (202). In selected experiments survival was fol-
lowed up to 10 days and it was found that deaths beyond the 48 hr in-
terval were seldom observed.
For lethality experiments the results were totaled for each day that a particular protocol was repeated. When the number of groups prohibited all groups being tested on the same day a fraction of the experimental groups were evaluated along with the appropriate control groups. By including controls for the experimental groups on any one day it was considered justifiable to total all animals in any one group when a particular experimental series was completed. The totals were used for all further data analysis.

The significance of difference between groups was assessed using the chi-square test with the correction factor of Yates for small samples included where appropriate (603). The chi-square tests were run on a PDP-12 digital computer (Digital Equipment Co., Maynard, Mass.) using the programs appended (p. A2). These programs compute the value of chi-square and the degrees of freedom. The statistical significance of the chi-square values for the specified degrees of freedom was read from conventional tables (158, 545). A "p" value of 0.050 or less was considered statistically significant.

D. Measurements of Gluconeogenesis and Glycogenesis in vivo

The gluconeogenic capacity and hepatic glycogenic function was studied in endotoxin treated and control rats in vivo. For this test rats were anaesthetized with an ip dose of approximately 12.5 mg of pentobarbital or a somewhat smaller dose if their condition dictated. The pentobarbital solution used in this and subsequent experiments consisted of 1 part Nembutal (pentobarbital sodium, 50 mg/ml, Abbot Laboratories, North Chicago, Illinois) mixed in 3 volumes of normal saline on the morning of the experiment.
A control sample of 0.1 ml of blood was collected from the cut end of the tail in a spot plate which had been prepared by drying one drop of 5,000 U per ml heparin (Hog gut, Sigma, St. Louis, Missouri) in water in each spot. Each rat then received an iv load of 100 mg L-alanine (Sigma) containing 4 μCi of uniformly labeled L-alanine-U-C-14 (Amersham/Searle, Arlington Heights, Illinois) in a volume of 1 ml saline adjusted to pH 7.4. The dose of L-alanine-U-C-14 was verified by counting an aliquot of the solution prepared for injection on each occasion the experiment was repeated. Thirty minutes after the alanine load the rat was bled by cardiac puncture. The abdomen was rapidly opened and the majority of the large right lobe of the liver was excised and placed in ice cold saline. The excised liver lobe was then rapidly blotted, two samples were cut, weighed on a Roller Smith balance, and immediately immersed in 15 ml graduated centrifuge tubes each containing 2 ml of 30% KOH. The sample for chemical glycogen determination weighed between 100 and 200 mg while the sample for C-14 labeled glycogen weighed about 500 mg.

Blood glucose was determined on a deproteinized aliquot of the initial control sample and on one portion of the cardiac blood by a glucose oxidase endpoint colorimetric method (Glucostat 4X, Worthington Biochemicals, Freehold, New Jersey). Carbon-14 incorporation into blood glucose was determined in \( \text{Ba(OH)}_2 - \text{ZnSO}_4 \) supernatants extracted in a mixed resin slurry as originally described by Exton and Park (176). The resins employed were 100 mg Dowex-50X and 200 mg of Dowex-1X and were washed twice in distilled deionized water before use. They were purchased through Sigma Chemical Company (St. Louis, Missouri).
In view of the results of Herrera, Knopp, and Freinkel (305) who performed essentially identical experiments with the exception of using a 1 millimole dose of C-14 pyruvate in female rats, further purification of blood glucose from the resin supernatant was deemed unnecessary. Using intervals between isotopic labeled substrate injections to time of sampling as long as 30 min, as used in the experiments of the present study, Herrera et al., showed that non-resin extracted C-14 activity other than glucose accounted for less than 0.1% of the radioactivity present in the initial resin eluates. In their study less than 0.07% of the alanine-C-14 applied to the resin was found in the eluate. Based on their data these authors also argued that an interval of up to 30 min was sufficiently soon after injection to minimize artifacts due to reutilization of labeled glucose synthesized from the labeled precursors.

The loss of labeled glucose in the resin was determined by processing a sample of glucose-U-C-14 in the exact same manner as the blood samples and results were corrected accordingly.

Liver glycogen was isolated for chemical analysis by a slight modification of the method of Good et al. (268), hydrolysed in 1 N H$_2$SO$_4$ in a boiling water bath for 3 hrs, and the liberated glucose determined on a neutralized aliquot by the glucose oxidase technique employing Tris buffer as described by Dalqvist (147) as was used in the blood glucose determinations. Carbon-14 incorporation into liver glycogen was determined by isolating the liver glycogen by a sodium sulfate-ethanol method (261, 498), suspending the washed glycogen in water and determining the radioactivity.

Both blood glucose and liver glycogen samples for $^{14}$C
determination were incorporated into a commercial solubilizer (PCS, Amersham/Searle) and counted in a Searle/Analytic (Des Plaines, Ill.) IsoCap 300 liquid scintillation system. The blood glucose samples which correspond to the glucose recovered from one twenty-second of a ml blood were counted for 20 minutes. The liver glycogen samples which corresponded to the glycogen recovered from one-sixteenth of a gram of liver were counted for 100 minutes. Background count rate was subtracted from the observed count rates. Counting efficiency was determined using the samples channels ratio method and a commercial (Amersham/Searle) set of C-14 quenched standards. Each sample was corrected to its corresponding DPM. All analyses were carried out in duplicate.

The means and standard errors of mean were calculated for the various parameters measured or calculated from measured values for all rats in each experimental treatment group using the computer program in the appendix (p. A4 ). Differences between groups were evaluated for statistical significance by the unpaired Students "t" test using the program given in the appendix which calculates both the "t" value and the corresponding "p" value for the specified degrees of freedom. A "p" value of less than 0.050 was taken as a statistically significant difference.

E. Intraperitoneal Glucose Tolerance and Insulin Response

Overnight fasted rats were given an iv injection of 1 ml volume at time zero which consisted of either isotonic saline or endotoxin in saline. At two hours after the iv injection all rats were given an ip injection of 2 ml saline or 400 mg glucose in that volume of saline. At intervals from the time of iv injection to 12 hours,
subgroups of the 4 groups of treated rats were anaesthetized with approximately 12.5 mg of pentobarbital ip, prepared in saline as described for the gluconeogenesis experiments. Immediately upon loss of the righting reflex the rats were bled by cardiac puncture into disposable 10 cc plastic syringes. Large (18 gauge) needles were used to avoid hemolysis. Only the first 3 to 4 ml of blood which could be rapidly withdrawn was collected. The needle was removed from the syringe and a few drops of blood were placed in a heparinized spot-plate, the remainder being expelled and allowed to clot in disposable glass culture tubes on ice. One tenth ml of the heparinized blood was pipetted from the spotplate for glucose determination by a glucose oxidase technique after hemolysis and deproteinization with Ba (OH)$_2$ and Zn SO$_4$. The blood samples in the glass culture tubes were kept on ice prior to centrifugation at not more than 2 hrs after collection. Serum was prepared by centrifuging the clotted blood in a refrigerated centrifuge at 4°C. The serum was aspirated into small glass vials, immediately frozen, and stored at -45°C.

**Immunoreactive Insulin Assay**

Immunoreactive insulin was determined on duplicate 100 µl aliquots of the serum samples through the employment of commercial radioimmunoassay kits in which guinea pig anti-porcine insulin antibodies are covalently coupled to Sephadex particles (Phadebas Insulin Test, Pharmacia Laboratories, Uppsala, Sweden). Since rat insulin was not available for use as a standard, porcine insulin was used as a relative reference. A single batch of anti-insulin antibody was used in all studies to avoid possible differences in immunologic cross
reactivity of rat and porcine insulin.

Seven insulin standards of 3.2, 8, 16, 32, 80, 160, and 320 microunits per ml were prepared using the lyophilized porcine insulin standard supplied with the commercial kits and following the dilution procedure described by the manufacturer (Phadebas Insulin Test, Clinical and Technical Information, 1973) which utilizes a combination of single and serial dilution steps. All details of the assay procedure were as described by the manufacturer. Serum samples, radioiodinated insulin, and standards were pipetted with Oxford Samplers. One ml aliquots of the Sephadex anti-insulin serum were pipetted with a 2 ml Cornwall syringe dispenser. The assay mixtures were incubated for 18 to 24 hrs at room temperature in 12 x 55 mm plastic tubes purchased from Pharmacia on a horizontal RIA Shaker (Pharmacia). After incubation the capped tubes were centrifuged for 2 minutes in a 96 position IEC swinging bucket head at 1,500 xg for 2 minutes. The caps were removed and the tubes were centrifuged at 1,500 xg for 5 minutes to sediment the Sephadex coupled anti-insulin serum and bound insulin. All except the bottom 5 mm of fluid was aspirated from each tube using a pipette with an adjustable collar coupled to a water vacuum pump. The sedimented Sephadex was washed 3 times using 2 ml volumes of 0.9% saline adjusted to pH 7.0-7.5 with 1 N Na OH. Each tube was counted for 10 minutes in a Searle/Analytic model 1185 gamma crystal counter with preset discriminator windows for Iodine-125.

Each time the test was repeated, duplicates of the buffer blank, the 7 insulin standards, a Pharmacia lyophilized pooled human serum, and aliquots of 2 different pools of rat serum and plasma which had been
stored at 45°C were run both preceding the experimental samples and again following the samples. This permitted assessment of both the intraassay and interassay variation. All experimental samples were run non-diluted in duplicate. If the coefficient of variation for any pair of samples was greater than 20% the results were discarded from further consideration.

A linear transform for the assay was found through the limits of 3.2 and 320 μUnits insulin per ml when the insulin concentration was expressed on a log scale on the abscissa and the percent of the amount of I-125 labeled insulin bound in the absence of added non-labeled insulin was expressed on a linear ordinate. Using this transform a computer program was written to evaluate the experimental results. This program calculated the apparent amount of insulin in each tube of the replicates. The values for each of the replicates of any one sample were then used to calculate the mean insulin concentration, the standard deviation, the standard error of the mean, and the coefficient of variation for that sample. Using this method of calculation the calculated mean insulin concentration of the replicates is not biased by the binding of the iodinated insulin being a function of the logarithm of the non-labeled insulin concentration. The computer program is given in the appendix (p. A7).

This immunoassay system was also evaluated to be certain that endotoxin itself did not influence the antigen-antibody reaction or influence the apparent level of insulin in rat serum.

The mean and standard error of the mean were calculated for each of the subgroups using the mean insulin value for the serum of
each rat. The non-paired Students "$t$" test was used to evaluate the
significance of differences between groups. The programs used for these
calculations appear in the appendix (p. A5).

F. Recovery of Expired $^{14}$C-$\text{CO}_2$ from Rats given Uniformly Labeled $^{14}$C-D-Glucose

The recovery of glucose carbon label as expired CO$_2$ was studied
in rats for a 4 hour period immediately following the ip injection of
20 $\mu$Ci of uniformly labeled D-glucose-C-14. The glucose dose consisted
of either a tracer amount (less than 16 micrograms) of D-glucose-U-C-14
(Amersham/Searle) in 2 ml of normal saline or of this amount of labeled
glucose mixed with 400 mg of carrier glucose in 2 ml of saline.

All rats were fasted overnight preceding the experiment to
minimize glycogen stores. Immediately after receiving the 2 ml ip in-
jection containing the labeled glucose, the rats were placed in 2 liter
volume bottles containing about 750 ml of wood shavings to absorb
moisture. The bottle had 2 fittings for the attachment of tubing to
holes in its cover. The inlet had approximately 15 cm of tubing at-
tached to the inner side of the cover to aid in mixing the air in the
bottle during the experiment. The outlet of the jar was connected by
approximately 45 cm of Tygon tubing (3/8" o.d., 1/4" i.d.) to the in-
let of a commercial CO$_2$ trapping bubble apparatus (liver perfusion
accessory, MRA Corp., Boston, Massachusetts). The inlet of the CO$_2$
trapping apparatus was led to a frittedglass cone submersed in the
bottom of a column of the trapping solution about 17 cm in depth.
The outlet of the CO$_2$ bubble trap was connected by Tygon tubing to a
suitable gas flow meter. The outlet of the flow meter was connected
through a needle valve to the laboratory vacuum source. The rate of airflow through the system was regulated at 500 ml per min. by adjustment of the needle valve and determined on the gas flow meter in series with the bottle and the CO₂ trap.

In another series of experiments on 34 rats with two of the CO₂ bubble traps in series, it was found that essentially no C-14 labeled CO₂ passed into the second trap. In almost all cases the count rate of the samples were near that of background. The activity found in the second trap was always less than 0.3% of that in the first trap even at the time of the final sample. Thus, in the experiments reported here only one CO₂ trap was used.

The CO₂ trapping solution consisted of 2 volumes of 2-methoxy-ethanol and 1 volume of ethanolamine as described by Jeffay and Alvarez (338). The initial volume in the CO₂ traps was 52 ml. Duplicate 1 ml samples were collected just before and at the designated times after the rats were placed in the bottles. The trapping fluid remaining in the system was thoroughly mixed prior to each sample collection. A 1 ml Oxford Sampler with disposable tips was used to pipette the samples. The samples collected immediately prior to placing the rat in the bottle served as the background count rate which was subtracted from all subsequent samples. The mean count rate of the background samples rarely exceeded 25 CPM demonstrating that the C-14 label was not carried over from one experiment to the next. The other pairs of samples were collected at 15 min. intervals for the first 60 min. and then at 30 min. intervals until 240 min. after the rat had been placed in the bottle.
Into each one ml sample of CO₂ trapping solution contained in a liquid scintillation vial (Spectravial, Amersham/Searle) was added 10 ml of a fluor consisting of 70% (by volume) toluene (AR grade, Mallinckrodt, St. Louis, Missouri), 20% 2-methoxyethanol (99%, Aldrich Chemical Company, Milwaukee, Wisconsin), and 10% ethanolamine (95%, Aldrich) plus 7.0 grams per liter PPO (2,5-Diphenyloxazole, Scintillation Grade, Amersham/Searle) and 0.15 gm per liter POPOP (p-bis-2-(5 phenyloxazolyl) benzene, Scintillation Grade, Amersham/Searle). This fluor efficiently counts C-14 CO₂ trapped in ethanolamine; C-14 CO₂ was not liberated from the ethanolamine even when the CO₂ content of the samples was high. Both commercial fluores (PCS, Amersham/Searle) and simple toluene fluores were found to liberate ethanolamine bound CO₂ when they are added to high concentrations of CO₂. Furthermore, commercial fluores containing Triton are not suitable for they give rise to a long life chemiluminescence when they are added to strong bases including ethanolamine.

Counting efficiency was determined by the method of internal standards utilizing toluene-C-14 which had been calibrated against a National Bureau of Standards certified standard (Amersham/Searle). In representative experiments 50 μl aliquots were pipetted into samples after they had been counted as well as into vials containing only fluor. The samples were recounted and the efficiency was calculated. There was no change of counting efficiency with time of sample collection and the mean efficiency of 77.87% was used for all further data analysis.

The actual dose of C-14 glucose administered to each rat was
determined by counting aliquots of the solution injected and correcting for counting efficiency and fraction of the dose counted. Thirty 10 μl replicates of each glucose solution were pipetted in scintillation vials using an Oxford sampler. Ten ml of PCS (Amersham/Searle) was added to each vial and they were each counted to 800,000 counts. Efficiency was estimated by the samples channel ratio method and the disintegration rate was multiplied by 200 to give the amount of C-14 per 2 ml dose expressed as DPMs.

All experimental samples were counted for at least 10 minutes or usually for 20 minutes or to 800,000 counts with the low count per minute reject set to terminate the counting program of samples that did not reach 100 counts in either channel within the first minute. Repeated countings of the same samples over a matter of days showed there to be no detectable phosphorescence or chemiluminescence. Results were expressed as the percent of the injected dose of C-14 recovered in the CO₂ trapping fluid at each specified sampling time. The percent recovery was calculated from the mean count rate of the duplicate one ml samples on a specially written computer program which incorporates corrections for background subtraction, counting efficiency, removal of label in previous samples, and volume of trapping fluid remaining at the time a particular pair of samples were drawn. This program is given in the appendix (p. A8).

The mean and the standard error of the mean were calculated for each group of rats at each of the specified times of sample collection. These were entered onto a magnetic tape storage system along with the rats weights before and after an overnight fast and with the blood
glucose values for the diabetic rats. By means of specially written computer programs unpaired Student's "t" tests were performed on the percent Carbon-14 label recovered at each of the corresponding times. The programs for the storage of data and its recall and the "t" test computations appear in the appendix (p. A9 and p. A10).

G. Induction of Streptozotocin Diabetes and Evaluation of the Diabetic State

Rats of a mean body weight of 330 gm in the fed state at the time of treatment were made diabetic by the intravenous injection of 25 mg streptozotocin per rat. This experimental agent was kindly supplied by W.E. Dulin of the Upjohn Company, Kalamazoo, Michigan. The streptozotocin was dissolved in citrate buffer (0.1 Molar in saline) at pH 4.5 as described by W.E. Dulin (personal communication). The streptozotocin was dissolved at a concentration of 100 mg per ml not more than 2.5 minutes before its injection into rats lightly anaesthetized with pentobarbital to facilitate rapid handling. At either 6 or 7 days following the streptozotocin treatment and after an overnight fast the rats were of a mean body weight of about 270 gm when used in the experiments. The polydipsia, polyphagia, and polyuria evident in these rats as well as the mean weight loss of 60 grams suggested a diabetic state. Urine from all rats so treated was positive for glucose by the Ames test tape evaluation. Only those rats were used which had a blood glucose of greater than 300 mg per 100 ml after an overnight fast as judged by the Ames Dextrostix reflectance meter (Ames Company, Division of Miles Laboratories, Elkhart, Indiana) method of estimating blood glucose. The blood sample used was obtained from the freshly
cut tip of the tail. The applicability of the use of this reflectance meter method of blood glucose analysis on rat blood has been documented in the literature (336).

H. Carbon Clearance Evaluation of RES Phagocytic Function

Carbon clearance in both fed and overnight fasted rats was evaluated by the method of Biozzi et al. (61) as modified by Filkins and Di Luzio (205). A special preparation of biological ink (C11/143a, Guenther Wagner, Hanover, West Germany) was diluted with normal saline to a concentration of 32 mg of carbon per ml. The carbon concentration was determined in a Beckman DU spectrophotometer using 1 x 1 cm cuvettes at a wavelength of 675 millimicrons. The previously published (205) conversion factor of 0.040 optical density units per mg carbon per liter of solution was used in calculating the carbon concentration of dilutions of the stock solution. All experiments reported were conducted using the same diluted stock solution of 32 mg per ml carbon. All rats were anaesthetized with approximately 12.5 mg of pentobarbital ip at least 15 min. prior to carbon injection. This anaesthesia does not alter the rate of carbon clearance in rats (348). A dose of 8 mg of carbon per 100 gm body wt. was given by iv injection via the dorsal vein of the penis. All doses were calculated from the weight of the rat on the evening before the test irrespective of whether they were fed or fasted overnight. Following carbon injection tail blood samples were collected in a spot plate containing dry heparin at 2 or 3 minute intervals until 30 minutes after the carbon injection. Samples for the densitometric determination of carbon consisted of 25 μl of fresh heparinized blood lysed in 5 ml of 0.1% sodium carbonate in
distilled water in 13 x 100 mm Hycel cuvettes. Samples were read against a blank consisting of 25 μl of tail blood from that rat obtained just prior to carbon injection and similarly lysed in 5 ml of the sodium carbonate solution. A Klett-Summerson photoelectric colorimeter (Klett Mfg. Co., New York, New York) was used to read samples with a red filter (#66, approximate spectral range 640 to 700 millimicrons) in place. Semilogarithmic plots of the densitometric values expressed in Klett Units were constructed with the time after carbon injection plotted on the linear abscissa. The best straight line was fitted through the 10 to 15 points by visual inspection disregarding any single aberrant points. The rate of disappearance was expressed as a half-time in minutes in the conventional manner.

Tail blood samples of 0.1 ml were collected immediately prior to carbon injection and at the time of collection of the final sample 30 minutes later. These samples were analyzed for glucose by the glucose oxidase method previously described. A mean blood glucose value of 30 mg per 100 ml or less was defined as hypoglycemia.

The statistical significance of the differences in carbon clearance half-times were evaluated by the unpaired Student's "t" test. The tendency of the blood glucose to fall or rise in each group was assessed for statistical significance by the paired Student's "t" test. The relationship between the parameters of carbon clearance half-time and mean blood glucose was assessed by calculating Pearson's coefficient of correlation and computing the p value for the appropriate number of degrees of freedom. The computer programs for each of these statistical tests or calculations are given in the appendix (p. A11 and p. A13).
I. Evaluation of Endotoxin Clearance in vivo

The clearance of biologically active endotoxin from the vascular compartment of the rat was assessed through the employment of the lead treated rat lethality bioassay as described by Filkins (198). Following an initial iv injection of either 250, 500, or 1,000 \( \mu \text{g} \) of endotoxin in saline freshly prepared on the day of the experiment at a concentration of 500 \( \mu \text{g} \) per ml, blood samples were collected at various times from 15 min. to 240 min. after the endotoxin injection. In one series of experiments repeated 0.5 ml samples were collected from the tail of each clearance rat at 15, 30, 60, 120, and 240 min. after the injection of 500 \( \mu \text{g} \) endotoxin. In all other experiments single samples were collected from each clearance rat by open chest cardiac puncture after rapid induction of ether anaesthesia. All clearance rats were of a weight of 300 \( \pm \) 1 grams and were in the fed state with the exception of the insulin hypoglycemic rats and their controls which were of a body weight of 300 \( \pm \) 1 gm on the evening preceding their overnight fast.

The blood samples were either collected directly into heparinized spot plates (tail blood) or collected in glass syringes prepared by rinsing in 5,000 units per ml heparin in water and dried in an oven. The blood samples were appropriately diluted to 2.5% to 10% blood in saline prior to assay in groups of 300 \( \pm \) 20 gm fed rats which received an iv injection of 13 micromoles of lead acetate in 1 ml of distilled water immediately following 1 ml of the diluted endotoxin containing blood. Other groups of assay rats received known doses of endotoxin in saline immediately preceding the dose of lead acetate.
Lethality in all assay groups was tabulated at 48 hrs after the injections.

The probit transform as described by Finney (211, 212) was found to give a linear relationship between the log of the endotoxin dose and the probit of the assay group lethality throughout the range of endotoxin doses of interest in lead treated rats. Using this transform the mean dose of endotoxin per assay rat was estimated from the lethality in that group of assay rats as compared to the probit line determined in other assay groups given known doses of endotoxin. The endotoxin content of the blood samples was then calculated from the dilution factor for those samples.

Using a semilogarithmic analysis comparable to that used in the carbon clearance experiments the mean half-time for the disappearance of biologically active endotoxin from the vascular compartment was calculated. Regression by the least squares method and interpolation programs used in these calculations appear in the appendix (p. A11, A13, A14, A15, A16, and A17).

Tolerance to endotoxin was induced by the iv injection of 1 mg of endotoxin in 1 ml of saline on days 1, 3, and 5. These rats were used for clearance experiments on day 7.

Bilateral adrenalectomy was performed through a single dorsal midline incision one hour before the clearance dose of 500 μg endotoxin. Surgical trauma control rats underwent a similar operation with the exception of extripation of the adrenal glands. Both procedures were conducted with the rats anaesthetized with ether.

Insulin hypoglycemia was produced by the injection of 0.5 units
of Lente insulin subcutaneously into overnight fasted rats one hour before the clearance of endotoxin. Control overnight fasted rats received 12.5 μl of saline sc one hour prior to the same 500 μg dose of endotoxin. In this group the statistical significance of difference in lethality of the assay rats receiving similarly diluted blood samples was assessed by the chi-square test at 3 hours after the test dose of endotoxin.

J. Evaluation of Endotoxin Clearance in vitro by the Perfused Rat Liver

Livers were surgically isolated from approximately 300 gm fed male rats and perfused in a commercial modification (Metalloglass Corporation, Boston, Massachusetts) of the recirculating liver perfusion apparatus first described by Miller (448). The basal perfusion media consisted of a balanced salt solution formulated by Schimassek (563) without pyruvate or lactate but with glucose at a concentration of 100 mg per 100 ml. When the perfusate consisted of Schimassek's solution only, 150 ml containing endotoxin at a concentration 2 μg per ml was the initial media. In a second series of experiments 10% rat serum was incorporated into the perfusate. On the day before the experiment 50 ml of fresh rat blood was collected by cardiac puncture without the use of heparin. After the blood had begun to clot in the glass centrifuge tubes they were ringed and stored overnight in a refrigerator at 4°C. On the day of the experiment the tubes were centrifuged and the serum collected. In a final series of perfusion experiments 50% heparinized rat blood in Schimassek's solution was used. Fed rats of about 300 gm body weight were anaesthetized with ether.
They were given an iv injection of 500 Units of heparin in saline and after 2 minutes for mixing of blood they were bled by cardiac puncture on the morning of the perfusion experiment.

All samples of perfusate which originally contained 2 μg per ml of endotoxin were diluted one to one with saline before being assayed for endotoxin in lead sensitized rats as described above. Throughout these experiments samples were kept on ice from the time they were removed from the perfusate and diluted with saline until they were bioassayed in the lead treated rats. When it was specified that samples were incubated they were incubated in the perfusion chamber at 37°C for the specified time. In each experiment appropriate controls were included to be certain that the endotoxin did not adhere to the perfusion apparatus or tubing and that the endotoxin was not inactivated by any of the perfusion media independent of the liver.

In these experiments never more than 3 minutes elapsed between the time of cannulation of portal vein and initiation of perfusion in the chamber. During the major part of this period the liver was being perfused with glucose free Schimassek's solution from a Mariette bottle. The temperature of the perfusion system was monitored with a small thermistor probe placed between the lobes of the liver and maintained at 37°C. The viability of the liver was assessed by its gross appearance, the rate of perfusate flow, the rate of bile secretion from the duct cannulated at the time of preparation of the liver for perfusion, and by histological slides prepared from each liver after the perfusion experiment was completed.

The significance of difference between assay group lethality
was assessed in two ways. First, chi-square comparisons were made using raw lethality data. Secondly, the method of probit analysis was applied using 95% confidence intervals about the best regression line calculated as described by Finney (212).
CHAPTER IV
RESULTS

A. Effects of Alterations of Insulin Status on Endotoxin Shock Lethality

Initial experiments evaluated the influence of insulin on endotoxin shock lethality. Fasting and treatment with mannoheptulose were used to depress insulin secretion; while glucose (Chapter IV, Section C) was used to increase insulin secretion. The experimental design, results, and a statistical analysis are presented in Table 1.

As shown in both Table 1 and Figure 1 doubling the endotoxin dose (i.e., from 1 to 2 mg/rat) significantly increased lethality in both fed (Groups 1 vs 6) and fasted (Groups 11 vs 16) rats. Overnight fasting significantly reduced lethality compared to fed rats; the decrement was significant \( p<0.001 \) at the 2 mg dose but not significant \( p=0.374 \) at the 1 mg dose.

The influence of an insulinogenic dose of glucose on endotoxin shock lethality in fed rats given 1 mg endotoxin is presented in Table 1 (Groups 1-4) and depicted in Figure 2. Glucose treatment significantly increased lethality when given either at 1 or 2 hours after endotoxin. The weighted mean of all glucose treated rats (Group 21) also reflected a significant sensitizing effect of glucose treatment. As shown in Table 1 in fed rats given the 2 mg dose (Groups 6-9), the increase in lethality in glucose treated animals,
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<td>Mannoheptulose</td>
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<td>Glucose (400mg)</td>
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<td>0,1,2 0 1 2 (b)</td>
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</table>
Footnotes to Table 1

a. Groups of rats were fed or fasted overnight and on the following morning were given the indicated dose of endotoxin intravenously. They were treated at 0, 1, or 2 hrs after endotoxin with either 2 ml of saline, or 400 mg of glucose dissolved in saline, or with mannoheptulose as indicated below. Lethality was recorded at 48 hrs.

b. Mannoheptulose treatment of fed rats consisted of subcutaneous injections of 50 mg at -2 hr, 100 mg at -15 min., and 25 mg at +2 hr. Fasted mannoheptulose treated rats followed the same schedule with the exception that the -15 min. dose was reduced to 50 mg. The mannoheptulose was prepared in saline at a concentration of 50 mg per ml.

c. Weighted mean of glucose treated consists of summation of the 3 groups of glucose treated animals irrespective of time after endotoxin within anyone dose of endotoxin and fed or fasted state.

d. Arbitrary group number assigned for reference in text and statistical analysis.

e. Significance of difference by 2 tailed test.

f. Change in percent lethality, + indicates increase in experimental group over control group, - indicates decrease in lethality of experimental group compared to control.
FIGURE 1

INFLUENCE OF FASTING ON ENDOTOXIN SHOCK LETHALITY

Rats were either allowed food ad libitum or fasted overnight prior to an iv challenge of 1 or 2 mg endotoxin per rat as described in Table 1. The two fed groups are shown on the left. The endotoxin doses (mg/rat) and the number of rats in each group (N) are indicated below each bar. LPS indicates endotoxin in this and in the following figures.
Fed rats were treated with 1 mg of endotoxin (LPS) iv and then an ip injection of 400 mg glucose at 0, 1, or 2 hr. The weighed mean (wt mean) lethality in the 3 glucose treated groups was computed as the total number of deaths per total number of rats treated with glucose and endotoxin. The number of rats in each group (N) is given below each bar.
although consistent in all 3 groups, did not reach statistical significance (0.1 > p > 0.05).

Similarly, the influence of glucose on lethality in overnight fasted rats treated with a 1 mg endotoxin is presented in Table 1 (Groups 11-14) and depicted in Figure 3. Lethality was increased over three-fold at all 3 glucose regimens. At the 2 mg endotoxin dose glucose treatment inconsistently increased lethality (Table 1, Groups 16-19).

The influence of mannoheptulose - a selective depressant of insulin secretion - on endotoxin lethality is presented in Table 1 (Groups 5, 10, 15, and 20) and depicted in Figure 4. At the 1 mg endotoxin dose mannoheptulose decreased lethality from 22% to 9% in fed rats and from 12% to 0% in fasted rats; these decrements were however not statistically different. In contrast, mannoheptulose significantly reduced lethality to 2 mg endotoxin from 69% to 48% in fed rats (p=0.028; Table 1, Groups 6 vs 10) and from 37% to 6% in fasted rats (p<0.001; Table 1, Groups 16 vs 20).

