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Effect of Traumatic Injury on Sensitivity to Insulin

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EFFECT OF TRAUMATIC INJURY ON SENSITIVITY TO INSULIN

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VITA

Karl M. Nelson was born on September 15, 1950 in Albert Lea, Minnesota. He attended Pacific Lutheran University in Tacoma, Washington, from 1968 to 1972 and graduated with a Bachelor of Arts degree in Biology. He attended the University of Washington, Seattle, Washington where he studied Zoology prior to beginning his graduate education.

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CHAPTER I

INTRODUCTION

Caloric homeostasis refers to the coordinated, physiological processes which maintain tissue fuel nutrition. Major traumatic injury, such as crushing injury with fractured bones, severe burns, prolonged hemorrhagic hypotension or severe infection, elicits a period of systemic metabolic instability, i.e., caloric dyshomeostasis. A host of complex factors--blood loss, pain, fear, anesthesia, starvation, immobilization, bacterial contamination and tissue necrosis--comprise the total injury. After the wound has been inflicted, these components of the injury continue to exert deleterious effects until they have been identified and corrected.

The consensus of many investigators is that in contrast to the wide variety of injuries which may compromise the metabolic machinery of the organism, the essential features of the generalized metabolic response to injury are the same (136). The cardinal feature of the response of a small mammal to injury is a fall in the temperature of the body surface and internal organs without evidence of

increased heat loss (153; 156). Therefore, the fall in body temperature must be due to decreased energy production. Since glucose is responsible for much of the production of useful energy, investigation of glucose metabolism should reveal important information about the metabolic response to injury. The present study was designed to investigate certain aspects of the regulation of insulin-glucose interactions following injury. Temporal changes in plasma glucose and serum insulin concentrations were evaluated after traumatic injury. In addition to assessing the role of glucose in regulating insulin levels, insulin action was evaluated both *in vivo* and *in vitro*.

CHAPTER II

REVIEW OF THE LITERATURE

To appreciate the pivotal role played by glucose in caloric homeostasis following injury--trauma, burn, hemorrhage or sepsis--it is necessary to understand the regulation of glucose in different models of injury, as well as different species. Because of the importance of blood insulin levels in the metabolism of glucose in health, it is also important to know something of its regulation and activity following different forms of injury. After a brief discussion of the various models of injury used for "a rude unhinging of the machinery of life" (65), the changes in blood levels of glucose and insulin, the regulation of insulin secretion by glucose, and the efficacy of insulin in promoting glucose utilization will be considered. The purpose is to arrive at a view of the essential changes in glucose-insulin interactions which appear to be common to the various forms of injury.

A. Models of Injury.

Clinically, physical trauma produces tissue damage

by direct damage to cell membranes, through infection arising from within or without, and by interference with the functional integrity of the circulatory system. Because patients recovering from the deleterious effects of traumatic injury do not lend themselves to more than a minimal amount of manipulation, progress in understanding the generalized response to injury and assessment of the role of individual components in the complex of factors that are so frequently involved requires taking the problem to the experimental laboratory. As far as possible, the different factors of ischemia, hemorrhage and infection should be examined separately. Sacrificing clinical realism in order to achieve this is helpful in understanding the regulation and integration of homeostatic mechanisms after physical trauma.

The experimental model must use an actual injury. The main requirements of such an injury are: it must be reproducible; it must be measurable and its intensity must be controllable. The injuries used in the past--shooting limbs with bullets, detonating explosives pellets applied to the body, crushing limbs in vices--are no longer useful because the open wound adds an indeterminate degree of infection to an indeterminate amount of tissue injury.

Suitable injuries which have contributed a great deal to understanding the generalized metabolic response to injury include hemorrhage, ischemia produced by tourniquets, burns, scalds, infection and generalized contusion.

