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GLUCOSE HYPERCATABOLISM IN ENDOTOXIC ADIPOSE TISSUE

by

Dianne Figlewicz

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

Master of Science

February

1977

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INTRODUCTION

Deranged carbohydrate homeostasis as manifest in progressive hypoglycemia is a significant feature in the pathogenesis of endotoxin shock. Insufficient glucose provision for energy metabolism contributes to organ dysfunction and circulatory collapse which are the more appreciated hallmarks of shock pathology. Three basic mechanisms have been proposed to account for the endotoxic defects in carbohydrate metabolism regulation: first, liver glycogen stores are rapidly released early in the onset of endotoxemia; second, hepatic gluconeogenesis is depressed; and lastly, glucose uptake and utilization by peripheral tissues are significantly enhanced in endotoxemia.

Whereas glycogen mobilization is attributed to enhanced sympathetic activity, the depression of gluconeogenesis and enhanced glucose utilization have been recently associated with endotoxic hyperinsulinemia. Since glucocorticoids antagonize the actions of insulin on carbohydrate metabolism, the present study evaluated glucocorticoid influences on various parameters of endotoxic glucose metabolism both *in vivo* and *in vitro*. In particular, glucose hypercatabolism in the endotoxic epididymal fat pad was characterized and the mechanism of dexamethasone's influence on the enhanced glucose utilization was evaluated.

REVIEW OF LITERATURE

Role of Altered Glucose Homeostasis in Endotoxin Shock

The depletion of carbohydrate stores and inability to maintain blood glucose levels are key metabolic lesions underlying endotoxin lethality. As outlined in a recent review by Hinshaw (29), the evidence for this relation of altered glucose homeostasis with the pathogenesis of endotoxin shock is of three types: (i) the loss of glycogen stores correlates with the progression of shock and agents which maintain glycogen stores prevent lethality; (ii) hypoglycemia develops in a progressive manner and correlates with the cardiovascular decompensation of shock; and (iii) prevention of hypoglycemia either by extrinsic glucose infusion therapy or by intrinsic manipulation of the mechanisms regulating glucose levels are generally beneficial to the organism and blunt the progression of shock. These three lines of evidence will now be considered.

A. Glycogen Depletion and Endotoxin Shock

Berry, Smythe, and Young (4) determined total body carbohydrate content in the carcasses of mice administered Salm. typhimurium ip and found that at the time of death these mice retained an average of only 0.9 mg./gm. body weight of carbohydrate as compared with control mice whose total body carbohydrate averaged 6.5 mg./gm. Evaluation of liver glycogen

levels in mice receiving identical treatment revealed a marked depletion of these glycogen stores. Administration of the dose of endotoxin which was responsible for these alterations in carbohydrate homeostasis resulted in almost 100% lethality. Comparable results were obtained by Menten and Manning (43) who reported hyperglycemia in rabbits administered lethal doses of killed S. enteriditis bacteria. Zeckwer and Goodell (70) correlated the initial hyperglycemic response with glycogenolysis in a later study. Hamosh and Shapiro (27) reported enhanced liver glycogenolysis by liver slices and homogenates prepared from rats treated with Chromobacterium prodigiosum endotoxin. Liver glycogen depletion in fed mice administered lethal doses of E. coli or Shigella flexneri endotoxin was documented by Sanford, Barnett, and Gott (54).

Carbohydrate store depletion is generally reflected in the blood glucose changes characteristic of endotoxemia. Within an hour following endotoxin administration, a hyperglycemia as described by Menten and Manning (43) results, followed by a hypoglycemia with blood glucose levels often essentially zero at the time of death.

B. Hypoglycemia and Endotoxin Shock

Endotoxin hypoglycemia has been documented by a number of investigators in several models. Berk (1) demonstrated two patterns of hypoglycemia in dogs receiving 0.25 to 2.0 mg./kg. E. coli endotoxin iv. While 58% of the dogs demonstrated an initial modest rise in blood glucose (although not hyperglyce-

mic) followed by a fall;in the remainder of the dogs, blood glucose levels fell steadily from the time of endotoxin administration. Berk (1) also reported hypoglycemia in the human patient with bacteremic shock and suggested that both glucose production and glucose utilization regulation may represent mechanisms whereby endotoxin affects blood glucose levels. Burrows (9) reported hyperglycemia followed by hypoglycemia in both the conscious and unconscious Shetland pony administered E. coli endotoxin iv. Griffiths, Groves, and Leung (25) demonstrated hypoglycemia in fasted dogs following the iv injection of live E. coli bacteria. Both McCallum and Berry (41) and Berry, Smythe and Young (4) reported the development of hypoglycemia in mice administered S. typhimurium endotoxin ip. Finkelstein (22) reported a marked hypoglycemia following administration of endotoxin to chick embryos. Filkins, Buchanan, and Cornell (21) demonstrated hypoglycemia in the rat treated with 5 mg. S. enteriditis endotoxin iv. Spitzer et al. (62) documented hypoglycemia in the baboon (Papio cynocephalus) injected with 4 to 5 mg./kg. E. coli endotoxin iv.

C. Maintainence of Blood Glucose and Lethality Protection

That hypoglycemia--and the depletion of carbohydrate stores which it represents--is an important metabolic lesion in the endotoxic animal was suggested in studies of McNamara, Mills, and Aaby (42). Endotoxic rabbits were maintained on one of three treatments: normal saline; 25% mannitol; or 50% glucose. Glucose solution administration resulted in significantly greater increases in mean blood pressure and pulse

pressure than administration of equivalent osmolar doses of saline or mannitol. Survival among the glucose-treated rabbits was also significantly greater. Berk (1) reported that maintenance of the blood glucose levels in his dogs receiving low doses of endotoxin significantly increased survival and commented that hypoglycemia is a factor which probably affects survival at all doses of endotoxin, but with larger doses, additional lethal factors play a key role as well. Hinshaw et al. (30) provide further evidence for the deleterious effect of hypoglycemia in the endotoxic animal. Eight of eleven dogs treated with 1 mg./kg. of E. coli endotoxin died with marked hypoglycemia, while the remaining three maintained their blood glucose levels and survived. Nine additional dogs were given iv 50% dextrose at rates sufficient to maintain blood glucose levels at control values, and all of these dogs survived. A positive correlation was shown to exist between endogenously maintained blood glucose levels and survival time. Exogenously administered glucose, in addition to maintaining blood glucose levels, was also found to improve additional metabolic, and certain cardiovascular parameters. Maintenance of adequate carbohydrate stores for energy metabolism thus can provide significant protection for the animal in shock..

Mechanisms Underlying Altered Glucose Homeostasis

Three contributory mechanisms have been delineated to account for inability of the endotoxic organism to maintain carbohydrate homeostasis. First, liver glycogen stores are

depleted relatively rapidly. Second, a defect in hepatic gluconeogenesis results in a decreased output of glucose for the organism. Third, glucose uptake and utilization by peripheral tissues are enhanced. Evidence exists to substantiate each of these alterations in carbohydrate metabolism, and each will be considered in turn.

A. Liver Glycogen Depletion

The first characteristic alteration in carbohydrate metabolism, which occurs within a few hours following endotoxin administration to the animal, is rapid liver glycogenolysis. Menten and Manning (43) reported a hyperglycemia in guinea pigs and rabbits with spontaneous Salmonella infections. Zeckwer and Goodell (70) injected suspensions of killed gram-negative bacteria of several species (B. proteus; B. proteus vulgaris; B. coli communis; B. paratyphosus B; and B. paratyphosus A) into male rabbits and followed the changes in blood sugar over a course of several hours. The possibility that sympathetic stimulation of the adrenals, resulting in glycogenolysis, served as the underlying mechanism for the hyperglycemia was tested by Evans and Zeckwer (17). E. coli suspensions were injected into adrenalectomized rabbits; this resulted in hypoglycemia accompanied by convulsions and ultimately lethality. McCallum and Berry (41) demonstrated a significant diminution of liver glycogen in fasted female mice administered a lethal dose of Salm. typhimurium endotoxin as early as one hour after treatment. In addition, liver glycogen synthase activity was significantly decreased and the

incorporation of the ^{14}C-label from glucose-U-^{14}C into liver glycogen was substantially impaired. McCallum and Berry (41) suggested that the reduction in carbohydrate synthetic ability served as a probable cause for rapid sugar loss during endotoxemia. Holper et al. (31) administered LD_{25}-LD_{50} doses of endotoxin to baboons and were able to demonstrate that the initial endotoxic hyperglycemia was dose-related, and was followed by a hypoglycemia correlated with the liver glycogen depletion. In a recent review by Stoner and Heath (65), however, it is noted that not all body glycogen stores become depleted in shock. Citing their own 1958 study, as well as the 1954 study of Cordier and Dessaux, they reported no change in brain levels of glycogen with the onset of shock, and a simultaneous increase in cardiac glycogen levels. Berry, Smythe, and Young (4) have reported a significant diminution of muscle (abdominal body wall) glycogen in Salm. typhimurium treated mice. The seeming inaccessibility of some glycogen stores, concomitant with the rapid depletion of liver and muscle glycogen thus represent a significant contribution to the deranged carbohydrate metabolism of shock.

B. Impaired Hepatic Gluconeogenesis

Defective liver gluconeogenesis has been demonstrated in the endotoxic animal. LaNoue, Mason, and Daniels (35) evaluated gluconeogenesis from sodium pyruvate by liver slices from rats injected with an LD_{50} dose of E. coli endotoxin. In addition, the activities of key gluconeogenic enzymes--glucose-6 phosphatase, fructose-1,6 diphosphatase, and phos-

phoenolpyruvate carboxykinase--were quantitated. Results indicated an impairment in the overall rate of glucogenesis and significantly lowered glucose-6 phosphatase activity, while other enzyme activities appeared unaffected. Shands, Miller, Martin and Senterfitt (58) measured gluconeogenesis in BCG-infected mice administered S. typhimurium endotoxin in vivo following an intraperitoneal injection of sodium pyruvate and sodium pyruvate-2-^{14}C, and concluded that the major defect leading to endotoxic hypoglycemia is in glucose synthesis from noncarbohydrate sources as reflected by the failure of these animals to raise their blood glucose levels following a pyruvate load. Filkins, Buchanan, and Cornell (21) demonstrated impaired gluconeogenesis in vivo in rats treated with 3 mg. S. enteriditis endotoxin by measuring both the hyperglycemic response of the rat to alanine loading and the conversion of ^{14}C-alanine to ^{14}C-glucose. An in vitro assessment of gluconeogenesis using isolated hepatocytes from rats treated with 1 mg. of endotoxin with alanine, lactate, and pyruvate as substrates also revealed that gluconeogenesis was defective. McCallum and Berry (41) measured the incorporation of the ^{14}C-label from alanine-U-^{14}C and pyruvate-2-^{14}C into blood glucose and reported a substantial impairment in mice administered S. typhimurium endotoxin. Evidence points to the inability of the endotoxic organism to maintain blood glucose levels by the synthesis of glucose from appropriate metabolic precursors. Thus, after utilization of the glucose supplied by initial glycogenolysis, hypoglycemia may result.

C. Peripheral Glucose Hypercatabolism

In addition to defective provision of glucose for peripheral tissue utilization, an enhanced glucose uptake and oxidation by peripheral tissues have been reported, and serve as a third contributory mechanism underlying endotoxic hypoglycemia and depletion of carbohydrate stores. Of all three mechanisms, glucose hypercatabolism is the least well-investigated and documented. In an attempt to clarify the mechanism of the hypoglycemic activity of endotoxin, Shands, Miller, Martin, and Senterfitt (58) ruled out the possibility of an endotoxin-induced hypermetabolic state. In mice administered endotoxin, they were unable to demonstrate glucose hypercatabolism as represented by ^{14}C-glucose clearance from the blood over a 20-minute time course, or by $^{14}CO_2$ recovery *in vivo* over a 60-minute time course. More recent investigations, however, have suggested that enhanced glucose uptake and utilization do occur in the endotoxic animal. Buchanan and Filkins (8) reported significantly enhanced $^{14}CO_2$ recovery *in vivo*, following intraperitoneal administration of U-^{14}C-glucose, in endotoxic rats over a four hour time course; significant enhancement was not observed until 150 minutes after rats received the ^{14}C-glucose. This latter finding may be helpful in interpreting the seemingly conflicting data of Shands *et al.*: evidently a lag period must occur before glucose hypercatabolism is demonstrable. Enhanced glucose utilization by the non-hepatic splanchnic area in baboons, as reflected by significant lowering of blood glucose levels in the mesenteric

vasculature, as reported by Spitzer et al. (62). A doubling of oxidative glucose metabolism was documented in a canine experimental peritonitis model by Bierenbaum, Bova, and Moss (5). Enhanced glucose utilization has also been demonstrated to occur in septicemia. Long, Spencer, Kinney, and Geiger (38) reported that glucose utilization more than doubled in septicemic men, while their total metabolic rates were only slightly increased; a dramatic increase in peripheral glucose utilization in septicemic men was also demonstrated by Gump, Long, Killian, and Kinney (26).

To date, few attempts have been made to localize specific tissue sites of the glucose hypercatabolism. Ryan, Blackburn, and Clowes (52) induced peritonitis in rats, then removed epididymal fat pads and diaphragms for an in vitro evaluation of ^{14}C-glucose oxidation. A threefold increase in glucose oxidation in fat pads from the rats with peritonitis, as compared to normal rats, with no concomitant change in diaphragm glucose oxidation was demonstrated. Drucker and Dekiewiet (15) reported significantly enhanced glucose uptake by hemidiaphragms of rats subjected to severe hemorrhagic shock. In contrast to the latter results, Chaudry, Sayeed, and Baue (11) were unable to demonstrate significant changes in glucose uptake by soleus muscle in rats after a hemorrhagic shock procedure. These studies do not serve to provide a complete or conclusive definition as to which peripheral tissues contribute to the glucose hypercatabolism which has been demonstrated in vivo.

Influence of Glucocorticoids and Insulin on Endotoxin Lethality and Hypoglycemia

The ability of endotoxin to enhance glucose uptake and utilization, and to inhibit gluconeogenesis has led investigators to describe its actions as "insulin-like." Insulin has indeed been shown to play critical roles in the caloric homeostasis alterations which occur in the endotoxic animal. Since the metabolic actions of insulin in the normal animal can be antagonized by the adrenal glucocorticoids, it may be anticipated that glucocorticoids protect the endotoxic animal not only against the derangements in carbohydrate homeostasis, but against ensuing lethality as well.

Glucocorticoid protection against lethality has been documented by several investigators. Jeffries and Wilkins (34) administered Amphenone B and metyrapone to suppress corticosteroid production in mice, and demonstrated a ten-fold increase in susceptibility to endotoxin lethality. Injection of corticosterone, cortisone, and hydrocortisone provided marked protection in both endotoxin- and endotoxin-steroid inhibitor-treated mice. Marecki (40) demonstrated significant protection against E. coli endotoxin in lead-sensitized BALB/c mice following administration of 6-alpha-methylprednisolone; such protection was found to be related to the time of administration of the methylprednisolone in relation to the endotoxin. Administration of hydrocortisone, prednisolone, or dexamethasone simultaneously with endotoxin was reported by Mills (44) to have a significant protective effect in mice, mortality

being reduced from 83% in control groups to 20-25% in treated groups at the optimum glucocorticoid dose level. Woodruff, Caridis, Cuevas, Koizumi, and Fine (69) demonstrated glucocorticoid protection in a trauma endotoxic model--30% immersion burn--in rabbits. Berry and Smythe (3) administered cortisone acetate to mice simultaneously with either an LD_{50} or twice the LD_{50} dose of endotoxin, producing a significant reduction in lethality. Delaying the injection of cortisone 1, 2, or 4 hours after that of endotoxin resulted in a loss of the lethality protection. The latter is in agreement with Marecki's data and is consistent with the hypothetical mechanism of glucocorticoid action which requires a definitive lag period of time, as a result of glucocorticoid-specific stimulation of protein synthesis. Berry and Smythe (3) further demonstrated that inhibitors of protein synthesis--such as actinomycin D and 2-thiouracil--potentiated the lethal action of endotoxin and prevented the protective effect of cortisone. That adrenal cortical hormones must play a role in the response of the organism to an endotoxic stress was also documented by Berry and Smythe (2). Cholesterol content of adrenal glands removed from endotoxic mice was nearly depleted, indicating a maximal response of the adrenals to endotoxin prior to their removal.