In order to determine if it was the insulin response to glucose treatment or the glucose per se which sensitized rats to endotoxin combined treatments with glucose and mannoheptulose were evaluated (Table 2 and Figure 5). As in the previous experiment (Table 1) glucose treatment significantly increased lethality (p=0.008; Table 2, Groups 1 vs 2). Mannoheptulose provided a striking protection with lethality reduced to 6%. In Group 4 treatment with both mannoheptulose and glucose reduced endotoxin shock lethality to 12%. This finding supports the notion that glucose sensitization to lethal
Overnight fasted rats were treated with endotoxin (LPS) iv and then an ip injection of 400 mg glucose at 0,1, or 2 hr. The weighted mean (wt mean) lethality in the 3 glucose treated groups was computed as the total number of deaths per total number of rats treated with glucose and endotoxin. The number of rats in each group (N) is given below each bar.
Fed or overnight fasted rats were challenged with 1 or 2 mg of endotoxin (LPS) iv as indicated in the figure and then treated with either saline or mannoheptulose as described in Table 1. The number of rats in each group is indicated below each bar. The corresponding saline and mannoheptulose treated groups (with respect to fed or fasted and endotoxin dose) are coded with bars of the same pattern.
<table>
<thead>
<tr>
<th>Group Number</th>
<th>1</th>
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<th>4</th>
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<tbody>
<tr>
<td>2mg LPS iv plus ip treatment&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Saline</td>
<td>Glucose</td>
<td>Saline</td>
<td>Glucose</td>
</tr>
<tr>
<td>sc treatment&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Saline</td>
<td>Saline</td>
<td>Mannoheptulose</td>
<td>Mannoheptulose</td>
</tr>
<tr>
<td>Number Tested</td>
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<td>65</td>
<td>50</td>
<td>50</td>
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<tr>
<td>% Lethality</td>
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<td>56.9</td>
<td>6.0</td>
<td>12.0</td>
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<tr>
<td>Chi Square and p value vs. Group 1</td>
<td>--</td>
<td>7.00&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.30</td>
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</tr>
<tr>
<td></td>
<td>--</td>
<td>0.008&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.020</td>
</tr>
<tr>
<td>Vs. Group 2</td>
<td>--</td>
<td>--</td>
<td>30.10&lt;sup&gt;p&lt;0.001&lt;/sup&gt;</td>
<td>22.48&lt;sup&gt;p&lt;0.001&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vs. Group 3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.49&lt;sup&gt;0.484&lt;/sup&gt;</td>
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</table>

a. After fasting overnight rats received 2 mg of endotoxin and the treatment indicated below.

b. Either 400 mg glucose in 2 ml of saline at time zero or an equivalent volume of saline.

c. Either 2 ml saline at -2 hr and 1 ml of saline at -15 min. or 100 mg mannoheptulose in 2 ml of saline and 50 mg mannoheptulose in 1 ml saline.

d. Chi-square value using Yates' correction factor.

e. Level of statistical significance for two tailed test corresponding the indicated Chi-square value.
Overnight fasted rats were treated with either saline or mannoheptulose sc and saline or glucose ip as indicated in the figure using the protocol described in Table 2. All rats were given 2 mg endotoxin (LPS) iv. The number of animals in each group is given to the left of each bar.
endotoxin shock is related to insulin secretion.

To further evaluate the possible role of insulin in the response to endotoxin, shock lethality was evaluated after treatment with the beta cell cytotoxic sulfonylurea agents, tolbutamide or tolazamide or with insulin per se. Both tolbutamide and tolazamide significantly increased lethality to 84% and 100% respectively. Insulin administered at the same time as endotoxin increased lethality to 88% (250 mU/rat) and 94% (500 mU/rat). When given one hour after endotoxin insulin (500 mU/rat) increased lethality to 100%. None of these agents, which produced a four-fold increase in lethality in endotoxin challenged rats, were lethal when given to saline treated rats (Table 3 and Figure 6).

B. Effect of Glucose Treatment on Gluconeogenesis and Glycogenesis after Endotoxin Treatment

Since profound hypoglycemia and near total hepatic glycogen depletion are characteristic of endotoxemia, the influence of glucose treatment on gluconeogenesis and hepatic glycogenesis was studied in endotoxin treated rats (Table 4). Gluconeogenic capacity and hepatic glycogenic function were evaluated 4 to 5 hours after iv saline or endotoxin.

Prior to the alanine load mean blood glucose was 77.81 ± 1.45 mg/dl in the saline control rats (Group 1). Blood glucose was increased by 21 mg/dl following glucose treatment (Group 2). Endotoxin treated rats (Groups 3 and 4) were significantly hypoglycemic.

Following alanine injection blood glucose increased in Group 1 controls by an average of 26 mg/dl. No further increment in blood
### TABLE 3
EFFECTS OF SULFONYLUREA AGENTS AND INSULIN ON ENDOTOXIN SHOCK LETHALITY\(^a\)

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<th>4</th>
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<th>6</th>
<th>7</th>
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<th>9</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2mg Endotoxin</td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>ip or sc treatment</td>
<td>Saline(^c)</td>
<td>Tolbut.(^d)</td>
<td>Tolaz.(^e)</td>
<td>Insulin(^f)</td>
<td>Tolbut.</td>
<td>Tolaz.</td>
<td>Insulin</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>250mU</td>
<td>500mU</td>
<td>500mU</td>
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<tr>
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<td>35</td>
<td>15</td>
<td>20</td>
<td>20</td>
<td>20</td>
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<tr>
<td>% Lethality</td>
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<td>84.0</td>
<td>100.0</td>
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TABLE 3 (cont'd)
STATISTICAL ANALYSIS OF DATA OF TABLE 3

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<td>Group 6</td>
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<td>Group 5 vs.</td>
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<td>0.689</td>
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<td>Group 6</td>
<td>0.02</td>
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a. Overnight fasted rats were given iv injection of either 2 mg endotoxin in 2 ml saline or that volume of saline alone. They were subsequently treated ip or sc with the agents shown at the indicated times. Lethality was recorded at 48 hrs.
b. ip or sc treatment given at the indicated time after endotoxin.
c. 1 ml ip or 12.5 µl sc.
d. 50 mg Sodium Tolbutamide (Orinase Diagnostic) in 1 ml, ip.
e. 50 mg Tolazamide (Tolinase) in suspension in 1 ml saline ip.
f. Lente crystaline zinc insulin, 40 Units per ml, 6.25 µl per 250 mU, or 12.5 µl per 500 mU.
Footnotes for Table 3 (cont'd)

g. Time after iv injection when ip or sc agent was administered.

h. Chi-square value using Yates' Correction Factor.

i. Statistical significance of difference for two tailed test of difference.

j. Increase (+) or decrease (-) in lethality where statistically significant difference.
Overnight fasted rats were treated with 2 mg endotoxin (LPS) iv or saline iv followed by ip tolbutamide, tolazamide, or saline; sc insulin or saline were given as indicated in the figure and described in Table 3. Sc or ip treatments were given at the same time as endotoxin except in the one group given sc insulin at 1 hr after endotoxin. The number of rats in each group is shown at the left of each bar.
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<tr>
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<td>—Saline, 1ml——</td>
<td>—Endotoxin, 1mg—</td>
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<tr>
<td><strong>ip treatment</strong></td>
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<tr>
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<td>16</td>
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<td>(\pm 2.35)</td>
<td>(\pm 1.88)</td>
<td>(\pm 2.32)</td>
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<tr>
<td>(grams)</td>
<td>9.40</td>
<td>9.99</td>
<td>8.69</td>
<td>8.64</td>
</tr>
<tr>
<td></td>
<td>(\pm 0.13)</td>
<td>(\pm 0.18)</td>
<td>(\pm 0.13)</td>
<td>(\pm 0.13)</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>16</td>
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<tr>
<td>(% Body Weight)</td>
<td>3.27</td>
<td>3.46</td>
<td>3.05</td>
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</tr>
<tr>
<td></td>
<td>0.04</td>
<td>(\pm 0.05)</td>
<td>(\pm 0.05)</td>
<td>(\pm 0.04)</td>
</tr>
<tr>
<td><strong>Blood Glucose</strong></td>
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<td>16</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>(mg per 100ml)</td>
<td>77.81</td>
<td>98.39</td>
<td>64.80</td>
<td>60.60</td>
</tr>
<tr>
<td>at Alanine inj.</td>
<td>(\pm 1.45)</td>
<td>(\pm 2.99)</td>
<td>(\pm 5.62)</td>
<td>(\pm 8.17)</td>
</tr>
<tr>
<td>at 30 min. post</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>16</td>
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<tr>
<td>Alanine</td>
<td>103.33</td>
<td>105.46</td>
<td>83.70</td>
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</tr>
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<td>(\pm 2.14)</td>
<td>(\pm 3.18)</td>
<td>(\pm 5.40)</td>
<td>(\pm 7.42)</td>
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<tr>
<td>increase in 30 min.</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>following Alanine</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>25.51</td>
<td>6.82</td>
<td>18.89</td>
<td>10.62</td>
</tr>
<tr>
<td></td>
<td>(\pm 2.35)</td>
<td>(\pm 4.65)</td>
<td>(\pm 4.90)</td>
<td>(\pm 4.09)</td>
</tr>
<tr>
<td>Group Number</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>--------------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
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<tr>
<td><strong>Blood Glucose C-14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(DPM per ml x 10³)</td>
<td>16.23</td>
<td>11.92</td>
<td>19.32</td>
<td>14.79</td>
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<td>+0.29</td>
<td>+0.43</td>
<td>+0.90</td>
<td>+0.71</td>
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<tr>
<td>(DPM per mg glucose x 10³)</td>
<td>15</td>
<td>16</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>+0.45</td>
<td>+0.46</td>
<td>+1.59</td>
<td>+1.76</td>
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<tr>
<td><strong>Liver Glycogen</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>(mg glucose equivalents/gm)</td>
<td>1.08</td>
<td>13.43</td>
<td>0.08</td>
<td>0.12</td>
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<td>+0.19</td>
<td>+0.70</td>
<td>+0.01</td>
<td>+0.02</td>
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<tr>
<td>(mg glucose equivalents per liver)</td>
<td>10.18</td>
<td>136.34</td>
<td>0.72</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>+1.85</td>
<td>+11.01</td>
<td>+0.12</td>
<td>+0.18</td>
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<tr>
<td><strong>Liver Glycogen C-14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPM/gm wet weight x 10³)</td>
<td>19.62</td>
<td>18.30</td>
<td>2.34</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td>+2.94</td>
<td>+1.56</td>
<td>+0.23</td>
<td>+0.37</td>
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<tr>
<td>(DPM/liver x 10³)</td>
<td>184.65</td>
<td>182.53</td>
<td>20.36</td>
<td>18.93</td>
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<td></td>
<td>+27.97</td>
<td>+15.38</td>
<td>+2.13</td>
<td>+3.31</td>
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<td><strong>Liver Glycogen Specific Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPM/mg glucose equivalents)</td>
<td>26.19</td>
<td>1.25</td>
<td>34.29</td>
<td>23.50</td>
</tr>
<tr>
<td></td>
<td>+5.57</td>
<td>+0.16</td>
<td>+5.61</td>
<td>+3.59</td>
</tr>
<tr>
<td><strong>Mean % of Alanine C-14 recovered as Glycogen C-14 (%)</strong></td>
<td>2.08</td>
<td>2.06</td>
<td>0.23</td>
<td>0.21</td>
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### TABLE 4 (cont'd)
STATISTICAL ANALYSIS OF DATA PRESENTED IN TABLE 4

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<tr>
<th>Groups Compared</th>
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<th>1 and 3</th>
<th>2 and 4</th>
<th>3 and 4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(grams)</td>
<td>29,043</td>
<td>0,722</td>
<td>1,060</td>
<td>0,168</td>
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<tr>
<td>(grams)</td>
<td>0,661</td>
<td>0,476</td>
<td>0,298</td>
<td>0,868</td>
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<td><strong>Liver weight</strong></td>
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<tr>
<td>(grams)</td>
<td>2,672</td>
<td>1,305</td>
<td>6,070</td>
<td>0,106</td>
</tr>
<tr>
<td>(grams)</td>
<td>0,012</td>
<td>0,202</td>
<td>&lt;0,001</td>
<td>0,916</td>
</tr>
<tr>
<td><strong>(% Body weight)</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3,046</td>
<td>3,525</td>
<td>6,587</td>
<td>0,426</td>
</tr>
<tr>
<td></td>
<td>0,005</td>
<td>0,002</td>
<td>&lt;0,001</td>
<td>0,673</td>
</tr>
<tr>
<td><strong>Blood Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg per 100ml)</td>
<td>6,185</td>
<td>2,240</td>
<td>4,338</td>
<td>0,423</td>
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<tr>
<td>at Alanine inj.</td>
<td>&lt;0,001</td>
<td>0,033</td>
<td>&lt;0,001</td>
<td>0,675</td>
</tr>
<tr>
<td>at 30 min. post alanine</td>
<td>0,556</td>
<td>3,380</td>
<td>4,242</td>
<td>1,360</td>
</tr>
<tr>
<td></td>
<td>0,583</td>
<td>0,002</td>
<td>&lt;0,001</td>
<td>0,184</td>
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<tr>
<td><strong>increase in 30 min. following alanine</strong></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>3,590</td>
<td>1,218</td>
<td>0,614</td>
<td>1,295</td>
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<tr>
<td></td>
<td>0,0012</td>
<td>0,233</td>
<td>0,544</td>
<td>0,206</td>
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<td><strong>Blood Glucose C-14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPM per ml)</td>
<td>8,391</td>
<td>3,281</td>
<td>3,448</td>
<td>3,954</td>
</tr>
<tr>
<td>x 10^3</td>
<td>&lt;0,001</td>
<td>0,003</td>
<td>0,002</td>
<td>&lt;0,001</td>
</tr>
<tr>
<td>Groups Compared</td>
<td>1 and 2</td>
<td>1 and 3</td>
<td>2 and 4</td>
<td>3 and 4</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
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<tr>
<td><strong>Blood Glucose C-14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPM per mg glucose) x 10^3</td>
<td>29 6.884 &lt;0.001</td>
<td>28 4.016 &lt;0.001</td>
<td>27 4.769 &lt;0.001</td>
<td>26 1.007 0.323</td>
</tr>
<tr>
<td><strong>Liver Glycogen</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg glucose equivalents/gm)</td>
<td>20 17.029 &lt;0.001</td>
<td>25 5.105 &lt;0.001</td>
<td>22 19.035 &lt;0.001</td>
<td>27 1.437 0.162</td>
</tr>
<tr>
<td>(mg glucose equivalents per liver)</td>
<td>20 11.299 &lt;0.001</td>
<td>25 5.106 &lt;0.001</td>
<td>22 12.284 &lt;0.001</td>
<td>27 1.505 0.144</td>
</tr>
<tr>
<td><strong>Liver Glycogen C-14</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DPM/gm wet weight) x 10^3</td>
<td>29 0.395 &lt;0.001</td>
<td>27 5.862 &lt;0.001</td>
<td>30 10.071 &lt;0.001</td>
<td>28 0.403 0.690</td>
</tr>
<tr>
<td>(DPM/liver) x 10^3</td>
<td>29 0.090 &lt;0.001</td>
<td>27 5.857 &lt;0.001</td>
<td>30 10.398 &lt;0.001</td>
<td>28 0.360 0.721</td>
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<tr>
<td><strong>Liver Glycogen Specific Activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>x 10^3</td>
<td>20 4.476 &lt;0.001</td>
<td>24 1.024 &lt;0.001</td>
<td>22 6.187 &lt;0.001</td>
<td>26 1.619 0.118</td>
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<tr>
<td>(DPM/mg glucose equivalents)</td>
<td>&lt;0.001</td>
<td>0.316 &lt;0.001</td>
<td>&lt;0.001</td>
<td>0.118</td>
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</table>
TABLE 4 (cont’d)
STATISTICAL SIGNIFICANCE OF CHANGES IN BLOOD GLUCOSE IN 30 MINUTES FOLLOWING ALANINE SHOWN IN TABLE 4

<table>
<thead>
<tr>
<th>Group</th>
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<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degrees of Freedom</td>
<td>28</td>
<td>30</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>&quot;t&quot; value</td>
<td>9.874</td>
<td>1.619</td>
<td>2.424</td>
<td>0.962</td>
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<tr>
<td>&quot;p&quot; value</td>
<td>&lt;0.001</td>
<td>0.116</td>
<td>0.022</td>
<td>0.344</td>
</tr>
</tbody>
</table>

a. Overnight fasted rats were given an iv injection of 1 ml saline or 1 mg endotoxin as indicated. At 120 minutes following the iv injection all rats received an ip injection of 2 ml of saline or 2 ml of 20% glucose (400 mg) in saline. In the period between 4 to 5 hrs after the iv injection (2 to 3 hrs after the ip injection) gluconeogenic capacity and hepatic glycogenic function were evaluated and a 100 mg load of L-alanine plus 4 micro Curies of L-alanine-U-C-14 as described in the methods section.

b. Body weight at time of iv injection
c. Liver weight at 30 minutes following alanine injection
d. Number of animals in group; so indicated throughout the Table
e. Mean value
f. Standard error of mean
g. Comparison of groups presented in Table 4 by unpaired Student's "t" test. Group numbers correspond to those given in Table 4.
h. Parameters as given in Table 6
i. Degrees of freedom
j. T value
k. p value
l. Student's "t" test of difference in blood glucose before and 30 minutes after alanine injection within 4 groups shown in Table 4.
glucose was observed in manifestly hyperglycemic Group 2 rats. Rats which had received endotoxin without glucose (Group 3) had an increase in blood glucose which averaged 19 mg/dl which was not significantly different from the control group (Group 1). In contrast to the Group 3 rats, the rats treated with both endotoxin and glucose (Group 4) showed no significant change in blood glucose at 30 after alanine.

In correspondence to the blood glucose determinations, glucose treatment of saline iv treated rats (Group 2) significantly decreased both the net conversion of alanine-C-14 to blood glucose-C-14 and the blood glucose specific activity. However, in the Group 3 rats, blood glucose-C-14 activity and specific activity were both increased over the values observed in controls (Group 1). In the Group 4 rats blood glucose-C-14 activity and specific activity were both increased over the values in Group 2 rats. However, blood glucose activity was depressed in the Group 4 rats to values significantly below the values observed in Group 3 rats.

Liver glycogen content and C-14 activity were determined 30 minutes after alanine injection. As shown in Table 4 the mean liver weight of saline treated rats which had received glucose was significantly increased. In endotoxin treated rats liver weight was reduced below that of their corresponding saline controls and no difference in liver weight was evident when the ip glucose (Group 4) and ip saline (Group 3) rats were compared. In view of these significant differences in liver weight all estimates of liver glycogen content and liver glycogen-C-14 were calculated both per gram and
per total organ. In Group 1 rats liver glycogen averaged 10 mg of glucose equivalents per total liver and 2.08% of the alanine label was present as liver glycogen. In the Group 2 rats 125 mg of the glucose load was recovered as liver glycogen which was increased to 136 mg glucose equivalents per liver. Similar to Group 1, 2.06% of the injected alanine dose was found in liver glycogen. In Group 3 there was near total depletion of liver glycogen—mean 0.7 mg glucose equivalents per liver—and only 0.23% of the alanine label was incorporated into liver glycogen. In Group 4 there was no significant increase in liver glycogen over the values found in rats given endotoxin and saline (Group 3). The value found was less than 1% of that observed in glucose treated rats not given endotoxin (Group 2). Both liver glycogen specific activity and the % of the alanine carbon label recovered as glycogen were insignificantly less than the values in Group 3 rats.

C. Glucose Tolerance and Insulin Response in Endotoxemia

Prior to evaluating the glucose tolerance and insulin response during endotoxemia, the insulin radioimmunoassay procedure (Pharmacia, Phadebas Insulin Test) was evaluated (Table 5). Seven point standard curves were linear with a mean Pearson's r of -0.9970 and showed minimal deviations in slope (M) and intercept (B) on a semi-logarithmic plot. The mean intra-assay coefficients of variation were 6.75% and 6.10% for the Pharmacia lyophilized human serum and the pooled rat serum respectively. Pooled heparinized rat plasma consistently showed a higher coefficient of variation. The inter-assay coefficients of variation for the serum pools were respectively
TABLE 5
INTRA- AND INTER-ASSAY VARIATION OF INSULIN RADIOIMMUNOASSAY PROCEDURE a

<table>
<thead>
<tr>
<th>Date Sample</th>
<th>Intra-assay variation</th>
<th>Inter-assay variation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean IRI (μU/ml)</td>
<td>Standard Deviation (μU/ml)</td>
</tr>
<tr>
<td>5-7-74 PLHS b</td>
<td>4</td>
<td>102.82</td>
</tr>
<tr>
<td>5-7-74 S-3-7-74 c</td>
<td>4</td>
<td>72.64</td>
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<tr>
<td>5-7-74 P-4-12-74 d</td>
<td>4</td>
<td>47.28</td>
</tr>
<tr>
<td>5-9-74 PLHS</td>
<td>4</td>
<td>84.34</td>
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<tr>
<td>5-9-74 S-3-7-74</td>
<td>4</td>
<td>66.84</td>
</tr>
<tr>
<td>5-9-74 P-4-12-74</td>
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<td>39.44</td>
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<tr>
<td>5-13-74 PLHS</td>
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<td>93.41</td>
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<tr>
<td>5-13-74 S-3-7-74</td>
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<td>67.56</td>
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<tr>
<td>5-13-74 P-4-12-74</td>
<td>4</td>
<td>36.15</td>
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<tr>
<td>5-14-74 PLHS</td>
<td>4</td>
<td>85.09</td>
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<td>5-14-74 S-3-7-74</td>
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<td>67.87</td>
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<td>5-14-74 P-4-12-74</td>
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<td>43.72</td>
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<td>5-15-74 PLHS</td>
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<td>91.73</td>
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<td>5-15-74 P-4-12-74</td>
<td>4</td>
<td>40.91</td>
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<tr>
<td>Inter-assay variation</td>
<td>Mean IRI (μU/ml)</td>
<td>Standard Deviation (μU/ml)</td>
</tr>
<tr>
<td>PLHS</td>
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<td>S-3-7-74</td>
<td>5</td>
<td>70.98</td>
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<tr>
<td>P-4-12-74</td>
<td>5</td>
<td>41.50</td>
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### TABLE 5 (cont'd)

#### Mean intra-assay parameters from 5 runs of assay

<table>
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<tr>
<th>Date</th>
<th>Sample N</th>
<th>Mean IRI (KU/ml)</th>
<th>Standard Deviation (KU/ml)</th>
<th>Coefficient of Variation (%)</th>
<th>SEM (KU/ml)</th>
<th>SEM as % Mean (%)</th>
<th>Standard Curve Parameters</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PLHS 5</td>
<td>6.75</td>
<td>6.10</td>
<td>6.75</td>
<td>3.06</td>
<td>2.32</td>
<td>6.46</td>
</tr>
<tr>
<td></td>
<td>S-3-7-74 5</td>
<td>6.10</td>
<td>2.32</td>
<td>11.59</td>
<td>3.06</td>
<td>2.32</td>
<td>6.46</td>
</tr>
<tr>
<td></td>
<td>P-4-12-74 5</td>
<td>11.59</td>
<td>6.46</td>
<td>11.59</td>
<td>3.06</td>
<td>2.32</td>
<td>6.46</td>
</tr>
</tbody>
</table>

Coefficients of variation (%s)

- PLHS: 6.75, 3.06, 1.37
- S-3-7-74: 6.10, 2.32, 1.04
- P-4-12-74: 11.59, 6.46, 2.89

#### Mean inter-assay

<table>
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<th>Standard Curve Parameters</th>
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<td>r 5 5 -0.9970 0.0009</td>
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<tr>
<td>M 5 5 -35.393 0.7212</td>
</tr>
<tr>
<td>B 5 5 112.294 1.4320</td>
</tr>
</tbody>
</table>

- a. Two pools of serum and one pool of plasma were assayed in duplicate both before and following the experimental samples on each of the 5 occasions on which the assay was repeated to obtain the data presented in Table 7.
- c. Pooled rat serum stored at -45°C.
- d. Pooled heparinized rat plasma stored at -45°C.
- e. Pearson's r of correlation of 7 point standard curve.
- f. Slope for equation \((B/Bo) \times 100 = \left[ M \times \ln(\mu U/ml) \right] + B \).
- g. Intercept for equation \((B/Bo) \times 100 = \left[ M \times \ln(\mu U/ml) \right] + B \).
- h. Mean and variation about the mean for the IRI values found in the 5 repetitions of the assay.
- i. Mean and variation for coefficients of variation for each of the 5 assay repetitions.
- j. Mean and variation for standard curve parameters of each of the 5 assay repetitions.
8.19% and 7.80% which are within 95% confidence limits of the mean intra-assay coefficients of variation for these samples.

Blood glucose responses following 1 mg of endotoxin iv are presented in Table 6 and depicted in Figure 7. As indicated in Figure 7 no marked changes in blood glucose were manifest following control saline injections (Group 1, Table 6) throughout the 12 hour observation period. Glucose treatment of saline control rats (Group 2, Table 6) resulted in a prompt hyperglycemia which steadily declined within 2 hrs to a plateau of 90 to 100 mg/dl. Blood glucose then remained significantly greater than the values for the Group 1 rats until 10 hrs after glucose treatment. Administration of 1 mg of endotoxin (Group 3) elicited a hyperglycemia which was not manifest at 15 min. but was pronounced by 45 min. The peak mean value was observed at 90 min. when blood glucose approached twice control values and remained significantly elevated until 240 min. after which it did not differ from control values in Group 1. In endotoxemic rats which received glucose at 120 min. (Group 4, Table 6) the initial hyperglycemic zenith was similar to that observed in Group 2. However, after 240 min. glucose values of Group 4 declined to values not different from those of Group 1 or Group 3 rats.

Serum insulin responses following 1 mg endotoxin are presented in Table 7 and depicted in Figure 8. The saline - saline control group (Group 1, Table 7) had mean insulin values in the range of 15 ± 2.5 U/ml. Serum insulin in rats which received saline iv and glucose ip (Group 2, Table 7) was increased to 383% of the corresponding value in Group 1 within 15 min. after glucose treatment.
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*TABLE 6*

BLOOD GLUCOSE FOLLOWING ENDOTOXIN TREATMENT AND COMBINED TREATMENT WITH ENDOTOXIN AND GLUCOSE

Group

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1. iv treatment at time zero: 1 ml Saline, 1 mg Endotoxin
2. ip treatment at 120 min.: 2 ml Saline, 400 mg Glucose
3. Time after (min.): 0, 15, 45, 90, 120, 135
4. Carbohydrates: 2 ml Saline, 400 mg Glucose
TABLE 6 (cont'd)

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STATISTICAL COMPARISONS OF BLOOD GLUCOSE DATA PRESENTED IN TABLE 6e

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Footnotes to Table 6

a. Overnight fasted rats were injected iv with one ml saline or 1 mg endotoxin as indicated. At 120 minutes following the iv injection all remaining rats received either 2 ml saline or 400 mg glucose in this volume of saline as shown. At the designated times after iv injection the indicated numbers of rats of each treatment group were bled by cardiac puncture and blood glucose and serum insulin were determined as described in the methods section. The blood glucose data are shown in this table; Table 7 presents the insulin data.

b. Number of samples.

c. Mean blood glucose (mg/100 ml).

d. Standard error of mean of blood glucose.

e. Comparison of data presented in Table 6 by two tailed non-paired Student's "t" test.

f. Groups, numbered as shown above, evaluated for statistical significance of differences in mean blood glucose values.

g. Degrees of freedom.

h. Student's "t" value.

i. Corresponding p value for 2 tailed test.
Overnight fasted rats were given an iv dose of saline or 1 mg endotoxin (LPS) at time 0 min. and an ip dose of saline or 400 mg glucose at 120 min. after the iv injection as indicated in the figure and described in Table 6. The four combinations of treatment shown on the figure correspond from top to bottom to Groups 1-4 of Table 6. The standard error of mean blood glucose values are shown as a vertical line. The mean number of animals for each point in time for each of the 4 Groups is shown following the treatment schedule in the upper right corner of the figure.
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* TABLE 7

**EFFECT OF GLUCOSE TREATMENT ON SERUM INSULIN FOLLOWING ENDOTOXIN TREATMENT**

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STATISTICAL COMPARISONS OF SERUM INSULIN DATA IN TABLE 7

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Footnotes to Table 7

a. Assays of serum insulin were performed on the same blood samples from which blood glucose
data were presented in Table 6.
b. Treatment groups are designated as in Table 6.
c. Number of rat serum samples.
d. Mean immunoreactive insulin (IRI) value
e. Standard Error of Mean serum IRI.
f. Data were analyzed by means of the 2 tailed non-paired Student's "t" test.
g. Evaluated for statistical significance of difference in mean serum IRI.
h. Degrees of freedom
i. Student's "t" value
j. Corresponding p value for 2 tailed test
SERUM INSULIN RESPONSES TO ENDOTOXIN AND GLUCOSE TREATMENT

Mean and standard error of the mean of serum insulin values are shown. See Table 7 and legend to Figure 7 for details.
As blood glucose declined serum insulin similarly declined. Serum insulin was not different from the values in Group 1 at 300 min. and beyond despite the presence of a significant hyperglycemia (Table 6). Rats treated with endotoxin (Group 3, Table 7) developed an insulinemia which paralleled the endogenously generated hyperglycemia i.e., as blood glucose rose to a peak value at 90 min. and subsequently declined until 180 min., serum insulin likewise reached a peak at 90 min. and declined until the 180 min. sampling period. However, despite the return of blood glucose to values not different from Group 1, serum insulin remained significantly elevated throughout the 12 hr observation period in the endotoxemic rats. In endotoxemic rats which received glucose at 120 min. (Group 4, Table 7) serum insulin was markedly elevated to a peak mean value of 182 \( \mu \text{U/ml} \). Subsequently, serum insulin declined as blood glucose decreased; however in the period from 60 to 240 min. after glucose treatment, blood glucose declined while serum insulin remained elevated at 50-65 \( \mu \text{U/ml} \). As in Group 3 the persistent elevation of serum insulin in Group 4 was associated with blood glucose values not different from those of Group 1 and significantly less than those of Group 2.

In order to evaluate the relationship of serum insulin to blood glucose in experimental endotoxemia in the rat, the glucose to insulin ratio and the product of blood glucose times serum insulin were calculated (Table 8). As seen in Figure 9 the glucose to insulin ratios of Group 1 ranged from 4.73 to 6.77 and later plateaued at 5.00 ± 0.25. In Group 2 the ratio initially decreased following glucose treatment but then increased during the ensuing 6 hr to values
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<th>Glucose X Insulin</th>
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TABLE 8
BLOOD GLUCOSE TO SERUM INSULIN RATIO AND PRODUCT FOLLOWING ENDOTOXIN TREATMENT
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<th>Blood Glucose</th>
<th>Serum Insulin</th>
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Footnotes to Table 8

a. Blood glucose to serum insulin ratio in mg per 100 ml per Unit per ml or mg per 100 Units. These values were derived from the mean values presented in Tables 6 and 7.

b. Blood glucose time serum insulin product in mg per 100 ml times Units per ml or mg x Units per 100 ml². These values were derived from the mean values presented in Tables 6 and 7.
FIGURE 9

EFFECT OF GLUCOSE TREATMENT ON THE BLOOD GLUCOSE TO
SERUM INSULIN RATIO IN ENDOTOXEMIA

The mean blood glucose to mean serum insulin ratios were
computed for the 4 groups listed in Tables 6 and 7 using the data
of Table 8.
greater than those seen in Group 1. During endotoxemia (Group 3, Table 8) the ratio was not changed initially but with the onset of hyperglycemia it declined abruptly to a nadir of 1.21 at 90 min. and remained depressed throughout the experimental period. During endotoxemia glucose administration (Group 4, Table 8) maintained the ratio at the low value of about 1.5.