1. Hemorrhagic Hypotension.

Partly because of technical ease of application, the production of injury by prolonged hemorrhagic hypotension has been widely used in a number of laboratories (52; 143; 175; 185). In experiments on hemorrhage, a basic decision is to choose between the Wiggers fixed hypotension model and the single volume withdrawal type of hemorrhagic shock. In the fixed hypotension model, blood is withdrawn to reduce mean arterial pressure to some definite level. This method has been criticized for eliminating any effective homeostatic response to restore arterial pressure and for being a study of the effects of prolonged hypotension. The "single-withdrawal" model attempts to provide a more realistic counterpart of the clinical condition. A fixed volume is withdrawn, regardless of changes in blood pressure, and inherent homeostatic mechanisms are permitted to come into play.

2. Ischemia.

A simple type of tissue injury is that caused by release of a tourniquet applied to an extremity for a given number of hours (153). Tourniquet shock is a standardizable lesion because total obstruction to flow in one or both lower extremities for a known period of time is a precisely defined degree of trauma. The amount of tissue injured can be measured and the changes in it readily investigated. The intensity can be controlled by varying the amount of tissue damaged or by altering the duration of ischemia. In addition to being a major cause of tissue damage after physical injury, ischemia is also the cause of many pathological lesions occurring in natural disease processes and this increases the interest in its effect.

3. Burns and Scalds.

The functional consequences following extensive burns and scalds depend on the degree of heat applied, the duration of exposure and the surface area involved. Scalding by immersion in hot water for measured short intervals of time has proved to be a satisfactory method for achieving consistent results. Burns are a mixed type of injury, for while the depth and area of tissue burned can be standardized, the consequent bacterial activity cannot.

Still the procedure has yielded a great deal of information with regard to the management of burns.

4. Endotoxin-Sepsis.

The most frequent causative organisms of shock secondary to sepsis are gram-positive and gram-negative bacteria, although any agent capable of producing infection may initiate septic shock. Gram-negative sepsis as a cause of shock is a more frequent and difficult problem than gram-positive sepsis. Septic conditions have been produced experimentally by ligation and puncture of the cecum, thereby allowing enteric organisms to infect the peritoneal cavity; by transient occlusion of the superior mesenteric artery or celiac axis producing visceral ischemic anoxia and rendering the bowel-blood barrier more permeable to enteric organisms; or by injection of the infectious organism. The abnormalities produced are probably initiated by endotoxins from the cell walls of the gram-negative bacteria. Intravenous injection of this lipopolysaccharide-protein complex into experimental animals produces a response which is highly similar to the response of low flow sepsis in humans.

5. Tumbling Trauma - Generalized Contusion.

In 1942, Noble and Collip devised an apparatus which produced a graded, reproducible type of generalized contusion. The apparatus consists of a rotating drum. The interior surface is equipped with two shelves on which the animals are carried during rotation to a definite height and then dropped. By varying the number of revolutions, traumatic injury of varying severity can be produced. The advantage of this model is that it is not complicated by infection or hemorrhage. It has been used in work on trauma tolerance (93; 103; 105; 117). By giving the animals repeated exposure to sublethal amounts of drumming, the animals can be conditioned so that they are tolerant to what is considered a supralethal amount of drumming. The assumption is made that those homeostatic mechanisms that contribute to the survival of the unconditioned animal are enhanced in the conditioned animal and that their identification would prove beneficial in the care and treatment of the injured patient.

B. Changing Blood Levels of Glucose.

As an immediate reaction to injury, the blood levels of glucose change. Bernard (11) is credited with being

the first to demonstrate hyperglycemia immediately after hemorrhage. The hyperglycemic response to injury has subsequently been confirmed with great regularity by many investigators utilizing a wide variety of illnesses and injuries in diverse species (47; 96; 154). The presence of hyperglycemia and its quantitative and temporal relationship have been studied relative to the experimental model, feeding and fasting, the presence or absence of the liver, and other conditions of the animal.