Several mechanisms have been postulated for the protective action of the glucocorticoids. These mechanisms appear to center around the known glucocorticoid abilities to suppress the immune system, stabilize membranes, and contribute to

caloric homeostasis through their influence on carbohydrate metabolism. Spath, Gorczynski and Lefer (59) were unable to demonstrate significant effects of dexamethasone on the characteristic splanchnic vasoconstriction of canine shock following hemorrhage, nor could they demonstrate a direct inotropic action of methylprednisolone or dexamethasone on papillary muscles removed from hemorrhaged cats. Latour and Renaud (36) were able to demonstrate inhibition of blood rheology changes following administration of triamcinolone prior to a non-lethal dose of endotoxin in rats. Characteristic fluctuations in thrombocytosis, platelet adhesiveness, and clotting time, as well as falling albumin levels were reduced by administration of high doses of triamcinolone two hours prior to endotoxin. Reilly and Schayer (49) reported that administration of cortisol in normal mice resulted in increased histamine formation in the gut, with lowered histamine formation in most other tissues and marked excretion of histamine. Schumer, Obernolte, and Erve (57) evaluated plasma and peritoneal histamine concentration in endotoxic and dexamethasone-treated endotoxic rats. Their results showed an increase in plasma and peritoneal exudate histamine in the endotoxic rat; simultaneous administration of dexamethasone decreased plasma histamine below control levels, and reduced peritoneal exudate histamine 40%. Such changes may well be accounted for by either decreased formation or enhanced excretion of histamine as noted by Reilly and Schayer.

Stabilization of lysosomal membranes by glucocorticoids

has been documented in shock models. Spath et al. (59) reported significantly higher levels of the lysosomal enzyme beta glucuronidase in dogs subjected to hemorrhagic shock but not treated with methylprednisolone or dexamethasone. Lefer and Martin (37) noted a low level of myocardial depressant factor in hemorrhaged cats treated with cortisol or dexamethasone and were able to demonstrate an inverse correlation between MDF activity and survival time. The lowered MDF was associated with the stabilization of lysosomal membranes: release of lysosomal proteases is postulated to result in the breakdown of plasma proteins, one or several of which may be MDF. Warren, Ferguson, and Wangensteen (68) were unable to demonstrate lysosomal stabilization in endotoxic dogs treated with massive doses of methylprednisolone (30 mg./kg.). However, they were also unable to significantly protect against lethality with that dose; both the endotoxic and the endotoxic-methylprednisolone treated groups (N=10) had only one long-range survivor. In an in vitro study by Ferguson, Warren, and Wangensteen, (20), lysosome suspensions from canine livers released significantly less cathepsin D following the addition of several glucocorticoids, providing further evidence for stabilization of lysosomal membranes as a protective mechanism. Glucocorticoid influence on immune and inflammatory reactions has also been cited as a mechanism underlying their lethality protection. In a study by Fukuda and Hata (23), antiendotoxic potencies of different glucocorticoids administered to endotoxic rats almost paralleled their respective anti-inflamma-

tory potencies. As a more direct approach to this issue, Schumer, Erve, and Obernolte (56) evaluated complement fixation in an *in vitro* system to which *S. typhimurium* endotoxin alone, or with dexamethasone, was added. They reported a dexamethasone-induced decrease in either complement levels or complement-fixation by endotoxin, and proposed that this would ultimately interfere with the production of anaphylatoxin or other shock toxins.

Carbohydrate and liver metabolism changes induced by the glucocorticoids have been investigated; it may be anticipated that glucocorticoid mobilization of precursors to the liver for glucose synthesis as well as inhibition of glucose uptake and utilization would counteract the endotoxic depletion of carbohydrate stores described above. The specific influence of adrenal corticosteroids on hepatic detoxification of endotoxin has not been extensively investigated. Filkins (as cited by Buchanan (6)) demonstrated sensitization to endotoxin-shock in adrenalectomized rats; however, liver homogenates from either acute or chronic adrenalectomized rats did not differ significantly from sham-operated controls in their ability to detoxify endotoxin as evaluated in the lead-sensitized rat bioassay. In contrast, Woodruff *et al.* (69) reported that intravenous injections of corticosteroids in rabbits accelerated the clearance rate of endotoxins which were injected as well as those which entered from the intestine following an ischemic injury. More conclusive results have been

obtained in regard to glucocorticoid influence on hepatic carbohydrate metabolism. That glucocorticoids can significantly protect against depletion of body carbohydrate stores was demonstrated by Berry, Smythe, and Young (4). Administration of 5 mg. cortisone to mice simultaneously with S. typhimurium endotoxin not only provided significant lethality protection but also markedly inhibited depletion of carbohydrate stores as represented by blood sugar, liver and muscle glycogen, and total body carbohydrates. Berry and Smythe further demonstrated the specific ability of cortisone to influence hepatic enzyme levels in endotoxic mice (3). Endotoxin-poisoned mice showed normal levels of liver tryptophan pyrrolase activity if administered cortisone along with the endotoxin. Holtzman, et al. (32) assessed changes in key glycolytic intermediates in the liver from rats receiving endotoxin, endotoxin plus dexamethasone, or saline. Administration of dexamethasone increased glycogen and blood glucose concentrations, decreased blood lactate levels, and returned liver fructose diphosphate and phosphoenolpyruvate levels to essentially control values, thus implicating a hepatic involvement of the glucocorticoids in maintainence of body carbohydrate stores. A similar study by Schuler, Erve, and Schumer (55) in endotoxin-shocked monkeys demonstrated, first, significant lethality protection by dexamethasone, and, second, the following changes in hepatic metabolite levels: increased glucose-6-phosphate, fructose-6-phosphate, phosphoenolpyruvate, ATP, ADP and glycogen; and decreased fructose-1,6-diphosphate, lactate and AMP. All

metabolite levels were returned to essentially control levels. The possible influence of the glucocorticoids in inhibiting endotoxic glucose hypercatabolism has been evaluated by Buchanan (6) in vivo in endotoxic dexamethasone-treated rats administered ^{14}C-glucose. $^{14}CO_2$ collection for four hours was significantly depressed, thus reflecting a depression of endotoxin-induced glucose hypercatabolism; dexamethasone concomitantly inhibited development of hypoglycemia in endotoxic rats (6). Thus, glucocorticoid administration can contribute to the maintenance of carbohydrate metabolism by influencing all three of the mechanisms which contribute to carbohydrate depletion as discussed previously.

The actions of endotoxin on hepatic gluconeogenesis and peripheral glucose utilization can be said to be "insulin-like." The possibility that insulin levels may be responsible for the alterations in carbohydrate metabolism and its regulation which occur in endotoxic shock has been investigated. Actual elevations in endogenous insulin levels within the endotoxic animal have been documented. Ryan, Blackburn, and Clowes (52) reported threefold higher insulin concentrations in their peritonitis rat model, which was associated with a threefold elevation of the insulin-sensitive adipose pyruvate dehydrogenase complex as well as the threefold increase in glucose oxidation to CO_2 by epididymal fat pad fragments. Buchanan (6) demonstrated a long duration hyperinsulinemia (IRI) in response to endotoxin administration, and an exaggerated insulin response to a glucose tolerance test (400 mg.

glucose ip) in the endotoxic rat. In addition, Buchanan and Filkins (7) demonstrated enhanced sensitization to endotoxin lethality in rats with administration of insulin, or with stimulation of insulin secretion, such as administration of beta cell stimulants or glucose; fasting rats or administration of mannoheptulose--either of which treatment would lower endogenous insulin levels--provided significant protection against endotoxin lethality. Spitzer et al. (63) reported enormous increases in plasma IRI levels in dogs administered an LD_{70} dose of E. coli endotoxin. The above data suggest that insulin is involved in the development of deranged carbohydrate homeostasis in endotoxicosis. Since adrenal glucocorticoids can be considered "anti-insulin" in their metabolic actions, it might be anticipated from the results of previous investigations discussed above that they would have significant protective effects in the endotoxic animal.

Endotoxic Adipose Tissue Metabolism

Adipose tissue lipolysis in vitro and plasma lipid changes in vivo have been studied in order to delineate the role of adipose in the metabolism of the endotoxic animal. Printen, Keefe, Foster, and Brown (48) reported a significant rise in free fatty acid levels in dogs with endotoxemia, but not with hemorrhage. Since blood pressure changes in the hemorrhage model were regulated by the investigators so that pressure drops were equal in severity and rapidity with endotoxic dogs, they suggest that endotoxic alterations in adipose metabolism must be mediated through a more complex pathway than a simple

response to norepinephrine or epinephrine, released as a result of hypotension. Griffiths, Groves and Leung (25) reported a rise in plasma triglycerides associated with a rise in norepinephrine in dogs infused with live E. coli bacteria. Gallin, Kaye, and O'Leary (24) noted significant elevations in total serum lipids, free fatty acids, and triglycerides in patients with gram-negative bacteremia. The enhanced lipolysis which is found in endotoxic but not hemorrhagic shock, in spite of catecholamine release in both conditions may perhaps be explained by the results of Spitzer, Kovach, Rosell, Sandor, Spitzer and Storck (60). The norepinephrine-stimulated lipolysis of isolated fat cells removed from several different depots in the endotoxic dog was evaluated; endotoxic shock in vivo increased the norepinephrine-induced lipolytic response in subcutaneous, omental, and mesenteric fat cells as reflected by in vitro increased free fatty acid and glycerol release. Spitzer et al. (60) were also able to demonstrate a higher norepinephrine-stimulated lipolytic response from isolated canine fat cells incubated with endotoxin in vitro. Assessment of cAMP content in endotoxin-treated fat cells in the same system revealed that they had significantly higher cAMP levels, which were then further elevated by subsequent norepinephrine stimulation. Confirmation of the in vitro enhancement of adipose cAMP levels by endotoxin comes from a study by Hewlett, Guerrant, Evans, and Greenough (28) which demonstrated enhanced responsiveness of epididymal fat cell membrane adenyl cyclase to epinephrine following in vitro

addition of E. coli endotoxin. Results from the studies summarized above do not seem to fit in with the concept of an insulin-like action on peripheral tissue metabolism; insulin inhibits lipolysis as well as lowering cAMP levels in the normal adipocyte. To date, the only study assessing endotoxic glucose utilization by adipose is that of Ryan, Blackburn, and Clowes (52) discussed above. How their results--significant glucose hypercatabolism by epididymal fat pad fragments--fit in with the well-documented endotoxic lipolysis by adipose has yet to be elucidated.

Influence of Glucocorticoids and Insulin on Adipose Metabolism

Although no specific investigations of the influence of the adrenal glucocorticoids or insulin on the metabolism of endotoxic adipose have been carried out, the influence of these two hormones on normal adipose tissue deserve some discussion: presumably they will exert the same qualitative influences over endotoxic adipose metabolism although quantitative differences may exist, as was documented by Spitzer in regard to norepinephrine sensitivity. Munck (46) reported a reduction in net glucose uptake by epididymal adipose isolated from adrenalectomized rats injected with cortisol, corticosterone, and deoxycorticosterone. The same steroids in vitro displayed similar actions, the effects occurring mainly after the adipose incubation had proceeded for over two hours. These results thus demonstrated that adipose can serve as a direct target for glucocorticoids. Fain, Scow, and Chernick (19) demonstrated enhanced fatty acid release from rat adipose in-

cubated in vitro with dexamethasone, corticosterone, or 2-alpha-methylcortisol; again a 2-hour lag period was required before the glucocorticoids had any significant effects on lipolysis. Addition of insulin completely antagonized lipolysis. Further evidence for the apparent antagonism between the glucocorticoids and insulin on glucose utilization is provided by Plager and Matsui (47). Cortisol addition to an in vitro system of mouse ear epidermis significantly reduced metabolism of ^{14}C-glucose to $^{14}CO_2$; the cortisol effect could be completely antagonized by insulin at several different concentrations. Treatment of isolated rat white fat cells with a maximally stimulating dose of insulin, in an incubation medium with a high concentration of glucose was reported by Czech and Fain (13) to completely antagonize the inhibitory action of dexamethasone on 1-^{14}C-D-glucose conversion to $^{14}CO_2$; it was suggested that dexamethasone--or glucocorticoids in general--inhibits glucose oxidation in the presence or absence of insulin when transport of glucose into fat cells is rate-limiting. In the latter situation, abundance of glucose and high insulin concentrations would assure the absence of any limitations on the glucose transport process. Data supporting the direct interaction of insulin with superficial membrane structures of isolated fat cells was provided by Cuatrecasas (12); direct membrane interactions resulted in enhanced glucose utilization and suppressed lipolysis.

The above discussion of the actions of insulin and the glucocorticoids on adipose metabolism is in no way a complete

literature review of the extensive research in these areas. However, these results do provide sufficient insight into the effects of these hormones on normal metabolism in adipose tissue; perhaps one may extrapolate from these, and predict what influence the adrenal glucocorticoids and insulin may have on endotoxic adipose metabolism.

Statement of Purpose

The purposes of the following study were fourfold. First a systematic assessment of glucose oxidation by a number of endotoxic tissues was carried out in an attempt to define the specific tissue sites of glucose hypercatabolism which was demonstrated *in vivo*. Second, the possibility that dexamethasone both *in vivo* and *in vitro* may have a direct influence on endotoxic epididymal adipose glucose utilization, in light of its ability to protect against lethality and hypoglycemia in endotoxicosis, was evaluated. Third, the ability of endotoxin *in vitro* to induce glucose hypercatabolism by epididymal adipose was assessed. Finally, the influence of insulin on endotoxic adipose metabolism, and the possibility that it may mediate *in vivo* endotoxic glucose hypercatabolism was investigated.

METHODS

A. Animals

Animals used in lethality and all subsequent experiments were male albino Holtzman rats (Holtzman Company, Madison, Wisconsin). Rats remained in the animal quarters for approximately seven days post-shipment, and prior to experimental use in order to assure adjustment to animal quarter conditions of a 12-hour, light-dark cycle (light from 7 a.m. to 7 p.m.) and unlimited access to Purina rat chow (Ralston Purina, St. Louis, Missouri) and tap water. All rats were fasted overnight prior to experimentation; food was removed at 4 p.m. but access to water was permitted.

B. Endotoxin

Endotoxin used in all experiments was the Boivin preparation of Salmonella enteriditis lipopolysaccharide (LPS), batch #505153 from Difco Laboratories, Detroit, Michigan. The lyophilized endotoxin was prepared in the appropriate concentration in pyrogen-free 0.9% saline (Baxter, Travenol Laboratories Inc., Deerfield, Illinois). It was administered intravenously under light ether anesthesia by two routes. Large rats (approximately 280-320 grams and 70-80 days of age) as used in the lethality and hypoglycemia studies received the endotoxin via the dorsal vein of the penis. Small rats (ap-

proximately 90-110 grams and 26-30 days of age) as used in the *in vivo* and *in vitro* $^{14}CO_2$ collection studies received the endotoxin via a tail vein. Endotoxin concentrations were adjusted so that the larger rats received the appropriate dose in a total volume of 1 ml. of saline, whereas the smaller rats received the appropriate dose in a volume of 0.5 ml. of saline.

C. Dexamethasone

Dexamethasone acetate (Sigma Laboratories, St. Louis, Missouri) was prepared in pyrogen-free saline in a concentration of 100 µg./ml. and was dissolved by an electric magnetic stirring apparatus (Thermolyne, Sybron Corp., Dubuque, Iowa). The resulting suspension was then sonicated for 60 seconds with a Biosonik IV sonicator (Bronwill, Rochester, New York). The suspension was mixed by hand prior to each injection since some settling of dexamethasone would occur. 1 ml. was injected intraperitoneally in the abdominal region of the rat.

D. Lethality Protocols

Fasted rats weighing 320±3 grams received 1, 2, 4, or 8 mg. of endotoxin iv and either 1 ml. saline or 1 ml. (100 µg.) dexamethasone acetate ip. Both the iv endotoxin and the appropriate ip injectate were administered simultaneously under light ether (Anaesthesia Grade, Mallinckrodt, St. Louis, Missouri). Ensuing lethality was followed for 48 hours, with rats living beyond 48 hours considered survivors. Most deaths occurred between 12 and 24 hours. All endotoxic-saline treated and endotoxic-dexamethasone treated groups were run simul-

taneously on two separate occasions.