The glucose-insulin product in endotoxemia is shown in Figure 10. Group 1 displayed little variation over the 12 hr period. Group 2 manifested an increase in the product following glucose treatment with a return to values not distinguishable from the rats given only saline (Group 1) at 3 hr after glucose injection. After endotoxin treatment (Group 3, Table 8) the product increased in a pattern similar to the serum insulin values (Figure 8) and also remained elevated throughout the 12 hrs of observation. During endotoxemia glucose administration (Group 4, Table 8) resulted in a large increase in the product, i.e., over 3 times that of Group 2, with elevated levels throughout the ensuing 10 hrs.

Table 9 and Figure 11 indicate the linear regression of serum insulin on blood glucose in the rats which received saline followed by ip glucose (Group 2). No difference in the regression existed whether all 12 points were included or if only those points after glucose treatment were used in determining the equation (Table 9). The regression lines for Groups 3 and 4 are given in Table 9 and shown on Figure 12; as in Group 2, the 12 point relationship was used for Group 4 since there was no difference between this and the relationship defined by the 8 points following glucose treatment.
The mean blood glucose and mean serum insulin products were computed for the 4 groups listed in Tables 6 and 7 using the data in Table 8. Note the change in scale of the ordinate.
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<th>B&lt;sup&gt;c&lt;/sup&gt;</th>
<th>r&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Dr&lt;sup&gt;e&lt;/sup&gt;</th>
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STATISTICAL COMPARISON OF REGRESSION EQUATIONS GIVEN IN TABLE 9

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<tr>
<td>Group 2, points after ip glucose vs. Group 4, points after ip glucose</td>
<td>12</td>
<td>12.918</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 3, all 12 points vs. Group 4, all 12 points</td>
<td>20</td>
<td>0.998</td>
<td>0.330</td>
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<tr>
<td>Group 4, all 12 points vs. Group 4, points after ip glucose</td>
<td>16</td>
<td>0.317</td>
<td>0.755</td>
</tr>
</tbody>
</table>
Footnotes to Table 9

a. Number of points
b. Slope of regression line of serum insulin on blood glucose
c. Y-axis (serum insulin) intercept of regression line of serum insulin on blood glucose
d. Pearson's coefficient of correlation for indicated regression lines
e. Degrees of freedom of regression line i.e., N-2
f. p value for indicated value of Pearson's r for the number of degrees of freedom shown.
g. X-axis (blood glucose) intercept of regression line of serum insulin on blood glucose
h. Lines for groups indicated in table were compared for significance of difference using the computer program given in the appendix.
i. Degrees of freedom for test of significance of difference of lines.
j. t value of significance of difference between lines
k. p value corresponding to the indicated value of t at the specified number of degrees of freedom.
l. Groups as defined in Tables 6 and 7.
The relationship of mean blood glucose to mean serum insulin in the rats of Group 2 of Tables 6 and 7 at the 12 times after iv saline is shown. The equation and the significance of the coefficient of correlation are given in Table 9.
Mean serum insulin is plotted against mean blood glucose for the rats of Groups 3 and 4 of Tables 6 and 7. Both groups were given endotoxin and Group 3 was treated with saline at 120 min. while Group 4 was given 400 mg glucose ip at 120 min. There is no difference in the 2 lines as shown in Table 9 where the significance of the coefficients of correlation is also given.
The regression line for Group 2 was significantly different from that of either Group 3 or Group 4 (Table 9). There were no differences in the regression lines for Groups 3 and 4 (Table 9) which indicates that glucose treatment did not significantly alter the relationship between serum insulin and blood glucose in endotoxin treated rats. As shown in Table 9 the slopes of the regression lines were increased to 261% to 295% of control (Group 2) in the endotoxin treated rats (Groups 3 and 4, respectively). A greater than doubling of the increase in serum insulin for any given increment in blood glucose occurred in endotoxemia. In marked contrast to the changes in slope (gain), the intercept of the regression lines on the blood glucose abscissa showed no difference after endotoxin (Table 9) with all values within the range of 48 to 51 mg/dl. This set of findings, i.e., increased slope and unaltered intercept on the blood glucose axis, may signify that while the threshold for insulin remains unaffected, the responsivity (gain) of the system to hyperglycemia is enhanced by endotoxin.

D. Glucose Oxidation after Endotoxin as Evaluated by $^{14}$C-CO$_2$ Collection

In order to examine the possible role of enhanced glucose oxidation in the derangements of carbohydrate metabolism in endotoxemia, glucose oxidation was studied by recovery of expired $^{14}$CO$_2$ from uniformly labeled glucose in 26 experimental treatments (Table 10). The data for these groups with selected key statistical comparisons are indicated in Table 11 and for ease of interpretation the data are also depicted in Figures 13 to 23.
<table>
<thead>
<tr>
<th>Group Symbol</th>
<th>Carrier Glucose Dose (mg)</th>
<th>Further Experimental Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0</td>
<td>1 ml saline iv at t=2 hr or at t=0 hr.</td>
</tr>
<tr>
<td>B</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>1 mg LPS iv at t=0 hr</td>
</tr>
<tr>
<td>D</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>1 mg LPS iv at t=-2 hr</td>
</tr>
<tr>
<td>F</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>100 mg MH sc at t=-1 hr; 100 mg MH sc at t=0 hr; 1 ml saline iv at t=0 hr</td>
</tr>
<tr>
<td>H</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>100 mg MH sc at t=-1 hr; 100 mg MH ss at t=0 hr; 1 mg LPS at t=0 hr</td>
</tr>
<tr>
<td>J</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>0</td>
<td>100 mg MH sc at t=-4 hr; 50 mg MH sc at t=-2 1/4 hr; 1 ml saline at t=-2 hr; 50 mg MH at t=-15 min.</td>
</tr>
<tr>
<td>L</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>100 mg MH sc at t=-4 hr; 50 mg MH sc at t=-2 1/4 hr; 1 mg LPS iv at t=-2 hr; 50 mg MH sc at t=-15 min.</td>
</tr>
<tr>
<td>N</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>0</td>
<td>Diabetic Rats 1 ml saline iv at t=0 hr</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>Diabetic Rats 1 mg LPS iv at t=0 hr</td>
</tr>
<tr>
<td>Q</td>
<td>0</td>
<td>3 mg LPS iv at t=0 hr</td>
</tr>
<tr>
<td>R</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>3 mg LPS iv at t=-2 hr</td>
</tr>
<tr>
<td>T</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>0</td>
<td>100 g Dexamethasone Acetate ip at t=-15 min; 1 ml saline at t=0</td>
</tr>
<tr>
<td>V</td>
<td>0</td>
<td>100 g Dexamethasone Acetate ip at t=-15 min; 1 mg LPS iv at t=0</td>
</tr>
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</table>
TABLE 10 (cont'd)

<table>
<thead>
<tr>
<th>Group Symbol</th>
<th>Carrier Glucose Dose (mg)</th>
<th>Further Experimental Treatmentc</th>
</tr>
</thead>
<tbody>
<tr>
<td>W</td>
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<td>Trauma Controls, feet taped at -30 min.</td>
</tr>
<tr>
<td>X</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>0</td>
<td>Trauma in Noble-Collip Drumi, 425 revolutions</td>
</tr>
<tr>
<td>Z</td>
<td>400</td>
<td></td>
</tr>
</tbody>
</table>

ga. Prior to experiments all rats were fasted overnight. Uniformly labeled C-14-D-glucose (20μCi) was then given ip in 2 ml saline as either a tracer dose or with 400 mg carrier D-glucose immediately prior to the collection of expired CO2.

b. Designation for each group of rats used in Table 11, text, and figures 13-23.

c. Groups of rats were treated with the indicated agents at the specified time relative to ip injection of C-14 labeled glucose and initiation of CO2 collection. All injections were given without anaesthesia.

d. Endotoxin (LPS) prepared daily and given in 1 ml of saline.

e. Mannoheptulose (MH) prepared as 50 mg per ml solution in saline as injected sc.

f. Diabetic rats treated with 25 mg streptozotocin 6 or 7 before CO2 collection experiment.

g. Dexamethasone acetate prepared as 100μg per ml suspension in saline immediately before ip injection.

h. Feet taped at 30 min. prior to ip C-14 glucose.

i. Tumbled 425 revolutions in a Noble-Collip tumbling apparatus at 33 revolutions per minute 30 to 15 min. prior to ip C-14 glucose.
<table>
<thead>
<tr>
<th>Group Symbola</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
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<td>400</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>400</td>
<td>0</td>
<td>400</td>
<td>0</td>
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<tr>
<td>Number in Groupc</td>
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<td>9</td>
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<td>9</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Weight (grams)</td>
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<td>299.45</td>
<td>297.50</td>
<td>298.00</td>
<td>300.12</td>
<td>298.75</td>
<td>298.12</td>
<td>297.29</td>
<td>297.60</td>
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<td>1.30</td>
<td>0.47</td>
<td>1.34</td>
<td>1.06</td>
<td>1.32</td>
<td>1.70</td>
<td>1.50</td>
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<td>283.00</td>
<td>281.56</td>
<td>283.38</td>
<td>283.88</td>
<td>281.00</td>
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<tr>
<td>% Dose Recoveredg</td>
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<td>15.00</td>
<td>14.50</td>
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<td>16.75</td>
<td>14.88</td>
<td>17.12</td>
<td>18.00</td>
<td>15.20</td>
</tr>
</tbody>
</table>

<p>| % Dose Recoveredg (min. after ip glucose) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 15              | 0.232           | 0.114           | 0.318           | 0.143           | 0.594           | 0.240           | 0.160           | 0.087           | 0.094           |
| 30              | 0.021           | 0.016           | 0.028           | 0.021           | 0.042           | 0.037           | 0.026           | 0.019           | 0.010           |
| 45              | 1.542           | 0.860           | 1.749           | 0.912           | 3.651           | 1.701           | 0.612           | 0.349           | 0.466           |
| 60              | 0.148           | 0.033           | 0.162           | 0.085           | 0.158           | 0.266           | 0.087           | 0.042           | 0.098           |
| 90              | 3.964           | 2.449           | 4.420           | 2.549           | 8.260           | 4.399           | 1.402           | 0.970           | 1.318           |
| 120             | 0.250           | 0.121           | 0.274           | 0.194           | 0.210           | 0.507           | 0.156           | 0.084           | 0.197           |
| 180             | 0.397           | 0.303           | 0.402           | 0.303           | 0.462           | 0.797           | 0.254           | 0.188           | 0.326           |
| 240             | 0.555           | 0.503           | 0.582           | 0.743           | 0.781           | 0.894           | 0.494           | 0.299           | 0.629           |
| 330             | 0.712           | 0.518           | 0.733           | 1.106           | 0.788           | 0.726           | 0.706           | 0.430           | 0.903           |
| 390             | 0.995           | 0.764           | 0.767           | 1.283           | 0.871           | 0.507           | 0.778           | 0.382           | 1.126           |
| 420             | 33.007          | 20.779          | 37.440          | 30.494          | 44.060          | 36.051          | 18.592          | 13.536          | 18.170          |
| 450             | 0.977           | 0.787           | 0.711           | 1.353           | 0.888           | 0.393           | 0.913           | 0.560           | 1.384           |
| 510             | 1.070           | 0.963           | 0.539           | 1.417           | 0.886           | 0.668           | 0.889           | 0.743           | 1.867           |
| 540             | 40.949          | 27.078          | 46.461          | 43.102          | 51.584          | 44.945          | 27.072          | 21.364          | 26.830          |
| 570             | 1.047           | 1.050           | 0.512           | 1.492           | 0.962           | 0.763           | 0.923           | 0.819           | 1.640           |</p>
<table>
<thead>
<tr>
<th>Group Symbol&lt;sup&gt;a&lt;/sup&gt;</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>P</th>
<th>Q</th>
<th>R</th>
</tr>
</thead>
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<td>0</td>
<td>400</td>
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<td>0</td>
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<td>400</td>
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<td>6</td>
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<td>8</td>
</tr>
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<td>Weight (grams) Before&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>299.12</td>
<td>298.43</td>
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<tr>
<td>% Dose Recovered&lt;sup&gt;g&lt;/sup&gt; min. after ip glucose</td>
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<td>T</td>
<td>U</td>
<td>V</td>
<td>W</td>
<td>X</td>
<td>Y</td>
<td>Z</td>
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</tr>
<tr>
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<td>0</td>
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<td>400</td>
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<tr>
<td><strong>Number in Group&lt;sup&gt;c&lt;/sup&gt;</strong></td>
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<td>6</td>
<td>9</td>
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<td>9</td>
<td>6</td>
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<tr>
<td><strong>Weight (grams)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Before&lt;sup&gt;d&lt;/sup&gt;</strong></td>
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<td>297.83</td>
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<td>0.078</td>
<td>1.413</td>
</tr>
<tr>
<td>420 min.</td>
<td>0.001</td>
<td>0.262</td>
<td>0.004</td>
<td>0.016</td>
<td>0.001</td>
<td>0.001</td>
<td>0.034</td>
<td>0.061</td>
<td>0.939</td>
<td>0.183</td>
</tr>
<tr>
<td>450 min.</td>
<td>4.290</td>
<td>1.440</td>
<td>3.250</td>
<td>3.627</td>
<td>4.702</td>
<td>6.780</td>
<td>1.099</td>
<td>2.091</td>
<td>0.172</td>
<td>1.797</td>
</tr>
<tr>
<td>480 min.</td>
<td>0.001</td>
<td>0.172</td>
<td>0.006</td>
<td>0.003</td>
<td>0.001</td>
<td>0.001</td>
<td>0.289</td>
<td>0.057</td>
<td>0.866</td>
<td>0.098</td>
</tr>
<tr>
<td>-----------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
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<td>-----</td>
</tr>
<tr>
<td>Degrees of Freedom</td>
<td>11</td>
<td>14</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>11</td>
<td>13</td>
<td>15</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Weight before</td>
<td>0.263</td>
<td>0.519</td>
<td>0.725</td>
<td>0.374</td>
<td>0.618</td>
<td>0.070</td>
<td>1.670</td>
<td>1.151</td>
<td>1.681</td>
<td>1.564</td>
</tr>
<tr>
<td>Weight on day</td>
<td>0.798</td>
<td>0.612</td>
<td>0.485</td>
<td>0.715</td>
<td>0.547</td>
<td>0.945</td>
<td>0.119</td>
<td>0.268</td>
<td>0.114</td>
<td>0.146</td>
</tr>
<tr>
<td>Change in weight</td>
<td>0.434</td>
<td>0.859</td>
<td>1.470</td>
<td>0.399</td>
<td>2.131</td>
<td>1.352</td>
<td>0.544</td>
<td>1.683</td>
<td>0.894</td>
<td>1.432</td>
</tr>
<tr>
<td>% Recovery after C-14-Glucose</td>
<td>0.673</td>
<td>0.405</td>
<td>0.172</td>
<td>0.697</td>
<td>0.053</td>
<td>0.204</td>
<td>0.596</td>
<td>0.113</td>
<td>0.486</td>
<td>0.180</td>
</tr>
<tr>
<td>15 min.</td>
<td>0.607</td>
<td>2.845</td>
<td>0.913</td>
<td>0.846</td>
<td>4.083</td>
<td>2.075</td>
<td>0.048</td>
<td>0.536</td>
<td>1.181</td>
<td>0.430</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.556</td>
<td>0.013</td>
<td>0.383</td>
<td>0.414</td>
<td>0.001</td>
<td>0.062</td>
<td>0.962</td>
<td>0.600</td>
<td>0.256</td>
<td>0.676</td>
</tr>
<tr>
<td>45 min.</td>
<td>0.798</td>
<td>0.519</td>
<td>0.725</td>
<td>0.374</td>
<td>0.618</td>
<td>0.070</td>
<td>1.670</td>
<td>1.151</td>
<td>1.681</td>
<td>1.564</td>
</tr>
<tr>
<td>60 min.</td>
<td>0.263</td>
<td>0.519</td>
<td>0.725</td>
<td>0.374</td>
<td>0.618</td>
<td>0.070</td>
<td>1.670</td>
<td>1.151</td>
<td>1.681</td>
<td>1.564</td>
</tr>
<tr>
<td>90 min.</td>
<td>0.037</td>
<td>0.006</td>
<td>0.153</td>
<td>0.242</td>
<td>0.003</td>
<td>0.026</td>
<td>0.026</td>
<td>0.316</td>
<td>0.022</td>
<td>0.240</td>
</tr>
<tr>
<td>120 min.</td>
<td>1.168</td>
<td>4.499</td>
<td>1.592</td>
<td>3.771</td>
<td>3.496</td>
<td>3.296</td>
<td>0.695</td>
<td>0.553</td>
<td>3.187</td>
<td>1.422</td>
</tr>
<tr>
<td>150 min.</td>
<td>0.268</td>
<td>0.001</td>
<td>0.142</td>
<td>0.003</td>
<td>0.004</td>
<td>0.007</td>
<td>0.499</td>
<td>0.588</td>
<td>0.006</td>
<td>0.183</td>
</tr>
<tr>
<td>180 min.</td>
<td>0.336</td>
<td>5.051</td>
<td>0.302</td>
<td>4.247</td>
<td>3.720</td>
<td>4.262</td>
<td>1.176</td>
<td>0.129</td>
<td>3.699</td>
<td>3.021</td>
</tr>
<tr>
<td>210 min.</td>
<td>0.743</td>
<td>0.001</td>
<td>0.769</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.261</td>
<td>0.899</td>
<td>0.002</td>
<td>0.012</td>
</tr>
<tr>
<td>240 min.</td>
<td>0.223</td>
<td>5.257</td>
<td>0.155</td>
<td>4.258</td>
<td>3.583</td>
<td>4.473</td>
<td>2.826</td>
<td>0.391</td>
<td>1.508</td>
<td>3.404</td>
</tr>
<tr>
<td>15 min.</td>
<td>0.828</td>
<td>0.001</td>
<td>0.880</td>
<td>0.001</td>
<td>0.003</td>
<td>0.001</td>
<td>0.014</td>
<td>0.701</td>
<td>0.152</td>
<td>0.006</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.460</td>
<td>5.924</td>
<td>1.394</td>
<td>5.836</td>
<td>3.793</td>
<td>4.604</td>
<td>2.318</td>
<td>0.856</td>
<td>0.536</td>
<td>3.408</td>
</tr>
<tr>
<td>45 min.</td>
<td>0.655</td>
<td>0.001</td>
<td>0.194</td>
<td>0.001</td>
<td>0.002</td>
<td>0.001</td>
<td>0.037</td>
<td>0.406</td>
<td>0.600</td>
<td>0.006</td>
</tr>
<tr>
<td>60 min.</td>
<td>0.076</td>
<td>6.260</td>
<td>0.700</td>
<td>6.296</td>
<td>3.473</td>
<td>4.179</td>
<td>1.846</td>
<td>1.174</td>
<td>1.160</td>
<td>3.288</td>
</tr>
<tr>
<td>90 min.</td>
<td>0.941</td>
<td>0.001</td>
<td>0.500</td>
<td>0.001</td>
<td>0.004</td>
<td>0.002</td>
<td>0.088</td>
<td>0.259</td>
<td>0.306</td>
<td>0.007</td>
</tr>
<tr>
<td>120 min.</td>
<td>0.057</td>
<td>6.574</td>
<td>0.058</td>
<td>6.285</td>
<td>3.072</td>
<td>3.385</td>
<td>1.684</td>
<td>1.774</td>
<td>2.203</td>
<td>2.272</td>
</tr>
<tr>
<td>150 min.</td>
<td>0.956</td>
<td>0.001</td>
<td>0.955</td>
<td>0.001</td>
<td>0.009</td>
<td>0.006</td>
<td>0.116</td>
<td>0.096</td>
<td>0.044</td>
<td>0.044</td>
</tr>
<tr>
<td>180 min.</td>
<td>0.255</td>
<td>6.078</td>
<td>0.075</td>
<td>5.293</td>
<td>2.167</td>
<td>2.103</td>
<td>1.701</td>
<td>1.960</td>
<td>2.151</td>
<td>1.913</td>
</tr>
<tr>
<td>210 min.</td>
<td>0.804</td>
<td>0.001</td>
<td>0.942</td>
<td>0.001</td>
<td>0.049</td>
<td>0.059</td>
<td>0.113</td>
<td>0.069</td>
<td>0.048</td>
<td>0.082</td>
</tr>
<tr>
<td>240 min.</td>
<td>0.076</td>
<td>6.187</td>
<td>0.047</td>
<td>3.982</td>
<td>1.585</td>
<td>0.860</td>
<td>1.409</td>
<td>2.072</td>
<td>3.504</td>
<td>1.320</td>
</tr>
<tr>
<td>15 min.</td>
<td>0.940</td>
<td>0.001</td>
<td>0.963</td>
<td>0.002</td>
<td>0.137</td>
<td>0.408</td>
<td>0.182</td>
<td>0.056</td>
<td>0.003</td>
<td>0.214</td>
</tr>
<tr>
<td>30 min.</td>
<td>0.129</td>
<td>6.020</td>
<td>0.249</td>
<td>2.771</td>
<td>0.909</td>
<td>0.191</td>
<td>1.241</td>
<td>1.629</td>
<td>3.407</td>
<td>0.051</td>
</tr>
<tr>
<td>45 min.</td>
<td>0.900</td>
<td>0.001</td>
<td>0.808</td>
<td>0.017</td>
<td>0.380</td>
<td>0.852</td>
<td>0.236</td>
<td>0.124</td>
<td>0.004</td>
<td>0.960</td>
</tr>
</tbody>
</table>
Footnotes to Table 11

a. Group symbol of treatment protocol as given in Table 10.
b. Dose of carrier D-glucose given with 20 μCi of uniformly labeled C-14-D-glucose at time zero
c. Number of animals contributing to all mean values in that column
d. Weight on evening before overnight fast or before streptozotocin treatment for groups 0 and P
e. Weight on morning of experiment after overnight fast
f. Change in weight during overnight fast except for groups 0 and P where mean blood glucose after overnight fast is presented.
g. Percent of C-14 label recovered as C-14 CO₂ at each specified time
h. Mean value of indicated number of rats
i. Standard error of mean
j. Groups compared using unpaired two tailed Student's "t" tests for each corresponding pair of data points
k. Degrees of freedom for Student's "t" test
l. t value
m. p value of significant difference
n. 0.001 indicates "p" value equal to or less than 0.001
Recovery of expired $^{14}$CO$_2$ in saline treated control groups given either a tracer dose (Group A) or a 400 mg load (Group B) was significantly greater in Group A at each sampling time (Figure 13). Similar treatment with 1 mg endotoxin and tracer glucose injection (Group C) resulted in no difference from Group A before the 150 min. samples; however thereafter significantly more label was recovered. Endotoxin with a 400 mg glucose load (Group D) resulted in significantly more label recovered such that at 240 min. 43.1% of the glucose dose was recovered as expired $^{14}$CO$_2$. When glucose injection and CO$_2$ collection were withheld until 2 hr after the 1 mg endotoxin dose (Groups E and F), increased recovery was manifest and all values were significantly greater than the corresponding values for Groups A and B, respectively (Figure 14). The marked increase in glucose oxidation after endotoxin (Table 11) was completely abolished by mannoheptulose suppression of insulin secretion (Groups G vs I and H vs J, Figure 15). Using a 4 hr pretreatment protocol without endotoxin (Groups K and L) mannoheptulose was less effective in reducing glucose oxidation when compared to 1 hr pretreatment (Groups G and H) for both the tracer dose (Figure 16) as well as the glucose load (Figure 17). In contrast, glucose oxidation was actually depressed in endotoxemic rats if mannoheptulose was administered 4 hrs prior to glucose (Groups M and N, Figure 18). In further support of an insulin involvement, streptozotocin induced diabetes both suppressed glucose oxidation (Group O, Figure 19) and prevented the endotoxin mediated increments in glucose oxidation (Groups P vs O, Figure 19).

Since low lethality occurs in overnight fasted rats given
Groups of rats were treated as described in Table 10. Immediately following the specified dose of carrier glucose plus $20\muCi$ of uniformly labeled C-14-D-glucose the collection of respiratory CO$_2$ was begun. The mean values plotted are those listed in Table 11 where the standard error of mean and the significance of difference of the mean values for pairs of groups at corresponding times are indicated. Duplicate samples were collected from each rat at each of the times indicated on the figure. The legend in the upper left identifies the groups as specified in Tables 10 and 11.
FIGURE 14

EFFECT OF PREVIOUS ENDOTOXIN TREATMENT ON
RECOVERY OF GLUCOSE CARBON LABEL AS CO₂

Legend as in Figure 13.
FIGURE 15

EFFECT OF MANNOHEPTULOSE TREATMENT ON RECOVERY OF GLUCOSE CARBON LABEL AS CO₂ IN RATS ACUTELY TREATED WITH ENDOTOXIN

Legend as in Figure 13.
FIGURE 16

GLUCOSE CARBON LABEL RECOVERY AS CO\textsubscript{2} USING A TRACER DOSE OF
GLUCOSE AND TWO PROTOCOLS OF MANNOHEPTULOSE TREATMENT

Legend as in Figure 13.
FIGURE 17

GLUCOSE CARBON LABEL RECOVERY AS CO₂ USING A 400 mg DOSE OF LABELED GLUCOSE AND TWO PROTOCOLS OF MANNOHEPTULOSE TREATMENT

Legend as in Figure 13.
FIGURE 18

EFFECT OF PREVIOUS ENDOTOXIN TREATMENT ON RECOVERY OF GLUCOSE CARBON LABEL AS CO₂ IN MANNOHEPTULOSE TREATED RATS

Legend as in Figure 13.
FIGURE 19

RECOVERY OF GLUCOSE CARBON LABEL AS CO₂ IN
STREPTOZOTOCIN DIABETIC RATS TREATED WITH ENDOTOXIN

Legend as in Figure 13.
1 mg endotoxin (Table 1), the influence of a high dose of endotoxin (3 mg per rat) on glucose oxidation was evaluated (Figures 20 and 21). Enhanced glucose oxidation was manifest earlier in response to 3 mg endotoxin using a tracer glucose dose (Group Q, Figure 20) when compared to 1 mg endotoxin (Group C); however, statistical significance was not achieved when a glucose load was used (Groups R vs D, Figure 20). No differences in glucose oxidation occurred when endotoxin at either 1 or 3 mg per rat iv was given 2 hr prior to either a tracer glucose dose or a load (Groups E vs S and Groups F vs T, Figure 21). It should be noted that while 3 mg endotoxin + tracer glucose resulted in 77% lethality (N=29), glucose (400 mg) treatment increased lethality to 100% (N=18).

Dexamethasone acetate significantly depressed glucose oxidation in both saline iv controls (Groups A vs U) and endotoxemic rats (Groups C vs V) such that no differences were measurable in the dexamethasone treated groups (Groups U vs V, Figure 22).

In order to extend these observations from endotoxin to traumatic shock, glucose oxidation was evaluated after Noble-Collip tumbling stress. In contrast to the enhanced glucose oxidation of endotoxemia, tumbling trauma actually depressed label recovery at the tracer dose (Groups Y vs W) but had only minimal influence when a glucose load was used (Groups Z vs X, Figure 23).

E. Carbon Clearance in Insulin Hypoglycemia

To assess the possible influence of blood glucose on the phagocytic activity of the RES the clearance of colloidal carbon from the blood was studied (Tables 12 and 13, Figures 24 and 25).
COMPARISON OF GLUCOSE CARBON LABEL RECOVERY AS CO₂ IN RATS ACUTELY TREATED WITH 1 OR 3 mg OF ENDOTOXIN

Legend as in Figure 13.
FIGURE 21

COMPARISON OF GLUCOSE CARBON LABEL RECOVERY AS CO₂ IN RATS PREVIOUSLY TREATED WITH 1 OR 3 mg OF ENDOTOXIN

Legend as in Figure 13.
FIGURE 22

INFLUENCE OF DEXAMETHASONE TREATMENT ON GLUCOSE CARBON LABEL

RECOVERY AS CO₂ IN CONTROL AND ENDOTOXEMIC RATS

Legend as in Figure 13.
RECOVERY OF GLUCOSE CARBON LABEL AS CO₂
IN IMMOBILIZED AND TRAUMATIZED RATS

Legend as in Figure 13.
The insulin treated rats were divided into two groups using 30 mg per dl as the hypoglycemic criterion. In addition, the data of all insulin treated rats was combined and treated as one group (Table 12, Group 2 + 3).

In control rats treated with saline a significant negative correlation between carbon clearance half-time and blood glucose was observed (Figure 24). Insulin treated rats which had blood glucose values above 30 mg per dl had carbon clearance half-times significantly longer than in saline controls, i.e., 19.9 min. vs 16.8 min. (Table 12). Figure 25 depicts a linear regression of carbon clearance half-time on blood glucose. This relationship was not different from that shown in Figure 24. Insulin hypoglycemia markedly prolonged the intravascular carbon half-time i.e., 30.8 min. vs 16.8 min. (Table 12). The correlation of half-time on blood glucose was not significant in this restricted blood glucose range. A significant correlation of clearance half-time and blood glucose occurred when all the insulin treated rats were grouped together; with a slope of -26 min. per 100 mg glucose per dl (Table 13 and Figure 25). This regression line is significantly different from that of the saline treated controls (Table 13).