The consensus reached by early investigators was that the concentration of blood glucose rose to maximum levels in about 30 minutes and continued at a high plateau for 3-4 hours after injury (1975). Hyperglycemia following a somewhat similar time course was seen after burn (37) or after tumbling injury by the rotating drum technique developed by Noble and Collip (36; 117).

Most of the excess glucose is derived from hepatic glycogen. Robertson (131) showed that mobilization of hepatic glycogen plays a major role in the hyperglycemic response to injury, for in cats after ligating the hepatic arteries and veins, there was no rise in blood sugar level. Preinjury depletion of hepatic glycogen by fasting not only prevents the post-injury rise in blood glucose but

usually results in hypoglycemia (48; 51; 96; 163).

The initial hyperglycemic phase may be followed by hypoglycemia. For many years it has been known that endotoxins, in the form of crude vaccines or as purified cell wall lipopolysaccharides, produce an early hyperglycemia and eventual hypoglycemia (12; 20; 79). Menten and Manning (107) first described the characteristic hyperglycemic response appearing soon after injection of endotoxin; followed by a severe hypoglycemia in animals given a lethal dose of endotoxin. Hypoglycemia following hyperglycemia was observed in tourniquet shock. During the period of hindlimb ischemia when the tourniquets were still in place, blood sugar levels were found to rise in fed rats (67; 152). Following removal of the tourniquets, blood sugar levels increased still further, rising to maximum levels about 3 hours later. They then fell progressively, reaching hypoglycemic levels in fatal cases.

From studies with various injury producing processes, it can be concluded that with the moblization of hepatic glycogen, an injury soon causes hyperglycemia. After most injuries the blood glucose concentration rises rapidly and steadily to a peak. The high concentrations will persist for some hours, with a gradual decline in blood glucose

levels. The fall in blood glucose increases with the first signs of peripheral vascular collapse and hypoglycemia occurs terminally in the severely injured. The fall in blood glucose is attributed to depletion of hepatic glycogen and failure of gluconeogenesis to keep pace with glucose utilization. The hypoglycemia occurring in the decompensatory stages of shock is not merely a terminal event but is correlated with the pathogenesis of irreversible shock. Its development is found to bear a close association with systemic hypotension, acidosis and impending death.

C. Utilization of Blood Glucose.

In non-injured animals an elevated blood glucose level is associated with increased uitlization of glucose (149). This has been demonstrated for cardiac muscle (110) skeletal muscle (29; 84) and for adipose tissue (42). Therefore, following injury with its concomitant hyperglycemia, one would predict an enhanced utilization of glucose as being responsible for the development of the terminal hypoglycemia. This appears to be the case for endotoxin and perhaps for hemorrhage as well. The situation with other models of injury is not as clear.

When rats were eviscerated by removing the entire gastrointestinal tract from esophagus to rectum as well as the spleen and pancreas and if the circulation to the liver was excluded, the remaining tissues would continue to utilize glucose. As no new source of glucose was available, there was a progressive fall in blood glucose and the rats eventually died of hypoglycemia. When an eviscerated rat was subjected to hemorrhagic hypotension, the blood glucose fell at a more rapid rate. This was ascribed to increased anaerobic utilization of glucose by the remaining tissues (135).

Beatty (8) attempted to determine by simultaneous arterial and venous sampling, whether or not hemorrhage was followed by changes in the magnitude of peripheral glucose utilization. She found the femoral arterial-venous glucose difference rose to a maximum and then progressively decreased until death supervened. Without any measurement of blood flow, the changes in the A-V glucose difference were interpreted as an initial increase in peripheral glucose utilization, followed by a decrease. Based on a progressive fall in venous glucose concentration compared to arterial blood, Seligman et al. (142) also concluded hemorrhage was followed by an increased utilization of

glucose. The arterial-venous difference in glucose concentration reflects the amount of glucose extracted or released, and can only permit qualitative assessment of regional glucose metabolism.