E. Data Analysis

Statistical analysis for significant differences was evaluated by the chi-square test with the correction factor of Yates for small samples. P-values for the comparison of appropriate chi-square values were obtained from a statistical table (50). One degree of freedom was used in the analysis of this data. A p-value of less than 0.050 was considered statistically significant.

Measurements of Plasma Glucose

A. Glucose Analyzer

Plasma glucose levels were determined by a glucose analyzer (Yellow Springs Instrument Model 23A). The analyzer permits reaction of glucose--as contained in 25 microliter plasma samples injected into the analyzer--with glucose oxidase. Sample determinations were carried out in duplicate. Blood samples were collected in 250 µl. heparinized microcentrifuge tubes (Beckman Co., U.S.A.). They were refrigerated approximately 24 hours before analysis. Immediately prior to analysis, samples were centrifuged in a Beckman microcentrifuge for five minutes, then placed on ice. Plasma glucose values, expressed in mg./dl., were obtained directly from the glucose analyzer.

B. Experimental Protocols

Fasted rats were subjected to one of the following treatments:

1. 1 ml. dexamethasone acetate ip (100 µg./ml. in saline)

1 ml. saline iv;

2. 1 ml. dexamethasone acetate ip,
   1 ml. endotoxin (5 mg./ml. in saline) iv;
3. 1 ml. saline ip,
   1 ml. endotoxin iv; or
4. 1 ml. saline ip,
   1 ml. saline iv.

Injections were given under ether anesthesia. The intravenous route was via the dorsal vein of the penis. Initial blood samples were obtained, simultaneous with the injections, by tail snip. A second blood sample was obtained from ether anesthetized rats by cardiac puncture 180 minutes after treatments. In the case of those rats which died prior to 180 minutes, blood samples were obtained by cardiac puncture at the time of death.

C. Data Analysis

Differences between the initial and 180 minute glucose levels in the individual rats as well as differences between 180 minute glucose levels of rats receiving different treatments were evaluated by the Student's unpaired "t" test with p-values less than 0.050 considered significant. P and t values were calculated by a PDP-12 computer programmed for statistical analysis.

In vivo Evaluation of Glucose Oxidation to CO_2

In order to evaluate glucose oxidation in vivo, fasted rats weighing 100±10 grams were administered a dose of ^{14}C-U-D-glucose (Amersham/Searle, Arlington Heights, Illinois) then

placed in individual containers. Their total expired CO_2 and $^{14}CO_2$ was trapped in a column containing CO_2 trapping fluid. The percent of the administered label which was recovered served as an index of glucose oxidation by the whole rat.

A. Treatment of Rats

In order to evaluate glucose oxidation in vivo, rats received a dose of 7 μCi of uniformly labeled ^{14}C-D-glucose at time 0 minutes. The labeled glucose was prepared in pyrogen-free saline at a concentration of 10 μCi/ml.; each rat was injected with 0.70 ml. intraperitoneally.

The dose of C-14-glucose administered to each rat was determined by counting 10 μl. aliquots of the ^{14}C-glucose solution to be injected in ten ml. of PCS (Amersham/Searle) in an Isocap 300 Liquid Scintillation System (Amersham/Searle). This count was corrected for counting efficiency by the Sample Channels Ratio method. The calculated disintegration rate was multiplied by 70 to give the corrected ^{14}C dose in a 0.70 ml. injection.

In addition to the administration of labeled glucose, rats received one of the following sets of injections:

1. 0.5 ml. of endotoxin (1.5 mg./ml. in pyrogen-free saline) iv;
2. 0.5 ml. of saline iv;
3. 0.5 ml. saline iv and 0.75 ml. saline ip three hours prior to glucose ip;
4. 0.5 ml. endotoxin iv and 0.75 ml. saline ip at three hours prior to glucose ip; or

5. 0.5 ml. endotoxin iv and o.75 ml. dexamethasone acetate (100 µg./ml. in pyrogen-free saline) three hours prior to glucose ip.

B. CO_2 Trapping Apparatus

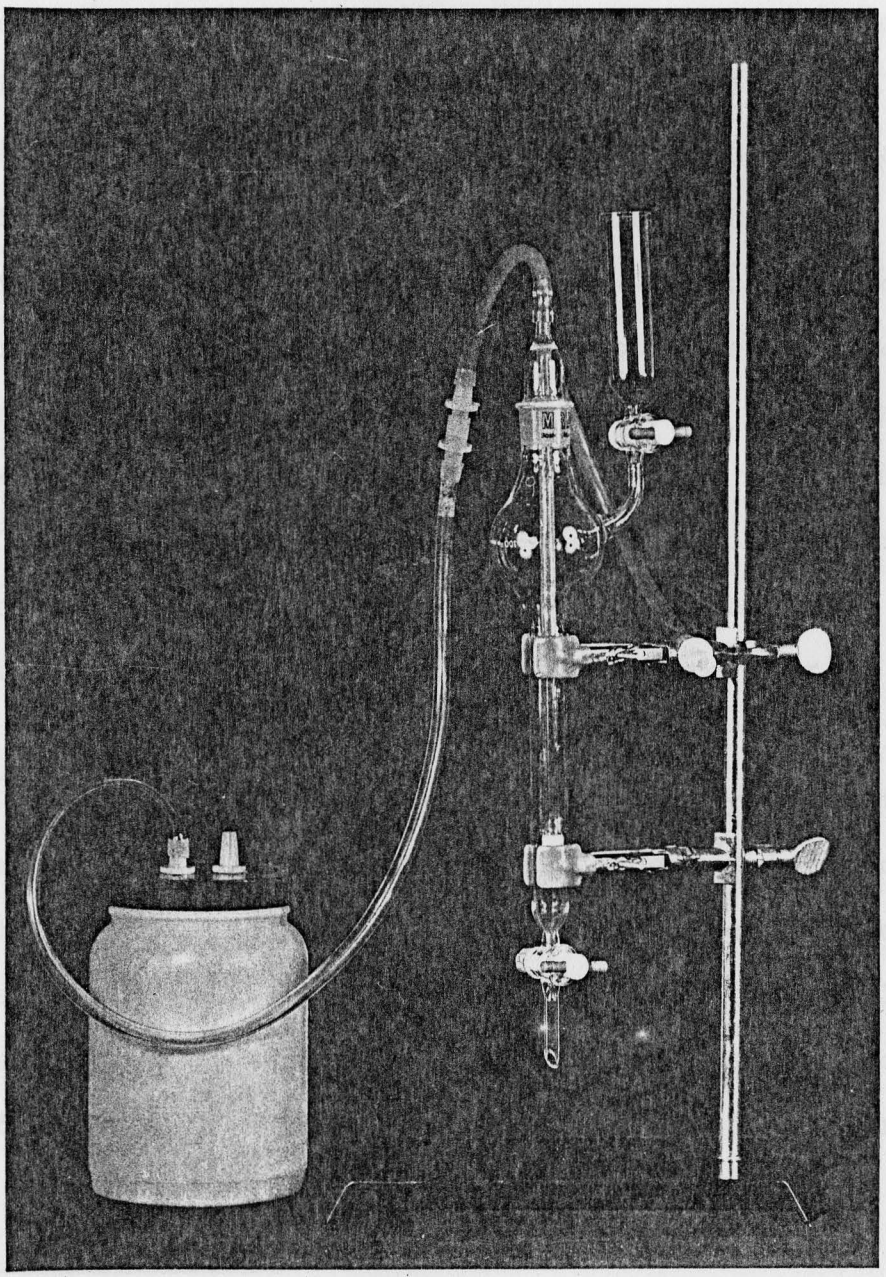
Immediately after receiving the labeled glucose injection and other treatments, rats were placed in individual bottles, each containing about 750 ml. of wood shavings to absorb moisture. The bottles had two outlets: one for drawing and mixing room air within the bottle for the rat to breathe, and one connected by tubing to a gas inflow attachment of a CO_2 bubble trap (Figure 1) from MRA Corp., Boston, Massachusetts. The gas inflow tube was submersed in 52 ml. of a CO_2 trapping fluid (33)(2 volumes 2-methoxyethanol: 1 volume ethanolamine). A gas outflow side arm of the CO_2 trap was connected to a vacuum source. Flow through each CO_2 trap was regulated by a needle valve connected in series with the trap and the vacuum source. Also connected in series between the trap and the valve was a flowmeter (Fisher Scientific Co., Chicago, Illinois); flow was adjusted to 500 ml. per minute as determined by the flow meters. Two stopcocks set in the main body of the CO_2 trap could be adjusted to permit vacuum-induced mixing of CO_2 with the trapping fluid, or sampling of the trapping fluid.

C. Sample Processing

Duplicate 1 ml. samples of the trapping fluid were taken immediately before and at specified times after placing the rats in their bottles. A 1 ml. Oxford Sampler (Scientific

FIGURE 1

IN VIVO $^{14}CO_2$ TRAPPING APPARATUS



Products, Oxford, California) with disposable pipette tips was used. Samples were taken at 15 minute intervals for the first hour of CO_2 collection, and subsequently at 30 minute intervals for the remaining three hours of the experiment. Ten ml. of liquid scintillation counting fluor was mixed in a scintillation vial with each 1 ml. aliquot of trapping fluid. The fluor composition was 70% (by volume) toluene (AR grade, Mallinckrodt); 20% 2-methoxyethanol (Aldrich Chemical Company, Milwaukee, Wisconsin); and 10% ethanolamine (Aldrich Chemical Company). To each liter of this solution, 7.0 grams of PPO (2,5-diphenyloxazole, Scintillation Grade, Amersham/Searle) and 0.15 gram POPOP (p-bis-2-(5-phenyloxazolyl)benzene, Scintillation Grade, Amersham/Searle) were added (6). Samples were counted in the liquid scintillation counter for 20 minutes or to a maximal count of 800,000.

Counting efficiency was determined by the method of internal standards using calibrated toluene-C-14 (Amersham/Searle). 50 µl. aliquots of the labeled toluene were added to samples which had been counted previously as well as into vials containing only fluor. These samples were counted and an efficiency of 76.47% was calculated and used for data analysis.

D. Data Analysis

Data are expressed as the percent of the injected 14-C dose recovered cumulatively at each time point. Count rates obtained from samples taken prior to placing the rats in their bottles served as background count rates. The background count

rate was then subtracted from the cumulative count rate at each time point. Data calculations were performed by means of a computer program (6) which could accumulate the mean count rate of the duplicate 1-ml. samples for each time point, simultaneously correcting for background, counting efficiency, removal of the ^{14}C-label in previous samples, and the volume of trapping fluid remaining at the time a particular set of samples was taken. The cumulative disintegrations per minute (DPM) data was then converted to percent recovery of the ^{14}C-label by dividing the DPM recovered at each time point by the initial DPM in the administered dose of ^{14}C-glucose.

Means and standard errors of the ^{14}C-recovery data for each experimental group were obtained with the use of a computer program designed to calculate these statistics. A computer program also evaluated statistical differences by the unpaired Student's "t" test.

In vitro Evaluation of Glucose Oxidation to CO_2

A. Tissue Preparations

Fasted rats (100±10 grams) were decapitated and a variety of tissues removed for an *in vitro* evaluation of glucose oxidation. Blood from the carcass was collected in a 20-ml. beaker containing 5000 units of heparin (Sigma). A 1 ml. aliquot of the blood was pipetted into a metabolic flask (see below). The right hemidiaphragm and left epididymal fat pad were removed in their entirety and placed in individual metabolic flasks. A portion of the stomach was re-

moved and everted, serving as a sample of gastric mucosa. Portions of the liver, spleen, ventricle, kidney, lungs, and brain were removed and slices approximately 500 micrometers thick were prepared using the Stadie-Riggs method (64). All tissue specimens were rinsed in iced physiological saline, blotted, and weighed before being placed in metabolic flasks. The number of slices of each tissue was varied such that a total sample of 200-500 mg. was generally maintained. Metabolic flasks containing incubation medium (see below) were kept on ice throughout the period of tissue sample preparation. Blood and samples to be sliced were also kept on ice.

B. Rat Treatments

For the series of experiments in which glucose oxidation by ten different tissues was evaluated, two groups of rats were used. One group was untreated, serving as controls. The second group received 0.5 ml. endotoxin (1.5 mg./ml. in saline) iv three hours prior to sacrifice.

A series of $^{14}CO_2$ recovery experiments were also carried out using only epididymal fat pads. The specific treatment of each group of rats or fat pads is detailed in the Results section. Bovine crystalline insulin (Sigma) and $ATP \cdot Mg^{+2}$ (Sigma) were used for a series of experiments at the concentrations specified. Each experimental group had its own corresponding control group. When fat pads were removed from treated rats, control fat pads were obtained from rats from the identical shipment as the experimental animals. In fat pad experiments in which a constituent of the incubation

medium was being manipulated, one epididymal fat pad served as an experimental fat pad and the second epididymal fat pad served as a control. In the latter instance, use of the right and left fat pad as experimental or control was varied in order to minimize possible intrinsic differences in right versus left fat pad weight or metabolism.

C. $^{14}CO_2$ in vitro Trapping Apparatus

The in vitro method of Saba and DiLuzio (53) was used for the collection of expired tissue CO_2 and $^{14}CO_2$. Tissues were placed in 50 ml. metabolic flasks (Kontes Glass Co., Vineland, New Jersey) with Teflon adapters screwed onto the side arms. Scintillation vials (Wheaton Scientific, Millville, New Jersey) with a cap diameter of 24 mm. were fitted onto the side arm. A 4.0 by 0.75 inch strip of Whatman No. 52 filter paper soaked in 0.3 ml. of hyamine hydroxide (1.0 molar in methanol, Amersham/Searle) was placed in each scintillation vial. Vials were attached to the metabolic flasks to trap the expired CO_2.

Five ml. of incubation medium was added to each flask shortly before the tissue specimen was added. The incubation medium was a modified Krebs-Ringer-Phosphate solution (67) (101.5 ml. 0.9% NaCl: 4 ml. 1.15%KCl: 1.5 ml. 1.22%$CaCl_2$: 1 ml. 3.82% $MgSO_4.7\ H_2O$: 20 ml. 0.1 M phosphate buffer, pH 7.4). It was maintained at a pJ of 7.38-7.39. One hundred mg. of D-glucose and 50 µl. of U-^{14}C-glucose (1 µCi/µl., Amersham/Searle) were added to the modified Krebs-Ringer-Phosphate to a final volume of 100 ml. The resultant medium had a

glucose concentration of 1 mg./ml. and an activity of 0.5 μci/ml. After tissues were placed in the flask, and while still on ice, each flask was gassed for 60 seconds with 100% O_2. The flasks were placed in a Dubnoff metabolic shaker bath maintained at 37° C and shaken at a rate of 60 cycles per minute.

The efficiency of the $^{14}CO_2$ trapping process was tested by adding a 17 μl. aliquot of a calibrated ^{14}C-$NaHCO_3$ solution (Amersham/Searle) to 100 ml. of saline. 1 ml. of this solution was combined with 4 ml. of saline in a metabolic flask. One ml. of 62.5% citric acid was injected into the flask. $^{14}CO_2$ recovery was evaluated at 30, 60, 90, and 120 minutes in separate flasks. About 90% of the ^{14}C-label was recovered after 30 minutes; percent recovery was fairly constant for 60, 90, and 120 minutes. It was concluded that essentially all $^{14}CO_2$ being evolved would be recovered.

D. $^{14}CO_2$ Sample Processing

In experiments designed to evaluate $^{14}CO_2$ recovery in ten different tissues, incubation was carried out for 120 minutes. At this time the scintillation vials on the flasks were replaced and 1 ml. of 62.5% citric acid was injected into each. Incubation was continued for 15 minutes, in order to recover $^{14}CO_2$ trapped within the medium. In those experiments in which only epididymal fat pad oxidation was evaluated, scintillation vials were replaced at 30, 60, 90, and 120 minutes after initiation of the incubation period. Again at 120 minutes, 1 ml. of citric acid was injected and incubation was continued for 15 minutes more. 15 ml. of liquid scintillation fluor

(1 liter toluene: 4.0 gm. PPO: 0.1 gm. POPOP) was added to each vial. The vials were counted in a liquid scintillation counter for 10 minutes, after a dark-adaptation period of about 30 minutes.