F. Endotoxin Clearance in vivo

This phase of the study focused on the influence of hypoglycemia on the disappearance of biologically active endotoxin from the vascular compartment. However, initial experiments were required in order to substantiate the use of the lead sensitized rat bioassay for measurement of intravascular endotoxin clearance. In particular
Carbon clearance half-time is plotted against the mean blood glucose for the 40 saline treated rats shown in Table 12. The best line through the points was computed by the method of least squares.
<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>N</th>
<th>Body Weight (gm)</th>
<th>Mean Blood Glucose (mg/dl)</th>
<th>Carbon Clearance Half-time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^b</td>
<td>Saline</td>
<td>40</td>
<td>306.45f ±3.14f</td>
<td>90.57 ±2.29</td>
<td>16.80 ±0.39</td>
</tr>
<tr>
<td>2^c</td>
<td>Insulin</td>
<td>16</td>
<td>309.81 ±3.20</td>
<td>45.44 ±4.70</td>
<td>19.94 ±0.80</td>
</tr>
<tr>
<td>3^d</td>
<td>Insulin</td>
<td>15</td>
<td>308.07 ±4.47</td>
<td>21.00 ±1.78</td>
<td>30.80 ±1.71</td>
</tr>
<tr>
<td>(2 + 3)^e</td>
<td>Insulin</td>
<td>31</td>
<td>308.91 ±3.41</td>
<td>34.84 ±3.61</td>
<td>25.19 ±1.34</td>
</tr>
</tbody>
</table>

Student's t tests of above data^h

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Degrees of Freedom</th>
<th>Blood Glucose</th>
<th>Half-time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>54</td>
<td>8.632^i</td>
<td>3.528</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001^j</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>53</td>
<td>23.986</td>
<td>7.982</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 vs (2 + 3)</td>
<td>69</td>
<td>13.044</td>
<td>6.012</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>29</td>
<td>4.863</td>
<td>5.752</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

a. Carbon clearance was evaluated at a dose of 8 mg per 100 gm body weight. Forty fed or fasted control rats treated with saline were paired with the insulin treated rats, each pair was treated with the same injection volume at the same time before the carbon clearance test. The insulin dose ranged from 0.5 to 4.0 units and was given at 0 to 4 hrs before the carbon clearance test to produce a wide spectrum of blood glucose values.

b. All saline treated rats

c. Insulin treated rats not hypoglycemic i.e., mean blood glucose > 30 mg per dl

d. Insulin hypoglycemic rats i.e., mean blood glucose of 30 or less mg per dl

e. All insulin treated rats combined and analyzed as one group

f. Mean

g. Standard error of mean
Footnotes to Table 12 (cont'd)

h. Two tailed Student's "t" test for non-paired data
i. Value of Student's "t"
j. $p$ value for corresponding value of $t$ at specified number of degrees of freedom
### TABLE 13

REGRESSION OF CARBON CLEARANCE HALF-TIME ON BLOOD GLUCOSE IN CONTROL AND INSULIN TREATED RATS\(^a\)

<table>
<thead>
<tr>
<th>Group (^b)</th>
<th>N</th>
<th>Pearson's r</th>
<th>Degrees of Freedom</th>
<th>p(^c)</th>
<th>M(^d)</th>
<th>B(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>-0.3911</td>
<td>38</td>
<td>0.0126</td>
<td>-0.0666</td>
<td>22.8347</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>-0.5893</td>
<td>14</td>
<td>0.0163</td>
<td>-0.0952</td>
<td>24.4868</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>-0.2435</td>
<td>13</td>
<td>0.3818</td>
<td>-0.2335</td>
<td>35.7042</td>
</tr>
<tr>
<td>(2 + 3)</td>
<td>31</td>
<td>-0.6423</td>
<td>29</td>
<td>0.001</td>
<td>-0.2560</td>
<td>33.8238</td>
</tr>
</tbody>
</table>

Statistical Comparison of above Regression Lines\(^f\)

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Degrees of Freedom</th>
<th>Student's t</th>
<th>p Value(^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>52</td>
<td>1.1514</td>
<td>0.2549</td>
</tr>
<tr>
<td>1 vs (2 + 3)</td>
<td>67</td>
<td>6.1230</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(a\). Regression and correlation parameters for data from groups shown in Table 12 and in Figures 24 and 25.

\(b\). Groups as designated in Table 12

\(c\). p value associated with indicated value of Pearson's coefficient of correlation at specified number of degrees of freedom

\(d\). Slope in 100 min x ml per mg for regression equation

Carbon Clearance Half-time (min = \(M\) (M\(xB\) Blood glucose in mg per 100 ml) + B

\(e\). Y axis intercept in min. for regression equation

Carbon Clearance Half-time (min) (M\(xB\) Blood glucose in mg per 100 ml) + B

\(f\). Evaluation of significance of difference of regression lines using program given in the Appendix.

\(g\). p value associated with indicated Student's "t" value for 2 tailed test at the indicated number of degrees of freedom
Carbon clearance half-time is plotted against mean blood glucose for the 31 insulin treated rats. Rats with a mean blood glucose value of or below 30 mg/dl are represented by ▲; rats with a mean blood glucose value above 30 mg/dl are represented by ●. The broken line extending across the figure is the best fit line through all 31 points. The cross slashed line extending to the right of the 30 mg/dl line is the best fit line through the 16 points corresponding to rats with blood glucose values of 30 or more mg/dl. Both lines were fitted by the method of least squares. The 2 uppermost ▲'s are off the scale of the axis at values of 43 and 45 minutes.
it was necessary to establish the relationship between endotoxin dose and lethality. Two hundred rats were divided into 5 groups and given 0.4, 0.5, 0.6, 0.75, or 1.0$\mu$gm of endotoxin simultaneously with a sensitizing dose of lead. As shown in Figure 26 a significant linear relationship was found between the logarithm of the dose and the probit of the assay group lethality in the range of probits 4 to 6 i.e., 16% to 84% lethality.

The clearance of a 500$\mu$gm endotoxin dose from the vascular compartment of non-pretreated 300 gm fed rats is presented in Table 14 and Figure 27; in this study tail blood for analysis was obtained by serial sampling. The half-time for disappearance of endotoxin from the blood was 68 min. and demonstrated a significant ($p<0.001$) linear relationship of the logarithm of blood endotoxin concentration to time (Figure 27). A more extensive series of experiments using only one blood sample from each clearance rat was designed to evaluate this method of determining the clearance of endotoxin (Table 15). Figure 27 compares the results obtained in the single sample experiments with a 500$\mu$gm endotoxin dose with the results obtained in the serial sampling experiments (Table 14). The half-time using the single sample technique was comparable to that of the previous experiment - 58 vs 68 minutes - and the coefficient of correlation was significant at the 0.01 level (Figure 27). For comparison with other clearance groups the clearance line for the single sample rats receiving 500$\mu$gm endotoxin appears on Figures 28 through 31. When the dose was doubled to 1,000$\mu$gm per rat, endotoxin disappearance appeared proportional to the blood endotoxin concentration in the period from 60 to 240 min.
FIGURE 26

DOSE-LETHALITY CURVE FOR 5 DOSES OF
ENDOTOXIN IN LEAD SENSITIZED RATS

The probits of the lethality of 5 groups of lead sensitized assay rats are plotted against the dose of endotoxin. The coefficient of correlation and the significance are indicated in the upper left for the probit range of 4.0-6.0
**TABLE 14**
**ENDOTOXIN CLEARANCE in vivo USING A SERIAL SAMPLING TECHNIQUE**

Endotoxin Assay Standards

<table>
<thead>
<tr>
<th>Dose (µg/rat)</th>
<th>N&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% Lethality</th>
<th>Probit&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>60</td>
<td>36.7</td>
<td>4.660</td>
</tr>
<tr>
<td>0.50</td>
<td>72</td>
<td>70.8</td>
<td>5.548</td>
</tr>
</tbody>
</table>

Unknown Diluted Blood Samples

<table>
<thead>
<tr>
<th>Time after Endotoxin (min)&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Number of Clearance Rats&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Number of Assay Rats&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Blood Endotoxin (µg/ml)&lt;sup&gt;g&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>3</td>
<td>25</td>
<td>31.2</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>26</td>
<td>25.6</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>41</td>
<td>18.0</td>
</tr>
<tr>
<td>120</td>
<td>5</td>
<td>50</td>
<td>9.8</td>
</tr>
<tr>
<td>240</td>
<td>5</td>
<td>50</td>
<td>3.0</td>
</tr>
</tbody>
</table>

---

a. Normal fed rats of 300 gm body weight were given 500µgm of endotoxin in 1 ml of saline iv at time zero. Aliquots of tail blood were collected serially from each of the 5 rats at the 5 indicated times. The blood was diluted in normal saline and bioassayed for endotoxin in lead sensitized rats. To permit estimation of the amount of endotoxin 132 rats received known doses of endotoxin.

b. Number of assay rats given specified dose of endotoxin.

c. Probit transform of lethality

d. Time after iv endotoxin at which blood samples were collected from clearance rats.

e. Number of clearance rats from which blood samples were bioassayed for endotoxin.

f. Number of rats used to assay diluted blood from indicated number of clearance rats.

g. Endotoxin content calculated from probit of assay rats and dilution factor.
Blood endotoxin content is plotted against time after injection of a 500 mg test clearance dose in 300 gram rat. X = clearance rats from which tail blood samples were obtained serially. O = clearance rats from which single samples were obtained by cardiac puncture. N_C = number of clearance rats. N_A = number of assay rats used for clearance samples. T_{1/2} = calculated half-time of disappearance of endotoxin from the blood assuming monoeXponential clearance and using the slope of best line through the points fitted by the method of least squares. The dashed line appears on Figs. 28-31 for comparison with other groups.
## TABLE 15
ENDOTOXIN CLEARANCE IN VIVO USING SINGLE CARDIAC BLOOD SAMPLES

### Endotoxin Assay Standards

<table>
<thead>
<tr>
<th>Dose (µgm/rat)</th>
<th>N</th>
<th>Lethality</th>
<th>Probit</th>
<th>Correlation (Probit analysis) ( r^c )</th>
<th>( r^d )</th>
<th>( p^e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>70</td>
<td>37.1</td>
<td>4.671</td>
<td>0.9942</td>
<td>9.2444</td>
<td>0.0686</td>
</tr>
<tr>
<td>0.50</td>
<td>100</td>
<td>57.0</td>
<td>5.176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>100</td>
<td>82.0</td>
<td>5.915</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Unknown Diluted Blood Samples

<table>
<thead>
<tr>
<th>Clearance Group</th>
<th>Dose per Clearance Rat (µgm)</th>
<th>Time after iv Endotoxin (min)</th>
<th>Number of Clearance Rats</th>
<th>Number of Assay Rats</th>
<th>Blood Endotoxin (µgm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>500</td>
<td>60</td>
<td>9</td>
<td>90</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>9</td>
<td>90</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>6</td>
<td>60</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240</td>
<td>9</td>
<td>90</td>
<td>2.5</td>
</tr>
<tr>
<td>Normal</td>
<td>1,000</td>
<td>60</td>
<td>4</td>
<td>40</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>4</td>
<td>40</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240</td>
<td>3</td>
<td>30</td>
<td>3.0</td>
</tr>
<tr>
<td>Normal</td>
<td>250</td>
<td>60</td>
<td>4</td>
<td>40</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>4</td>
<td>40</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240</td>
<td>4</td>
<td>40</td>
<td>2.1</td>
</tr>
<tr>
<td>Tolerant</td>
<td>500</td>
<td>15</td>
<td>3</td>
<td>30</td>
<td>2.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>2</td>
<td>20</td>
<td>2</td>
</tr>
</tbody>
</table>
TABLE 15 (cont'd)

<table>
<thead>
<tr>
<th>Clearance Group</th>
<th>Dose per Clearance Rat (μg/ml)</th>
<th>Time after iv endotoxin (min)</th>
<th>Number of Clearance Rats</th>
<th>Number of Assay Rats</th>
<th>Blood Endotoxin (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16-18 hr after 500 g endotoxin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500</td>
<td>15</td>
<td>3</td>
<td>30</td>
<td>5.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>3</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>4 hr after 500 g endotoxin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>500</td>
<td>15</td>
<td>4</td>
<td>40</td>
<td>26.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>5</td>
<td>50</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>5</td>
<td>50</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>240</td>
<td>3</td>
<td>30</td>
<td>2.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Rats were given the specified dose of endotoxin iv in saline at a concentration of 500 gm per ml in saline at time zero. At the indicated times the clearance rats were bled by cardiac puncture and their blood was diluted to 25 to 100 1 of blood per ml in normal saline and bioassayed for endotoxin as described in lead treated rats. Symbols used in the table are the same as those in Table 22 with the following additions.

<sup>b</sup> Linear correlation of probit of lethality and logarithm of endotoxin dose.

<sup>c</sup> Pearson's r for correlation

<sup>d</sup> t value corresponding to indicated value of r

<sup>e</sup> p value corresponding to indicated t value

<sup>f</sup> Treated with 1 mg endotoxin on days 1, 3, and 5 with clearance evaluated on day 7.

<sup>g</sup> Treated with 500 μg/ml endotoxin 16-18 hr prior to administration of the test dose.

<sup>h</sup> Treated with 500 μg/ml of endotoxin 4 hrs prior to the test dose of endotoxin.
after endotoxin (Figure 28); there was a significant correlation of
the logarithm of blood endotoxin and time. The half-time was 57 min.
which was almost identical to the 500μg/ml dose clearance value. At a
lower dose of 250μg/ml endotoxin no significant linear relationship was
obtained on a semi-logarithmic plot. From the points plotted and the
expected blood level of endotoxin at zero time it appeared that the
rate of disappearance decreased with time, at an estimated half-time
of 103 min. To further evaluate this method of endotoxin clearance
measurement, rats were pretreated with endotoxin prior to analysis
(Table 15 and Figures 29 and 30). Tolerance induced by 3 injections
of 1 mg endotoxin on alternate days resulted in no detectable endo-
toxin after 15 min. (Figure 29); this finding is in agreement with the
well-known rapid clearance of endotoxin in the tolerant state. Further-
more endotoxin pretreatment only 16 to 18 hr before a 500μg/ml test dose
resulted in only 5μg/ml per ml of bioassayable endotoxin at 15 min. As
in the tolerant rats less than 2 gm per ml of blood were found at 60
and 120 min; the slope of the line between the 15 min. value and the
maximum value at 60 min. corresponded to a half-time of 33 min.
Lastly, rats were pre-treated with 500μg/ml endotoxin 4 hr before the
clearance study. In this group clearance appeared to be multiphasic
and the slope of a line between the 15 and 60 min. points corresponded
to a half-time of 30 min., in the 60 to 120 min. interval the apparent
half-time was increased to 75 min., and in the 120 to 240 min. period
the slope of a line connecting the points corresponded to a 151 min.
half-time. Since endotoxin sensitivity accompanies adrenal in-
sufficiency, endotoxin clearance was also studied after acute
FIGURE 28

ENDOTOXIN CLEARANCE in vivo AT THREE DOSES OF ENDOTOXIN

Blood endotoxin contents at 60, 120, and 240 minutes after a dose of 1,000 micrograms or 250 micrograms per 300 gm rat were used to determine the line of best fit (least squares). The "Single Samples" group given a 500 microgram dose is shown for comparison (---).
FIGURE 29

INFLUENCE OF TOLERANCE AND PRIOR ENDOTOXIN TREATMENT ON ENDOTOXIN CLEARANCE in vivo

$X$ = 7 Day Tolerance $N_c=8$
$N_A=80$

$O$ = 16-18 hour Pre-Treated $T_{1/2}<33$ minutes (-----)

$N_c=9$
$N_A=90$

$X$ = tolerance induced by 3 injections of 1 mg endotoxin on alternate days with a 500 microgram test clearance dose administered on day 7.

$O$ = 500 microgram endotoxin at 16 to 18 hrs prior to a 500 microgram test clearance dose.
FIGURE 30

INFLUENCE OF ENDOTOXIN PRETREATMENT ON

ENDOTOXIN CLEARANCE in vivo

- Pre-Treated 4 hr. Prior
  \( r = -0.9263 \)  \( P > 0.05 \)
  \( T_{1/2} = 75\text{min.} \)  \( N_c = 17 \)
  \( N_A = 170 \)

\( \bullet = 500 \) micrograms of endotoxin 4 hrs prior to a 500 microgram test clearance dose.

\( \circ = \) estimated blood endotoxin content at time zero based on 500 microgram control group of Table 15.
adrenalectomy (Table 16 and Figure 31). Sham-adrenalectomy resulted in no significant change in endotoxin clearance as compared to untreated controls. In contrast, adrenalectomy significantly augmented endotoxin clearance as reflected in a half-time of 38 min. (Figure 31). The influence of insulin hypoglycemia on endotoxin clearance is depicted in Figure 32 and Table 17. As depicted, insulin hypoglycemia depressed endotoxin clearance as evidenced in the blood endotoxin concentration at 3 hr of 3 times control values. Fasted controls had a half-time for endotoxin clearance similar to fed rats i.e., 51 min.

G. Endotoxin Clearance by the Perfused Rat Liver

As a complement to the in vivo studies of endotoxin clearance (Section F, Chapter 4) the uptake of bioassayable endotoxin by the isolated perfused rat liver was also studied. In order to quantitate endotoxin a 5 point standard dose lethality curve was employed and the probit transform of the lethality scores with 95% confidence limits about the best line (least squares fit) through the points is shown in Figure 33. The line and the confidence limits were computed as described by Finney (212). The coefficient of correlation for this line is significant at the p<0.001 level.

Endotoxin clearance during liver perfusions with balanced salt solution are shown in Table 18. The probit of lethality for the initial perfusate before addition to the perfusion apparatus was not different than would have been expected for an endotoxin dose of \( \mu \text{gm} \) per assay rat; this corresponds to \( 2 \mu \text{gm} \) per ml of perfusate since each perfusate sample was diluted two-fold with normal saline before assaying 1 ml aliquots in lead sensitized rats (Sample 1).
### TABLE 16
ENDOTOXIN CLEARANCE AFTER SURGICAL STRESS OR ACUTE ADRENALECTOMY

#### Endotoxin Assay Standards

<table>
<thead>
<tr>
<th>Dose (μg/m/rat)</th>
<th>N</th>
<th>Lethality %</th>
<th>Probit</th>
<th>Correlation (Probit analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>50</td>
<td>36.7</td>
<td>4.660</td>
<td>0.9968</td>
</tr>
<tr>
<td>0.25</td>
<td>50</td>
<td>52.0</td>
<td>5.050</td>
<td>17.6353</td>
</tr>
<tr>
<td>0.50</td>
<td>50</td>
<td>74.0</td>
<td>5.643</td>
<td>0.0032</td>
</tr>
<tr>
<td>1.00</td>
<td>50</td>
<td>88.0</td>
<td>6.175</td>
<td></td>
</tr>
</tbody>
</table>

#### Unknown Diluted Blood Samples

<table>
<thead>
<tr>
<th>Clearance Group</th>
<th>Dose per Group Clearance</th>
<th>Time after IV endotoxin (min)</th>
<th>Number of Clearances Rats</th>
<th>Number of Assay Rats</th>
<th>Blood Endotoxin (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Sham Adrenalectomy</td>
<td>500</td>
<td>60</td>
<td>4</td>
<td>40</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>4</td>
<td>40</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>4</td>
<td>40</td>
<td>2.3</td>
</tr>
<tr>
<td>Acute Adrenalectomy</td>
<td>500</td>
<td>60</td>
<td>5</td>
<td>50</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>120</td>
<td>6</td>
<td>60</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>4</td>
<td>40</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>5</td>
<td>50</td>
<td>1.8</td>
</tr>
</tbody>
</table>

---

*a.* Rats were either adrenalectomized or sham operated 1 hr before the test dose of endotoxin. All other symbols as in Table 23.
FIGURE 31

INFLUENCE OF SURGICAL TRAUMA AND ACUTE ADRENALECTOMY

ON ENDOTOXIN CLEARANCE in vivo

- Surgical Shams
  $r = -0.9734$
  $T_{1/2} = 41 \text{ min.}$
  $N_C = 12$
  $N_A = 120$

- Acute Adrenalectomy
  $r = -0.9992$
  $P < 0.001$
  $N_C = 17$
  $N_A = 170$
  $T_{1/2} = 38 \text{ min.}$

$\bullet = \text{surgical sham adrenalectomy 1 hr prior to a 500 microgram test clearance dose of endotoxin.}$

$X = \text{acute adrenalectomy 1 hr prior to a 500 microgram test clearance dose of endotoxin.}$
### TABLE 17
**ENDOTOXIN CLEARANCE IN FASTED AND INSULIN HYPOGLYCEMIC RATS**

#### Endotoxin Assay Standards

<table>
<thead>
<tr>
<th>Dose (µg/rat)</th>
<th>N</th>
<th>% Lethality</th>
<th>Probit</th>
<th>Correlation (Probit analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.10</td>
<td>20</td>
<td>15</td>
<td>3.964</td>
<td>r = 0.9843, t = 7.8866, p = 0.0157</td>
</tr>
<tr>
<td>0.25</td>
<td>50</td>
<td>32</td>
<td>4.532</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td>60</td>
<td>70</td>
<td>5.524</td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>50</td>
<td>82</td>
<td>5.915</td>
<td></td>
</tr>
</tbody>
</table>

#### Unknown Diluted Blood Samples

<table>
<thead>
<tr>
<th>Clearance Group</th>
<th>Dose per Clearance (µg)</th>
<th>Time after iv endotoxin (min)</th>
<th>Mean Blood Glucose (mg/dl)</th>
<th>Number of Clearance Rats</th>
<th>Number of Assay Rats</th>
<th>Blood Endotoxin (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasted +</td>
<td>500</td>
<td>60</td>
<td>154</td>
<td>2</td>
<td>20</td>
<td>10.3</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>180</td>
<td>87</td>
<td>5</td>
<td>50</td>
<td>2.04</td>
</tr>
<tr>
<td>Fasted +</td>
<td>500</td>
<td>60</td>
<td>30</td>
<td>3</td>
<td>30</td>
<td>19.9</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>120</td>
<td>30</td>
<td>6</td>
<td>60</td>
<td>17.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180</td>
<td>32</td>
<td>7</td>
<td>70</td>
<td>6.5</td>
</tr>
</tbody>
</table>

a. Rats weighing 300 ± 1 gm were fasted overnight. At 1 hr prior to the clearance dose of 500 gm endotoxin rats were given either 500 milli Units of Lente insulin sc or 12.5 µl of saline. All symbols are as indicated in Table 23.

b. Mean blood glucose at time sample was obtained for endotoxin bioassay.
ENDOTOXIN CLEARANCE in vivo IN INSULIN HYPOGLYCEMIC RATS

\[ \Delta = 500 \text{ milliunits of insulin sc 1 hr prior to a 500 microgram test clearance dose of endotoxin.} \]

\[ \bigcirc = \text{saline control group, 12.5 microliters of saline sc 1 hr prior to a 500 microgram test clearance dose of endotoxin.} \]
95% confidence limits of the dose lethality curve for endotoxin clearance by the perfused liver.

The 95% confidence limits about the best line for the 5 endotoxin standards is depicted. Two hundred rats were distributed in the 5 dosage groups of 0.4, 0.5, 0.6, 0.75, and 1.00 micrograms per assay rat. Endotoxin dose per assay rat is plotted on a logarithmic scale.
### TABLE 18
**ENDOTOXIN CLEARANCE in vitro BY RAT LIVER PERFUSED WITH BALANCED SALT SOLUTION**

<table>
<thead>
<tr>
<th>Sample Assayed</th>
<th>Number of Livers Perfused</th>
<th>Number of Assay Rats</th>
<th>% Lethality</th>
<th>Probit(^e) Endotoxin(^f) (µgm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Initial perfusate(^b)</td>
<td>6</td>
<td>60(^g)</td>
<td>90.0</td>
<td>6.282 (2.0)</td>
</tr>
<tr>
<td>2 Circulated 1 hr(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without liver in apparatus</td>
<td>6</td>
<td>61</td>
<td>80.3</td>
<td>5.852 1.81</td>
</tr>
<tr>
<td>3 After 1 hr of(^d) perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>through liver</td>
<td>6</td>
<td>60</td>
<td>56.7</td>
<td>5.169 1.19</td>
</tr>
</tbody>
</table>

Chi-Square Test of above groups

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Chi-Square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>1.5361</td>
<td>0.2152</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>15.3835</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>6.8010</td>
<td>0.0091</td>
</tr>
</tbody>
</table>

---

* Rat livers were perfused using an initial volume of 150 ml of Schimassek's balanced salt solution containing 2 micrograms per ml of endotoxin. The perfusate was circulated in the perfusion apparatus for 1 hr prior to addition of a liver. All samples were diluted with an equal volume of saline before assay for endotoxin in lead-sensitized rats. This table incorporates data from the first hour of perfusion of the livers which were also perfused with 10% rat serum (Table 19).
Footnotes to Table 18 (cont'd)

b. Samples of perfusate evaluated prior to addition to the liver perfusion apparatus.

c. Perfusate samples evaluated after circulating in the apparatus 1 hr in the absence of a rat liver.

d. Perfusate samples evaluated after 1 additional hour of perfusion through the rat liver.

e. Probit value corresponding to assay group lethality.

f. Endotoxin concentration estimates from probit of assay lethality; for initial perfusate the known amount of 2 micrograms per ml is indicated in parentheses.

g. Ten or eleven assay rats per sample
In addition, the mere circulation of the perfusate in the perfusion apparatus *per se* had no significant effect on bioassayable endotoxin, i.e., a probit analysis endotoxin content of 1.81μg per ml which is within the 95% confidence limits of 2.0μg per ml (Sample 2). After 1 hr of liver perfusion the perfusate endotoxin concentration was significantly reduced to an estimated mean concentration of 1.19μg per ml and the 95% confidence for the observed lethality were 1.01 to 1.38μg per ml (Sample 3). Chi-square comparisons confirmed that the endotoxin concentration of the 1 hr perfusate was significantly less than observed with either Sample 1 or 2 (Table 18). During the entire experiment an estimated 142μg of biologically active endotoxin disappeared from the perfusate and 115μg of this loss was attributed to liver uptake. Additional experiments employed 10% rat serum in the perfusion media (Table 19). No significant effect of serum on endotoxin clearance was demonstrated (Sample 4 vs 3, p>0.4). Furthermore, incubating the endotoxin with 10% rat serum in balanced salt solution had no significant effect on bioassay lethality (Sample 5 vs 3, p>0.1). Lastly, livers were perfused with 50% heparinized rat blood and endotoxin clearance evaluated (Table 20). The lethality of the initial perfusate was not different from 2.0μg per ml (Sample 1). During the 1 hr of circulation of the endotoxin containing blood perfusate no loss of bioassayable endotoxin was evident (Sample 2). After 30 min. or 90 min. of perfusion of the liver no loss of endotoxin from the perfusate was measured (Samples 3 and 4); though the probit value of the 90 min. blood perfusate was near the 95% confidence limit of 2.0μg per ml of perfusate. Study of blood detoxification indicated
### TABLE 19

**ENDOTOXIN CLEARANCE BY THE RAT LIVER PERFUSED WITH 10% RAT SERUM**

<table>
<thead>
<tr>
<th>Sample Assayed</th>
<th>Number of Perfusions or Incubations</th>
<th>Number of Assay Rats</th>
<th>% Lethality</th>
<th>Probit</th>
<th>Endotoxin (µgm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Initial Perfusate</td>
<td>3</td>
<td>30</td>
<td>90.0</td>
<td>6.282</td>
<td>(2.0)</td>
</tr>
<tr>
<td>2 Circulated 1 hr without liver in apparatus</td>
<td>3</td>
<td>30</td>
<td>76.7</td>
<td>5.729</td>
<td>1.67</td>
</tr>
<tr>
<td>3 After 1 hr of perfusion with balanced salt solution</td>
<td>3</td>
<td>30</td>
<td>40</td>
<td>4.747</td>
<td>0.91</td>
</tr>
<tr>
<td>4 After 2nd hr with 10% rat serum</td>
<td>3</td>
<td>30</td>
<td>53.3</td>
<td>5.083</td>
<td>1.12</td>
</tr>
<tr>
<td>5 1 hr perfusate + 10% serum incubated for period of liver perfusion with 10% serum</td>
<td>3</td>
<td>30</td>
<td>60</td>
<td>5.253</td>
<td>1.25</td>
</tr>
</tbody>
</table>
### TABLE 19 (cont'd)

<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Chi-Square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>1.0800</td>
<td>0.2987</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>14.3590</td>
<td>0.0002</td>
</tr>
<tr>
<td>1 vs 4</td>
<td>8.2079</td>
<td>0.0042</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>6.8571</td>
<td>0.0088</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>0.6027</td>
<td>0.4376</td>
</tr>
<tr>
<td>3 vs 5</td>
<td>1.6667</td>
<td>0.1967</td>
</tr>
<tr>
<td>4 vs 5</td>
<td>0.0679</td>
<td>0.7944</td>
</tr>
</tbody>
</table>

**a.** After perfusion for 1 hr with balanced salt solution, two 10 ml aliquots were withdrawn from the reservoir; one for endotoxin bioassay, and the other for incubation after the addition of 1.1 ml of pooled rat serum. Fourteen and one half ml of rat serum containing 2 micrograms per ml of endotoxin was then added to the remaining 130 ml of perfusate and the liver was perfused for an additional 1 hr. All samples were diluted with an equal volume of saline and bioassayed in lead sensitized rats. The remainder of the legend is as for Table 18.

**b.** Sample of perfusate evaluated after second hour of perfusion with pooled rat serum (10%) added.

**c.** Sample evaluated after first hour of liver perfusion--10% pooled rat serum added and incubated 1 hr at 37°C prior to bioassay in lead sensitized rats.
<table>
<thead>
<tr>
<th>Sample Assayed</th>
<th>Number of Livers Perfused or Samples Incubated</th>
<th>Number of Assay Rats</th>
<th>% Lethality</th>
<th>Probit</th>
<th>Endotoxin (μgm/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Initial 50% blood perfusate</td>
<td>3</td>
<td>31</td>
<td>77.4</td>
<td>5.752</td>
<td>(2.0)</td>
</tr>
<tr>
<td>2 Circulated 1 hr without liver in apparatus</td>
<td>3</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95</td>
<td>6.645</td>
<td>-</td>
</tr>
<tr>
<td>3 After 30 min of 7.50 blood perfusion</td>
<td>3</td>
<td>30</td>
<td>90.0</td>
<td>6.282</td>
<td>-</td>
</tr>
<tr>
<td>4 After 90 min of blood perfusion</td>
<td>3</td>
<td>30</td>
<td>73.3</td>
<td>5.622</td>
<td>1.57</td>
</tr>
<tr>
<td>5 50% blood-50% balanced salt solution incubated for 2 hr</td>
<td>3</td>
<td>30</td>
<td>90</td>
<td>6.282</td>
<td>-</td>
</tr>
<tr>
<td>6 100% blood incubated 2 hr</td>
<td>3</td>
<td>30</td>
<td>86.7</td>
<td>6.112</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in parentheses.
<sup>b</sup> Number of rats assayed.
<sup>c</sup> Value not available.
<table>
<thead>
<tr>
<th>Groups Compared</th>
<th>Chi-Square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>1.6672</td>
<td>0.1966</td>
</tr>
<tr>
<td>1 vs 3</td>
<td>0.9623</td>
<td>0.3266</td>
</tr>
<tr>
<td>1 vs 4</td>
<td>0.0053</td>
<td>0.9420</td>
</tr>
<tr>
<td>1 vs 5</td>
<td>0.9623</td>
<td>0.3266</td>
</tr>
<tr>
<td>1 vs 6</td>
<td>0.3673</td>
<td>0.5445</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>0.0113</td>
<td>0.9153</td>
</tr>
<tr>
<td>2 vs 4</td>
<td>2.4898</td>
<td>0.1146</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>1.7811</td>
<td>0.1820</td>
</tr>
<tr>
<td>4 vs 5</td>
<td>1.7811</td>
<td>0.1820</td>
</tr>
<tr>
<td>4 vs 6</td>
<td>0.9375</td>
<td>0.3329</td>
</tr>
<tr>
<td>5 vs 6</td>
<td>0.0001</td>
<td>0.9920</td>
</tr>
</tbody>
</table>

a. Perfusion of livers with 50% fresh heparinized rat blood in Schimassek's balanced salt solution containing 2 micrograms of endotoxin per ml of perfusate. The initial perfusate (150 ml) was circulated in the perfusion apparatus 1 hr prior to addition of a rat liver after which a 10 ml aliquot was withdrawn for endotoxin bioassay. The rat liver was perfused for 90 min. with a 10 ml aliquot taken at each 30 and 90 min. from the perfusion reservoir. Ten ml of additional perfusate was incubated for 2 hr at 37°C. An additional 10 ml of undiluted rat blood containing 20 micrograms of endotoxin was incubated at 37°C for 2 hr. All samples were diluted with an equal volume of saline before bioassayed for endotoxin in lead sensitized rats. The remainder of the legend is as for Table 18.

b. Ten assay rats per one sample; 5 rats each for the remaining two.

c. Probit beyond range at which linear relationships of probit of assay lethality and logarithm of endotoxin dose was demonstrated.
no detectable change in bioassayable endotoxin when endotoxin was incubated in either 50% rat blood in Schimassek's solution or incubated in whole rat blood for 2 hr at 37°C (Samples 5 and 6).