Shearburn et al. (146) coupled blood flow measurements with arterial-venous glucose determinations to quantitatively calculate glucose uptake by an "isolated" dog hindlimb. They found a tripling of glucose uptake by the limb during hemorrhage. The work of Wiener and Spitzer (173; 174) also indicated metabolic utilization of glucose was enhanced in the early period of hemorrhage hypotension.

The marked hypoglycemia after an initial hyperglycemia, a response which is characteristic to endotoxin has been interpreted to be the result of the combined effects of depressed gluconeogenesis and increased peripheral utilization of glucose (20; 51; 178). The tissues responsible for the endotoxin glucose hypercatabolism have been identified as muscle, adipose tissue, and liver and spleen (50).

The utilization of glucose during tumbling trauma in the Noble-Collip drum as well as 90 minutes after its termination was investigated by Nemeth and Vigas (115) with the aid of intravenous glucose tolerance tests.

Fasted male rats were given 0.5 g glucose per kg and the blood glucose was then measured at the beginning, at the end of, and 10 minutes after the end of Noble-Collip drum trauma. The blood glucose was lower in the injured rats at the end of the trauma period than in the non-injured animals. In later experiments, blockade of the glycolytic pathway with 2-deoxyglucose prevented the hypoglycemia (116). It was concluded that during the first period of Noble-Collip drum trauma, when the animals are in the drum, a true metabolic disappearance of glucose from the blood occurred. This enhanced glucose utilization was not a long lasting phenomenon, for by 90 minutes after the termination of tumbling, glucose utilization was depressed. Nemeth and Vigas (115) considered the presence of glucose intolerance 90 minutes after trauma as evidence of a decreased glucose utilization.

In recent years, many of the studies of carbohydrate metabolism in shock have dealt with experimental limb ischemia. Stoner, Heath and Collins (155) gave intravenous injections of ^{14}C glucose, ^{14}C fructose and 2-^{14}C-pyruvate to rats 1-½ hours after a 4 hour period of bilateral hindlimb ischemia. Hyperglycemia was maximal from 1-½ to 3-½ hours after removal of the tourniquets; this provided the

steady state required for interpretation of tracer studies. During this period the concentration of labeled glucose in the plasma fell more slowly in the injured rats than in the controls. The rate of loss of glucose from the plasma, liver water and interstitial fluid fell from 1.30 mg/min/100 grams body weight in the controls to 0.885 mg/min/100 grams body weight in the injured rats. Similarly, the cumulative excretion of $^{14}CO_2$ after injection of any of the labeled compounds was severely depressed. The fraction of glucose lost from the plasma and ECF-liver water pool which was rapidly oxidized to CO_2 resulted in a fall in the rate of glucose oxidation from 1.22 mg/min/100 grams body weight in control rats to 0.638 mg/min/100 grams body weight in injured rats. Ashby, Heath and Stoner (4) also investigated carbohydrate metabolism after hindlimb ischemia and found that injury reduced the rate of glucose oxidation. They concluded that inhibition of pyruvate oxidation was responsible.

Using the primed constant infusion of (6-^{3}H) glucose, Wolfe and his colleagues (179) evaluated the role of the liver and of peripheral glucose uptake in the development of postburn hyperglycemia. They reported that the plasma glucose concentration was significantly elevated

at 30 min postburn and continued to increase throughout the 4 hour experiment until it reached a value that was more than double the preburn value. The early hyperglycemia was due to an increased rate of appearance of glucose, presumably from the liver. The metabolic clearance rate of glucose, an indicator of the ability of the tissues to remove glucose from the plasma, was significantly depressed from 45 minutes postburn until the end of the experiment. It was probably this decreased ability of the tissues to extract glucose that accounted for the continued hyperglycemia.