E. Data Calculation and Analysis

Data was expressed as total DPM of $^{14}CO_2$ recovered per gram wet weight of the appropriate tissue, or per one ml. of blood. Due to the occasional vaporization of water in the scintillation vial during incubation, which caused a decrease in the counting efficiency, conversion of CPM to DPM was calculated individually for each sample. A computer program was used which constructed a sample channels ratio vs. efficiency curve based upon a C-14 standard quench set (Amersham/Searle) count rate. The problem of water vaporization in the scintillation vials was very minor. Most samples counted with an efficiency of approximately 85%.

The DPM from the 120 minute incubation and from the $^{14}CO_2$ collection period were added and this total divided by the tissue weight. Thus data for the "tissue survey" experiments is represented as total DPM per 120 minutes of incubation per gram wet weight of the tissue (or per 1 ml. blood). For experiments in which only epididymal fat pads were used, recovered DPM were totalled at each time point (i.e., 30, 60, 90, and 120 minutes). Data is expressed as cumulative DPM per gram wet weight, at each time point. Means, standard errors, and statistical differences between groups were evaluated with the use of computer programs described above.

RESULTS

Protection by Dexamethasone against Endotoxin Shock Lethality

Experiments were carried out in order to evaluate the ability of dexamethasone to significantly blunt endotoxic shock lethality. Experimental results and a statistical analysis are presented in Table 1, and are depicted in Figure 2.As depicted, administration of 100 μgm. of dexamethasone acetate simultaneously with the administration of endotoxin resulted in a significant depression of the percent lethality. In groups administered 4 (groups 5 vs. 6) or 8 (groups 7 vs. 8) mg. of endotoxin, lethality was significantly reduced from 70% to 5%, and from 80% to 10%, respectively. Groups administered 1 (groups 1 vs. 2) or 2 (groups 3 vs. 4) mg. of endotoxin demonstrated a reduction in lethality from 15% to 0%, and from 30% to 15%, respectively; reduction of lethality in these groups was not significant as reflected in p-values in the range of 5-50%.

Dexamethasone Protection Against Endotoxic Hypoglycemia

Administration of 5 mg. of endotoxin to fasted rats resulted in the development of a severe hypoglycemia (Table 2). By 180 minutes post-endotoxin, plasma glucose levels dropped from 86.8 to 24.0 mg./dl. Concomitant administration of 100 μg. of dexamethasone acetate not only served to prevent the

TABLE 1
PROTECTION BY DEXAMETHASONE AGAINST ENDOTOXIN SHOCK LETHALITY[a]

Group Number	1	2	3	4	5	6	7	8
Endotoxin dose[b] (mg./rat)	1	1	2	2	4	4	8	8
Treatment	Saline[c]	DXM[d]	Saline	DXM	Saline	DXM	Saline	DXM
Number tested	20	20	20	20	20	20	20	20
Rat weight (grams)	317.9[e]	318.6	320.3	321.2	320.3	323.3	320.4	323.7
	5.9[f]	3.8	3.5	3.1	2.4	3.5	2.3	3.4
% Lethality	15%	0%	30%	15%	70%	5%	80%	10%

Statistical Analysis of Lethality Data

Control Group	Experimental Group	Chi-square Value	P-value
1	2	1.4414	5-50%
3	4	0.5735	5-50%
5	6	15.3600	0.1%
7	8	17.0707	0.1%

Footnotes to Table 1

a. Lethality was defined as death occurring within 48 hours after endotoxin administration.
b. Endotoxin was administered intravenously and was prepared in concentrations of 1, 2, 4, or 8 mg./ml. in pyrogen-free saline, each rat receiving 1 ml.
c. Saline treatment consisted of intraperitoneal injection of 1 ml. pyrogen-free saline.
d. Dexamethasone treatment consisted of intraperitoneal injection of 1 ml. dexamethasone (100 μg./ml. prepared in pyrogen-free saline).
e. Value represents mean.
f. Value represents standard error.

FIGURE 2

DEXAMETHASONE PROTECTION

AGAINST ENDOTOXIN SHOCK LETHALITY

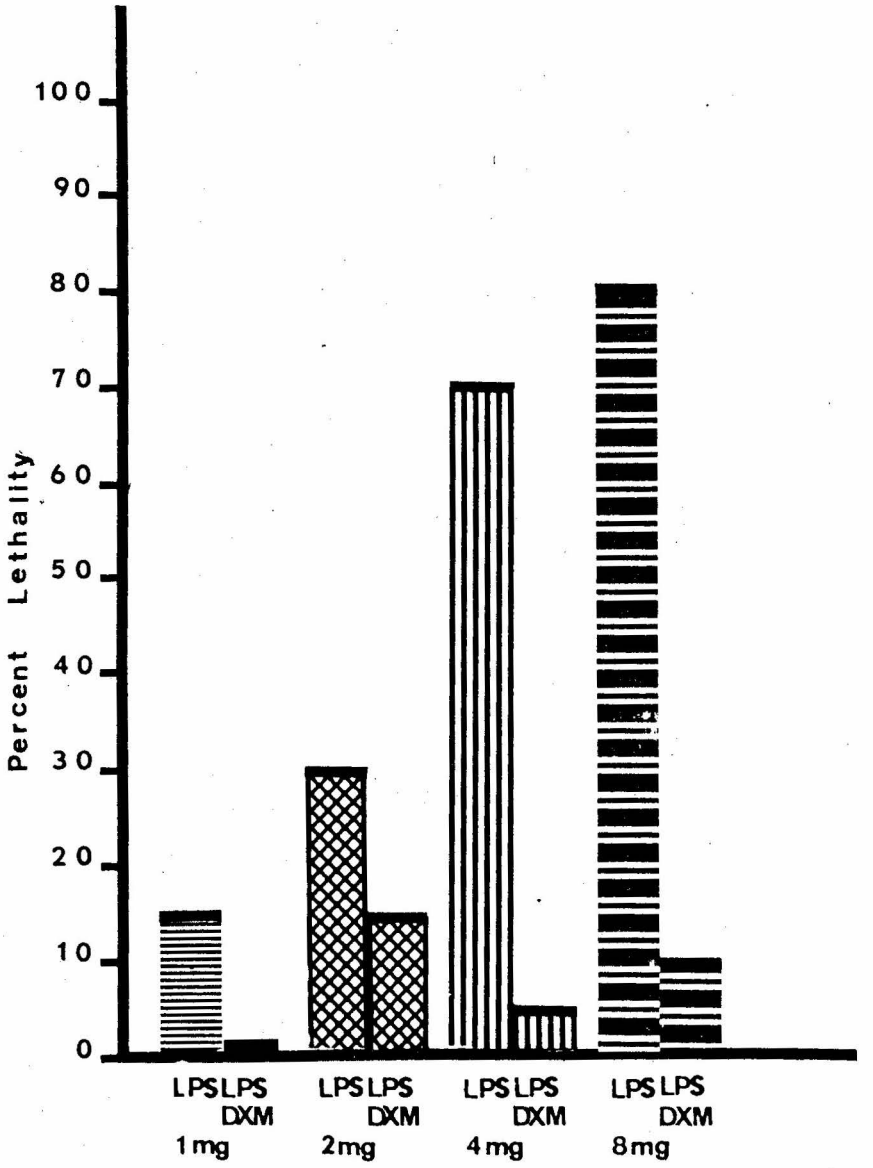


TABLE 2
PROTECTION BY DEXAMETHASONE AGAINST ENDOTOXIC HYPOGLYCEMIA

Group	A	B	C	D
Number in group	10	10	9	10
Rat weight (grams)	280.0[a]	282.6	273.0	268.3
	6.3[b]	3.7	2.6	4.0
Treatment	100 µg. DXM ip 1 ml saline iv	100 µg. DXM ip 5 mg. LPS iv	1 ml. saline ip 5 mg. LPS iv	1 ml. saline ip 1 ml. saline iv
Plasma Glucose (mg./dl.)				
at 0 minutes:	82.2	84.8	86.8	80.5
	2.9	3.3	3.5	2.3
at 180 minutes:	101.4	129.3	24.0	90.2
	2.2	3.4	9.0	1.8

Statistical Analysis

0 minutes		180 minutes		0 minutes--180 minutes	
A--B	0.6[c]	A--B	6.9	A	5.2
	0.6[d]		0.0		0.0
B--C	0.4	B--C	11.0	B	9.4
	0.7		0.0		0.0
		A--D	3.9	C	6.5
			0.0		0.0
				D	3.3
					0.0

Footnotes

a. Value represents mean.
b. Value represents standard error.
c. Value represents t-value.
d. Value represents p-value; value less than 0.050 considered significant.

development of hypoglycemia but elevated plasma glucose levels from 84.8 to 129 mg./dl. by 180 minutes post-treatment. Treatment of rats with 100 μg. of dexamethasone acetate alone resulted in a significant increase in plasma glucose levels from 82.2 to 101.4 mg./dl. within 180 minutes; however this increment was lesser than that observed in dexamethasone-treated endotoxic rats. Plasma glucose levels in saline-treated rats exhibited a small but significant increase, from 80.5 to 90.2 mg./dl., by 180 minutes post-treatment.

In vivo $^{14}CO_2$ Recovery following ^{14}C-D-Glucose Administration

Glucose oxidation in the endotoxic rat, and its modulation by dexamethasone acetate, was evaluated by recovery of $^{14}CO_2$ following intraperitoneal administration of a tracer dose of uniformly labeled ^{14}C-glucose. Experimental treatments for the five experimental groups are outlined in Table 3. Data for the five groups is presented in Table 4, and depicted in Figures 3 and 4, and is expressed as percent of the initial dose of ^{14}C recovered at each time point.

Statistical analysis (Table 5) of groups 1 and 2 reveals a significant increase in percent $^{14}CO_2$ recovered--hence, significant enhancement of glucose oxidation--by rats treated with 0.75 mg. endotoxin by 180 minutes post-treatment. When CO_2 collection was started 180 minutes following administration of endotoxin or saline (groups 3 and 4), significant glucose hypercatabolism by the endotoxic rats was demonstrated by 30 minutes after administration of ^{14}C-glucose. The ability of dexamethasone acetate to blunt the hypercatabolism was demonstrated

TABLE 3

EXPERIMENTAL TREATMENTS OF GROUPS OF RATS FOR $^{14}CO_2$ COLLECTION AFTER ^{14}C-D-GLUCOSE ADMINISTRATION

Group Number	Experimental Treatment
1	0.5 ml. saline iv at t. 0 min.; .7 ml. ^{14}C-glucose ip at t. 0 min.
2	0.5 ml. LPS[a] iv at t. 0 min.; .7 ml. ^{14}C-glucose ip at t. 0 min.
3	.75 ml. saline ip at t. -180 min.; .5 ml. saline iv at t. -180 min.; .7 ml. ^{14}C-glucose ip at t. 0 min.
4	.75 ml. saline ip at t. -180 min.; .5 ml. LPS iv at t. -180 min.; .7 ml. ^{14}C-glucose ip at t. 0 min.
5	.75 ml. dexamethasone[b] ip at t. -180 min; .5 ml. LPS iv at t. -180 min.; .7 ml. ^{14}C-glucose ip at t. 0 min.

[a] LPS is S. enteriditis lipopolysaccharide, 1.5 mg./ml.

[b] Dexamethasone is prepared at a concentration of 100 µg./ml.

TABLE 4

$^{14}CO_2$ RECOVERY AFTER IP ^{14}C-D-GLUCOSE

Group Number	1	2	3	4	5
Number in group	13	15	8	12	9
Weight (grams)	101.2[a]	102.4	116.1	114.8	120.0
	5.0[b]	4.8	4.2	2.6	2.0
% Dose recovered after ip glucose at 15 min.	1.12	0.83	1.30	1.29	1.26
	0.10	0.09	0.11	0.09	0.09
30 min.	2.84	2.66	3.14	4.17	3.53
	0.16	0.19	0.19	0.29	0.27
45 min.	5.52	5.39	5.49	7.68	6.41
	0.25	0.28	0.33	0.39	0.44
60 min.	8.51	8.82	8.15	11.50	9.59
	0.40	0.37	0.37	0.62	0.57
90 min.	14.93	16.20	13.69	18.36	15.09
	0.61	0.39	0.59	0.82	0.70
120 min.	20.59	21.94	18.03	22.94	19.08
	0.71	0.41	0.69	0.70	0.70
150 min.	25.16	27.13	21.42	26.46	21.85
	0.88	0.41	0.77	1.20	0.75
180 min.	28.16	30.81	23.84	28.88	23.99
	0.99	0.43	0.86	1.27	0.83
210 min.	30.55	33.39	25.75	30.89	25.34
	1.03	0.44	0.88	1.35	0.68
240 min.	32.39	35.18	26.94	32.45	26.69
	1.11	0.44	0.91	0.47	0.92

[a]Value represents mean.
[b]Value represents standard error.

TABLE 5
SELECTED STATISTICAL COMPARISONS
OF IN VIVO $^{14}CO_2$ RECOVERY DATA

Groups Compared	1--2	3--4	4--5	3--5
Degrees of Freedom	26	18	19	15
Rat weight	0.17[a]	0.26	1.59	0.84
	0.87[b]	0.79	0.13	0.42
% Recovery after C-14- Glucose at:				
15 min.	2.08	0.12	0.18	0.29
	0.05	0.91	0.85	0.78
30 min.	0.75	2.95	1.62	1.18
	0.46	0.01*	0.12	0.25
45 min.	0.33	4.32	2.15	1.67
	0.74	0.00*	0.03*	0.05
60 min.	0.57	4.67	2.27	2.11
	0.57	0.00*	0.01*	0.15
90 min.	1.74	4.64	3.02	1.52
	0.09	0.00*	0.01*	0.11
120 min.	1.65	3.87	3.03	1.07
	0.11	0.00*	0.01*	0.30
150 min.	2.03	3.54	3.26	0.40
	0.05	0.00*	0.00*	0.70
180 min.	2.46	3.27	3.22	0.12
	0.02*	0.00*	0.00*	0.90
210 min.	2.54	3.19	3.67	0.36
	0.02*	0.00*	0.00*	0.72
240 min.	2.35	3.18	3.32	0.20
	0.02*	0.00*	0.00*	0.84