Throughout these experiments the viability of the liver was evaluated by observing its gross appearance, recording its changes in perfusion resistance and perfusate flow, recording the rate of bile production, and examination of histological slides prepared from each liver. The mean rate of bile production for each of the 3 groups of experiments is listed in Table 21. The gross appearance and perfusate flow were not noticeably different than observed in livers perfused in the absence of added endotoxin. The histological appearance of the livers perfused in these experiments were not different from livers of control rats fixed at the time of removal from the body except for the absence of blood cells and slightly lighter staining cytoplasm in H and E stained sections.
**TABLE 21**

**BILE PRODUCTION BY RAT LIVER PERFUSED WITH ENDOTOXIN CONTAINING PERFUSATES**

Cumulative Bile Collected (1 per liver)

<table>
<thead>
<tr>
<th>Time Perfused (min)</th>
<th>Balanced Salt Solution Only(^b)</th>
<th>Balanced Salt Solution followed by 10% Rat Serum(^c)</th>
<th>50% Rat Blood in Balanced Salt Solution(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>186(^e) (\pm 7)</td>
<td>184 (\pm 8)</td>
<td>156 (\pm 44)</td>
</tr>
<tr>
<td>30</td>
<td>394 (\pm 22)</td>
<td>374 (\pm 42)</td>
<td>344 (\pm 104)</td>
</tr>
<tr>
<td>45</td>
<td>598 (\pm 16)</td>
<td>590 (\pm 12)</td>
<td>592 (\pm 128)</td>
</tr>
<tr>
<td>60</td>
<td>768 (\pm 20)</td>
<td>729 (\pm 5)</td>
<td>821 (\pm 140)</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>849</td>
<td>1,134 (\pm 220)</td>
</tr>
<tr>
<td>90</td>
<td>929 (\pm 35)</td>
<td>1,354 (\pm 256)</td>
<td></td>
</tr>
<tr>
<td>105</td>
<td>1,017 (\pm 44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1,121 (\pm 58)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Footnotes to Table 21

a. The bile duct of each rat was cannulated near the porta hepatis with a blunt piece of 27 gauge needle cannula attached to a length of PE-20 tubing (Clay-Adams Intramedic) just prior to surgical isolation of the liver for the perfusion experiments shown in Tables 18, 19, and 20. Bile was collected in macro-Wintrobe hematocrit tubes during the perfusions. The hematocrit tubes were calibrated for volume by weighing before and after addition of mercury to various division marks. Cumulative bile production was recorded at 15 min. intervals.

b. Cumulative bile production in 6 livers from which data is shown in Table 18.

c. Cumulative bile production in 3 livers from which data is shown in Table 19.

d. Cumulative bile production in 2 of the 3 livers from which data is shown in Table 20.

e. Mean bile production in microliters per liver.

f. Standard error of mean
CHAPTER V
DISCUSSION AND CONCLUSIONS

The present studies were undertaken to assess the relationships between host resistance to lethal endotoxemia, the marked alterations of carbohydrate metabolism characteristic of endotoxicosis, and endogenous insulin secretion. Three primary questions were posed:

1.) How is resistance to lethal endotoxin shock influenced by the insulin state?

2.) How does insulin affect and relate to the characteristic carbohydrate depletion of lethal endotoxin shock? and

3.) How does insulin affect the host-defense functions of the reticuloendothelial system during endotoxemia?

The ensuing discussion will focus on each of these three primary questions and relate the findings of this study to the results of previous investigators. Lastly a hypothesis which presents a unifying schema of the role of insulin in endotoxin shock is presented and discussed.
RELATION OF ENDOTOXIN SHOCK RESISTANCE AND THE INSULINEMIC STATE

The first major question posed in this study was - How is the insulinemic state related to resistance to lethal endotoxin shock? This question arose as a result of previous findings (57, 182, 200, 204, 574, 575) that the hypoglycemia of both lead-sensitized and non-sensitized animals given a lethal dose of endotoxin was so severe as to suggest a casual relationship to death. In a logical approach to remedy the hypoglycemia attempts were made to protect rats from lethal endotoxin shock by treatment with glucose. Although some subjective improvement in clinical condition occurred during the time of glucose therapy and indeed while glucose treatment may have lengthened the median survival time, a protective effect of glucose was not demonstrated by any of several protocols employed. The preliminary results suggested that glucose treatment may have actually increased endotoxin shock lethality; perhaps due to its insulinogenic actions. However, in common with previous investigators who failed in their attempts to protect animals from endotoxin by glucose replacement (45, 50, 57, 182, 574) the control lethality was so high (80-100%) that differences did not reach statistical significance. A second observation which suggested an insulin involvement was that overnight fasting resulted in significant protection from lethal endotoxin shock (83, 84, 204). Lastly, manipulations such as treatment with lead acetate or adrenalectomy, which acutely sensitize animals to endotoxemia (198, 199, 318, 571) and accentuate the hypoglycemic response to endotoxin (45, 200, 318) also sensitize to the hypoglycemic effect of insulin (78, 84, 151, 409). Based on these observations as well as the published reports of others
lethality experiments were conducted to assess the role of insulin in endotoxin shock.

A. Effect of Glucose Injection, Fasting, and Mannohexitolose Treatment on Endotoxin Shock Lethality

Overnight fasting, which markedly depresses insulin secretion (92, 460, 519, 528, 644, 669) provided a significant protection from lethal endotoxin shock (Table 1, Figure 1). This finding is consistent with the original reports from this laboratory (83, 84, 204) dealing with effects of fasting on endotoxin shock lethality. Since glucose is the dominant regulatory influence on insulin secretion (92, 103, 216, 224, 368, 400, 429, 519, 528, 609, 635, 669) both fed and fasted rats were treated with an insulinogenic dose of glucose. Glucose treatment increased endotoxin shock lethality in both fed and fasted rats whether it was given at the same time as endotoxin or later in the shock syndrome (Tables 1 and 2, Figures 2, 3, and 5). The increases in lethality were statistically significant only when the control shock lethality was not excessively elevated. As compared in Figures 2 and 3, the increase in lethality was greater and more consistent in the overnight fasted rats treated with glucose. This may be due to a greater relative effect of glucose on insulin levels in fasted rats, since fasting depresses control insulin levels and may attenuate the endogenous hyperglycemic response to endotoxin injection. These results with glucose sensitization and fasting resistance suggested that endotoxin shock lethality could be increased by stimulating insulin secretion.

To gain further insight into the possible role of insulin in animals treated with only endotoxin, mannohexitolose was employed to
depress insulin secretion. It should be noted that mannoheptulose selectively and reversibly depresses insulin secretion in vivo (223, 509, 588-590) in the rat and the degree of suppression is a function of the dose (369). However, this sugar is not metabolized in the rat (479). Furthermore, in vitro studies have shown that this inhibition of insulin secretion is a direct action on the pancreatic islets (128, 422, 424, 588, 645). Although most effective in suppressing glucose stimulated insulin release (588) mannoheptulose is also effective in depressing the insulinemic response to non-carbohydrate metabolites (415, 588). Mannoheptulose does not influence the metabolism of non-pancreatic rat tissues even at a 3:1 molar ratio to glucose and has no influence on the action of insulin of rat tissues (115). Thus any influence of mannoheptulose on endotoxin shock lethality would be expected to be a result of depressed insulinemia. Mannoheptulose treatment was found to significantly protect rats from lethal endotoxemia (Tables 1 and 2; Figures 4 and 5). The greatest protective effect was seen in fasted rats where the depressed hyperglycemic response to endotoxin (204) would not as strongly counteract mannoheptulose suppression of insulin secretion (588). To further evaluate the interpretation that glucose treatment sensitized rats to lethal shock by stimulating insulin secretion, the results of combined glucose and mannoheptulose treatment were evaluated (Table 2 and Figure 5). In this experiment with a larger number of animals the sensitization produced by glucose at the 2 mg endotoxin dose was found to be statistically significant. As in the previous experiment mannoheptulose protected rats from endotoxin shock. In the group which received both mannoheptulose and
glucose, a significant protection was observed which suggests mannoheptulose to be the dominant component of the combination. These results indicate glucose treatment had no significant effect on endotoxin shock lethality in rats in which insulin secretion had been suppressed by mannoheptulose treatment, and considerably strengthen the hypothesis that insulin is a key mediator in the mechanism by which glucose treatment leads to increased sensitivity to endotoxin shock.

In agreement with these studies Shands et al. (577) using mice made hyper-reactive to endotoxin by systemic BCG infection reported that an iv dose of glucose (10 mg/20 gm) given 90 min. after endotoxin produced a severe reactive hypoglycemia from which the mice did not recover. These workers (577) found that while mannoheptulose treatment (200 mg/100 g) prevented the severe reactive hypoglycemia, no increase in long term survival was observed in their infected mice. However, as stressed by Berry (50) and evident in the published data (56, 574, 575, 577, 623) endotoxin shock in BCG infected animals differs considerably from that in non-sensitized animals. BCG infected mice usually die within 4 hrs with hypoglycemic convulsions accompanying death; normal mice seldom die before 12 hrs after endotoxin (50). Shands et al. (575) concluded that these early convulsive deaths were due to impaired gluconeogenesis which was thought to be a result of acute hepatic necrosis following endotoxin treatment of BCG infected mice (576). In our experiments glucose treatment did not cause a reactive hypoglycemia (Table 6) and glucose treated animals seldom died before 12 hrs after endotoxin treatment; this sequence was similar to the time when the saline treated animals in these experiments died. This time of death
is also comparable to previous reports (198, 202). Thus although Shands et al. (577) found glucose to sensitize BCG infected mice to lethal endotoxemia, the above cited differences suggest that the mechanism was different from that observed in our experiments.

Boruchow et al. (74) found that tryptophan given in glucose solution orally increased endotoxin shock lethality from 52% to 100%, whereas the same dose of tryptophan had no effect when given iv (56% mortality). However, these authors attributed the difference to the route of administration of the amino acid and did not consider: (i) the variables involved with glucose administration, (ii) that tryptophan directly stimulates insulin secretion (180, 214) and potentiates glucose stimulated insulin secretion (40, 180, 519, 669), nor (iii) that amino acids elicit a greater insulin response when given orally than intravenously (180, 215, 669) due to the secretion of gastrointestinal hormones (180, 519, 669). Agarwal (6) has also shown that ip treatment of mice with leucine significantly increases endotoxin shock lethality. Agarwal's (6) finding also supports the hypothesis that increased insulin secretion sensitizes animals to lethal endotoxin shock; this is of special interest since as well as a potent stimulus to insulin release (170, 180, 181) leucine is unique in that it is not a gluconeogenic amino acid.

Theiss and Beller (629) found that when endotoxin was either infused into rats with glucose solution or if the endotoxin injection was followed by a dextrose infusion both treatments resulted in a decrease in plasma fibrinogen and an increase in plasma fibrin breakdown products compared to saline infused controls. These findings may be
a reflection of a more severe shock syndrome. Alternately the apparent increase in activation of intravascular coagulation may play a role in the pathogenesis of lethal shock in glucose treated rats.

Smith and Dubos (597) assessed the influence of fasting on the resistance of mice to lethal infection and found a significant effect in that glucose treatment of fasted mice decreased their resistance to lethal infections while lactate protected the mice. Glucose treatment did increase the number of bacteria which were recovered from the liver and spleen which suggested a possible defect in reticuloendothelial bactericidal function.

B. Effect of Insulin and Beta-cytotropic Agents on Endotoxin Shock Lethality

The findings of investigations cited above and the results presented in Tables 1 and 2 indirectly, suggested a role of insulin in resistance to lethal shock; therefore the influence of insulin per se on endotoxin shock sensitivity was examined. The experiment shown in Table 3 and Figure 6 assessed the sensitivity of endotoxin treated rats to insulin under conditions independent of any influence of feeding or treatment with glucose or mannoheptulose. Endotoxin shock lethality was increased at least four-fold to near 100% by treatment with either of the 2 beta-cytotropic sulfonylurea agents; exogenous insulin similarly augmented lethality. These agents proved effective at doses which were never lethal to control rats treated with iv saline (Table 3) or sodium acetate (84). These results suggested that endotoxin shock lethality could be strikingly increased by endogenously secreted insulin and demonstrated that small doses of insulin markedly sensitized
rats to lethal endotoxin shock. However, when pharmacological manipulations are used to assess physiological mechanisms due consideration must be given to the actions of the drug on the systems under study as well as other influences in the body. The most prominent pharmacological effect of tolbutamide and tolaзамide is their stimulatory influence on beta-cell insulin secretion which most effectively increases the insulinemic response to a glucose stimulus (411, 635). Extra pancreatic effects have been described in both in vivo and in vitro experiments. However, as revealed in the reviews of Feldman and Lebovitz (186), Madsen (420), and Loubatieres (411) the extra-pancreatic effects of sulfonylureas which have been demonstrated in vivo in acute experiments appear to be all either attributable to an insulin-like effect of these agents or to a sensitization of tissues to the actions of insulin. Also, experiments which have shown extra-pancreatic effects of sulfonylureas have almost exclusively used either adrenalectomized, pancreatectomized, or diabetic animals or patients (186, 187, 411, 420). It is thus unlikely that the sensitization due to tolbutamide was due to extra-pancreatic effects; and since no such effects of tolaзамide have been observed in similar in vivo experiments, it is likely that the sensitization produced by these agents was a direct result of their stimulation of endogenous insulin secretion. The similar results with small doses of exogenous insulin lend support to this view.

The finding of an influence of sulfanilamide derivatives on shock lethality has been previously reported. In 1942 Hunter and Zahl (331) investigated the influence of a number of such agents on endotoxin shock lethality in mice and suggested that these compounds
may compete for the enzymes concerned in the detoxification of bacterial toxins. More recently, Kovacs and coworkers (365, 366) found tolbutamide increased the sensitivity of rats to a number of vasoactive amines which have been postulated to be mediators of endotoxin toxicity (253, 314, 476, 530, 650, 651). Kovacs et al. (365, 366) postulated that the sensitizing action of tolbutamide was a direct consequence of insulin secretion. Pieroni and Levine (514) were the first to report that treatment with insulin sensitized animals to endotoxin. Using a dose of 33 Units per kg they observed increases in endotoxin lethality comparable to those observed in this study using a dose of 0.8 to 1.6 Units per kg. They suggested that the sensitization produced by insulin was the result of the combined hypoglycemic activity of endotoxin being potentiated by the well known hypoglycemic activity of insulin. The results of the present study do not support this contention since no insulin like activity of endotoxin was found in vivo when the insulinemic response to endotoxin had been inhibited by treatment with either streptozotocin or mannoheptulose (see below). Shands et al. (577) found that BCG infected mice were sensitized to insulin and that insulin sensitized BCG infected mice to lethal endotoxin shock. Unfortunately, no data were presented concerning the insulin sensitivity of non-infected mice treated with endotoxin. Finally, since endotoxin shock has long been known to resemble in many ways the hypersensitivity response to a common bacterial antigen (49, 612) and indeed has been postulated to be the result of the liberation of the same endogenous mediator substances (143, 253, 314, 450, 451, 476, 485, 530, 650, 651), these results might be interpreted in this frame of reference. The correlation between
glycemia and immune responses was first carefully defined by Adamkiewicz (3) in his provocative review of this literature and analysis of the complex relationships. Endotoxin shock would correspond to a parahypersensitivity phenomenon or "anaphylactoid shock" reaction in his classification and it was in this type of reaction that the correlation between glycemia and resistance were most strongly linked. Also included in this class is the sensitization produced by *Bordetella pertussis* vaccination and the shock-like states produced by histamine and serotonin. Adamkiewicz (3) suggested that procedures which increase the glycemic state such as growth hormone, cortisol, glucagon and adrenaline treatment or diabetes inhibit anaphylactoid reactions whereas decreased glycemia resulting from fasting, adrenalectomy, or insulin treatment sensitize to anaphylactoid reactions. It is interesting to note that those procedures which increase resistance to parahypersensitivity reactions also produce resistance to endogenous insulin (169, 669) and that adrenalectomy sensitizes to insulin (78, 151, 170, 403, 409). Adamkiewicz (4) also showed that endogenous insulin sensitized to anaphylactoid reactions and through the employment of tolbutamide he presented strong evidence against any non-beta cell effects by showing that it did not sensitize alloxan diabetic rats while insulin treatment was effective in sensitizing diabetic rats. Leme et al. (397) have recently affirmed Adamkiewicz's suggestion that the mechanism of tolbutamide sensitization was a result of insulin secretion in a study showing that insulin intensified the microcirculatory derangements of parahypersensitivity reactions. These findings are supported by Koltai et al. (364) who also found insulin to aggravate the disruption
of microcirculatory function as did the other endotoxin sensitizing agents, actinomycin D and cycloheximide (50, 513, 572). Gulbenkian and coworkers (284, 370, 624) have studied the metabolic basis by which pertussis vaccination sensitizes to anaphylactoid reactions and have suggested a prominent role of hyperinsulinemia accompanied by depressed glucose release in this phenomenon. Muszbek and associates (140, 471-474) also suggested a role of increased insulin secretion in the severe hypoglycemia observed in pertussis vaccinated rats in shock. These investigators suggested an increased sensitivity to endogenous insulin hypoglycemia to be an important mechanism in pertussis sensitization. Pieroni and Levine (515) suggested that the mechanism by which insulin treatment sensitized mice to anaphylactoid reactions was the same as that of pertussis vaccination. The striking increase in sensitivity of pertussis vaccinated animals to endotoxin was first observed by Parfentjev (505) and has since been repeatedly confirmed (50, 213, 470, 574, 575, 577, 623) however, metabolic studies in pertussis sensitized animals given endotoxin have not been reported (50). Since a significant factor in endotoxin-toxicity is believed to be the hypersensitivity-like response, the finding of a relationship between insulin, glycemia, and sensitivity to anaphylactoid reactions in the above cited literature lends credence to the postulated role of insulin in endotoxin shock and support the interpretation given the experimental results shown in Figures 4 and 6.

Although it is suggested that insulin enhances endotoxin shock lethality at least in part by potentiating the endotoxin induced derangements in carbohydrate metabolism (see below), other actions of
insulin may also contribute to the pathogenesis of endotoxin shock.

Endotoxin is known to activate the coagulation cascade (121, 461) and disseminated intravascular coagulation may thus play a role in shock (304, 320, 321, 476); this view is supported by the observation that heparin treatment protects rats from endotoxin (206, 320). As summarized by Martin and Tilsner (431) a growing body of evidence indicates that insulin increases thrombokinase activity and increases platelet aggregation. Insulin also depresses fibrinolytic activity in the rat (382, 383). These effects would be expected to potentiate the defects in coagulation observed in endotoxemia. This is not a pharmacologic effect or stress induced response. Insulin induced alterations in the coagulation - fibrinolysin system can be observed during oral glucose tolerance tests in non-diabetic patients (299).

Insulin treatment also causes intensive secretory activity of the adrenal medulla cells of the rat with increased in vitro endocytic activity (1), elevated levels of catecholamines are thought to be one of the mediators of endotoxin toxicity while their eventual depletion has been suggested to be one factor responsible for the terminal hypotensive phase (209, 253, 314, 476, 501).

Insulin is also the most potent natural inhibitor of the lung and plasma enzymes responsible for the degradation of bradykinin and conversion on of angiotensin I to II (332). Insulin may thus depress the degradation of bradykinin which is thought to be an important mediator of endotoxin shock (253, 314, 450, 451, 476, 485) and is known to cause severe circulatory derangements. Also, insulin may prevent the conversion of angiotensin I to II and the beneficial effects of
exogenous angiotensin I infusion (476) could not be expressed by elevated renin activity in shock. A potential positive feedback system exists since while insulin inhibits bradykinin degradation (332), bradykinin also inhibits insulin degradation by the insulin specific protease (81, 82) responsible for the degradation of insulin in the rat (87, 161, 363). The possible role of vasoactive substances in the development of lethal endotoxemia receives strong support from the laboratory of Fine (143, 144) where it has been shown that infusions of bradykinin or biogenic amines initiate the perpetuation of the shock syndrome and result in lethal endotoxemia.

Insulin may also play a role in the elevation of lysosomal enzymes observed in endotoxin shock. Insulin increases the activity of hippuryl-L-lysine hydrolase in the plasma of shocked dogs and this action can be suppressed by corticosteroid treatment (477). Insulin also increases the permeability of the blood brain barrier (22, 674, 691) and this increase in cellular membrane permeability may be a factor in the altered neurotransmitter metabolism observed in the brains of rats given endotoxin (518). Finally as well as aggravating the injuries produced by endotoxin insulin may also impair restorative processes since it delays wound healing in rats (546).
MECHANISM OF INSULIN SENSITIZATION TO LETHAL ENDOTOXIN SHOCK

The second major question posed in this study was, how does insulin depress endotoxin shock resistance and impair the maintenance of carbohydrate homeostasis in shock? Based on two well-documented hepatic effects of endotoxin and insulin, a working hypothesis was constructed and experimentally evaluated. The two key premises of this hypothesis were as follows: First, Berry and coworkers (49, 50, 51, 54, 55, 542) as well as others (598) using in vivo models and Agarwal and his associates (5, 6) using the isolated perfused liver have demonstrated an "actinomycin D-like" action of endotoxin. In the context of Berry this action alludes to the ability of endotoxin to inhibit the expected increases in hepatic enzyme activity in response to either physiological or pharmacological stimuli. Recent studies have confirmed that this inhibition of hepatic enzyme "induction", like that due to actinomycin D treatment, is largely due to a depression of specific enzyme protein synthesis (51, 542). However, unlike actinomycin D, endotoxin selectively inhibits the synthesis of enzymes with half-lives of as short as 2 hrs and which are normally at elevated levels after treatment with hormones which increase hepatic gluconeogenesis (50, 51, 542). Phosphoenolpyruvate carboxy kinase (PEPCK), a probable rate limiting enzyme of hepatic gluconeogenesis in the rat (173, 483, 567, 657), is an example of such a short half-life enzyme which is both stimulated by either treatment with hormones which increase gluconeogenesis (294, 357, 511) or by fasting (512, 620) and whose synthesis in response to these stimuli is prevented by prior endotoxin treatment (50, 51, 55, 167). It is important to
note that the inhibition of hepatic gluconeogenic enzyme synthesis is not an immediate effect of endotoxin but develops over a period of several hours (49, 50, 51, 54, 55). Furthermore, other enzymes such as tyrosine-ketoglutarate-transaminase and pyruvate kinase, which are stimulated by insulin treatment (250, 251, 357, 367) show increased levels after endotoxin treatment (49, 50, 54, 55, 598).

Second, insulin is known to be a suppressor of the biosynthesis of a series of hepatic gluconeogenic enzymes which are induced by treatment with hormones which increase hepatic gluconeogenesis (294, 661, 657-659). Insulin also prevents the normal rise in PEPCK in the post absorptive state (620). Insulin at concentrations as low as $2 \times 10^{-11}$ molar -- i.e., in vivo concentrations -- inhibits both the dibutyryl cyclic AMP and dexamethasone stimulation of hepatic PEPCK activity (661). Insulin's influence on hepatic gluconeogenic enzyme levels is not limited to altering their rates of synthesis, since glucose treatment is more effective than puromycin in depressing hepatic PEPCK activity in vivo. Foster et al. (219) ascribed this to an increased rate of enzyme degradation. This possibility has only recently been assessed by Hopgood et al. (322) and it now appears that the degradation rate of PEPCK in the liver is subject to hormonal-metabolic regulation; the fed state was associated with a 60% decrease in the enzyme's biologic half-life. The increased degradation rate could not be reversed for several hours despite treatments which increase the rate of synthesis (322). More recently Tilgham et al. (631) have shown that endogenous insulin is required for the alterations in PEPCK metabolism.
Given the above observations that (i) endotoxin will prevent the induction of hepatic gluconeogenic enzymes some hours after its administration and (ii) that insulin rapidly depresses the synthesis of hepatic gluconeogenic enzymes and increases the rate of degradation of preformed enzyme protein for a period of several hours, a mechanism by which insulin plays a role in the development of the altered carbohydrate metabolic homeostasis characteristic of endotoxemia was formulated. It was hypothesized that if increased levels of insulin occurred early in endotoxemia either in response to endogenously generated hyperglycemia or following treatment with glucose or other insulinemic stimuli, then carbohydrate metabolism would be perturbated in at least two important ways. First, in the early phase, elevated insulin levels would increase the rate at which the body pool of carbohydrate stores would be depleted, and second, at this early time insulin would also depress the synthesis of gluconeogenic enzymes and the existing enzyme protein would be more rapidly degraded before the onset of the secondary inhibition of enzyme synthesis due to endotoxin would become manifest. Thus an increase in insulin levels would depress the gluconeogenic capacity of the challenged host even after the insulin levels are no longer elevated. As will become evident the metabolic and endocrine alterations in endotoxemia are probably much more complex. However, this working hypothesis served as a conceptual basis in assessing the possible role of insulin in the pathogenesis of the altered carbohydrate metabolism secondary to endotoxemia.

A. The Influence of Glucose Treatment on Hepatic Gluconeogenesis and Glycogenesis in vivo
This hypothesis was first examined by an assessment of the effect of glucose treatment on gluconeogenesis and hepatic glycogenesis. A parallel study in non-endotoxin treated rats was required (Table 4) not only as an adequate control for the experimental groups but also since the effectiveness of glucose in suppressing these functions under the conditions of these experiments has not been previously reported. The first point worthy of note is that a significant acute reduction in liver weight was observed in endotoxemia (Table 4). Therefore, in order to assess the total contribution of the liver in host defense to endotoxemia the data in this study were reported both on the basis of total liver weight as well as in the more conventional per gram basis. In the glucose control rats (Group 2, Table 4) the significant increase in liver weight is attributed to glucose treatment since in all other ways they were treated as the Group 1 controls which received saline rather than glucose. The glucose treated rats which had received endotoxin showed no increase in liver weight over their endotoxemic controls (Group 3) which was consistent with their failure to exhibit significant increases in liver glycogen. The fasting blood glucose in the saline controls (Group 1) was comparable to that previously reported from this laboratory (83, 204) and other experiments in this study (Table 6). The significant rise in blood glucose following alanine treatment is consistent with previous studies using this and similar models (130, 204, 305, 594). In contrast to Group 1 the pretreatment of saline iv rats with glucose (Group 2) 2-3 hrs before the alanine produced the following changes: (a) hyperglycemia was present at the time of alanine injection--to
the same degree as in the experiment shown in Table 6, (b) alanine
treatment caused no increase in blood glucose, (c) incorporation of
alanine carbon label into blood glucose and blood glucose specific
activity was depressed, and (d) a marked depression in liver glycogen
specific activity occurred due to the high glycogen levels with un-
altered incorporation of alanine carbon label. Most of these changes
are attributable to the hyperglycemia and concomitant elevated insulin
levels. The above data are consistent with the early observation of
Bach and Holmes (30) in 1937 when they reported insulin to be a potent
inhibitor of alanine gluconeogenesis in rat liver. The depressive
effect of insulin on hepatic gluconeogenesis has been repeatedly con-
firmed (150, 173-175, 260, 339, 418, 443, 449, 452, 463, 475, 506, 507,
595, 609, 657) and dose-response studies have shown physiological
levels are effective (116, 173, 418, 595, 609, 643). Another impor-
tant mechanism by which insulin depresses hepatic gluconeogenesis
in vivo is by lowering the plasma levels of gluconeogenic amino acid
substrates (173, 192, 194-196, 609), as first reported by Luck, Morrison
and Wilbur (414) in 1928. Since in our experiments depressed gluco-
eogenesis was observed after an exogenous alanine load, an insulin
mediated inhibition of substrate release from peripheral tissues (93,
94, 173, 337, 524) is unlikely; however, an increased uptake of amino
acids into extrahepatic tissues (20, 92, 541, 618) and the stimula-
tion of protein synthesis (218, 407, 449, 681) may have influenced
the fraction of the alanine load available for gluconeogenesis. The
finding of depressed gluconeogenesis (Group 2, Table 4) in conjunction
with no difference in alanine carbon label incorporation into hepatic
glycogen may be attributed to the hyperglycemia as well as the expected insulinemia. Glucose, even in the absence of increased insulin can depress the net release of glucose from the perfused liver (86, 90, 550) as well as activate glycogen synthetase in vivo and promote the deposition of glycogen (154, 173, 260, 288, 307, 434) independent of changes in cyclic AMP levels (90, 621). Furthermore, glucose-6-phosphatase is inhibited by glucose (29, 39, 327, 328) which in turn preferentially directs hexose phosphates toward glycogen formation and depresses net glucose release by the liver (26, 27, 327, 328).

B. Influence of Endotoxin on Hepatic Gluconeogenesis and Glycogenesis in vivo

Blood glucose values at both the time of alanine injection and 30 min. after alanine were significantly depressed when rats were treated with endotoxin (Group 3 vs Group 1, Table 4). However, the blood glucose activity and specific activity were elevated by endotoxin treatment. At least two general mechanisms may account for these divergent results (288): (i) an increased turnover rate of blood glucose with relatively greater increase in the rate of disappearance than the rate of appearance occurred in endotoxemic rats, or (ii) a preferential utilization of the injected alanine as a gluconeogenic substrate occurred in the endotoxin treated rats. When both insulin and gluconeogenic hormones are elevated the turnover of blood glucose is markedly increased (116). Our data show that insulin levels are elevated at this time after endotoxin (Table 7) and increases in glucagon in stress (67, 68, 296, 380, 406, 416,
including infections (41, 544, 560) and endotoxemia (67, 68) and shock (406) are well documented. Similarly, elevations in sympathetic activity and circulating catecholamines are believed to occur in endotoxin shock (208, 209, 253, 314, 476, 530). The combination of elevated insulin, glucagon, and epinephrine would be expected to contribute to an increased rate of glucose turnover in shock and experimental evidence supports this contention (120, 160, 287, 319, 408, 410, 490, 605, 606, 664-666). Insulin is probably a necessary component of the increased uptake of glucose by peripheral tissues in shock as first reported in muscle by Drucker and DeKiewiet (160) since insulin is required for anoxia stimulated glucose permeation of muscle (275). Epinephrine also increases glucose turnover (312, 313) and it has recently been shown that epinephrine directly increases the permeability of muscle cell membranes to glucose through an alpha-adrenergic mediated mechanism (555). The possibility of preferential utilization of alanine as a gluconeogenic substrate is suggested by the observation that although alanine label incorporation into blood glucose was elevated, a significant hypoglycemia occurred at the time of alanine injection and persisted 30 minutes after alanine injection (Table 4). Further experiments would be required to assess the possibility that endogenous substrate supply to the liver was a factor limiting gluconeogenesis prior to alanine treatment. As expected from previous studies in this laboratory (130, 204) liver glycogen was near totally depleted in endotoxemic rats (Table 4). Similar findings in mice have been reported by McCallum and Berry (434).