D. Glucose Intolerance After Injury.

In spite of the conflicting reports regarding the peripheral utilization of glucose based on measurements of blood flow, A-V glucose differences and isotopic methods, it is correct to say that glucose intolerance exists after injury. When sufficient glucose is given to injured and non-injured animals to raise the plasma glucose concentration by about 150-200 mg/dl, the rate of fall of the plasma glucose concentration is usually slowed by injury.

Haist and Hamilton (67) found that glucose given either orally or intravenously to rats injured by a clamping

technique appeared to accumulate in the blood stream. Seligma et al. (142) reported that the disappearance of injected glucose from the blood of dogs in hemorrhagic shock was prlonged. Both Frayn (54) and Turinsky et al. (159) demonstrated pronounced intolerance to glucose in burned rats. There was a slight intolerance to glucose ninety minutes after termination of tumbling in the Noble-Collip drum (115).

Glucose intolerance has been shown many times in man (6; 21; 158). Hayes and Brandt (70) investigated carbohydrate metabolism in the immediate post-operative period, and concluded that a decreased tolerance to glucose is an integral part of the composite picture of metabolic alterations produced by surgical trauma. The degree of glucose intolerance can be related to the severity of the operation; patients undergoing more severe operations showed greater glucose intolerance (182). In his study of battlefield casualties, Howard (80) reported greater glucose intolerance with major injury than with minor injury. The same was true of infectious diseases (10; 53; 73; 176).

Glucose intolerance is greatest on the day of injury and improves during the convalescence from injury (80; 141; 157). The presence of a diabetic-type of

glucose tolerance curve for some time after an injury has given rise to the concept of diabetes of injury or traumatic diabetes. Thomsen (156) concluded that except for injuries which result in extensive destruction of the pancreas, traumatic injury was unable to cause diabetes mellitus. The condition is not permanent and probably represents severe examples of the changes described above or the development of complications which slow the recovery processes.

The association of a reduced rate of plasma glucose disposal with a high plasma glucose concentration requires that the normal hyperglycemic release of insulin is inhibited or there is resistance to the action of insulin. Both mechanisms probably are involved in the generation of a diabetic-type glucose tolerance curve.

E. Changing Blood Levels of Insulin.

The effect of several types of injury on the circulating levels of immuno-reactive insulin has been studied in a number of species including man. Studies of the temporal changes in the plasma concentration of insulin due to injury have variously suggested either an increase or a reduction in insulin secretion. Variable results regarding

glucose-stimulated insulin secretion have also been obtained from glucose tolerance tests.

In dogs made hypotensive by means of rapid exsanquination, there was an increase in glucose as well as in insulin levels (7; 101; 161). The hyperglycemia which occurs during hemorrhagic shock was not associated with increased circulating insulin concentrations in baboons. Within 5 minutes of hemorrhage, peripheral insulin levels and portal vein insulin declined substantially and remained below prehemorrhage levels during the first hour of shock (25; 26; 111; 112). Bleeding in sheep was followed by a progressive rise in blood glucose. Serum insulin levels remained unchanged during the bleeding induced hyperglycemia (68). Similar observations have been made following hemorrhage in the awake pig (23).

Within hours of injury by either burn or hindlimb ischemia, rats show hyperglycemia and an elevated plasma insulin concentration (54; 59). Turinsky et al. (159) reported that burned rats did not show an increase in serum insulin, while they did at 1 day after burn. Swine given a conflagration injury did not show either hyperglycemia or any change in serum insulin concentrations (170) at 24 hours after injury.

In patients hospitalized with severe traumatic injuries, plasma glucose levels were significantly elevated. Despite the hyperglycemia, plasma insulin concentrations were not elevated (24; 85; 101; 106; 148).

Glucose-stimulated insulin secretion has been studied in patients after burn, myocardial infarction, and during operations and infection. Glucose tolerance tests performed during the shock phase of burn injury show that there was glucose intolerance and failure of the insulin to rise in response to the glucose (2; 148). A similar observation was made on 12 patients within 15 hours of myocardial infarction (1). Studies of the changes in the plasma concentration of insulin due to operation have variously suggested either an increase (134) or a reduction in insulin secretion (3; 182).