[a] t-value
[b] p-value
* significant difference, with p-value less than 0.050

FIGURE 3

IN VIVO $^{14}CO_2$ RECOVERY

IMMEDIATELY FOLLOWING ENDOTOXIN ADMINISTRATION

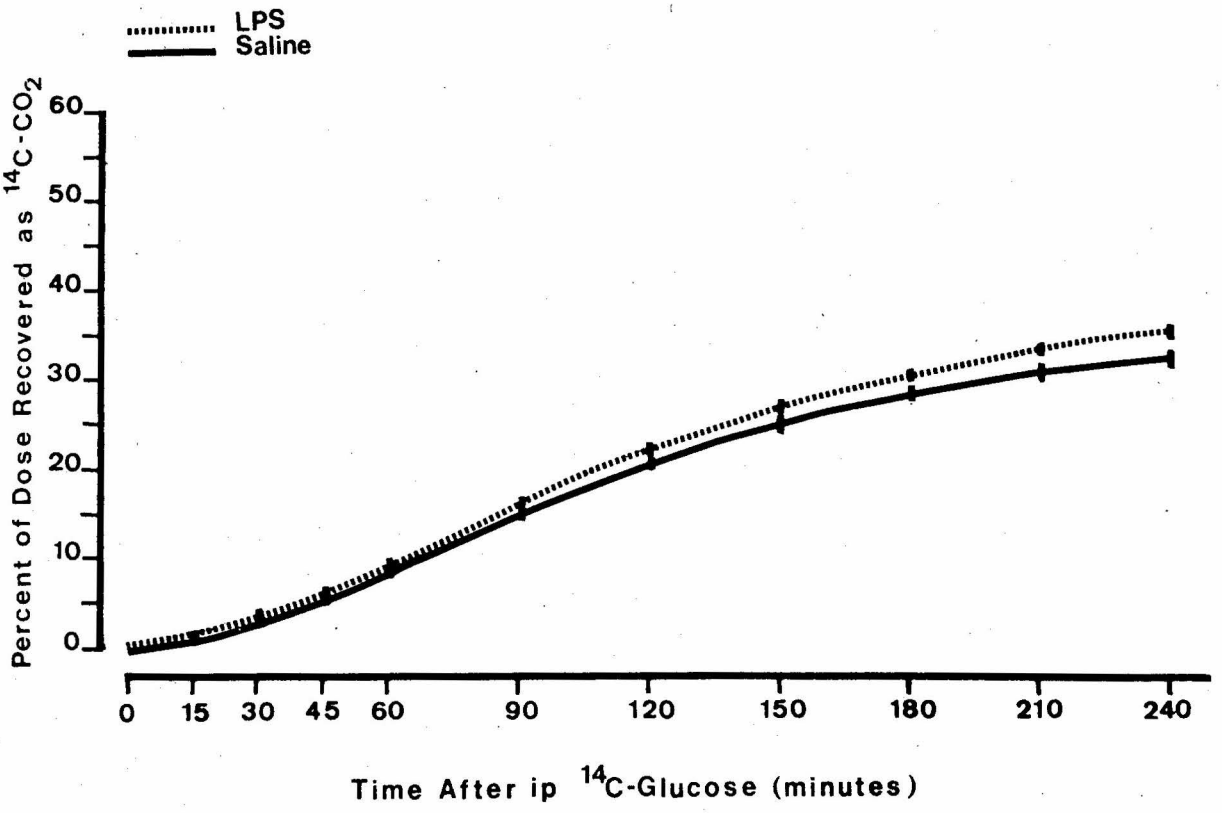
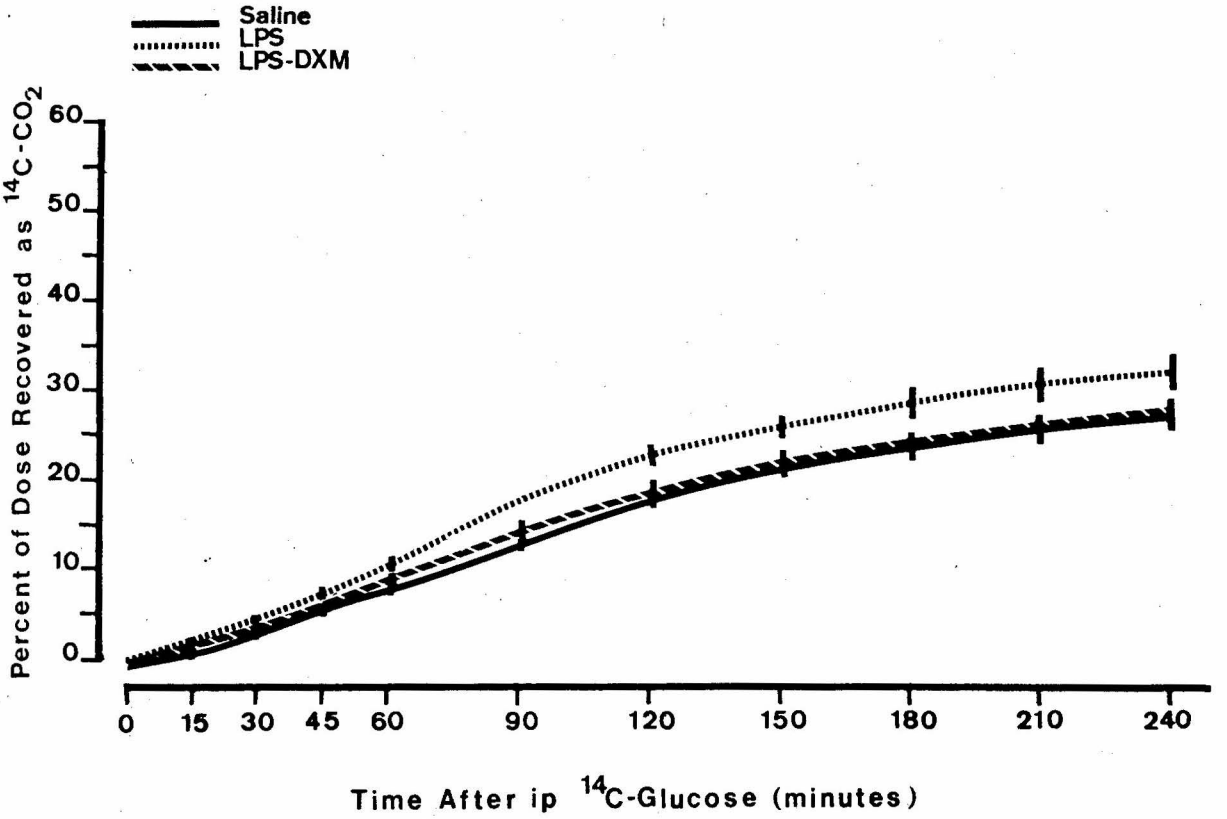


FIGURE 4

IN VIVO $^{14}CO_2$ RECOVERY

THREE HOURS AFTER ENDOTOXIN ADMINISTRATION



by group 5, administered dexamethasone acetate simultaneously with endotoxin. By 45 minutes after administration of ^{14}C-glucose, $^{14}CO_2$ recovery in dexamethasone-treated endotoxic rats was reduced to control levels.

In vitro $^{14}CO_2$ Recovery from Rat Tissues

In order to identify the specific tissue loci of endotoxic glucose hypercatabolism, $^{14}CO_2$ was recovered *in vitro* from ten tissues removed from either control or endotoxic rats. Results and a statistical analysis are presented in Table 6. Complete recovery of $^{14}CO_2$--including that released to the incubation medium--over a 120 minute incubation period was quantitated.

As depicted in Table 6, liver slices, spleen slices, hemidiaphragms and epididymal fat pads removed from endotoxic rats oxidized significantly more glucose than the respective tissues removed from untreated rats. Glucose oxidation by blood and gastric mucosa, and by ventricle, kidney, lung, and brain slices removed from endotoxic rats was not significantly enhanced.

In vitro $^{14}CO_2$ Collection from Epididymal Fat Pads

Characterization of endotoxic glucose hypercatabolism was carried out in a series of experiments using epididymal fat pads. The 48 experimental groups, their treatments, the data and statistical analyses are presented in Tables 7 through 11, and results are depicted in Figures 5 through 9.

The ability of endotoxin *in vivo* to significantly enhance glucose oxidation by epididymal fat pads was demonstrated

TABLE 6

IN VITRO $^{14}CO_2$ RECOVERY FROM RAT TISSUES

Tissue	N	Tissue Weight (mg.)	Treatment	$^{14}CO_2$ Recovery (DPM/gm.wet wt.)	P-values
Blood	9	1 ml.	--[a]	12346±1083[b]	0.13
	8	1 ml.	LPS	15988±2011	
Liver	9	344.1±44.0	--	82441±13970	0.00
	9	357.9±27.4	LPS	171180±9496	
Hemidiaphragm	8	117.8±8.3	--	278080±21172	0.01
	9	115.8±6.9	LPS	462270±63510	
Epididymal Fat Pad	8	243.3±12.6	--	76229±6528	0.03
	8	284.7±27.4	LPS	98171±6155	
Spleen	9	232.6±26.8	--	569470±54615	0.02
	9	255.1±16.5	LPS	758410±50599	

TABLE 6 CONT.

Tissue	N	Tissue Weight (mg.)	Treatment	$^{14}CO_2$ Recovery (DPM/gm.wet wt.)	P-values
Ventricle	9	244.1±22.5	--	299750±44175	0.92
	9	241.4±25.6	LPS	306280±43723	
Kidney	9	213.2±17.3	--	974270±92705	0.80
	9	245.0±14.3	LPS	944430±65995	
Lung	9	259.2±26.7	--	241240±27045	0.12
	9	258.0±18.8	LPS	312200±33012	
Brain	9	174.1±10.8	--	1698300±192790	0.58
	9	200.1±25.8	LPS	1847400±184640	
Gastric mucosa	9	756.3±37.7	--	101770±16453	0.44
	9	809.3±33.7	LPS	119860±15528	

[a]0.5 ml. LPS (1.5 mg./ml.) administered at t. -3 hours.
[b]Values represent mean±standard error.

by group 2 at all time points studied. Such data would appear to correspond to *in vivo* $^{14}CO_2$ collection data presented previously in which significant glucose hypercatabolism was demonstrated by 30 minutes after the administration of ^{14}C-glucose, when endotoxin had been administered 180 minutes prior to the ^{14}C-glucose.

That endotoxic glucose hypercatabolism could not be caused by direct interactions between endotoxin and the epididymal adipose was shown by the addition of endotoxin in concentrations of 50, 100, or 200 µg./ml. to the incubation medium. $^{14}CO_2$ recovery over a 120-minute incubation period from fat pads placed in a medium containing endotoxin was not significantly enhanced with any concentration of endotoxin, as compared to recovery from the matched fat pads (groups 7 vs. 8; 9 vs. 10; and 11 vs. 12).

Blunting of endotoxic glucose hypercatabolism by simultaneous administration of dexamethasone acetate with the endotoxin could be demonstrated *in vitro* by the epididymal fat pad (group 3). Significant depression of glucose hypercatabolism in fat pads removed from endotoxic dexamethasone-treated rats was demonstrable by 60 minutes after the onset of incubation. Such an inhibition of glucose oxidation by the *in vivo* administration of dexamethasone could not be demonstrated with fat pads removed from non-endotoxic dexamethasone-treated rats (group 5); no significant difference could be demonstrated at any time point when compared to a group of fat pads from untreated rats (groups 5 vs. 6).

TABLE 7
IN VIVO INFLUENCE OF ENDOTOXIN AND DEXAMETHASONE ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Group Number	Treatment
1	0.5 ml. LPS (1.5 mg./ml.) iv at t. -3 hr
2	0.5 ml. saline iv at t. -3 hr.
3	0.5 ml. LPS iv and 0.5 ml. dexamethasone acetate (150 µg./ml.) ip at t. -3 hr.
4	0.5 ml. LPS iv and 0.5 ml. saline ip at t. -3 hr.
5	0.5 ml. dexamethasone acetate ip at t. -3 hr.
6	0.5 ml. saline ip at t. -3 hr.

TABLE 7 CONT.

$^{14}CO_2$ Recovery from Epididymal Fat Pads						
Group Number	1	2	3	4	5	6
Rat Weight (grams)	107.5[a]	105.1	105.7	106.9	122.2	121.8
	5.1[b]	6.7	1.9	2.1	2.3	1.3
Fat Pad Weight (mg.)	307.5	287.7	305.6	301.9	316.8	341.6
	20.2	23.7	16.8	15.4	8.7	13.4
Number in Group	12	14	14	16	10	10
Cumulative DPM recovered per gram wet weight at:						
30 minutes	9722	5236	8942	11069	4695	4676
	1009	334	976	1177	589	287
60 minutes	32781	18485	27436	35941	14531	14568
	2514	1223	2957	3304	1555	843
90 minutes	58218	34902	48902	63995	27036	28558
	3468	2118	4809	5043	2477	1222
120 minutes	83983	52409	70247	92804	40520	43508
	4415	2884	6925	6448	3259	1642
CO_2 collection	6836	5412	6217	7857	4500	4840
	459	289	327	336	144	185

[a] represents mean value
[b] represents standard error ;
data presented in Tables 8 through 11 is arranged in similar fashion

TABLE 7 CONT.

Statistical Analysis			
Groups Compared	1--2	3--4	5--6
Rat weight	0.28[a] 0.78[b]	0.41 0.69	0.15 0.88
Fat pad weight	0.64 0.53	0.16 0.87	1.55 0.14
Degrees of Freedom	24	28	18
Cumulative DPM recovered per gram wet weight at:			
30 minutes	4.22 0.00	1.39 0.17	0.03 0.98
60 minutes	5.09 0.00	1.92 0.06	0.02 0.98
90 minutes	5.74 0.00	2.16 0.04	0.55 0.59
120 minutes	5.99 0.00	2.38 0.02	0.82 0.42
CO_2 collection	2.62 0.01	3.50 0.00	1.45 0.16

[a]represents t-value
[b]represents p-value; value less than 0.050 considered significant

TABLE 8
IN VITRO INFLUENCE OF ENDOTOXIN
ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Group Number	Treatment
7	LPS (50 μg./ml.) added to incubation medium
8	--
9	LPS (100 μg./ml.) added to incubation medium
10	--
11	LPS (200 μg./ml.) added to incubation medium
12	--

TABLE 8 CONT.

$^{14}CO_2$ Recovery from Epididymal Fat Pads						
Group Number	7	8	9	10	11	12
Rat Weight	114.8	114.8	118.8	118.8	123.0	123.0
(grams)	1.0	1.0	4.3	4.3	4.2	4.2
Fat Pad Weight	321.6	320.8	358.8	360.8	318.4	318.0
(mg.)	35.2	34.7	38.1	38.0	26.1	25.4
Number in Group	5	5	5	5	5	5
Cumulative DPM recovered per gram wet weight at:						
30 minutes	4843	5290	4547	4608	4583	4761
	677	1223	278	331	360	287
60 minutes	16406	18426	16348	16267	15918	16339
	2344	4449	1286	1171	1340	1283
90 minutes	31111	34273	31008	31000	30754	30915
	4189	7051	2200	1958	2124	2173
120 minutes	47417	51468	48277	48165	47892	47428
	5888	9010	3382	2884	3288	3415
CO_2 collection	4851	5061	5942	5482	5203	5042
	554	463	526	409	209	328

TABLE 8 CONT.

Statistical Analysis			
Groups Compared	7--8	9--10	11--12
Rat Weight	--	--	--
Fat Pad Weight	0.02 0.99	0.68 0.51	0.01 0.99
Degrees of Freedom	8	8	8
Cumulative DPM recovered per gram wet weight at:			
30 minutes	0.32 0.76	0.14 0.89	0.36 0.72
60 minutes	0.40 0.70	0.05 0.96	0.23 0.83
90 minutes	0.38 0.71	0.00 0.99	0.05 0.96
120 minutes	0.38 0.72	0.02 0.98	0.10 0.92
CO_2 collection	0.29 0.78	0.69 0.51	0.41 0.69

TABLE 9
IN VITRO INFLUENCE OF DEXAMETHASONE
ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Group Number	Treatment
13	dexamethasone acetate (50 μg./ml.) added to incubation medium
14	--
15	dexamethasone acetate (100 μg./ml.) added to incubation medium
16	--
17	dexamethasone acetate (200 μg./ml.) added to incubation medium
18	--
19	0.5 ml. LPS (1.5 mg./ml.) iv at t. -3 hr. dexamethasone acetate (50 μg./ml.) added to medium
20	0.5 ml. LPS iv at t. -3 hr.
21	0.5 ml. LPS iv at t. -3 hr. dexamethasone acetate (100 μg./ml.) added to incubation medium
22	0.5 ml. LPS iv at t. -3 hr.
23	0.5 ml. LPS iv at t. -3 hr. dexamethasone acetate (200 μg./ml.) added to incubation medium
24	0.5 ml. LPS iv at t. -3 hr.

TABLE 9 CONT.

$^{14}CO_2$ Recovery from Epididymal Fat Pads						
Group Number	13	14	15	16	17	18
Rat Weight	110.1	110.1	113.4	113.4	109.6	109.6
(grams)	4.0	4.0	2.6	2.6	3.3	3.3
Fat Pad Weight	262.7	272.2	308.9	306.2	295.6	293.1
(mg.)	25.5	23.5	19.3	17.6	20.9	23.3
Number in Group	9	9	9	9	9	9
Cumulative DPM recovered per gram wet weight at:						
30 minutes	5595	5530	4455	4573	4373	4494
	487	653	435	521	482	610
60 minutes	20539	20115	15479	17071	14576	15812
	1924	1998	1157	1343	1534	1743
90 minutes	37565	37499	29740	32337	27364	29740
	3034	3173	1868	2483	2361	2690
120 minutes	54743	54788	43507	46321	39806	44007
	4348	4618	3010	4029	3410	3858
150 minutes	72620	72718	59272	62427	54019	58548
	5477	5866	3855	5107	4207	5337
180 minutes	78321	92979	75512	79386	69051	76132
	9160	7033	4386	5943	4720	5107
CO_2 collection	4621	5081	4593	5236	4068	4688
	539	478	326	594	240	384

TABLE 9 CONT.