In the Group 4 rats the finding of a significant hypoglycemia
2 to 3 hrs after glucose treatment was unexpected (Table 4). The possibility that the hypoglycemia was due to depressed gluconeogenic capacity was supported by the finding of no significant increase in blood glucose after alanine injection and a depressed incorporation of label into blood glucose. Since liver glycogen values were not different from those in Group 3 (Table 4) the fate of the 400 mg glucose load was not evident and conversion to lactate and/or oxidation were suggested. The data in Table 4 were interpreted as demonstrating that glucose treatment impaired gluconeogenic capacity in endotoxemic rats. As discussed above with respect to the hyperglycemic Group 2 rats insulin could produce such effects.

C. Insulin Immunoassay

An assessment of the radioimmunoassay used in this study is shown in Table 5. The method used was essentially that of Velasco et al. (648) and employed anti-insulin-antibody covalently coupled to Sephadex as originally developed and described by Wide and co-workers (662, 663). Recently Bolton and Hunter (73) evaluated this technique and compared it with others and suggested it to be the method of choice for a number of radioimmunoassay procedures. Porcine insulin was used as the standard due to the limited availability of purified rat insulin. Possible differences in cross-reactivity of antisera were prevented by using a single batch of antiserum in all experiments reported in this study. The intra-assay coefficient of variation for the serum samples shown in Table 5 is comparable to that reported by others (136, 648) and lower than that found by Przybyla (527). The consistency of this technique was evident in
the observations that the standard curves were near identical and that
the inter-assay coefficient of variation was not different from the
intra-assay values. The inter-assay coefficient of variation of ap-
proximately 8% for the serum samples was similar to that reported by
Velasco et al. (648) and markedly better than that reported by Cryer
et al. (136). As has been previously reported there appeared to be
an influence of heparinized plasma which was not manifest using serum
(34, 185, 630).

D. Blood Glucose Levels in Endotoxemia

The data in Table 6 demonstrate that overnight fasted rats
develop a significant hyperglycemia in the early response to endo-
toxin. The slow onset of the hyperglycemia as well as the known de-
pletion of liver glycogen in overnight fasted rats suggests that the
hyperglycemia was probably due to enhanced gluconeogenesis or depressed
glucose utilization. The data in Table 4 support the former and
discount the latter at least later in the shock syndrome when blood
glucose returns toward control values. The rapid recovery of the
blood glucose values in the endotoxemic rats given glucose revealed
no apparent change in glucose tolerance after endotoxin treatment.
Although glucose tolerance does not appear to have been previously
assessed in the acute phase of endotoxemia, the observation of a
transient hyperglycemia followed by a return to normoglycemia even
following glucose treatment is consistent with the findings of
others (280, 285, 286, 342, 503, 526). It may be noted that pro-
nounced hypoglycemia was not evident in this experimental design.
Probably at least two factors biased this experiment against such
a finding. First—at the dose of endotoxin employed, death and the accompanying profound terminal hypoglycemia do not usually occur until after at least 12 hrs or more after endotoxin even when the rats are sensitized by glucose treatment. Second—blood samples were collected only from rats which survived until their pre-designated sampling time and rats were not substituted between sampling times.

E. Serum Insulin Levels in Endotoxemia

Endotoxemic rats given ip saline or glucose revealed a striking elevation of insulin levels and an exaggerated insulin response to glucose following endotoxin treatment (Table 7). It is important to note that serum insulin remained elevated in endotoxemic rats long after blood glucose had returned to or below control values. The possibility that elevated levels of immunoreactive insulin were an artifact of the assay system appeared remote for several reasons. (a) Endotoxin added to serum or to buffer in vitro had no measurable influence immunoassayable insulin values, (b) the insulinemic response paralleled the blood glucose elevations and dropped as blood glucose dropped, even if a second peak in blood glucose was provoked by glucose treatment, and (c) dilution of serum of endotoxemic rats showed the same dilution curve obtained with pooled rat serum.

The finding of elevated insulin levels in endotoxin shock carries profound implications concerning the metabolic-humoral response to endotoxemia. As recently stressed by Schumer (564) the evidence of a role of metabolic adaptation in determining survival to potentially lethal shock is increasing and the evidence of alterations in insulin and glucagon secretion, as playing a role in
resistance to lethal shock is now evolving. As recently as 1969 Cuthbertson and Tilstone (146) reviewed the literature on "Metabolism During the Postinjury Period" and only one reference to altered pancreatic hormone secretion appeared. One year later Kinney (359) found that conflicting reports had appeared concerning insulin secretion in shock. However, despite the growing interest in endocrine pancreatic function in shock our findings of elevated insulin levels in endotoxemia can not be compared to any existing literature since insulin levels in experimental endotoxemia have not previously been reported.

However, a number of reports have indicated altered insulin levels under conditions which are pertinent to the present study. Immunoreactive insulin levels in shock were first studied by Halmagyi et al. (240) using a hemorrhage model and it was reported that serum insulin levels were not necessarily related to fluctuations in blood glucose. Moss and coworkers (464, 465) have found serum insulin levels depressed in both dogs and baboons early in hemorrhagic shock. Cerchio et al. (105) confirmed the earlier results of Moss and coworkers and showed that tolbutamide had only a minimal effect on insulin levels in hemorrhaged baboons. However, in another study Cerchio et al. (104) found plasma insulin returned above the baseline values during the second hour of hemorrhage despite the maintenance of hypovolemia; a decrease in blood glucose accompanied the rise in plasma insulin. The mean half-time for clearance of exogenous immunoreactive insulin from the plasma was lengthened which suggested to Cerchio et al. (105) that insulin secretion was depressed. Vigas and coworkers (652-654) found that the insulin response to
glucose was depressed in tumbled rats and the suppression of insulin secretion diminished upon establishing trauma tolerance (654). Insulin levels were also depressed in humans during surgical stress (9, 10, 427, 683), after myocardial infarction (9, 162, 627) during experimental hemorrhage (293), and following traumatic shock (101). Cryer and co-workers (136-139) have reported depressed insulin levels in fulminating rapidly lethal *E. coli* sepsis in the baboon. Egdahl and coworkers (309-311, 435, 436) measured the actual rate of pancreatic insulin secretion and insulin clearance by the kidney in hemorrhagic shock and found that peripheral levels of insulin were not well correlated with the actual rate of insulin secretion and on this basis criticized the interpretation that increased plasma insulin always reflects increased beta-cell insulin secretion. A partial explanation for this discrepancy may lie in the depression of the absolute rate of insulin clearance by the kidney observed in their studies. Hiebert et al. (309) also reported the insulin secretory response of adrenalectomized baboons to iv glucose was maintained even during epinephrine infusion; however, the insulin response was diminished when intact animals were hemorrhaged.

In contrast to the above cited findings of depressed insulin secretion in acute shock, increased insulin levels have been measured in shock and stress situations under conditions which may more closely resemble the slower, progressive shock syndrome characteristic of endotoxemia under the conditions of the present investigation. Ross et al. (547) found much higher levels of plasma insulin and an increased insulinemic response to a glucose stimulus in post operative patients; this finding was corroborated by Johnston et al. (341) who
also attributed the insulinemia to increased ACTH secretion. Elevated insulin levels and a correlation of blood glucose and serum insulin have also been reported following acute myocardial infarction (375, 649) and in patients with cardiac disease (168). The insulin responses during hypovolemic shock are controversial. Bauer et al. (38) found that the hyperglycemia of acute hypotension was accompanied by elevations of serum insulin and that insulin levels were elevated during iv glucose tolerance testing. McCormick et al. (435) also observed increased insulin levels and hyperglycemia during hemorrhage in the dog and a portion of the elevation was attributed to depressed clearance of insulin. Lau et al. (384, 385) reported hyperinsulinemic incident to hemorrhage and noted a dissociation of insulin secretion and blood glucose in shock. Stake et al. (607) maintain that the hyperglycemia of acute hypovolemia is characteristically accompanied by an increase in insulin secretion. Increased insulin has also been found in trauma (107, 487). Hyperinsulinemia has consistently been observed in experimental infections in human volunteers and rhesus monkeys (247, 532, 533, 573) and in a recent review, Beisel (40) stressed the role of pancreatic hormones in the regulation of hepatic metabolism in infections. Clowes et al. (120) and O'Donnell et al. (490) have also demonstrated hyperinsulinemia in gram negative sepsis in pigs; septic patients in the "high flow" state also had marked hyperinsulinemia whereas after septic shock had progressed to the "low flow" state insulin levels were normal. Insulin levels were also found elevated in gram negative sepsis in dogs (280) especially in those animals which later developed hypoglycemia and those in which death ensued.
Gump and coworkers (285) studied insulin levels and iv glucose tolerance in patients very early in the course of sepsis before cardiovascular instability developed and found that control insulin levels were elevated and that iv glucose widened hepatic vein-arterial insulin gradients; the latter finding suggested increased insulin secretion.

Two additional factors which may account for some of the disparities in previous studies of insulin secretion in shock were the use of heparin and the influence of anaesthetic agents. Recently Orosz et al. (496) confirmed previous studies (495) and showed that heparin inhibition was a direct pancreatic effect. The use of anesthetics in many previous studies may have also influenced insulin secretion. Greene (277) recently concluded that many anaesthetics depressed insulin secretion in vivo. In a recent editorial Brunner (80) called attention to the depressive actions of anaesthetics on insulin secretion. The sympatho-excitatory and depressive action of anaesthetics on insulin secretion is well known (8, 29, 59, 60, 119, 277, 281, 353, 433, 497, 500).

F. Possible Factors Underlying Hyperinsulinemia in Endotoxemia

The hyperinsulinemia accompanying endotoxicosis in undoubtedly multi-factorial but six primary determinants are dominant and merit detailed discussion.

1) Depressed Insulin Clearance. One factor which may have contributed to the elevated serum insulin levels observed in endotoxemic rats is depressed insulin clearance. Using the immunoassay technique which accurately reflects endogenous insulin metabolism (224, 245, 335, 580) insulin clearance has been studied mainly in
hemorrhagic shock. McCormick et al. (435) found that the half-life of immunoreactive insulin was increased in hemorrhagic shock and the fractional clearance across the liver was unchanged. It was also reported that the arterial-venous difference across the kidney was unaffected by acute hemorrhage but the actual amount of insulin removed was severely depressed due to the large decrease in renal plasma flow (436). Similarly, Cerchio et al. (105) found that the half-life of immunoreactive insulin was doubled in baboons subjected to hemorrhagic hypotension and noted an important difference in the metabolism of insulin in hemorrhaged as compared to control baboons. While insulin steadily declined to control values after insulin infusion in control baboons, plasma insulin reached a plateau of about 75 µU per ml over control values in the shocked baboons. It was suggested that this was due to increased insulin secretion as shock progressed (105). The elevated plateau observed following treatment of rats with glucose (Figure 8) may be related to a similar depression of insulin clearance and increase in insulin secretion. Cryer et al. (138) also studied iv insulin during lethal septicemia in baboons and noted an increased mean half-time for insulin disappearance from 15.7 min. in controls to 33.1 min.; however, these results may have been influenced by the more rapid and severe hypotension, hypotension, and earlier deaths in the insulin treated septic baboons. In general the studies of insulin clearance in shock are readily explicable since the major organs responsible for insulin clearance are the liver (197, 345, 548) and kidneys (232, 354, 544, 669) and it is well known that blood flow to these organs is restricted in shock (476). Insulin extraction by the liver is also
altered by changes in blood glucose (197, 345) however, the possible influence of changes in blood glucose in insulin clearance in shock has not been investigated. Since epinephrine treatment prevents the changes in insulin clearance attributable to charges in blood glucose (493), the epinephrine response to shock may similarly suppress glucose mediated alterations in hepatic insulin extraction. Sepsis and trauma also alter the capacity of various tissues to degrade insulin (668). Since the reticuloendothelial cells of the liver are a major site of insulin accumulation (682) and RES activity is depressed in shock, the postulated involvement of RES elements in insulin degradation (682) may well contribute to the impaired insulin clearance found in shock (105, 138, 435).

ii) Alterations in Beta-Cell Responses to Metabolites. As reported in Table 7 insulin was elevated as early as 45 min. after endotoxin—a time when the cardiovascular decompensation incident to endotoxemia in the rat would not be expected to be severe (253). Insulin levels were also significantly correlated to blood glucose both when the hyperglycemia was endogenously generated or when accentuated by treatment with exogenous glucose (Table 9 and Figures 11 and 12). However, the insulin response in endotoxemia was probably abnormal since the regression analysis (Table 9) showed the increase in serum insulin per increase in blood glucose was significantly different in endotoxemic rats (Groups 3 and 4 vs Group 2). Beta-cell secretion of insulin is influenced by glucose as well as a variety of other metabolites, viz, amino acids (24, 180, 181, 214, 519, 669), free fatty acids (33, 102, 133, 134, 419, 519), and ketone bodies (298, 445, 516,
and these metabolites also potentiate the insulinogenic response to glucose (180, 224, 368, 422, 519, 669). However, the roles of these metabolites in regulating insulin secretion in shock has not been assessed despite the fact that changes in the plasma levels of these metabolites occur in shock and therefore they may well play a role in the regulation of insulin secretion in shock. Since it is well documented that proteolytic and lysosomal enzymes are released from the pancreas in shock (257, 292, 293, 295, 296, 604), it is interesting to note that Orci et al. (494) found that mild treatment of isolated pancreatic islets with proteolytic enzymes doubled the insulin secretory response to glucose and it may well be that enzymes released from hypo-perfused tissues such as the pancreas sensitize the beta-cells to glucose in vivo.

iii) Altered Hormonal Regulation of Insulin Secretion in Shock. Insulin secretion is influenced by alterations in hormonal levels--viz, gastrointestinal hormones, gut glucagon, pancreatic glucagon, or ACTH (85, 135, 180, 224, 368, 398, 519, 529, 535, 638, 669). The influence of gastrointestinal hormones in altering insulin secretion in shock has not been explored; however, the possibility exists that these hormones may well be released during splanchnic ischemia. A possible role of pancreatic glucagon as a mediator of the increased insulin secretion in shock is suggested by the observation that plasma pancreatic glucagon is elevated in stress (67, 68, 296, 380, 416, 417) including endotoxemia (67, 68), and sepsis and shock (67, 406, 439, 544, 560, 643, 671, 680). While glucagon is a potent stimulus to insulin secretion (180, 224, 264, 368, 519, 643, 669) and hyperglucagonemia
may therefore be a stimulus to insulin secretion in endotoxemia; no data however exists to substantiate this effect. Lastly, it is well recognized that acute stress rapidly evokes ACTH release (148, 172, 234, 267, 356, 403, 540, 562, 655). It is now recognized that ACTH may have acute effects on insulin secretion in vivo (246, 361, 362, 388, 491, 669) and in vitro (246, 413, 622). Geauth and Lebovitz (246) and others (388) found increases as great as ten-fold in plasma insulin within 20 min. after ACTH treatment which were antagonized by manno-heptulose (246). Unlike the responses with multiple doses of glucocorticoids which produce hyperglycemia (362, 562) the elevated insulin levels following acute ACTH treatment are accompanied by hypoglycemia (246, 388) suggesting that ACTH directly stimulates insulin release in vivo. Since shock (48, 330) as well as endotoxemia and sepsis (42, 91, 517) and putative mediators of endotoxin toxicity (82, 153, 562) stimulate ACTH release, insulin release may be altered by this mechanism; no evidence exists however to support this notion.

iv) Serotonin Modulation of Insulin Secretion. Similar to the possible role of hormones in modulating insulin secretion in endotoxemia, serotonin may play a role in stimulating insulin secretion. Numerous investigators have demonstrated that serotonin modifies insulin secretion (184, 188, 233, 389, 399, 628, 642, 672, 680) and hypothesized that serotonin may play a physiological role in regulating insulin secretion (389, 628, 632, 642, 672). Since doses of serotonin as low as 0.5 mg per kg per min. increase the insulin response to hyperglycemia (184), the finding of plasma levels as high as 30 μg per liter in shock (618) might be expected to produce a substantial
insulin response. The exact role of serotonin in the insulinemic response to endotoxemia however remains to be investigated.

v) Alterations in Autonomic Regulation of Insulin Secretion.

The autonomic nervous system has profound influence on the endocrine functions of the pancreas (169, 224, 225, 270, 271, 368, 519, 633, 669, 680). Since profound alterations in autonomic activity occur in endotoxemic stress and shock (143, 208, 209, 302, 314, 315, 393, 395, 476, 502, 518, 604, 634) the influence of these effects on insulin secretion is plausible. Adrenergic depression of insulin secretion was suggested as early as 1930 by Colwell and Bright (126; however Chidsey and Dye (117) concluded that epinephrine stimulated insulin secretion. La Barre (376) also recognized the role of the central nervous system in regulating insulin secretion and attributed the effects to both the release of an insulinogenic hormone (377) and direct vagal stimulation (376). It was also recognized by Masson (432) that stress influenced the glycemic response to both insulin and epinephrine. Cannon, McIver, and Bliss (96) indicated that the glycemic state had profound influences on the activation of the autonomic nervous system, however, the relationships between autonomic regulation of insulin secretion, glycemia, and stress were first systematically investigated by Gellhorn and associates (157, 189, 190, 236, 237, 240-244, 258, 686). Hypoxemic stress was found to sensitize rats and dogs to insulin hypoglycemia (241, 258) and it was shown that hypoxia stimulates the autonomic nervous system (241, 244). Subsequently it was concluded that stress stimulates the vago-insulin (parasympathetic) system causing an acetylcholine mediated rise in insulin secretion; however in intact animals the sympathetic centers
were also activated (189). Furthermore, it was concluded that stress induced by a variety of means resulted in a stimulation of both divisions of the autonomic nervous system with the vago-insulin stimulation masked by the sympathetic activation under severe stress (189, 236, 686). However, in later studies (243) it was suggested that insulin was a physiological antagonist to stress and thereby inhibited alterations in carbohydrate metabolism characteristic of stress. Gellhorn et al. (242) suggested that hyperglycemia played a physiological role in stress by increasing the availability of glucose to the nervous system and thus prevented sympathetic dominance while insulin antagonized these adaptive changes and further disturbed homeostasis (157). In studies of the physiological relationships of the sympathetico-adrenal and vago-insulin activation induced by stress, Gellhorn and coworkers (189) concluded that the response to stress was dependent on a "finely tuned" and balanced activation of both components of the autonomic nervous system and that reduced activity of either component produced a pathological alteration which was attributed to overdominance of the other division. Gellhorn's concepts of "autonomic tuning" and the simultaneous balanced activation of both divisions of the autonomic nervous system are still believed valid and have recently been extended (238, 239). The concept of reciprocal innervation is not believed to be applicable to the central autonomic regulatory process (189, 238, 239).

Despite the convincing demonstrations by Gellhorn and coworkers and LaBarre on the role of the autonomic nervous system in the regulation of the endocrine pancreas, this subject received little attention until sensitive radioimmunoassays were developed which permitted the measurement
of circulating plasma levels of insulin and glucagon. Porte and co-workers (520, 521) first directly demonstrated that epinephrine infusion depressed the insulinenic response to the accompanying hyperglycemia. However, epinephrine infusion did not depress basal insulin levels (271). Although Porte et al. (520, 521) used pharmacological epinephrine doses in their studies (103) other studies have since established a probable physiological role of catecholamines in the regulation of insulin secretion \textit{in vivo} (25, 95, 103, 217, 221, 224, 345, 350, 351, 371, 684). Catecholamine suppression of insulin secretion is believed to be mediated by alpha-receptor activation leading to a depression in beta-cell cAMP (25, 421, 522, 639, 640, 669). Similarly it has been shown that vagal stimulation and cholinergic agents increase insulin secretion (43, 149, 225, 226, 349-351, 412, 423, 480, 522, 679), and recent reviews support the above generalizations (169, 170, 225, 271, 368, 519, 523, 635, 669, 680). It should also be noted that the rat is not different from other species in that severe stress or the administration of catecholamines depress insulin secretion and plasma levels (217, 224, 421, 523, 639, 640, 680, 684). From the above cited literature the serum levels of insulin might be expected to be depressed during the stress of endotoxemia, however as shown in Table 7 serum insulin levels were elevated in endotoxemia and insulinenic response to hyperglycemia was increased (Tables 8 and 9). Although unexpected, the finding of increased serum insulin levels following endotoxin treatment is not inconsistent with limited literature on this topic. Feldman and Gellhorn (190) and Gellhorn (237) concluded that typhoid-paratyphoid vaccine caused an increase in serum insulin mediated by increased vagal
activity. More recently Bloom and coworkers (67, 68) reported no depression of plasma insulin following a pyrogenic dose of endotoxin which did elicit an increase in plasma glucagon. Bloom et al. (70, 71) ascribed increased glucagon release to stimulation of vagal efferents to the pancreas; a view supported by the observations of Iversen (333) and Kaneto et al. (349). It appears probable that increased parasympathetic activity may have contributed to the hyperinsulinemia observed following endotoxemia. This interpretation receives support by additional studies which have demonstrated increased vagal activity in stress and shock including endotoxemia (347, 557, 618, 651). In fact, recently Fukuda and Hata (230) suggested that most of the toxic manifestations of endotoxemia were a result of increased vagal activity and could be ameliorated by vagotomy. Strauss et al. (618) demonstrated that the large release of serotonin from the gastrointestinal tract of shocked dogs was vagally mediated; the possible role of serotonin as a mediator of the altered insulin secretion in endotoxemia has been discussed above (page ). Vagal activation may also be in part responsible for the hyperglucagonemia of stress and shock (67, 68, 70, 71, 296, 349, 406) since cholinergic stimulation has been demonstrated to increase glucagon secretion (70, 71, 333, 349). However, it must be kept in mind that there is also substantial evidence of adrenergic regulation of glucagon secretion (67, 69, 169, 170, 225, 248, 249, 296, 334, 390, 416, 417, 430, 680). There may also be alterations in beta-cell neuroreceptor mechanisms which cause a reversal of epinephrine action on insulin secretion as have been described after treatment with *Bordetella pertussis* vaccine (140, 284, 370, 471-474, 624); however, this possibility has
not been explored following endotoxin treatment. From the available evidence it appears that both glucagon and insulin levels may be elevated in endotoxemia (Table 7, and 67, 68, 190, 237).

G. Evidence of Insulin Resistance in Endotoxemia

The depressed glucose to insulin ratios in the endotoxin treated rats (Table 8) suggested the possibility of a resistance to insulin action in endotoxemia; a view supported by the literature on insulin action in infections and bacteremia (40, 41, 120, 125, 193, 247, 256, 285, 490, 532, 533, 544, 560, 573) and shock and injury (109-111, 146, 286, 287, 325, 340, 341, 408, 547, 614, 683). In accord with the above cited investigators, glucagon, epinephrine, norepinephrine, ACTH, glucocorticoids, growth hormone, elevated free fatty acids; or some other factor may have been in part responsible for this apparent resistance; the precise mechanism remains unresolved. The possibility of glucagon as an important insulin antagonist is intriguing and deserves further investigation.

As noted above, this hormone is profoundly elevated in stress and shock. Frey et al. (222) showed insulin resistance after infusing patients with glucagon, and it has been reported that glucagon produces peripheral resistance to insulin (151, 216, 222, 609) although the mechanism may not be direct (216, 391, 404, 428, 599, 600, 609, 643, 669). The glucose-insulin products calculated in Table 8 applies the criteria of Levine and Haft (400) to the data from endotoxemic rats in that insulin level and the blood glucose concentration are both important factors influencing glucose utilization and the higher this product, the greater is the resistance to the hypoglycemic action of insulin. By these criteria it also appears that endotoxemia induces insulin resistance.
However, although the data in Table 8 suggest insulin resistance in endotoxemic rats those in Table 3 paradoxically suggest that endotoxin treated rats are sensitized to insulin. Cryer et al. (139) found similar insulin sensitivity in acute \textit{E. coli} septicemia in baboons. As recently noted by Frey et al. (222) who found similar evidence of insulin resistance at near normal blood glucose levels in patients given long term glucagon infusions the possibility exists that the insulin measured by the radioimmunoassay technique employed was not of full biological activity. The apparent insulin resistance observed in this study may be due to the immunoassay rather than a defect at the tissue receptor level. Insulin may be altered by the proteolytic enzymes released into the extracellular fluid in shock or alternately the pancreas may release pro-insulin or some other intermediates which crossreact with many anti-insulin antibodies but have only a fraction of the biological activity of insulin in the rat (360, 690). This possibility can be assessed either by the radioreceptor assay of Neville and coworkers (274) or more rapidly by simple \textit{in vitro} bioassay techniques. While no evidence exists to suggest that insulin in the serum of shocked animals is in any way different from that of non-stressed animals, the possibility remains to be explored.

The analysis of the regression of serum insulin on blood glucose (Table 9) suggested that the threshold blood glucose concentration for an insulinemic response was not altered in endotoxemia, in that the value of blood glucose at which serum insulin would be predicted to fall to zero was similar for all three groups of rats. A three-fold increase in the slopes of the regression lines of the two endotoxin
treated groups over that observed in the controls suggests that an increased elevation in serum insulin per increment in blood glucose over basal was the cause of the elevated insulin levels in endotoxemia. Glucose treatment of endotoxemic rats had no effect on the regression line which indicates that the insulinemic response to endogenously generated hyperglycemia was not different from that in response to glucose treatment. The relationship in the Group 1 rats which received saline iv followed by saline ip (Table 8) was not analyzed since there were no changes in blood glucose or serum insulin of the same order of magnitude observed in the other 3 groups. This method of comparing the insulinemic responses of rats treated with different agents was described by Froesch et al. (223), and their extrapolated zero insulin blood glucose intercept was 50 mg per dl in rats treated only with glucose; this is in close agreement to the values found in this study (Table 9).

H. Glucose Utilization During Endotoxemia

The preceding experiments suggested that glucose utilization might be increased in endotoxemia and it was expected that elevated insulin levels may play a role in rapid disappearance of glucose in endotoxemic rats. Irreversible glucose utilization as evaluated by the recovery of C-14 label as expired $^{14}$CO$_2$ from rats given uniformly labeled $^{14}$C-D-glucose was studied in endotoxemic rats and the effects of treatments known to influence insulin secretion or action was evaluated (Tables 10 and 11). These data (Figures 13 and 14, Table 11) suggest that endotoxin treatment induces a hypermetabolic state with respect to glucose oxidation. This finding is in contrast to the conclusions of other investigators who have used less direct approaches. Berry
and Smythe (52) stated there was no evidence of an increase in carbohydrate utilization in endotoxemia and the hypothermia of lethal endotoxemia suggested a depressed metabolic rate; thus they elected to search no further. Berry (50) has recently affirmed his earlier view on this topic. Shands et al. (575) considered the possibility that increased glucose utilization may have contributed to the profound hypoglycemia which rapidly developed in BCG infected mice given a lethal dose of endotoxin but found no significant influence of endotoxin treatment on net oxygen consumption in either non-infected or BCG-infected mice and the disappearance of total $^{14}$C metabolites from the blood following iv $^{14}$C-U-glucose was not significantly altered by endotoxin treatment of either non-infected or BCG-infected mice. The oxygen consumption experiment is inconclusive with respect to carbohydrate utilization and the $^{14}$C metabolite disappearance experiment is difficult to interpret since $^{14}$C-glucose was not isolated from other labeled compounds and the disappearance of $^{14}$C-glucose from the blood does not adequately reflect $^{14}$C-glucose utilization (610). Finally, Shands et al. (575) collected $^{14}$CO$_2$ for 60 min. in non-infected and BCG-infected mice given endotoxin and reported that endotoxin did not significantly increase glucose oxidation. This finding is in accord with the results in Table 11 in that glucose oxidation using a tracer dose of glucose was not significantly elevated until after the 150 min. samples. However, using an acute septicemia model Long et al. (410) found a two-fold increase in $^{14}$CO$_2$ recovery from C-14 glucose injection in dogs which lends support to our findings.

When insulin secretion was acutely depressed by mannoheptulose
treatment, a significant depression in $^{14}$CO$_2$ recovery at both glucose doses occurred (Groups G vs A and Groups H vs B, Table 11). In insulin suppressed rats endotoxin treatment had no significant effect on glucose oxidation (Figure 15), a finding which lends support to the notion that endogenous insulin played a role in the increased glucose oxidation observed in endotoxemia (Table 11). When rats were pretreated with mannoheptulose 4 hr before ip glucose the suppression of glucose oxidation in non-endotoxin treated rats was very much reduced (Groups K vs G, Figure 16 and Groups L vs H, Figure 17). However, the 4 hr mannoheptulose pretreatment protocol was effective in suppressing endotoxin stimulated glucose oxidation (Figure 18); the greater reduction in glucose oxidation of endotoxemic rats probably was attributable to a depressed excretion of mannoheptulose since glomerular filtration is reduced in endotoxemia (53). The finding of no significant increase in $^{14}$CO recovery following endotoxin treatment of streptozotocin diabetic rats (Figure 19) lends further support to the hypothesis that increased serum insulin is a significant factor contributing to elevated rate of glucose oxidation observed in endotoxemic rats. The data in Figure 20 demonstrated that increased glucose oxidation following endotoxin treatment was not a low dose phenomenon since the increased $^{14}$CO$_2$ recovery was observed earlier after a high lethality dose of 3 mg endotoxin. Although glucose oxidation was increased earlier after the 3 mg dose than after the 1 mg endotoxin dose (Figure 20) no difference was observed when the glucose was given 2 hrs after either endotoxin dose (Figure 21); this suggests that the 1 mg endotoxin dose maximally stimulated glucose oxidation within 2 hrs after its administration.
Since glucocorticoids have been repeatedly shown to protect animals from lethal endotoxemia and it is believed that this protection is due at least in part to a inhibition of the carbohydrate depleting actions of endotoxin (49, 50, 52, 57, 319, 434, 564), glucose oxidation was studied in endotoxemic rats treated with dexamethasone (Figure 22). Dexamethasone alone in saline controls (Group U) depressed glucose oxidation--a finding in agreement with the insulin resistance secondary to glucocorticoid treatment (169, 271, 403, 426, 467, 468, 469, 519, 635, 669). When dexamethasone treated rats were given endotoxin (Group V) no significant increase in glucose oxidation above the depressed value observed in dexamethasone treated controls was observed (Groups V vs U, Figure 22). This finding suggests that a depression of glucose oxidation may be a significant factor in the mechanism by which glucocorticoids protect animals from the carbohydrate depleting actions of endotoxin and thereby prevent lethal endotoxin shock. From the data obtained using Noble-Collip drum traumatized rats (Figure 23) it was concluded that elevated glucose oxidation was not a common factor in the pathogenesis of the hypoglycemia observed in this shock model (481). This finding is supported by the observation of more severely and rapidly depressed gluconeogenic capacity in this shock model than in endotoxin shock (Filkins, unpublished observations) and by the reports of depressed insulin secretion in tumbled rats (652-654). The results obtained in the endotoxemic rats showing increased glucose oxidation in shock are supported by recently published results of others who have found increased glucose utilization in sepsis (120, 280, 358, 410, 490, 687, 688) trauma (481, 614), endotoxemia (605, 606), severe hemorrhage
(160, 664-666) and surgical stress (408). As noted above (p. ) the observation of increased glucose oxidative turnover is consistent with the hypothesis that there is a simultaneous elevation of both insulin and glucagon levels; a condition shown by Cherrington and Vranic (116) to produce similar alterations in blood glucose levels and blood glucose turnover.
INFLUENCE OF INSULIN ON THE HOST-DEFENSE FUNCTION OF THE RETICULO-ENDOTHELIAL SYSTEM

The third major question approached in this study was: does insulin and the resultant hypoglycemia of endotoxemia play a role in the perpetuation of the shock syndrome and to an ultimate demise of the challenged host. Since the reticuloendothelial system plays a role in resistance to lethal shock, the influence of insulin hypoglycemia on reticuloendothelial function was assessed.

The granulopectic activity of the RES was first studied by measuring the rate of carbon clearance (Table 12 and 13). In saline treated controls (Group 1) there was a significant negative correlation between blood glucose and carbon clearance half-time suggesting that blood glucose may be an important physiological regulator of RES functional activity. When rats were made moderately hypoglycemic (blood glucose greater than 30 mg per dl) by insulin treatment there was a significant impairment of carbon clearance (Group 2, Table 12) and the significant correlation between blood glucose and clearance half-time indicated that the severity of the hypoglycemia was a significant factor in the depression of RES phagocytic activity. In rats which were hypoglycemic (blood glucose of 30 or less mg per dl) the carbon clearance half-time was markedly increased to 183% of the saline control value indicating severely depressed RES phagocytic function. When the insulin-treated groups were combined regression analysis indicated that for each 10 mg per dl decrease in blood glucose carbon clearance half-time would be expected to be increased by an average of 2.6 minutes (Table 13). The depressed rate of removal
of carbon from the blood of insulin hypoglycemic rats was probably due to a direct action of insulin on macrophage function per se since in control experiments insulin had no significant influence on carbon clearance when blood glucose was maintained by glucose treatment. These experiments suggested that hypoglycemia is a significant depressant of RES functional activity as reflected by the depressed rate of removal of colloidal carbon from the blood. Hypoglycemia may thus increase susceptibility to lethal shock and play a role in the perpetuation of the shock syndrome at this level of host defense. The influence of hypoglycemia on the phagocytic activity of the RES in vivo does not appear to have been previously investigated. However, the influence of starvation has been studied (155, 343, 344, 438, 554) and depressed RES phagocytic activity has been found in all studies. In addition RES activity is depressed when animals are made hypoglycemic and insulin-sensitive by hypophysectomy (170, 355). RES function is also stimulated by steroidal analogues (484, 636) which have been reported to cause insulin resistance (169, 669). In addition metabolic defects which impair glucose utilization are known to have profound influences on RES function and host defense (31, 46, 159, 669) and it is believed that glucose may be necessary and play a key role in regulating of normal macrophage phagocytic function (283) as has been shown using polymorphonuclear leukocytes (558, 616). The results of this study and the above cited literature are consistent with the hypothesis that hypoglycemia may reduce host resistance to lethal shock by depressing RES activity.

To further assess the role of hypoglycemia in the progression
of the shock syndrome, a technique was devised to measure the clearance of biologically active endotoxin from the blood of rats in vivo and after this technique had been evaluated for its validity, the method was applied to measure endotoxin clearance in hypoglycemic rats. The bioassay employed was that of Filkins (198). However, to achieve truly quantitative estimates of biologically active endotoxin the method of data analysis was modified. Of several lethality transforms tested in combination with a number of potential metametric dose scales, the probit transform described by Finney (211,212) appeared to provide the most linear relationship when the probits were plotted against the endotoxin doses on a logarithmic scale (Figure 26). The significant linear relationship between the probit of lethality and the logarithm of the endotoxin dose in the probit range of 4 to 6 was also tested and verified in later experiments (Tables 15,16, and 17). The establishment of this relationship permitted direct computation of unknown doses from lethality scores in the range of 16% to 84% (211, 212).

When normal fed rats were given a 500 gm endotoxin dose and 5 samples of tail blood were collected at 15 to 240 min. after iv injection, the concentration of biologically active endotoxin in the blood was adequately described as a monoexponential function of time as shown by the significant correlation of the logarithm of blood endotoxin and time (Figure 27). This relationship i.e., the absolute rate of disappearance proportional to the blood concentration, is characteristically found when a sufficiently large dose of a material removed by the RES is administered (61,553). The rate of disappearance corresponded to a half-time of 68 min. (K = 0.0044). The estimated
blood endotoxin concentration immediately after the iv injection was calculated by extrapolating the curve back to time zero and a value of 34.8 gm per ml was obtained. This concentration corresponds to an endotoxin distribution space of 14.4 ml per 300 gm body wt or 4.9 ml per 100 gm body wt. This value is very near to the reported blood volume of similar size rats (13, 47, 326, 504, 574) which suggests that the endotoxin distribution space following iv injection is limited to the vascular compartment is also suggested by Milner, Rudbach and Ribi (453). When 500μgm endotoxin dose was given and blood samples were collected by cardiac puncture results similar to those obtained using tail-blood were obtained (Table 15 and Figure 27). The half-time for endotoxin clearance was not increased when the test clearance dose was increased to 1,000μgm per rat (Figure 28). This would not be expected if endotoxin clearance followed the same kinetics as classical RE test substances (61, 553); however, these results may well be within the error of the estimate with the limited number of points examined on the clearance curve. When the endotoxin test clearance dose was reduced to 250μgm per rat, clearance was multiphasic (Figure 28). By the criteria of Saba (553) and Biozzi et al. (61) this suggests that the critical dose of endotoxin to evaluate RE function in 300 gm fed rats is between 250 and 500μgm per rat. Rats made tolerant to endotoxin by three 1 mg doses cleared endotoxin very rapidly (Table 15). This is in agreement with previous studies of endotoxin clearance in endotoxin tolerant animals in vivo (75, 100, 112, 113, 152, 301, 306, 459, 510, 592). Accelerated endotoxin disappearance was also observed when rats were pretreated with endotoxin 16 to 18 hr before the test dose (Table
following a single endotoxin dose. Furthermore, resistance to lethal endotoxemia which accompanies accelerated endotoxin clearance is well documented following single dose pretreatment (11?). Endotoxin clearance was also influenced when rats were pretreated with endotoxin only 4 hrs before the test clearance dose (Table 15), however, although the initial rate of disappearance appeared accelerated the rate of clearance progressively decreased throughout the 4 hr observation period. Although these data are consistent with the literature on endotoxin clearance \textit{in vivo} the interpretation of the studies in which rats were pretreated with endotoxin 16 to 18 hrs or 4 hrs before the test clearance endotoxin is at present complicated by recent findings in our laboratory dealing with the appearance of blood detoxification of endotoxin subsequent to endotoxemia (201, 203). However, these data lend support to the validity of the technique used to measure endotoxin clearance in this study. Similarly, in acutely adrenalectomized rats the rate of endotoxin clearance was significantly increased over the non-treated controls (Table 16). This finding lends support to the data of Ribble et al. (539) which demonstrated cortisone depression of endotoxin clearance in mice; similarly RES stimulation was observed by Reichard et al. (537) after adrenalectomy. It is well known that corticosteroids alter host defense functions \textit{in vivo} and the results shown in Figure 31 suggest that possibly endogenous corticosteroids alter endotoxin clearance.

From the above results on endotoxin clearance \textit{in vivo} using the lead sensitized rat lethality bioassay and comparisons with the findings of others, it was concluded that the method of assessing endotoxin clearance used in this study was a valid measure of the
rate of disappearance of biologically active endotoxin from the blood. However, it deserves comment that the absolute values obtained for the half-times of endotoxin disappearance from the blood of non-pretreated rats were longer than the values found by in most previous investigations which almost exclusively employed radiolabeled endotoxin preparations. At least 2 factors probably contributed to this apparent discrepancy; (a) the majority of the previous studies used much smaller doses of endotoxin, and (b) there may have been a dissociation of radiolabel from biological activity in some studies. The influence of decreasing the endotoxin dose on the rate of clearance is shown in Figure 28 and suggests that the initial rate of disappearance of a small dose is more rapid than for either larger dose. Braude and coworkers (100) previously warned of this difference in clearance kinetics of a smaller or tracer dose as compared to a larger dose. The dissociation of biological activity of endotoxin from endotoxin radiolabel has been demonstrated by Chedid et al. (114) and others (592, 593) and such a dissociation has been suggested by many other studies. In fact recently Prigal et al. (525) found that incubation of endotoxin with chromium ions actually detoxified the endotoxin as measured using a mouse lethality bioassay. However, if a sufficiently large dose of repurified high molecular weight radiolabeled endotoxin or native endotoxin is given, prolonged residence of bioassayable endotoxin in the blood similar to that observed in the present study has been reported (75-77, 114, 123, 156, 202, 459, 513, 539, 572, 591, 592, 636, 667).

When rats were made hypoglycemic by treatment with insulin, endotoxin clearance was profoundly depressed (table 17, Figure 32).
The finding of depressed endotoxin clearance by insulin hypoglycemia suggests that the hypoglycemia characteristic of endotoxin shock (605) may play a role in the perpetuation of the endotoxin shock syndrome by impairing the first line of host defense to uncontrolled endotoxemia, i.e., the ability of the challenged host to clear endotoxin from the blood.

The perfused liver experiments on endotoxin clearance reported in this study were designed to further assess the method used in studying endotoxin clearance in vivo and to evaluate the role of serum opsonins in endotoxin clearance in the rat. In accord with their previous hypothesis (208, 209, 502) Fine and coworkers have recently presented endotoxin clearance data similar to those of this study and suggested that the systemic endotoxemia is not the result of slow RES clearance but rather is due to continual absorption of endotoxin from the gut and on into the systemic circulation (98, 141, 142, 145, 291, 675). The results of the perfused liver experiments do not support this interpretation of Fine and coworkers. When livers were isolated from the potential intestinal source of endotoxin and perfused in vitro endotoxin clearance remained slow with a maximum of 115 μg/m of endotoxin removed per hr (Table 18). The addition of 10% rat serum did not accelerate endotoxin clearance but rather appeared to retard it (Table 19). Even when 50% heparinized rat blood was used as the perfusate endotoxin clearance was not accelerated. These findings suggest that opsonins are not a significant factor in determining the rate of endotoxin clearance by the perfused rat liver. In what appears to be the only other published study of endotoxin clearance by the perfused
liver, Goodman et al. (269) found slow endotoxin clearance even when 5% normal rabbit serum was added to the perfusate used to assess endotoxin clearance by rabbit livers. More recently Moon has investigated endotoxin clearance by the perfused mouse liver using purified Cr-51 labeled endotoxin and also found extremely slow removal of endotoxin from the perfusate (Robert Moon, unpublished communication). Our findings and those of others using the perfused liver to measure endotoxin clearance support the notion that the rate of endotoxin clearance in vivo is not as rapid as if often stated and lend further support to our in vivo studies of endotoxin clearance in the rat.
PROPOSED ROLE OF INSULIN IN THE PATHOGENESIS OF ENDOTOXIN SHOCK

A proposed mechanism of the role of insulin in the pathogenesis of lethal endotoxin shock is presented in Figure 34. The components of this schema which are directly supported by the results of this dissertation appear in capital letters; those elements supported by the literature are shown in lower case type. The role of insulin and the consequent hypoglycemia, as they contribute to the severity of the shock syndrome, receive major emphasis. Each element of this schema is sequentially discussed below in the order indicated by the letters shown in the figure. For clarity not all of the inter-relations discussed and implied below are shown on Figure 34.

A. Endotoxin injection was the immediate cause of endotoxemia in the experiments reported in this dissertation; however as indicated, endotoxin absorption from the gut may also occur as a result of circulatory impairment and splanchnic ischemia, especially if coupled with depressed RES function (98, 141-145, 208, 209, 235, 291, 303, 501, 626, 675, 689).

B. Endotoxemia and shock lead to the release of bradykinin and possibly other vasoactive peptides (253, 314, 316, 450, 451, 461, 476, 485, 530, 538) as well as peptides which specifically depress myocardial contractility (257, 392-396, 492) and RES function (64, 65, 394).

C. It is well known that endotoxemia and shock lead to the release of biogenic amines including serotonin and histamine (253, 314, 476, 530, 618, 650, 651); a role of vagal discharge in the release of biogenic amines in shock has been suggested (618).

D. Endotoxemia and shock also lead to intra-vascular coagulation
FIGURE 34

PROPOSED SCHEMA OF THE ROLE OF INSULIN AND HYPOGLYCEMIA IN
THE PATHOGENESIS OF LETHAL ENDOTOXIN SHOCK

Please see text for discussion of this figure.
(17, 121, 302, 304, 320, 321, 461, 476); a derangement probably enhanced by elevated blood lactate (266, 378) and high levels of plasma free fatty acids (118, 656) produced by endotoxin treatment (641). Insulin may potentiate the coagulopathy of endotoxemia by increasing thrombokinase activity (431) and increasing platelet aggregation while at the same time depressing fibrinolytic activity (382, 383).

E. The stress of endotoxemia is known to activate the central nervous system producing a variety of neural and humoral responses (27, 28, 67, 68, 190, 208, 209, 237, 476, 641). ACTH is released from the pituitary as well as extra-pituitary stores (19) which leads to stimulated glucocorticoid secretion as well as effects attributable to ACTH per se (see below). The sympatho-adrenal axis is activated (208, 209, 253, 476) as is the vago-insulin system (67, 68, 190, 237, 618, 651), which lead to an activation of the endocrine pancreas (see below) and the secondary release of endogenous factors which influences the insulin response subsequent to the autonomic activation in endotoxemia and shock (347, 557, 618, 651), e.g. serotonin and glucagon or gut glucagon (180, 184, 216, 224, 368, 519, 642, 643).

F. It is well recognized that plasma pancreatic glucagon is elevated in stress (67, 68, 296, 380, 416, 417) including endotoxemia (67, 68) and shock and sepsis (67, 68, 406, 439, 544, 560, 671, 680). The hyperglucagonemia may then in turn enhance catecholamine secretion (561, 566, 617) as well as stimulate insulin secretion (180, 224, 368, 519, 643, 669) both of which may be deleterious in resistance to lethal shock.

G. Endotoxin induced ACTH secretion and subsequent adrenal
cortical activation are well recognized responses to endotoxin and shock (19, 148, 172, 234, 267, 356, 540, 562, 655). Elevated ACTH modulates insulin secretion (246, 361, 362, 388, 413, 491, 622) which produces hyperinsulinemia and hypoglycemia (246, 388). It is also well-recognized that ACTH stimulates lipolysis and elevates plasma free fatty acids; elevated free fatty acids have severe toxic effects on tissues including destruction of immuno-physiologic host-defense elements (637).

**H.** The sympatho-adrenal response to endotoxemia and shock is so severe that the initial catecholamine response has been suggested to be a cause of many of the pathophysiological disturbances observed in lethal shock (136-139, 208, 209, 253, 302, 314, 315, 392, 393, 395, 476, 501, 502, 641). The consequences of elevated catecholamine levels include: (i) altered insulin and glucagon secretion, (ii) glycogenolysis, (iii) enhanced tissue glucose utilization, (iv) depletion of carbohydrate stores, (v) impaired tissue perfusion, (vi) lactic acidosis, and (vii) the production of a hypermetabolic state. Catecholamines also enhanced platelet aggregation and blood coagulation in vivo.

**I.** The acute response to severe stress including endotoxemia is an initial hyperglycemia. Some factors contributing to this response include: (i) Glucagon induced hepatic glycogenolysis and gluconeogenesis (173, 177, 179, 216, 367, 368, 599, 600, 601, 643).


(iii) Peripheral tissue insulin resistance (see below).

(iv) Hepatic insulin resistance (285).
(v) Anoxic stimulation of glycogenolysis (568-570).

(vi) Endotoxin activation of hepatic adenylate cyclase (62, 254, 255).

(vii) Serotonin stimulation of hepatic glycogenolysis (402).

(viii) Release of cellular ATP into the extracellular fluid subsequent to tissue injury leading to hepatic glycogenolysis (401). In this context it is well to note that hyperglycemia is believed to exert a protective effect in impending shock (3, 4, 364, 397, 619) while hypoglycemia sensitizes to both lethal shock and the putative mediators of endotoxin toxicity (3, 4, 83, 84, 138, 210, 284, 364-366, 370, 397, 481, 514, 515, 574, 575, 577). In fact, in assessing the relationships between blood glucose and resistance to lethal hemorrhagic shock Stawitz and coworkers (619) concluded that "---ultimate death or survival of an animal in hemorrhagic shock is independent of its initial glucose reserves, but seems somehow to be related to its ability to maintain itself in a hyperglycemic state."

J. Insulin levels are increased in endotoxemia (67, 68, 190, 237). Some factors which may contribute to elevated insulin levels in endotoxin shock include:

(i) Depressed insulin clearance secondary to diminished perfusion of the major organs responsible for insulin degradation (105, 138, 435, 436, 556).

(ii) Failure of RES uptake of insulin.

(iii) Stimulated insulin secretion in response to the hyperglycemia of shock (38).

(iv) Alterations in levels of non-carbohydrate metabolites
including amino acids, free fatty acids, and ketone bodies.

(v) Inhibition of insulin degradation due to bradykinin inhibition of the protease responsible for insulin destruction.

(vi) ACTH stimulation of insulin secretion.

(vii) Glucagon stimulation of insulin secretion.

(viii) Serotonin modulation of insulin secretion.

(ix) Vagal stimulation of insulin secretion.

(x) Epinephrine stimulation of insulin secretion (99, 117), and

(xi) Pancreatic ischemia, cellular lysis and the release of insulin secretory granules.

In the context of the proposed role of insulin in the pathogenesis of shock, especially that directly associated with endotoxemia, we can not agree with the suggestion of Gunnar and coworkers (673) that insulin is a miscellaneous drug of no consequence when given to bacteremic patients with cardiovascular instability and clinical shock. The cardiovascular effects of insulin merit further investigation (see below).

K. It is well documented that a state of insulin resistance is observed in infection and bacteremia (40, 41, 120, 125, 193, 247, 256, 285, 490, 532, 533, 544, 560, 573, 647) and in shock and injury (110, 111, 146, 183, 286, 287, 325, 340, 341, 408, 547, 614, 683). Although the mechanism has not been resolved, recent studies suggest it is independent of either epinephrine secretion or elevated levels of glucocorticoids (110). Elevated free fatty acids produce such insulin resistance (32, 317, 528, 551) as do a number of hormonal factors (169, 669) including the stress induced insulin antagonist of Vargas (647) or the insulin binding alpha-2-macroglobulin found after thermal
injury (183). Furthermore, cellular injury may lead to insulin resistance through increased cell membrane permeability. ATP inhibits insulin stimulated but not basal glucose transport in both adipose tissue and striated muscle (106, 108) by a mechanism involving phosphorylation of a membrane protein (106). In fact this may be the mechanism by which iv ATP treatment increases resistance to lethal shock; a finding which has been repeated confirmed in controlled studies (578) since it was reported by Talaat et al. (625).

However, despite the apparent insulin resistance an enhancement of glucose utilization occurs in shock and sepsis (120, 408, 410, 490, 606, 664, 665, 666) and an accompanying hypermetabolic state ensues (289, 478). The results presented in this dissertation provide strong evidence that insulin is involved in the development of the state of enhanced glucose utilization observed following endotoxin treatment since this response was aborted either by treatment with mannoheptulose or by rendering the rats diabetic prior to endotoxin challenge. It should also be noted that insulin is required for anoxia stimulated glucose transport (275) as is believed to occur as a consequence of the tissue hypoperfusion of shock. A further mechanism by which insulin increases glucose utilization is by stimulating hepatic glucose oxidation (272, 273, 447) which will depress net hepatic glucose production. This mechanism reinforces the increase in peripheral tissue glucose utilization secondary to elevated insulin levels in plasma and extracellular fluid (300). Catecholamines may also play a role in the enhanced glucose utilization observed in shock through their effect on skeletal muscle alpha-adrenergic receptors (555).
M. Depressed hepatic gluconeogenesis is well documented in endotoxemia and shock (50, 200, 204, 319, 381, 434, 575, 576, 577, 670); however the mechanisms responsible for this response are not entirely understood. The results presented in this dissertation have shown that the insulin response to glucose treatment depressed hepatic gluconeogenesis in endotoxemic rats. It is not surprising that the glucose induced insulin response was effective in depressing gluconeogenesis in endotoxemia since the rat liver is sensitized to insulin inhibition of glucose production by hypoxia (457) and moderate elevations of gluconeogenic hormones (173, 177, 259, 457, 463) as occurs following endotoxin treatment.

N. It is well known that shock and endotoxemia lead to a severe depletion of body carbohydrate stores including both tissue glycogen and blood glucose (49, 50, 57, 160, 204, 280, 434, 619). Some factors enhancing glycogenolysis in endotoxemia may include:

(i) elevated plasma glucagon levels (67, 68)
(ii) elevated plasma serotonin (402)
(iii) catecholamine stimulation of glycogenolytic hormone release (178, 179, 248, 249, 296, 430, 599, 600, 601)
(iv) direct catecholamine stimulated muscle glycogenolysis, and
(v) elevated activity of the hepatic sympathetic nerves (35, 163-165, 581-587).

Regarding the depletion of hepatic glycogen stores and the hypoglycemia associated with endotoxemia it should be emphasized that the rat liver does not respond to hypoglycemia with increased glucose release either in vivo (124) or in vitro (602). Increased hepatic glucose release
is dependent on neural and hormonal stimulation as has been shown to occur in endotoxemia.

0. Hypoglycemia and lactic acidosis, the hallmarks of endotoxin shock (605), are the final results of the above discussed factors including (J) hyperinsulinemia, (L) enhanced glycolysis, (M) depressed hepatic gluconeogenesis, and (N) the depletion of carbohydrate stores. The remainder of the discussion of the hypothesis presented in Figure 34 focuses on the question of how the combination of (N) depletion of carbohydrate stores and (O) hypoglycemia and lactic-acidosis lead to a perpetuation of the shock syndrome and ultimate demise of the challenged host. At this point it should be noted that it is unlikely that glucose treatment will avert the lactic-acidosis and hypoglycemia secondary to hepatic damage in endotoxemia. Patients in shock with recurrent hypoglycemia have been given as much as 2.5 kg of iv glucose over a 24 hr period at which time they were stuporous with a blood glucose of 40 mg per dl; a subsequent 0.6 kg of glucose over the next 2 hr only raised glucose to 60 mg per dl while plasma insulin reached a level of 250 microunits per ml. There was no significant glycosuria (556). Hypoglycemia may also explain the hypothermia observed in endotoxemia in the rat (207) or after traumatic shock (613) for it is now well established that CNS glucopenia produces hypothermia due largely to peripheral vasodilation and increased heat loss (220).

P. Endotoxemia initially depresses RES function as the macrophages engulf the toxic lipopolysaccharide. Depletion of carbohydrate stores is believed to depress macrophage function (283), and depletion of Kupffer cell glycogen has been documented in patients with a hepatic
impairment in gluconeogenesis (193). Hypoglycemia also depressed RES function as assessed using colloidal carbon. Furthermore, hypoglycemia impairs the ability of rats to clear lethal endotoxin from their blood and, as stressed by Fine and coworkers (98, 141-45, 208, 209, 291, 501, 502, 626, 675) any defect in RES clearance of endotoxin leads to increased absorption of endogenous endotoxin and rampant endotoxemia which results in lethal shock. Thus, the RES failure secondary to the hypoglycemia of shock may perpetuate the shock syndrome by limiting RES clearance of endotoxin as well as other substances which it clears from the blood (e.g. fibrin, enzymes, tissue debris collagen, bacteria, etc). Fine is not alone in his belief that the unaltered RES activity is of paramount importance in resistance to lethal shock (14, 16, 18, 64, 65, 132, 308, 536, 694).

Q. Hypoglycemia and the depletion of carbohydrate stores may also be an important factor in the terminal failure of sympathetic nervous system function believed to occur in irreversible shock (208, 209, 253, 314, 476). It is well known that the function of the nervous system is dependent on adequate carbohydrate supplies and it has been shown that rat sympathetic ganglia first begin to fail in their role of transmitting neural impulses at the time they become depleted of glycogen stores (295). Alternately, sympathetic failure may lead to hypoglycemia. Since insulin release by the beta-cells of the pancreas is supposedly under constant alpha-adrenergic inhibition (95), a decrease in sympathetic activity may permit a release of insulin which causes hypoglycemia to which the hypoxic brain can no longer respond with a normal increase in blood flow (58) and thereby suffers irreversible
damage. In this context it is well to note that sympathectomy as a result of cervical spinal cord section or destruction causes profound hypoglycemia (646), inhibits the hyperglycemic response to epinephrine (178), and sensitizes to hypoglycemia (79). As noted above, central nervous system glucopenia will also lead to vasodilation a deleterious event in relative hypovolemia.

R. Tissue hypoperfusion is believed to be an important mechanism by which endotoxemia and shock lead to cellular damage (253, 302, 314, 315, 387, 476) and the generation of shock toxins (64, 65, 257, 314, 392-396, 476). Deranged circulatory function secondary to cardiac decompensation also leads to spontaneous hypoglycemia (66, 437) and lactic acidosis (437). The mechanism of the circulatory impairment secondary to endotoxemia is undoubtedly multifactorial. However, a role of insulin and hypoglycemia in the tissue hypoperfusion of shock is supported by the findings indicated below. Glucopenia in vivo initially potentiates the mesenteric arterial response to sympathetic stimulation (425) and this may cause a more severe splanchnic ischemia with its attendant deleterious effects. However, later in the shock syndrome when blood glucose levels fall profoundly to only 0-15 mg per dl (200, 204, Buchanan and Filkins, unpublished observations) vascular smooth muscle contraction is probably inhibited. Aerobic glycolysis is essential for vascular smooth muscle contractions since oxidative phosphorylation and tricarboxylic acid cycle activity do not support contraction (276). It has been repeatedly reported that lack of sufficient glucose inhibits vascular smooth muscle contractions 15, 122, 329, and see 72). In this context, insulin increases the rate at
which vascular smooth muscle degrades its available glucose to lactate (482). In this way the hypoglycemia of shock may contribute to the circulatory disturbances. This suggestion of deranged cardiovascular function subsequent to hypoglycemia receives support from experimental as well as clinical observations (2, 11, 12, 23, 166, 171, 265, 534). Furthermore, endotoxemia has been reported to "reset" the baroreceptor reflex to a new hypotensive level (634) and endotoxin has been shown to produce a vasodilation which was reversed by acute hemorrhage (450).

Insulin may also potentiate the circulatory derangements of endotoxin shock independent of blood glucose. It has been reported that insulin depresses the baroreceptor responses in patients (21, 446) and attenuates the pressor responses to carotid artery occlusion in dogs (88). In patients maintained hyperglycemic with iv glucose, insulin has been reported to cause syncope (446). By such effects on peripheral circulatory control it is believed that the hyperinsulinemia of shock may add to the deranged tissue perfusion subsequent to endotoxemia.

S. Splanchnic hypoxia is one of the many results of impaired tissue perfusion and may lead to the generation of any of a number of "shock mediators" which have been described. Splanchnic hypoxia may also be an important factor in the depressed hepatic gluconeogenic capacity of endotoxin shock. And, as repeatedly proclaimed by Fine and coworkers splanchnic hypoxia leads to increased intestinal permeability to endotoxin and by this action perpetuation of the shock syndrome leading back to endotoxemia which was the initiating event in this schema and the experiments described in this dissertation.
Based on the results and discussion thus far presented in this dissertation the relationships shown in Figure 35 are believed to be of key importance in the mechanism by which insulin plays a role in the pathogenesis of endotoxin shock and eventual death.

Endotoxemia leads to increased levels of circulating insulin, probably as a result of both (i) increased insulin secretion in response to early hyperglycemia of shock, and (ii) depressed insulin clearance, perhaps related to altered macrophage function. Elevated insulin levels depress hepatic gluconeogenesis both acutely and by a long lasting effect related to the suppression of hepatic gluconeogenic enzyme synthesis. Elevated insulin levels in endotoxemia are also a necessary component of the elevated rate of glucose utilization observed in endotoxin shock. The end result of depressed glucose synthesis and elevated glucose utilization is profound terminal hypoglycemia. In conjunction with its other deleterious effects hypoglycemia leads to depressed RES clearance function. When the phagocytic activity of the RES is depressed susceptibility to shock is increased; and as in the present experiments hypoglycemia may lead to depressed endotoxin clearance. When endotoxin clearance is depressed, the shock state is perpetuated by the actions of this toxic lipopolysaccharide remaining in the systemic circulation.

In summary it is concluded that insulin plays a role in the pathogenesis of lethal endotoxin shock. Under the conditions of the present experiments serum insulin levels were markedly elevated early in endotoxemia and remained elevated for at least 12 hours, during which time depressed gluconeogenesis and elevated glucose utilization were observed.
FIGURE 35
KEY ROLE OF INSULIN IN THE PATHOGENESIS OF LETHAL ENDOTOXIN SHOCK
CHAPTER VI

SUMMARY

1. Endotoxin shock lethality was studied in rats treated so as to influence insulin secretion:

   A. Lethality was significantly increased when insulin secretion was elevated, viz, the fed state and treatment with ip glucose.

   B. Lethality was significantly reduced when insulin secretion was depressed, viz, by overnight fasting or mannoheptulose treatment.

2. Glucose treatment per se did not sensitize rats to lethal endotoxin shock since when treatments with mannoheptulose and glucose were combined:

   A. Lethality in endotoxin treated rats administered both glucose and mannoheptulose was not different from rats treated with only mannoheptulose and endotoxin.

   B. Lethality in both mannoheptulose treated groups -- irrespective of glucose treatment -- was significantly less than the lethality in control endotoxin treated rats.

3. Both stimulation of endogenous insulin secretion with tolbutamide or tolazamide and treatment with small doses of insulin per se significantly increased endotoxin shock lethality above levels observed in controls treated with endotoxin and saline.
4. The influence of glucose treatment on in vivo gluconeogenic capacity and hepatic glycogenic function was studied in overnight fasted rats 4 to 5 hrs after iv endotoxin and 2 to 3 hrs after ip glucose (400 mg) using a 100 mg alanine dose, and:

A. In rats not given endotoxin, glucose treatment caused a hyperglycemia at the time of alanine injection while both endotoxin treated groups had blood glucose values significantly less than their saline controls.

B. Glucose treatment prevented the significant rise in blood glucose levels observed 30 minutes after alanine in endotoxin treated rats not treated with glucose.

C. Glucose treatment significantly depressed the incorporation of alanine carbon label into blood glucose.

D. Hepatic glycogen reserves were not increased by glucose treatment of endotoxemic rats.

E. Glycogen synthesis from alanine was severely depressed in both endotoxin treated groups as compared to the iv saline control groups.

5. Analysis of blood glucose and serum insulin responses in overnight fasted rats given iv endotoxin revealed:

A. A transient hyperglycemia reaching a peak at almost twice fasting blood glucose at 90 minutes after iv endotoxin,

B. A large increase in serum insulin accompanying the hyperglycemia and reaching a peak of 130 μU per ml at 90 minutes after iv endotoxin,
C. An elevation of serum insulin throughout the 12 hr observation period despite the return of blood glucose to fasting levels by 240 minutes, and

D. A depression of the glucose to insulin ratio and an elevation of the glucose-insulin product.

6. Evaluation of intraperitoneal glucose tolerance and the associated insulin response in overnight fasted rats 2 hr after endotoxin treatment revealed that:

A. Compared to controls, endotoxemic rats had no impairment in the rate of decline in blood glucose following a similar peak blood glucose concentration.