$^{14}CO_2$ Recovery from Epididymal Fat Pads						
Group Number	19	20	21	22	23	24
Rat Weight	110.8	110.8	109.2	109.2	115.4	115.4
(grams)	4.2	4.2	2.1	2.1	2.5	2.5
Fat Pad Weight	330.7	331.2	317.6	318.0	316.2	315.8
(mg.)	20.9	23.6	14.7	16.4	20.4	22.7
Number in Group	8	8	9	9	9	9
Cumulative DPM recovered per gram wet weight at:						
30 minutes	12111	14436	10063	13095	9020	15891
	1205	982	218	364	887	1372
60 minutes	32817	38921	25033	34586	23215	43182
	3294	2814	770	1607	2520	4428
90 minutes	50781	62781	41375	56979	37681	71325
	5124	4755	1298	2735	3873	6825
120 minutes	60831	85472	56749	79312	51023	98257
	10378	6138	1978	3835	5234	9297
150 minutes	94345	92253	70644	100390	64213	123880
	10279	15509	2690	5100	6402	11381
180 minutes	113520	129170	84656	120460	76677	148430
	12182	9066	3480	6279	7683	13621
CO_2 collection	5121	6747	3838	5991	3421	7094
	543	423	226	329	295	642

TABLE 9 CONT.

Statistical Analysis						
Groups Compared Rat Weight	13--14 --	15--16 --	17--18 --	19--20 --	21--22 --	23--24 --
Fat Pad Weight	0.27 0.79	0.10 0.92	0.08 0.94	0.01 0.99	0.02 0.98	0.01 0.99
Degrees of Freedom	16	16	16	14	16	16
Cumulative DPM recovered per gram wet weight at:						
30 minutes	0.08 0.94	0.17 0.86	0.15 0.88	1.50 0.16	7.13 0.00	4.21 0.00
60 minutes	0.15 0.88	0.90 0.38	0.53 0.60	1.41 0.18	5.36 0.00	3.92 0.00
90 minutes	0.01 0.99	0.81 0.43	0.66 0.52	1.72 0.11	5.15 0.00	4.29 0.00
120 minutes	0.01 0.99	0.56 0.58	0.82 0.42	2.04 0.06	5.23 0.00	4.43 0.00
150 minutes	0.01 0.99	0.49 0.63	0.67 0.51	0.11 0.91	5.16 0.00	4.57 0.00
180 minutes	1.27 0.22	0.52 0.61	1.02 0.32	1.03 0.32	4.99 0.00	4.59 0.00
CO_2 collection	0.64 0.53	0.95 0.36	1.37 0.19	2.36 0.04	5.40 0.00	5.20 0.00

TABLE 10
IN VITRO INFLUENCE OF INSULIN
ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Group Number	Treatment
25	insulin (2.5 μU/ml.) added to medium
26	--
27	insulin (5 μU/ml.) added to medium
28	--
29	insulin (10 μU/ml.) added to medium
30	--
31	insulin (20 μU/ml.) added to medium
32	--
33	0.5 ml. LPS (1.5 mg./ml.) iv at t. -3 hr insulin (2.5 μU/ml.) added to medium
34	0.5 ml. LPS iv at t. -3 hr.
35	0.5 ml. LPS iv at t. -3 hr. insulin (5 μu/ml.) added to medium
36	0.5 ml. LPS iv at t. -3 hr.
37	0.5 ml. LPS iv at t. -3 hr. insulin (10 μU/ml.) added to medium
38	0.5 ml. LPS iv at t. -3 hr.
39	0.5 ml. LPS iv at t. -3 hr. insulin (20 μU/ml.) added to medium
40	0.5 ml. LPS iv at t. -3 hr.

TABLE 10 CONT.

	$^{14}CO_2$ Recovery from Epididymal Fat Pads							
Group Number	25	26	27	28	29	30	31	32
Rat Weight	107.7	107.7	96.1	96.1	103.9	103.9	105.4	105.4
(grams)	5.4	5.4	4.2	4.2	2.5	2.5	2.9	2.9
Fat Pad Weight	299.1	289.6	222.8	221.3	264.0	265.8	277.6	273.8
(mg.)	33.5	34.0	13.6	13.4	9.4	12.8	18.4	19.1
Number in Group	9	9	9	9	9	9	9	9
Cumulative DPM recovered per gram wet weight at:								
30 minutes	12635	4982	9882	5449	21060	13883	23831	12081
	2129	589	1656	555	891	892	2415	2004
60 minutes	51661	16012	47448	15390	70447	29016	80069	30441
	8513	1305	7720	1286	7033	1606	11198	2584
90 minutes	100720	30539	97948	30851	130790	46093	142720	47329
	14183	2457	16983	2616	13712	2624	19239	3375
120 minutes	139300	48310	159760	48360	194710	62185	212210	63487
	24823	3515	25550	4003	23358	3481	28398	3885
CO_2 collection	14142	4413	13437	4124	14586	4150	17470	4253
	2088	434	2006	439	1866	374	3170	328

TABLE 10 CONT.

	$^{14}CO_2$ Recovery from Epididymal Fat Pads							
Group Number	33	34	35	36	37	38	39	40
Rat Weight	104.1	104.1	105.0	105.0	116.8	116.8	102.4	102.4
(grams)	5.1	5.1	5.5	5.5	1.6	1.6	2.9	2.9
Fat Pad Weight	268.0	275.2	270.0	267.3	326.0	337.3	249.3	246.2
(mg.)	29.3	33.6	25.3	28.8	18.8	13.2	16.2	18.7
Number in Group	8	8	9	9	8	9	9	9
Cumulative DPM recovered per gram wet weight at:								
30 minutes	22012	13026	26061	11283	47805	23575	38290	12023
	2285	1241	3726	1608	3503	1983	3324	1131
60 minutes	87178	39694	91499	36121	167140	57579	126820	32041
	10996	6091	14557	4445	14658	5658	10506	2698
90 minutes	159850	72910	173990	62178	291460	87636	228360	54479
	18175	11010	25112	6736	26518	8401	21036	4427
120 minutes	233560	101060	253510	89431	430800	117390	315600	78172
	25479	14754	34215	9669	39245	11166	42633	5540
CO_2 collection	15017	6697	17180	6770	35843	8089	24486	5006
	2189	664	3042	682	3369	686	2746	379

TABLE 10 CONT.

Groups Compared	Statistical Analysis							
Rat Weight	25--26	27--28	29--30	31--32	33--34	35--36	37--38	39--40
	--	--	--	--	--	--	--	--
Fat Pad Weight	0.20	0.08	0.11	0.14	0.16	0.07	0.49	0.12
	0.84	0.94	0.91	0.89	0.87	0.94	0.63	0.90
Degrees of Freedom	16	16	16	16	14	16	15	16
Cumulative DPM recovered per gram wet weight at:								
30 minutes	3.46	2.54	5.69	3.74	3.84	3.64	6.02	7.48
	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
60 minutes	4.14	4.10	5.74	4.32	3.78	3.64	6.97	8.74
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90 minutes	4.88	3.90	6.07	4.88	4.09	4.30	7.33	8.09
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120 minutes	3.63	4.31	5.61	5.19	4.50	4.62	7.68	5.52
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CO_2 collection	4.56	4.41	5.48	4.15	3.64	3.34	8.07	7.03
	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

TABLE 11
IN VITRO INFLUENCE OF ATP
ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

Group Number	Treatment
41	ATP (10^{-5}M) added to incubation medium
42	--
43	ATP (10^{-4}M) added to incubation medium
44	--
45	0.5 ml. LPS (1.5 mg./ml.) iv at t. -3 hr ATP (10^{-5}M) added to incubation medium
46	0.5 ml. LPS iv at t. -3 hr.
47	0.5 ml. LPS iv at t. -3 hr. ATP (10^{-4}M) added to incubation medium
48	0.5 ml. LPS iv at t. -3 hr.

TABLE 11 CONT.

	$^{14}CO_2$ Recovery from Epididymal Fat Pads							
Group Number	41	42	43	44	45	46	47	48
Rat Weight	116.3	116.3	109.9	109.9	107.1	107.1	107.6	107.6
(grams)	5.4	5.4	5.1	5.1	3.3	3.3	4.7	4.7
Fat Pad Weight	309.3	299.8	275.2	265.0	277.5	284.0	294.0	294.7
(mg.)	28.0	25.2	23.0	20.5	16.6	21.0	18.4	20.5
Number in Group	8	8	8	8	8	8	9	9
Cumulative DPM recovered per gram wet weight at:								
30 minutes	18982	18761	13475	17024	12745	10967	11081	9608
	4127	3807	1173	1575	1428	976	1170	1413
60 minutes	38506	37783	29611	35639	43332	38964	27481	33465
	5492	5401	2251	2819	3802	2853	2779	4068
90 minutes	57266	56788	46103	53074	75850	70089	45051	61113
	6699	6835	3133	3860	5238	5011	4200	5859
120 minutes	74976	74100	64266	71666	106940	100340	61748	87889
	7386	8035	4051	4407	7064	6377	5584	7599
CO_2 collection	4799	4729	4713	5136	7677	8611	4608	7374
	411	530	235	207	560	960	463	813

TABLE 11 CONT.

Statistical Analysis				
Groups Compared	41--42	43--44	45--46	47--48
Rat Weight	--	--	--	--
Fat Pad Weight	0.25 0.80	0.33 0.74	0.24 0.81	0.02 0.98
Degrees of Freedom	14	14	14	16
Cumulative DPM recovered per gram wet weight at:				
30 minutes	0.04 0.97	1.81 0.09	1.03 0.32	0.80 0.43
60 minutes	0.09 0.93	1.67 0.12	0.92 0.37	1.21 0.24
90 minutes	0.05 0.96	1.40 0.18	0.79 0.44	2.23 0.04
120 minutes	0.08 0.94	1.24 0.24	0.69 0.50	2.77 0.01
CO_2 collection	0.10 0.92	1.35 0.20	0.84 0.41	2.96 0.01

FIGURE 5

IN VIVO INFLUENCE OF ENDOTOXIN AND DEXAMETHASONE ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

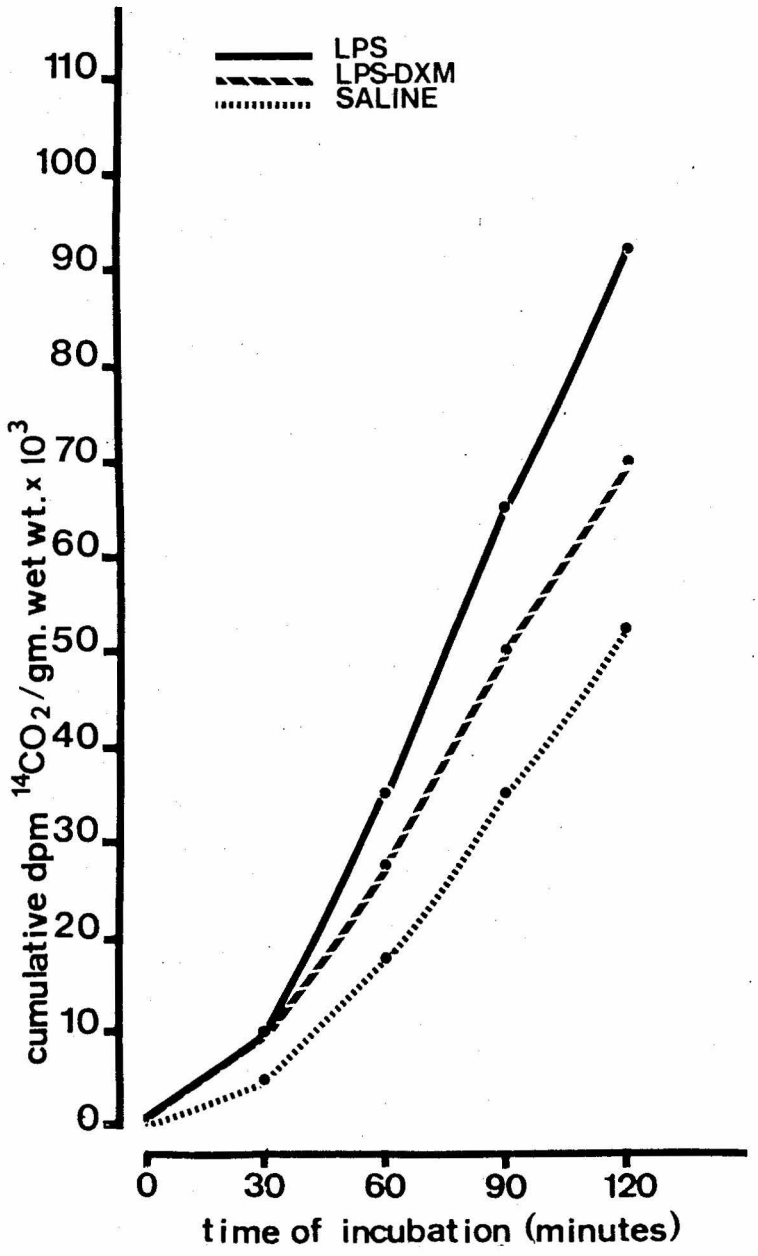


FIGURE 6

IN VITRO INFLUENCE OF DEXAMETHASONE

ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

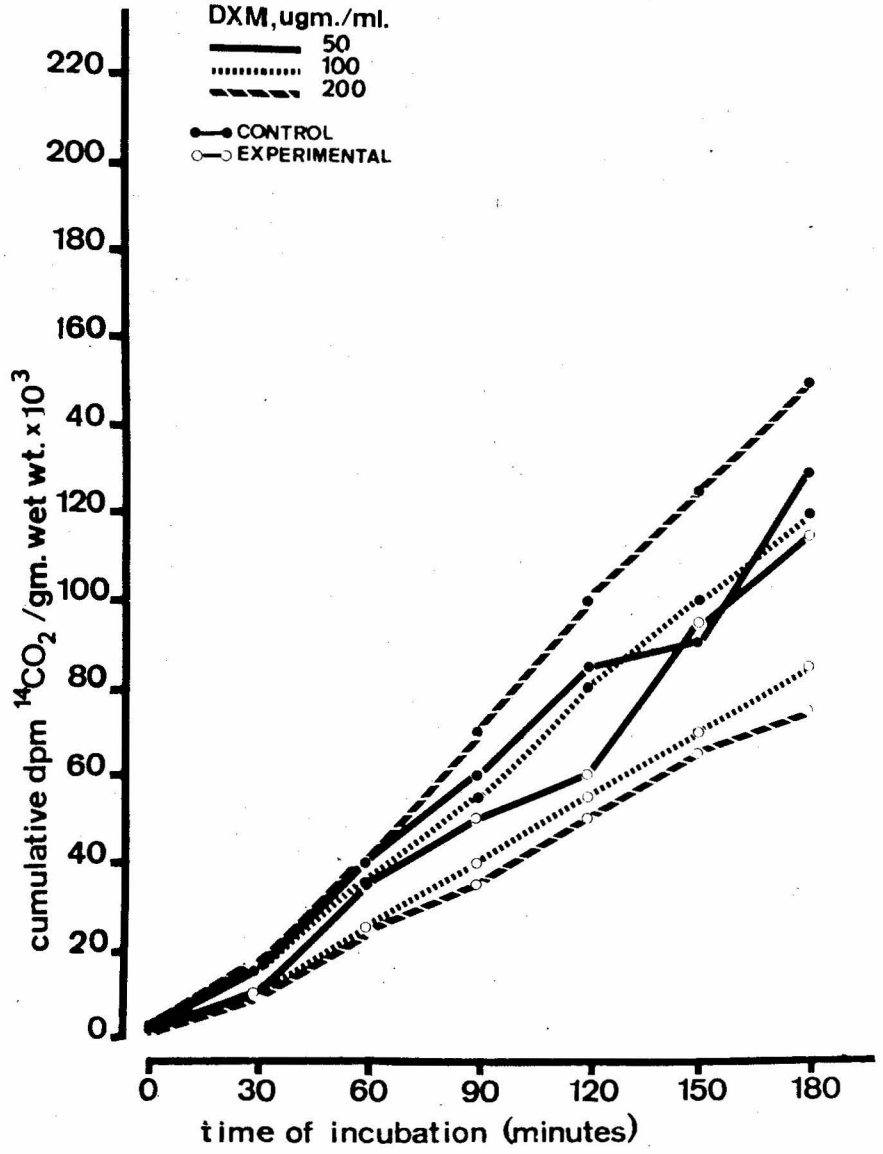


FIGURE 7

IN VITRO INFLUENCE OF INSULIN

ON EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

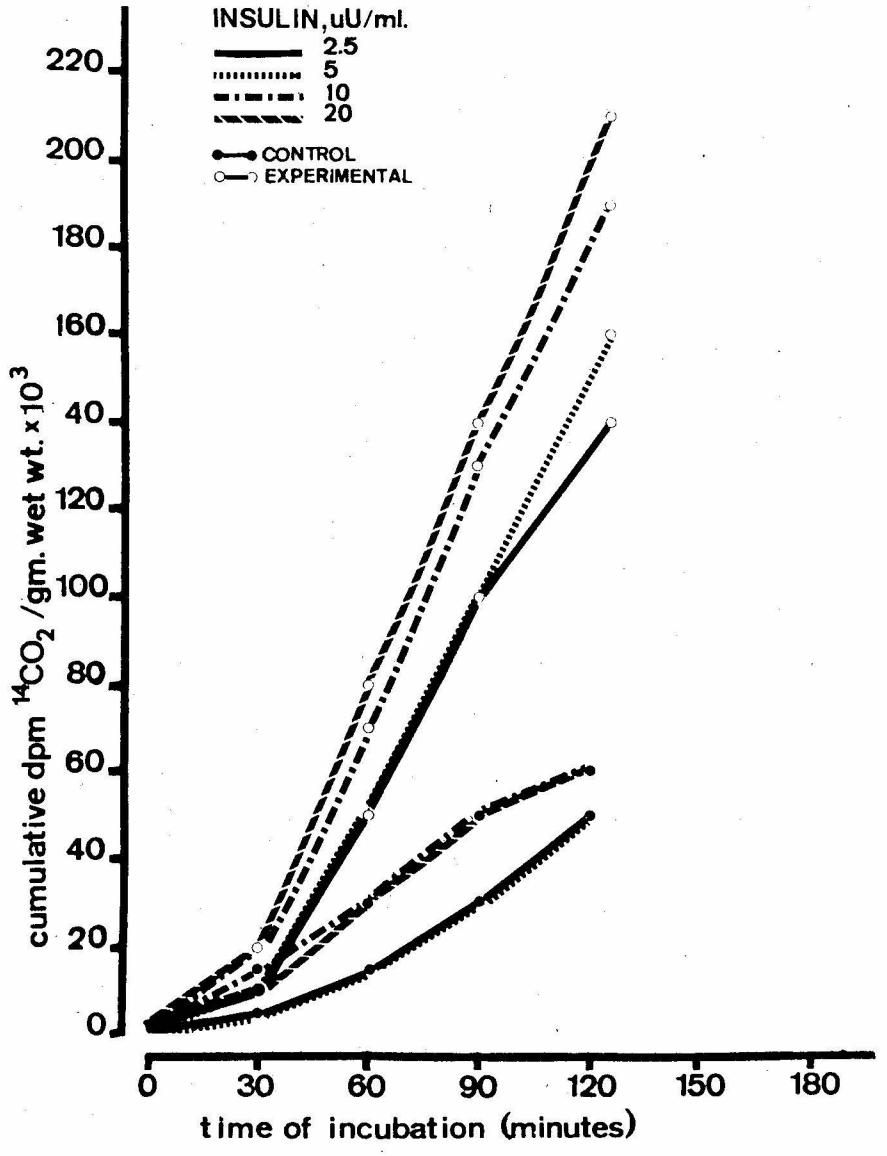


FIGURE 8

IN VITRO INFLUENCE OF INSULIN

ON ENDOTOXIC EPIDIDYMAL FAT PAD GLUCOSE OXIDATION

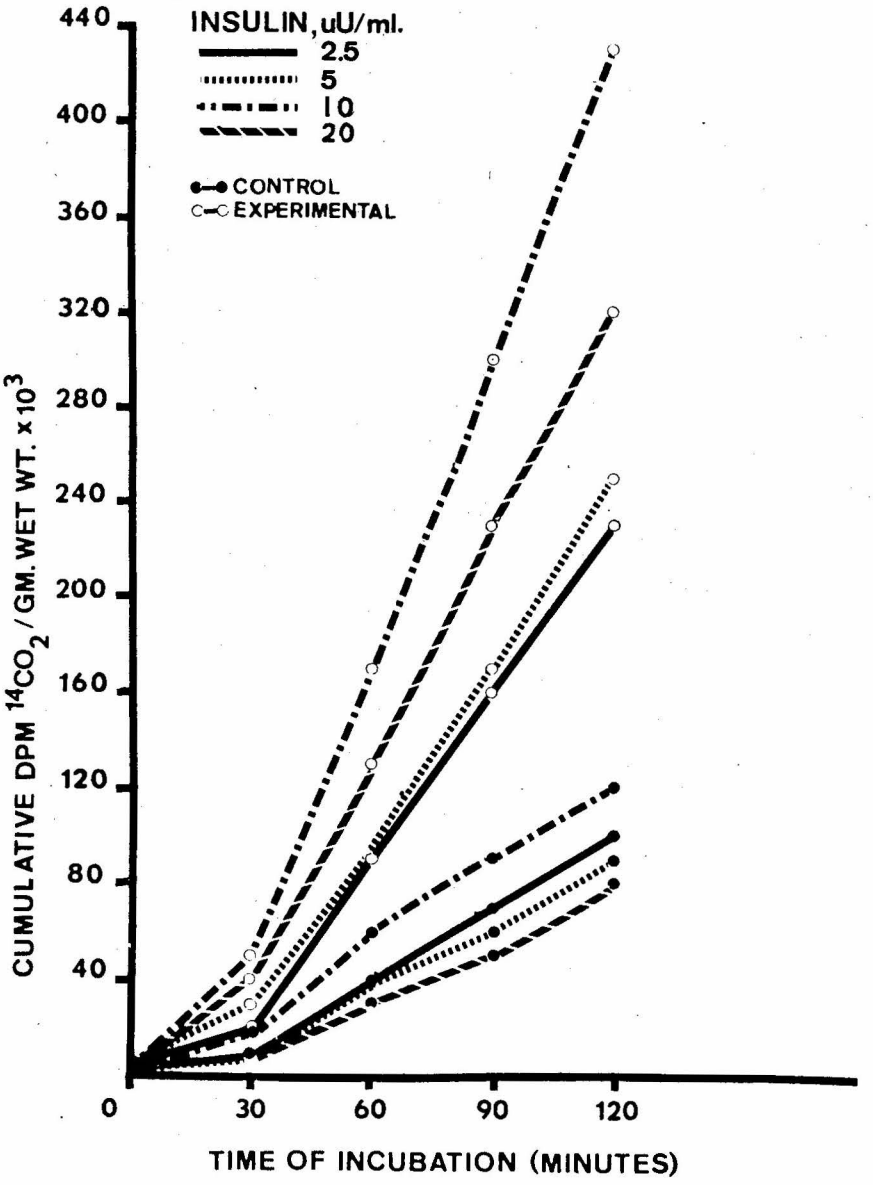
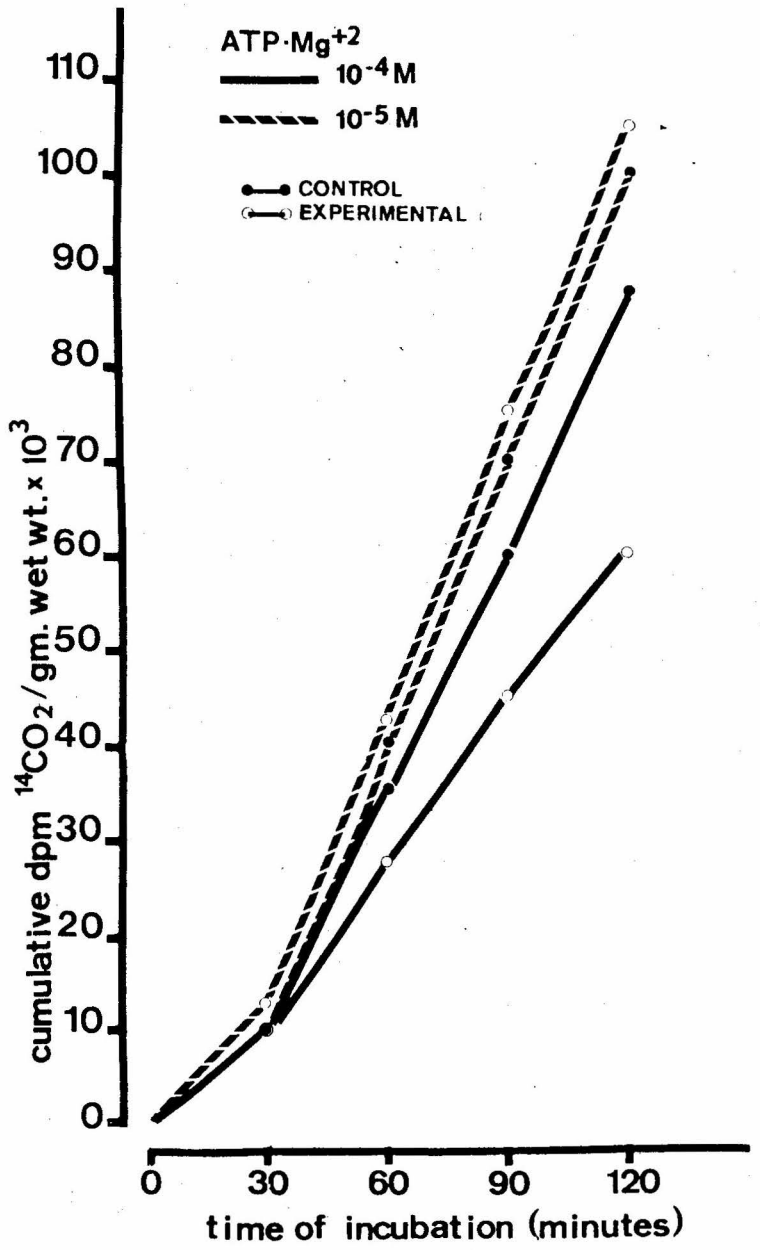


FIGURE 9

IN VITRO INFLUENCE OF ATP

ON ENDOTOXIC EPIDIDYMAL FAT PAD GLUCOSE OXIDATION



The effectiveness of dexamethasone in depressing endotoxic glucose hypercatabolism could be demonstrated by its in vitro administration as well. Dexamethasone acetate was added in concentrations of 50, 100, and 200 μg./ml. to incubation medium in which endotoxic fat pads were placed. Owing to the possibility of a necessary 'lag time' for the full development of dexamethasone's actions, CO_2 collection was carried out for 180 minutes. Results show that addition of dexamethasone acetate in the concentrations of 100 and 200 μg./ml. significantly depressed glucose oxidation by the endotoxic fat pads at all time points (groups 21 vs. 22, and 23 vs. 24); at the concentration of 50 μg./ml. only the $^{14}CO_2$ found in solution and released during the final collection period was significantly affected.

Dexamethasone added to the incubation medium in which control fat pads were placed was unable to significantly influence glucose oxidation at any time point over a 180-minute collection period. As indicated by groups 13, 15, and 17, dexamethasone acetate in the concentrations of 50, 100, or 200 μg./ml. respectively, did not blunt the basal glucose oxidation of untreated fat pads.

Endotoxin-stimulated glucose oxidation could be enhanced further by the in vitro administration of insulin. Addition of insulin in concentrations of 2.5, 5, 10, and 20 μU/ml. to the incubation medium in which endotoxic fat pads were placed resulted in a further enhancement of the glucose hypercatabolism, significant for all time points and at all concentrations

used (groups 33 vs. 34; 35 vs. 36; 37 vs. 38; and 39 vs. 40). Insulin-stimulated endotoxic glucose oxidation--as reflected by $^{14}CO_2$ recovery--was greater than insulin-stimulated glucose oxidation of fat pads removed from untreated rats (groups 25, 27, 29, and 31) at all concentrations used.

Addition of ATP in concentrations of 10^{-5} and 10^{-4} M did not significantly blunt glucose uptake and utilization by fat pads removed from untreated rats at any time point for a 120 minute incubation period. ATP in the concentration of 10^{-5} M also had no influence on glucose oxidation by endotoxic fat pads. However, 10^{-4} M ATP significantly decreased endotoxic glucose oxidation by 90 minutes after the onset of the incubation period.

DISCUSSION

Dexamethasone Protection against Endotoxin Lethality

The extensive use of corticosteroids in the treatment of shock, particularly endotoxic or septic shock, has been based on a substantial literature documenting a protective effect; however, specific mechanisms underlying this protective effect remain unclear. Current studies evaluated the ability of a potent glucocorticoid--dexamethasone--to alleviate the detrimental effects of bacterial endotoxin on carbohydrate homeostasis and thereby to impart resistance to the induction of lethality.

In agreement with a vast literature which is affirmative for a shock-protective capability of glucocorticoids, administration of 100 μg. of dexamethasone resulted in a significant diminution of lethality from 4 or 8 mg. of Salm. enteriditis endotoxin (Table 1 and Figure 2). These results concur with the findings of Marecki (40), Mills (44), and Berry and Smythe (3) in mice; with the results of Woodruff et al. (69) in rabbits; and with the findings of Fukuda and Hata (23) in rats. At the lower doses of 1 and 2 mg. of endotoxin, lethality was sufficiently low in untreated rats--15% and 30% respectively--that a significant protective effect of dexamethasone was not apparent. The lethality studies cited above,

however, have not served to isolate specific mechanisms whereby glucocorticoids could exert their protection; most studies to date which have evaluated specific protective mechanisms have been carried out using glucocorticoids *in vitro* in isolated systems such as cat papillary muscle (37) or lysosomal fractions (20). Based on the demonstration of a protective effect for dexamethasone, and on the significant role of altered glucose homeostasis in endotoxic shock (29), further investigations evaluated the specific influence of dexamethasone both *in vivo* and *in vitro* on select aspects of glucose metabolism.

Dexamethasone Protection against Post-endotoxin Hypoglycemia

The occurrence of a progressive and profound hypoglycemia following administration of endotoxin lipopolysaccharide, live or killed gram-negative bacteria, has been documented in several species. Results obtained in these studies represent yet another confirmation of the phenomenon of endotoxic hypoglycemia (Table 2). Rats treated with 5 mg. of endotoxin iv exhibited a drop in plasma glucose levels from 87 to 24 mg./dl. within 180 minutes. This rapid onset of hypoglycemia, which may serve either as a cause or as a contributing parameter of endotoxic lethality has been described previously by Berk (1), Hinshaw (29), Filkins *et al.*(21), Holper *et al.*(31) and others. In the studies described here, 7 out of 10 rats died in convulsions with plasma glucose levels around 5 mg./dl. The three rats which survived for 180 minutes had plasma glucose levels around 50 mg./dl. These levels concur with plasma glucose

levels reported by the investigators cited above which were obtained a few hours after the administration of endotoxin.

Both Berry, Smythe and Young (4) and Holtzman et al. (32) reported a significant increase in blood glucose levels in endotoxic mice and rats treated with a glucocorticoid. Results reported here indicate that rats are not only protected from hypoglycemia with the administration of dexamethasone, but become hyperglycemic. This somewhat exaggerated response was not reported by either Berry et al. or Holtzman et al; they noted a maintenance of blood glucose levels near control but did not report a significant hyperglycemia. Schuler, Erve, and Schumer (55) evaluated the influence of dexamethasone on various metabolite levels in the liver as well as lethality, and also reported a maintenance of these levels at control values. The exaggerated glucose response to dexamethasone reported here is also reflected in the alterations of glucose oxidation by endotoxic epididymal fat pads which were induced by dexamethasone, both in vivo and in vitro, and will be discussed below.

Administration of dexamethasone to untreated rats resulted in a small but significant increase in plasma glucose levels. The increase in plasma glucose is in agreement with findings cited by de Bodo and Altszuler in their review (14), and is consistent with current knowledge of glucocorticoid actions--i.e., their positive influence upon gluconeogenic enzyme activity, mobilization of gluconeogenic precursors, and inhibition of peripheral glucose uptake should result in an

increase in plasma glucose levels within a few hours of their administration. For the purpose of the present studies, glucocorticoid inhibition of glucose oxidation was the single mechanism evaluated.