B. Control rats -- not given endotoxin -- maintained significantly elevated blood glucose values for at least 6 hrs, while the endotoxemic rats returned to fasting blood glucose values within 2 hrs.

C. The insulin response to glucose was exaggerated in the endotoxin treated rats and insulin levels remained elevated throughout the 10 hr observation period despite the early return to normoglycemia.

D. In endotoxin treated rats the increase in serum insulin associated with a given increase in blood glucose was 3 times that observed in the glucose treated controls; treatment with exogenous glucose did not influence the significant linear relationship between blood glucose and serum insulin in endotoxemic rats, and,

E. The depressed glucose to insulin ratio appeared to be the result of an increased insulin response to the existing blood
glucose level with no change in the blood glucose threshold for a serum insulin response.

7. The rate of glucose oxidation in vivo, as evaluated by the recovery of carbon label as $^{14}\text{CO}_2$ from uniformly labeled $^{14}\text{C}-\text{D}-\text{glucose},$ was studied over a 4 hr period immediately following either a tracer dose or a 400 mg load of glucose and;

A. Label recovery was significantly increased when rats were given 1 mg endotoxin at the same time as either dose of glucose.

B. Label recovery was greater if endotoxin was given 2 hr before either glucose dose.

C. When insulin secretion was suppressed with mannheptulose, endotoxin treatment at the same time as either dose of glucose did not influence the recovery of label.

D. When mannheptulose treated rats were given endotoxin 2 hr before either glucose dose, there was no increase in label recovery.

E. Endotoxin treatment of streptozotocin diabetic rats did not increase label recovery above the depressed level seen in saline-treated diabetic rats.

F. Dexamethasone treatment depressed glucose oxidative turnover; endotoxin treatment did not increase label recovery above this depressed level.

G. Increasing the endotoxin dose from 1 mg (approximately LD$_{10}$-20) to 3 mg (approximately LD$_{90}$) per rat further increased the recovery of label at both glucose doses and decreased the time before the values were significantly above control, and,
H. Enhanced glucose oxidation did not appear to be common to all shock inducing stresses, since tumbling shock did not increase label recovery following either glucose dose.

8. Evaluation of reticuloendothelial phagocytic function by the rate of removal of colloidal carbon from the blood following an 8 mg per 100 gm dose revealed:

A. A significant linear correlation between carbon clearance half-time and blood glucose in saline treated controls.

B. A significant linear correlation between blood glucose and carbon clearance half-time in insulin treated rats; the slope of the regression line of half-time on blood glucose was -26 min. per 100 mg per dl.

C. A significant depression of intravascular phagocytosis in insulin hypoglycemic rats which was related to the severity of the hypoglycemia, and,

D. No alterations in reticuloendothelial phagocytic function which could be attributed to insulin per se.

9. The use of the lead-sensitized rat for the bioassay of endotoxin was evaluated and the relationship between the probit of endotoxin shock lethality and logarithm of the endotoxin dose was linear in the probit range of 4 to 6. This permitted accurate determinations of sub-microgram quantities of biologically active endotoxin using a shock death endpoints in groups of lead-treated rats with lethality scores between 16% and 84%.

10. Endotoxin clearance was studied in vivo by injecting endotoxin iv and measuring the amount of biologically active endotoxin
remaining in the blood at 15 to 240 min. after injection using the lead-
sensitized rat bioassay and;

A. In normal fed rats given a 500μg/m endotoxin dose a
linear relationship existed between the logarithm of the blood endo-
toxin concentration and time throughout the period of 15 to 240 min.
The half-time for disappearance of this dose was 63 min. in serially
sampled rats and 58 min. in a single sampling protocol.

B. Increasing the endotoxin dose to 1,000 gm had no in-
fluence on the half-time for endotoxin clearance and the semi-logarithmic
plots were still linear.

C. Decreasing the endotoxin dose to 250 gm produced mul-
tiphasic clearance curves with rapid clearance soon after the endotoxin
injection and a progressive decrease in the clearance rate with time.

D. In rats made tolerant to endotoxin by three 1 mg doses
on alternate days the rate of disappearance was markedly accelerated
and corresponded to a half-time of about 4 min. in the 0 to 15 min.
period.

E. In rats pre-treated with 500μg/m of endotoxin 16 to
18 hr before the 500μg/m clearance dose, the rate of endotoxin dis-
appearance was almost as fast as in the 3 dose tolerant rats.

F. In rats pre-treated with 500μg/m of endotoxin 4 hrs
before a subsequent dose of 500μg/m, clearance was multiphasic with
the rate of disappearance increased over controls in the first 60 min.
followed by a depressed rate of disappearance in the 120 to 240 min.
period.

G. Control experiments suggested that the disappearance
of biologically lethal endotoxin from the blood of both normal rats and rats pre-treated with endotoxin was not the result of detoxification of endotoxin in the blood.

H. Surgical stress 1 hr before a 500 μg test dose did not alter endotoxin clearance in the 120 min. following injection but did increase the rate of disappearance in the 120 to 130 min. period.

I. In rats adrenalectomized 1 hr before a 500 μg test dose the rate of disappearance was proportional to the blood endotoxin level and corresponded to a half-time of 38 min.

J. Blood endotoxin was compared in overnight fasted and insulin hypoglycemic rats 3 hrs after a 500 μg test dose; insulin hypoglycemia depressed the rate of disappearance of biologically active endotoxin with significantly more lethal endotoxin remaining in the blood.

11. Endotoxin clearance by the isolated perfused rat liver was studied using the lead sensitized rat bioassay to quantitate endotoxin.

A. Livers perfused for 60 min. with only a balanced salt solution removed about 115 μg endotoxin from the 140 ml of perfusate.

B. When livers were perfused with 10% rat serum in the balanced salt solution following 1 hr of perfusion with the salt solution alone, no further loss of endotoxin from the perfusate occurred.

C. When livers were perfused with 50% fresh heparinized rat blood in the salt solution no significant loss of endotoxin occurred in the first 30 min. The decreased lethality of the 90 min. perfusate indicated the liver had removed about 55 μg of endotoxin.
D. Control experiments showed the loss of biologically active endotoxin was not the result of inactivation of the endotoxin by any of the perfusion media used independent of the liver.
CHAPTER VII

SELECTED BIBLIOGRAPHY


a. The references appearing in this selected bibliography are in the format prescribed by the Council of Biology Editors, Committee on Form and Style. 1972. CBE style manual. Third edition. American Institute of Biological Sciences, Washington, D.C. As instructed in this manual the Journal abbreviations used are those of Index Medicus (Volume 14, 1973). In those cases where cited Journals did not appear in Index Medicus the complete titles are written out.


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CHI-SQUARE TEST WITH YATES CORRECTION FACTOR INCLUDED

WRITE
C focal-12

01.01 T 39.03J E
01.02 A "R"Rja "C"CI S DF=(R-1)X(C-1)J S L=R*C
01.03 A "S"N(K)I S RP=RP+N(K)J S CSJ=CS(J)+N(K)J S K=K+1
01.05 S J=J+1
01.06 I (C-J)1.07 J.04 J.04
01.07 S RS{I}=RP I S J=J S I=I+1 S RP=0
01.08 I (R-1)1.09 J.04 J.04
01.09 I (L-(K-1))1.10 J.2.01 J.2.01
01.10 T !!!T "E"J T !!! J G 1.01

02.01 F J=1,1, RJS T=T+RS(J)
02.02 S J=1 S I=1 S K=1
02.03 S E(K)=(RS(I)/T)*CS(J)J S J=J+1 S K=K+1
02.04 I (C-J)2.05 J.2.03 J.2.03
02.05 S J=1 S I=1+1
02.06 I (R-1)2.07 J.2.03 J.2.03
02.07 I (L-(K-1))2.08 J.3.01 J.3.01
02.08 T !!!T "E2"J T !!! T

03.01 F K=1,1, LJS X2=X2+((FABS(N(K)-E(K))-.5)*2)/E(K)
03.02 T !!! S K=1 S Y=1 S N=1 S I=1 D B*0JT !
04.02 F J=1 J CJD 6*0
04.03 T "*JT !!! F J=1 J CJD 5*0
04.04 T "*JT "JT "RS"RS{N}J S N=N+1JT !
04.05 F J=1 J CJD 7*0
04.06 T "*JT !!! B*0JT !!! S I=I+1 J (R-1)4.07 J.4.02 J.4.02
04.07 T !!! F J=1 J CJD 9*0
04.08 T !!! "JT "TJT !!! T "X2"X2JT "JT B.03JT "DF"DFJT !
04.09 A "M?"ZI I (Z-2)4.10 J.01 J.01
04.10 Q
05.01 T "*JT ">

06.01 T "*JT "JT "O"N(K)JT "J S K=K+1
07.01 T "*JT "JT "E"E(Y)JT "J S Y=Y+1
08.01 F M=1,1, (C+15+1)JT "*"
09.01 T "JT "CS"CS(J)JT ""
COMPUTATION OF "P" VALUE FOR CHI-SQUARE VALUE AT SPECIFIED DEGREES OF FREEDOM

WRITE
C FOCAL-12

01.10 0 SIG CJT "CHI SQ PROB",!!!E
01.20 A ?X?, ?DF?, !!!S BX=X

02.10 S A=S*XJS E=2*FTR(DF/2)-DF
02.15 I (E)2,2,2.27,2.2
02.20 I (DF-2)2,3,2,3,2,27
02.27 I (BX-40)2,29,2,3,2,3
02.29 S YY=FEXP(-A)
02.30 I (E)2,4JS S=YYG 2.6
02.40 S X=FSQT(X)*D 101 I (X)2.45,2.45JS P=(Z+1)/21G 2.5
02.45 S P=(1-Z)/2
02.50 S S=2*P
02.60 I (DF-2)2,7,2,7,3,1
02.70 O TI "6.4,!!!,"P =""Sx,"" WITH",Sx,DF," DF"!!!
02.90 G

03.10 S X=5*(DF-1)J1 (E)3,2JS Z=1JG 3,3
03.20 S Z=5
03.30 I (BX-40)3,8J1 (E)3,4JS E=0JG 3,5
03.40 S Ex=57265
03.50 S C=FLOG(A)
03.60 I (X-Z)3,7JS E=E+FLOG(Z)JS S=FEXP(C-Z-A-E)+SJS Z=Z+1JG 3,6
03.70 G 2,7
03.80 I (E)3,8JS E=1JG 3,87
03.85 S E=56,419/FSQT(A)
03.87 S C=0
03.90 I (X-Z)3,9JS E=E/A/ZJS C=C+EJS Z=Z+1JG 3,9
03.95 S S=C*YY+SIG 2,7

10.10 I (X)10,11,10,21D 11JR
10.11 D 11JR
10.20 S Z=0JR

11.10 S Y=FABS(X)/2
11.20 I (Y-)11,3JS Z=1JR
11.30 I (Y-1)11,4JD 12JR
11.40 S W=Y+Y
11.50 S Z=((.00124819*W-.000010752)*W+.00519877)W
11.60 S Z=((Z-.0191983)*W-.0598540)*W-.151969)*W
11.70 S Z=((Z+.319153)*W-.531923)*W+.797885)*Y2
11.80 R

12.10 S Y=Y-2
12.20 S Z=((-.0004526*Y+.000152529)*Y-.0000195381)*Y
12.30 S Z=((Z-.000676905)*Y+.0013906)*Y-.00079462)*Y
12.40 S Z=((Z-.00203425)*Y+.00654979)*Y-.0105576)*Y
12.50 S Z=((Z+.0116308)*Y-.00927945)*Y+.0053538)*Y
12.60 S Z=((Z-.00214127)*Y+.0053531)*Y+.999937
12.70 R
CALCULATION OF MEAN STANDARD DEVIATION & STANDARD ERROR OF MEAN

WRITE
C FOCAL-12

01.10 L O,F0,F,TAB,0
01.20 S S=0;S N=0;T "X"!
01.30 A X(!)S F(0(N+1)=X
01.40 I (X)1,5,1,6,1,5
01.50 S N=N+1;S S=S+X;G 1,3
01.60 S M=S/N;S S=0
01.70 F J=1,N;S S=S+(M-F(0(J)))*2
01.80 S S=S/(N-1)
01.90 I (S)2,1,3,05,2,1

02.10 S S=0.5*FLOG(S);S D=FEXP(S)
02.15 T !!,"N"="N
02.20 S N=0.5*FLOG(N);S N=FEXP(N)
02.30 G 3,1

03.05 S D=0
03.10 T !;T "MEN";M
03.20 T !;T "SD ",D
03.30 T !;T "SE ",D/N;T !!!
03.35 A A;I (A)3,40,1,20,3,40
03.40 L C,F0

*
UNPAIRED STUDENT'S "T" TEST

WRITE
C FOCAL-12

01.10 D 7
01.20 S C=.63661977
01.30 S D=.5*FLOG(DF); S D=FEXP(D)
01.40 S TH=FATN(T/D); S X=DF/(DF+T+2)
01.50 I (DF-1)1.6,1.6,2.1
01.60 S A=C*TH/JG 4.1

02.10 S TS=FTRC((DF/2); S TS=2*TS
02.20 I (DF-TS)2.3,2.3,2.4
02.30 S IX=11G 2.5
02.40 S IX=2
02.50 S A=1; S Y=1; S Z=IX; S W=Z+1
02.60 I (DF-W)3.1,3.1,2.7
02.70 S Y=X*Y*Z/W
02.80 S A=A+Y; S Z=Z+2; S W=W+2; G 2.6

03.10 I (2-IX)3.2,3.2,3.4
03.20 S N=.5*FLOG(X-X+2); S N=FEXP(N)
03.30 S A=C*(TH+A*N); G 4.1
03.40 S N=.5*FLOG(1-X); S N=FEXP(N); S A=A+N

04.10 S P=1-A

05.10 T !,,"DF",DF,,!,,"T","T",!;T %10.08;T "P",P;T %8.04;G 1.1

07.10 A !,,"NX",NX,,"MX",MX,,"SE",EX
07.20 A !,,"NY",NY,,"MY",MY,,"SE",EY
07.30 S DF=NX+NY-2
07.40 S T=FABS(MX-MY)/FSQT(EX+2+EY+2)
*
PAIRING STUDENT'S "T" TEST

WRITE C FOCAL-12

01.10 S C1=0; S C2=0; S R=0
01.20 A !; "NJ", N
01.30 F J=1, N; A !, V; S C1=C1+V; S C2=C2+V+2
01.40 S M1=C1/N; S K=(C2-C1+2/N)/(N-1); S DX=FSQTC(K)
01.50 S EX=DX/FSQTC(N); S DF=N
01.60 D 1; !; A !; "NY", NJD 1.3
01.70 S MY=C1/N; S K=(C2-C1+2/N)/(N-1); S DY=FSQTC(K)
01.80 S EY=DF/FSQTC(N); S DF=DF+N-2
01.85 A !; "PAIRED?", Y; I (Y-25)1.95,1.9,1.95
01.90 A !; "R", R; S DF=DF/2
01.95 S T=FABS((MX-MY)/FSQTC(EX+EY+2-2*R*EX*EY))

02.10 S C=.63661977
02.20 S D=FSQTC(DF)
02.30 S TH=FATN(T/D); S X=DF/(DF+T+2)
02.40 I (DF-1)2.5, 2.5, 3, 1
02.50 S A=C*TH+G 4.5

03.10 S TS=FIPTC(DF/2); S TS=2*TS
03.20 I (DF-TS)3.3, 3, 3, 3.4
03.30 S IX=1+G 3.5
03.40 S IX=2
03.50 S A=1; S Y=1; S Z=IX; S W=Z+1
03.60 I (DF-W)4.1, 4, 1, 3.7
03.70 S Y=X*Y*Z/W
03.80 S A=A+YS Z=Z+2; S W=W+2; S G 3.6

04.10 I (2-IX)4.2, 4.2, 4.4
04.20 S N=FSQTC(X-X+2)
04.30 S A=C*TH*A*N); G 4.5
04.40 S N=FSQTC(1-X); S A=A*N
04.50 S P=1-A

05.10 T !; "MX=" , MX, !; "SD=" , DX, !; "SE=" , EX
05.20 T !; "MY=" , MY, !; "SD=" , DY, !; "SE=" , EY
05.30 T !; "DF=" , DF, !; "T= " , T, !; "P= " , P, !; !; Q

*
INSULIN RIA CALCULATION; SAMPLE MEAN, S.D., S.E.M., & COEFFICIENT OF VARIATION

WRITE
C FOCAL-12

01.10 A "M",M,!,"B",B,!
01.20 A "BKIG",BG,!
01.30 A "CPM H0",H0,!!

02.05 A "N",N,;I (N)2.10,12.99,2.10
02.10 S CT=0; S UT=0; T " %H0 UG/ML",!!
02.20 F I=1,N; D 6
02.30 S UM=UT/N; S CM=CT/N
02.40 T " , ,CM,UM,!
02.50 F I=1,N; S X(I)=C(I)
02.60 S XM=CM+D 5; S CD=SD; S CE=SE; F I=1,N; S X(I)=U(I)
02.70 S XM=UM+D 5; S UD=SD; S UE=SE; T " , ,CD,UD,100*UD/UM,!!
02.80 T " , ,CE,UE,100*UE/UM,!!
02.99 G 2.05

05.10 S SX=0; F I=1,N; S SX=SX+(X(I)-XM)+2
05.20 S SD=FSQT(SX/(N-1))
05.30 S SE=SD/FSQT(N-1)

06.10 A "CPM",CP; S C(I)=100*(CP-BG)/H0; S CT=C(I)+CT
06.20 S Y=C(I); D 10; S U(I)=X; S UT=U(I)+UT
06.30 T C(I),U(I),!

10.10 S X=FEXP((Y-B)/(0.4343*M))

12.99 Q
*

A 7
COMPUTATION OF %INJECTED DOSE RECOVERED AS $^{14}\text{CO}_2$ AFTER $^{14}\text{C}$-UD-GLUCOSE

WRITE
C FOCAL-12

01.10 S T=0; S SX=0; S V=52; A "ENTER TOTAL DPM'S INJECTED/RAT", Z, !!
01.20 A "ENTER COUNTING EFFICIENCY", E, !!
01.25 A "ENTER BACKGROUND COUNT", BG, !!
01.30 T " T SCPM/ML TRVOL DPMO CUMDPMO DPMTR"
01.35 T " TOTDPM %INJDOSE", !!
01.40 T %3.0, T; A X; S X=X-BG; S X=100*X/E; T %8.0, V, 2*X; S SX=SX+2*X
01.50 T SX-2*X, V*X, V*X+SX-2*X, %4.02," (V*X+SX-2*X)*100/Z, !!
01.60 I (240-T)*1.80, 1.80, 1.65
01.65 I (T-60)*1.70; S T=T+15
01.70 S T=T+15; S V=V-2*G 1.40
01.80 Q
*

*
PROGRAM TO MAKE LIBRARY DATA FILES FOR UNPAIRED STUDENT'S "T" TEST

WRITE
C FOCAL-12

01.10 A "HOW MANY DATA TRIPLETS?" , NT, !
01.20 F I=1, 3, 3*NT; T !; F J=0, 2; A F0(I+J)
01.25 F I=1, 3, 3*NT; T !; F J=0, 2; T F0(I+J), %4.0, I+J, %5.04
01.27 A A; I (A)1.35, 1.30, 1.35
01.30 L C, F0; I0
01.35 *

*
PROGRAM FOR UNPAIRED STUDENT'S "T" TEST USING DATA FILES STORED ON TAPE USING LIBRARY MAKE PROGRAM

WRITE
C FOCAL-12

01.10 S C=0.63661977
01.20 A "HOW MANY PAIRS?",NP,!!
01.30 F I=1,3,NP+D 2,D 3
01.90 L C,F1IL C,F2
01.99 Q

02.10 S N(1)=F1(I); S N(2)=F2(I); S M(1)=F1(I+1); S M(2)=F2(I+1)
02.20 S S(1)=F1(I+2); S S(2)=F2(I+2); S DF=N(1)+N(2)-2
02.30 S ST=FSQTL(S(1)*2+S(2)*2); S T=FABS(M(1)-M(2))/ST

03.10 S D=FSQTL(DF); S TH=FATN(T/D); S X=DF/(DF+T+2); I (DF-1)3,2,3,2,3,3
03.20 S A=C*TH; G 3,96
03.30 S TS=FITR(DF/2); S TS=2*TS; S IX=2; I (DF-TS)3,4,3,4,3,5
03.40 S IX=1
03.50 S A=1; S Y=1; S Z=IX; S W=W+1
03.60 I (DF-W)3,8,3,8,3,7
03.70 S Y=X*Y*Z/W; S A=A*Y; S Z=Z+2; S W=W+2; G 3,6
03.80 I (2-IX)3,9,3,9,3,95
03.90 S N=FSQTL(X-X/2); S A=C*(TH+A*N); G 3,96
03.95 S N=FSQTL(1-X); S A=A*N
03.96 T "N1",N(1),!,"M1",M(1),!,"SE",S(1),!
03.97 T "N2",N(2),!,"M2",M(2),!,"SE",S(2),!
03.98 T "DF",DF,!,"T",T,!,"P",(1-A),!!

*
COMPUTATION OF PEARSON'S R FOR LINEAR & LOGARITHMIC DATA

(PROGRAM $LINE IS USED BY THIS PROGRAM AND IS ON THE NEXT PAGE OF THE APPENDIX)

WRITE
C FOCAL-12

01.05 L 0,F1,F,TAB,0
01.10 S L=0;F J=1,28;S F1(J)=0
01.20 T !JA "N",N;S F1(29)=N;T !;T "ENTER VALUES";T !
01.30 T !JA "X",X;A "Y",Y;S L=L+1
01.40 S LX=4.343*FLOG(X);S LY=4.343*FLOG(Y)

02.10 S F1(1)=F1(1)+X;S F1(2)=F1(2)+LX
02.20 S F1(3)=F1(3)+X+2;S F1(4)=F1(4)+LX+2
02.30 S F1(5)=F1(5)+Y;S F1(6)=F1(6)+LY
02.40 S F1(7)=F1(7)+Y+2;S F1(8)=F1(8)+LY+2
02.50 S F1(9)=F1(9)+X+Y;S F1(10)=F1(10)+X*LY
02.60 S F1(11)=F1(11)+LX*Y;S F1(12)=F1(12)+LX*LY
02.70 I (L-N)1,3,3,1,3,1

03.10 S F1(13)=F1(9)-F1(1)*F1(5)/N
03.20 S F1(14)=F1(10)-F1(1)*F1(6)/N
03.30 S F1(15)=F1(11)-F1(2)*F1(5)/N
03.40 S F1(16)=F1(12)-F1(2)*F1(6)/N
03.50 S F1(17)=(F1(3)-F1(1)+2/N)*(F1(7)-F1(5)+2/N)
03.60 S F1(18)=(F1(3)-F1(1)+2/N)*(F1(8)-F1(6)+2/N)
03.70 S F1(19)=(F1(4)-F1(2)+2/N)*(F1(7)-F1(5)+2/N)
03.80 S F1(20)=(F1(4)-F1(2)+2/N)*(F1(8)-F1(6)+2/N)
03.90 F J=17,20;S F1(J)=5*FLOG(F1(J));S F1(J)=FEXP(F1(J))

05.10 T !;T "R X,Y ",F1(13)/F1(17)
05.20 T !;T "R X,LY ",F1(14)/F1(18)
05.30 T !;T "R LX,Y ",F1(15)/F1(19)
05.40 T !;T "R LX,LY ",F1(16)/F1(20)
05.50 T !;L C,F1;L G,$LINE,0;Q
PROGRAM "$LINE" USED IN COMPUTATION FOR PROGRAM "$REGRESS"

WRITE
C FOCAL-12

01.10 L 0,F1,F,TAB,0
01.20 S N=F1(29)

04.10 S C1=F1(3)-F1(1)+2/N; S C2=F1(4)-F1(2)+2/N
04.20 S F1(21)=(F1(9)-F1(1)*F1(5)/N)/C1
04.30 S F1(22)=(F1(10)-F1(1)*F1(6)/N)/C1
04.40 S F1(23)=(F1(11)-F1(2)*F1(5)/N)/C2
04.50 S F1(24)=(F1(12)-F1(2)*F1(6)/N)/C2
04.60 S F1(25)=(F1(5)-F1(1)*F1(21))/N
04.70 S F1(26)=(F1(6)-F1(1)*F1(22))/N
04.80 S F1(27)=(F1(5)-F1(2)*F1(23))/N
04.90 S F1(28)=(F1(6)-F1(2)*F1(24))/N

05.15 T !;T "M",F1(21);T "B",F1(25)
05.25 T !;T "M",F1(22);T "B",F1(26)
05.35 T !;T "M",F1(23);T "B",F1(27)
05.45 T !;T "M",F1(24);T "B",F1(28)
05.50 T !!!;L C,F1;L L,$REGRES,0;Q

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COMPUTATION OF "P" VALUE FOR PEARSON'S R AT (N-2) DEGREES OF FREEDOM

WRITE
C FOCAL-12

01.10 A "N",N,"R",R
01.20 S DF=N-2; S D=FSQT(DF)
01.30 S T=FSQT(1-R+2); S T=R*D/T

02.10 S C=.63661977
02.20 S D=FSQT(DF)
02.30 S TH=FATN(T/D); S X=DF/(DF+T+2)
02.40 I (DF-1)2.5,2.5,3.1
02.50 S A=C*TH*G 4.5

03.10 S TS=FITR(DF/2); S TS=2*TS
03.20 I (DF-TS)3.3,3.3,3.4
03.30 S IX=1; G 3.5
03.40 S IX=2
03.50 S A=1; S Y=1; S Z=IX; S W=Z+1
03.60 I (DF-W)4.1,4.1,3.7
03.70 S Y=XY*Z/W
03.80 S A=A+Y; S Z=Z+2; S W=W+2*G 3.6

04.10 I (2-IX)4.2,4.2,4.4
04.20 S N=FSQT(X-X+2)
04.30 S A=C*(TH+A*N); G 4.5
04.40 S N=FSQT(1-X); S A=A*N
04.50 S P=1-A

05.10 T ";DF=";DF; ";T= ";T
05.20 T ";%10.10;"P= ";P; ";8.04
05.30 G 1.1

*
INTERPOLATION OF "X" FROM SLOPE & INTERCEPT OF LINE & VALUE OF "Y" (USED FOR LINEAR, SEMILOGARITHMIC, & LOGARITHMIC RELATIONSHIPS)

WRITE
C FOCAL-12

01.10 T !;A "M";M;A "B";B
01.20 T !;A "Y";Y
01.30 S A=(Y-B)/M
01.40 S C=(.4343*FLOG(Y)-B)/M

02.10 T !;T A
02.20 T !;T C
02.30 T !;T FEXP(A/.4343)
02.40 T !;T FEXP(C/.4343)
02.50 G 1.2
*

A 14
INTERPOLATION OF "Y" FROM SLOPE & INTERCEPT OF LINE & VALUE OF "X"
(USED FOR LINEAR, SEMILOGARITHMIC, & LOGARITHMIC RELATIONSHIPS)

WRITE
C FOCAL-12

01.10 T !;A "M",M;A "B",B
01.20 T !;A "X",X
01.30 S A=M*X+B
01.40 S C=M*4343*FLOG(X)+B

02.10 T !;T A
02.20 T !;T FEXP(A/.4343)
02.30 T !;T C
02.40 T !;T FEXP(C/.4343)
02.50 G 1.2
*

A 15
COMPUTATION OF PARAMETERS OF LINEAR REGRESSION LINE

WRITE
C FOCAL-12

01.10 S C1=0;S C2=0;S C3=0;S C4=0;S C5=0
01.20 A !!,"N",N
01.30 F J=1,N;A !,"X","X","Y","Y;D 5

02.10 S DX=C2-C1+2/N;S DY=C4-C3+2/N
02.20 S NU=C5-C1*C3/N;S R=NU/FSQT(DX*DY)
02.30 S M=NU/DX;S B=(C3-C1*M)/N
02.40 S S=DX-DX*M+2;S E=FSQT(S/(N-2))
02.50 S XM=C1/N;S YM=C3/N
02.60 S XD=FSQT(DX/(N-1));S XE=XD/FSQT(N)
02.70 S YD=FSQT(DY/(N-1));S YE=YD/FSQT(N)

03.10 T !!,"LIN REG- Y=B+MX+E"
03.20 T !!,"SX ",C1!,"SX2",C2!,"SY ",C3
03.30 T !!,"SY2",C4!,"SXY",C5
03.40 T !!,"MNX",XM!,"SDX",XD!,"SEX",XE
03.50 T !!,"MNY",YM!,"SYD",YD!,"SEY",YE
03.60 T !!,"DX ",DX!,"DY ",DY!,"S ",S
03.70 T !!,"M ',M!,"B '",B!,"E ",E
03.80 T !!,"R ",R!,"R+2",R+2,!!!

05.10 S C1=C1+X;S C2=C2+X+2;S C3=C3+Y
05.20 S C4=C4+Y+2;S C5=C5+X+Y

*
COMPUTATION OF SIGNIFICANCE OF DIFFERENCE OF TWO REGRESSION LINES

WRITE
C FOCAL-12

01.10 A !,"N1",N1,"N2",N2
01.20 A !,"DX1",D1,"DX2",D2
01.30 A !,"S1",S1,"S2",S2
01.40 A !,"M1",M1,"M2",M2
01.50 S DF=N1+N2-4 S S=(S1+S2)/DF
01.60 S DI=FSQ(T/(D1+D2))
01.70 S T=ABS((M1-M2)/DI)

02.10 S C=.63661977
02.20 S D=FSQ(T/DF)
02.30 S TH=FATN(T/DF); S X=DF/(DF+T+2)
02.40 I (DF-1)2.5,2.5,3.1
02.50 S A=C*TH; G 4.5

03.10 S TS=FITR((DF/2)3 S TS=2*TS
03.20 I (DF-TS)3.3,3.3,3.4
03.30 S IX=I*G 3.5
03.40 S IX=2
03.50 S A=I; S Y=I; S Z=IX; S W=Z+1
03.60 I ·(DF-W)4.1,4.1,3.7
03.70 S Y=X*Y*Z/W
03.80 S A=A+Y; S Z=Z+2; S W=W+2; G 3.6

04.10 I (2-IX)4.2,4.2,4.4
04.20 S N=FSQ(T-X+2)
04.30 S A=C*(TH+A*N); G 4.5
04.40 S N=FSQ(T-1-X); S A=A*N
04.50 S P=1-A

05.10 T !!,"DF=";DF,!,"T= ";T,!,"P= ";P,!!!
*
The dissertation submitted by Bernard J. Buchanan has been read and approved by the following Committee:

Dr. James P. Filkins, Chairman of Committee
Associate Professor, Physiology, Loyola

Dr. Nicholas R. DiLuzio
Professor and Chairman, Physiology, Tulane University, School of Medicine, Louisiana

Dr. Hugh J. McDonald
Professor and Chairman, Biochemistry and Biophysics, Loyola

Dr. Walter C. Randall
Professor and Chairman, Physiology, Loyola

Dr. James J. Smith
Professor and Chairman, Physiology, Medical College of Wisconsin, Milwaukee

Dr. Robert D. Wurster
Associate Professor, Physiology, Loyola

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.

January 8, 1975

Director's Signature