Studies pertaining to glucocorticoid protection against endotoxic hypoglycemia suggest that this may represent a significant mechanism underlying lethality protection. Hinshaw has demonstrated the benefits of glucose infusion in the endotoxic dog (30); administration of glucocorticoids may have similar therapeutic influence over altered carbohydrate homeostasis, since they can maintain plasma glucose levels, and since their influence can be exerted within the first few hours after an endotoxic insult, during which alterations of carbohydrate metabolism seem to be critical to the eventual outcome of the animal. The positive influence of glucocorticoids over blood glucose levels would thus seem to be of clinical as well as experimental interest.

Influence of Dexamethasone on in vivo Glucose Oxidation

Two major mechanisms have been suggested to account for the hypoglycemia of endotoxicosis: impaired ability of the liver to synthesize glucose, and enhanced glucose uptake and utilization by peripheral tissues. Studies presented here evaluated the latter mechanism (Table 4 and Figures 3 and 4). Glucose oxidation by the whole animal in vivo was significantly enhanced within a few hours following the administration of a non-lethal dose of endotoxin. Such a finding is important since it highlights the ability of endotoxin to seriously alter

carbohydrate metabolism in an animal which appeared normal and which would probably exhibit no significant changes in its cardiovascular parameters. Although cardiovascular alterations in endotoxicosis have served as the center of investigation previously, results such as the present findings emphasize the probability that metabolic lesions precede, and therefore underlie the cardiovascular changes which are often considered the cause for endotoxic lethality.

Studies assessing glucose oxidation in the endotoxic or septic organism have been limited, and few reports document an *in vivo* glucose hypercatabolism. The results discussed here are in agreement with results of Buchanan and Filkins (8) in the rat; with Bierenbaum *et al.* (5) in the dog; and with Long *et al.* (38) and Gump *et al.* (26) in septicemic men. These findings suggest that tissue glucose hypercatabolism represents an important mechanism contributing to endotoxic hypoglycemia, and thus endotoxin can exert its influence on peripheral tissue as well as liver metabolism. The questions of whether or not endotoxin can directly effect peripheral tissue glucose metabolism, as well as which tissues exhibit glucose hypercatabolism, are two important issues which deserve consideration once *in vivo* glucose hypercatabolism has been established, and will be considered below.

Since dexamethasone protected the endotoxic rat against hypoglycemia, it was of interest to evaluate dexamethasone's influence over endotoxic glucose oxidation. Studies revealed that dexamethasone administration resulted in a lowering of

endotoxic glucose oxidation to a level attained by normal rats. These studies implicate inhibition of glucose uptake and utilization as a significant contributory mechanism underlying the hyperglycemic action of the glucocorticoids. Previous studies have stressed the ability of glucocorticoids to support liver carbohydrate metabolism in the endotoxic animal; these results indicate that glucocorticoids attack the development of hypoglycemia at both sites which contribute to it, i.e., the liver and peripheral tissues.

Effects of Endotoxin on Glucose Oxidation by Excised Tissues

Although endotoxic glucose hypercatabolism *in vivo* has been documented, no attempts have been made to characterize it or define which peripheral tissues contribute to it. The results of an *in vitro* study (Table 6) evaluating glucose oxidation by ten endotoxic tissues presented here represent the first systematic assessment of specific tissue contributions to *in vivo* glucose hypercatabolism.

A few studies have examined glucose uptake and oxidation by shock tissues, using other shock models. The epididymal fat pad from a rat peritonitis model (52) demonstrated glucose hypercatabolism; diaphragms removed from the same rat peritonitis model demonstrated no hypercatabolism, but demonstrated enhanced glucose uptake when removed from hemorrhaged rats (15). Such data is inconclusive, since different models were utilized. The advantage of the studies pursued here lies not only in the number of tissues studied, but also in the consistency of the endotoxic model used: all ten tissues were removed from each

rat administered endotoxin. Demonstration of a significant glucose hypercatabolism by epididymal fat pad and hemidiaphragm is in general agreement with the studies cited above. Although the liver is generally not considered a "peripheral" tissue, its glucose hypercatabolism is significant in view of its central role in glucose metabolism and carbohydrate homeostasis. In particular, glucose consumption by the liver Kupffer cells involved in the phagocytosis of endotoxin may be significantly enhanced; demonstration of glucose hypercatabolism by the endotoxic liver may serve as a starting point for further investigations into liver--endotoxin interactions. The significance of glucose hypercatabolism by the spleen has yet to be explored.

It is interesting to note that the insulin-like action of endotoxin on peripheral tissue glucose oxidation is exerted on the three tissues noted for their sensitivity to insulin: the liver, muscle, and adipose. Hyperinsulinemia in the endotoxic animal has been reported; however, the missing links between administration of endotoxin, development of hyperinsulinemia, and derangements in carbohydrate homeostasis have not been found. In an attempt to clarify these phenomena somewhat, further *in vitro* studies were undertaken to evaluate the responsivity of endotoxic tissue to insulin, and to test the responsivity of normal rat tissue to endotoxin *in vitro*.

Lack of in vitro Effect of Endotoxin on Fat Pad Glucose Oxidation

The ability of endotoxin to directly affect tissue meta-

bolism has been tested by a number of investigators. For instance, Rush and Hsieh (51) evaluated oxygen consumption in canine liver slices and reported that *in vitro* addition of endotoxin had no significant effect on oxygen consumption, but liver slices from endotoxic dogs demonstrated a depressed oxygen consumption. That the endotoxin-induced derangements in carbohydrate metabolism are mediated rather than direct has also been suggested by Moore, Goodrum, and Berry (45) in their study on endotoxic inhibition of PEPCK induction. These studies are in agreement with results presented here on epididymal fat pad glucose oxidation (Table 8). *In vitro* addition of endotoxin in concentrations up to 200 μg./ml. did not influence glucose oxidation, while epididymal fat pads removed from rats administered 0.75 mg. of endotoxin demonstrated a marked hypercatabolism.

The results obtained in these studies, however, are in contrast to those of Hewlett *et al.* (28) in which rat epididymal fat cells were able, first, to respond to endotoxin directly by increasing their cAMP levels, and second, to alter their sensitivity to epinephrine added to the incubation medium. Spitzer's demonstration (60) of altered sensitivity to norepinephrine by isolated fat cells placed in a medium containing endotoxin also is in opposition to results presented here. Not only is the *in vitro* responsivity to endotoxin in opposition to the data presented here, but the concept of endotoxin raising cAMP levels is in contrast to the concept of its insulin-like nature described here; i.e., one would anticipate

that in vitro endotoxin would lower cAMP levels in adipose.

If all of the above results were to be considered together, it would seem reasonable to conclude that endotoxin's insulin-like stimulation of glucose oxidation is not due to a direct interaction with peripheral tissues. The only direct effects which have demonstrated to date cannot be considered "insulin-like". This leads to the possibility that insulin itself mediates endotoxic glucose hypercatabolism, and thus the in vitro hypercatabolism demonstrated by endotoxic fat pads is due to the fact that while they were within the endotoxic rat, they were exposed to a higher "environment" of insulin than the normal fat pad.

In vivo and in vitro Dexamethasone Prevention of Fat Pad Glucose Hypercatabolism

The significant blunting of in vivo glucose hypercatabolism by administration of dexamethasone was demonstrated in vitro in the epididymal fat pad (Table 7 and Figure 5). The data serves as an in vitro confirmation of glucocorticoid inhibition of glucose oxidation as an important mechanism underlying its protective effects as outlined above. No significant influence was exerted by dexamethasone administration to untreated rats on fat pad glucose oxidation; dexamethasone did not suppress basal glucose uptake and utilization. The latter results do conflict with in vitro studies by Czech and Fain (13) which demonstrated dexamethasone's ability to inhibit glucose oxidation in the presence and absence of insulin when glucose transport is rate-limiting; in

addition, Fain (18) proposed that a unique uptake process exists for glucose uptale by the adipocyte which is independent of insulin, but can be suppressed by dexamethasone. Although Czech and Fain based their glucose transport model on *in vitro* glucocorticoid effects, it seems reasonable to expect that endogenous adrenal glucocorticoids or glucococrticoids administered *in vivo* would exert their influence by mechanisms similar to those elucidated *in vitro*. Data obtained here does not support the concept of glucocorticoid-induced suppression of basal glucose uptake and utilization by adipose tissue.

In vitro addition of dexamethasone at several dosages to the incubation medium (Table 9 and Figure 6) gave results which confirmed those obtained with *in vivo* administration of dexamethasone. Endotoxic glucose hypercatabolism was blunted by *in vitro* addition of dexamethasone in concentrations of 100 or 200 µg./ml. These results can be considered with other protective mechanisms of the glucocorticoids which have been demonstrated in an *in vitro* system: lysosomal membrane stabilization (20) and decreased complement-fixation by endotoxin (56), and emphasize the ability of glucocorticoids to exert their protective effects through direct interactions with endotoxic tissues.

Studies of *in vitro* dexamethasone influence over glucose oxidation by fat pads from control animals provide evidence conflicting with the work of Czech and Fain. In doses up to 200 µg./ml. within the incubation medium, dexamethasone did not suppress glucose oxidation. This is in direct contrast

to the *in vitro* results of Fain which were obtained using much smaller doses of dexamethasone.

In vitro results obtained with endotoxic fat pads are in agreement with the postulate of Czech and Fain that dexamethasone can inhibit (insulin) stimulated glucose uptake, and provide a direct demonstration of a protective mechanism of the glucocorticoids in maintaining caloric homeostasis.

Insulin Influence over Fat Pad Glucose Oxidation

Based on the documentation of endotoxic hyperinsulinemia, and the alterations in the sensitivity of adipose to norepinephrine and epinephrine, *in vitro* studies were carried out to evaluate the influence of high levels of insulin on endotoxic fat pad glucose oxidation.

In vitro addition of insulin to the medium of control fat pads stimulated glucose oxidation at all doses used (Table 10 and Figures 7 and 8). Insulin addition to endotoxic fat pads significantly stimulated glucose oxidation although the fat pads were already hypercatabolic. Endotoxic fat pads did not exhibit altered sensitivity to insulin. Insulin enhanced glucose oxidation approximately three-fold in the normal fat pad as well as in the endotoxic fat pad at the doses employed. However, owing to the intrinsically higher rate of glucose utilization by the endotoxic fat pad, insulin enhancement appeared to be much greater.

These studies do not rule out the possibility that an altered sensitivity to insulin is manifest by endotoxic adipocytes; rather, such an alteration seems reasonable in light

of the facts that, first altered sensitivity to another hormone has been demonstrated, and, second, that endotoxin administration induces lipolysis. A significant reduction in fat content within the adipocyte may result in a significant reduction in size. An inverse relationship between cell size and hormone sensitivity has been documented by Storck and Spitzer for norepinephrine-stimulated lipolysis (66). Thus the hypothesis that endotoxic adipose exhibits altered sensitivity to insulin seems tenable and another *in vitro* approach to the problem may be warranted.

In vitro manipulations with adipocyte responsivity to insulin were attempted in the endotoxic animal in order to demonstrate that insulin is directly responsible for glucose hypercatabolism. Chang and Cuatrecasas (10) were able to demonstrate a reduction in the oxidative response of isolated fat cells to insulin by the *in vitro* addition of ATP in concentrations of 10^{-4} and 10^{-5} M to the incubation medium. At the concentration of 10^{-5} M, no influence on basal glucose uptake and oxidation was demonstrated. Glucose oxidation was inhibited when glucose transport served as a rate-limiting step. In the current studies, addition of ATP in concentrations of 10^{-5} and 10^{-4} M to the medium of normal fat pads did not significantly alter their glucose oxidation (Table 11 and Figure 9). ATP addition in a concentration of 10^{-4} M resulted in a significant depression of endotoxic glucose hypercatabolism. If ATP-inhibition is specific for the insulin-mediated glucose transport process, then it seems reasonable to conclude

that endogenous insulin release is responsible for endotoxic glucose hypercatabolism. Inhibition of in vivo insulin release as would occur following administration of mannoheptulose in the endotoxic animal, followed by in vitro evaluation of glucose oxidation may provide a simple and direct test of the possibility that endotoxic peripheral tissue glucose hypercatabolism occurs as a result of endogenous hyperinsulinemia. Should such experimentation verify this hypothesis, then further studies for the connections between endotoxin and the carbohydrate homeostasis derangements it causes would have to shift their focus to the pancreas and the mechanisms responsible for endotoxin-induced insulin secretion.

Ultimately, tissue glucose hypercatabolism is a phenomenon involving interactions--eg., insulin with its receptor--at the cell membrane level. Figure 10 depicts possible interactions occurring at the endotoxic adipocyte's membrane. Following administration of endotoxin, a hyperinsulinemia results. Insulin molecules can bind to their receptors, resulting in an increased glucose uptake and oxidation. ATP interferes with the stimulated glucose transport by phosphorylating the insulin receptor, as suggested by Chang and Cuatrecasas (10). Dexamethasone may blunt the enhanced glucose uptake by one of two mechanisms: an immediate and direct interaction with the insulin receptor, or possibly at another external membrane site; or by the well-characterized mechanism of action involving a lag period of one or two hours, and obligatory protein synthesis. Blunting of basal glucose uptake has not been demonstrated in these studies.

Conclusions

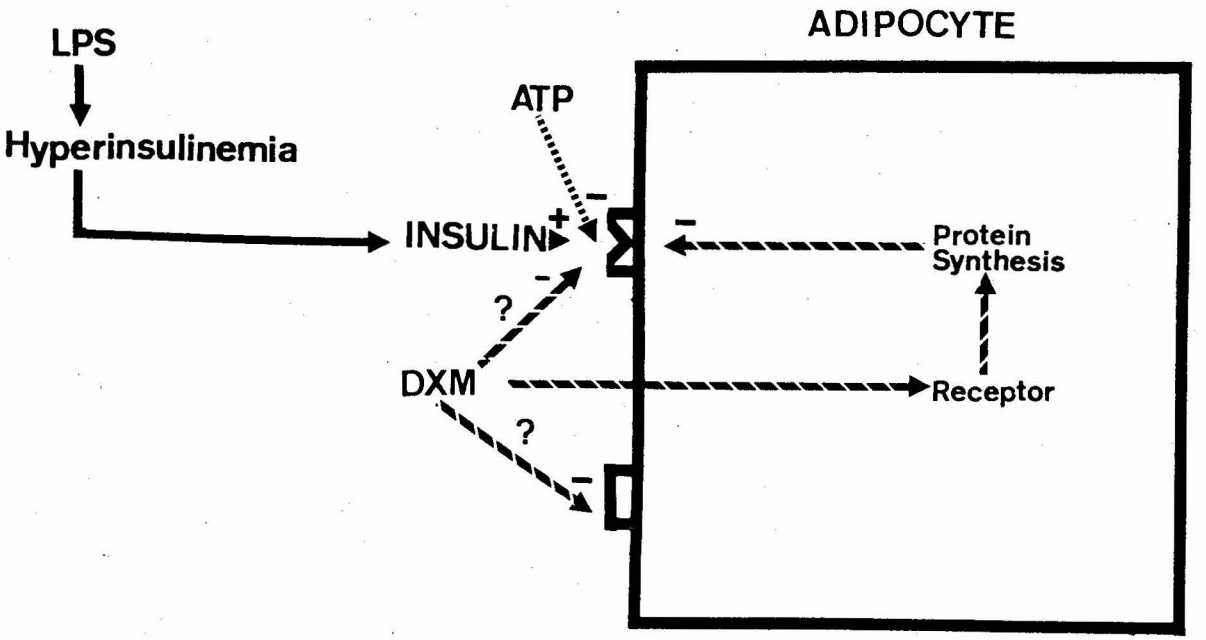
In summary, the studies presented here have demonstrated:

1. that protection against the development of hypoglycemia is a significant mechanism underlying dexamethasone protection against endotoxic lethality.
2. that endotoxic glucose hypercatabolism can be blunted both *in vivo* and *in vitro* by the administration of dexamethasone, thus protecting against the development of hypoglycemia.
3. that liver, epididymal adipose, diaphragm, and spleen are tissues responsible for glucose hypercatabolism.
4. that endotoxin does not exert its insulin-like actions on peripheral tissues through direct interactions, and
5. that insulin--fat pad interactions may be responsible for endotoxic glucose hypercatabolism.

FIGURE 10

ROLE OF INSULIN AND DEXAMETHASONE

IN ENDOTOXIC GLUCOSE HYPERCATABOLISM



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APPROVAL SHEET

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

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

Aug 13 1976
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