Infection and high-flow sepsis is characterized by a tripling of basal insulin levels. If a glucose tolerance test is performed in patients with an early febrile infection, the resultant insulin secretion appears to be exaggerated both in magnitude and duration. Clowes and his colleagues (39; 40; 41; 121; 137) have repeatedly demonstrated elevated basal levels of insulin in septic patients as well as experimental models. Rats infected with

Diplococcus pneumoniae have elevated portal insulin levels (45; 184). Glucose administration brings out clear cut differences between infected and non-infected patients. Septic patients or patients with a mild viral illness demonstrate both glucose intolerance and elevated insulin levels (66; 128; 144). An exaggerated insulin response to glucose is seen after endotoxin administration in rats (20). The response of baboons to septicemia is similar to their response to hemorrhage in that they respond with hyperglycemia and hypoinsulinemia (43; 44).

Spitzer et al. (150) made observations concerning the response of pancreatic β cells to glucose stimulation after endotoxin administration. Eight dogs were given E. coli endotoxin and 50% glucose was infused at a rate sufficient to prevent endotoxin-induced hypoglycemia. Glucose was measured with glucose oxidase and immunoreactive insulin was measured by a double antibody technique. These eight dogs showed a marked hyperinsulinemia; insulin levels of this magnitude were not seen in dogs given endotoxin alone or in dogs with comparable glucose levels without endotoxin administration. Further studies were designed to characterize the hyperinsulinism in response to hyperglycemia in endotoxin-treated dogs (14). Beta blockade

with propranolol failed to inhibit the hyperinsulinism. Tolbutamide administration resulted in the expected elevation of plasma IRI concentration but no excessive release comparable to that observed following glucose stimulation was noted.

The concentration of a hormone in venous or arterial blood may not give an accurate reflection of actual secretion of the hormone. This is particularly true in the case of insulin, which is secreted into the veins which collect to form the hepatic portal system and which is affected by passage through the liver. More information concerning pancreatic function can be obtained by sampling blood for insulin determinations from the hepatic portal vein. In this way the effects of dilution first in portal and then in systemic blood pools can be partially evaded. Furthermore, the influence of uptake by the liver can be overcome.

The ideal parameter to measure is the net amount of insulin secreted into the pancreatic veins per unit time per weight of the pancreas. The net secretion on insulin by the pancreas is given by the difference between the amount of insulin leaving the pancreas and the amount entering. This is described by the formula:

$$ISR = \frac{Qp(I_{pv} - I_{pa})}{100 \text{ g pancreas}}$$

where:

ISR = insulin secretory rate (uU/min/100 g pancreas)

Qp = pancreatic plasma flow (ml/min)

I_{pa} = pancreatic arterial IRI concentration (uU/ml)

I_{pv} = pancreatic venous IRI concentration (uU/ml)

This necessitates measurement of pancreatic blood flow as well as sampling from pancreatic veins. Because of the abundance of anastomoses between the branches of the pancreatico-duodenal, splenic, gastric, omental and epiploic vessels, the classical approach of measuring the blood coming from one of the larger veins cannot be considered reliable. A number of groups have estimated the pancreatic output of insulin by measuring hepatic portal flow or splanchnic flow and multiplying that by the portal-arterial insulin gradient. The assumptions are that portal plasma flow and portal insulin concentrations are an accurate reflection of pancreatic blood flow and insulin concentration in the pancreatic venous drainage.

Drucker and his colleagues (46; 92) attempted to determine directly the secretion of insulin from the body of the pancreas during successive stages of hemorrhagic

shock. They reported a pronounced and abrupt output of insulin from the pancreas with hypovolemia when there was minimal hyperglycemia; this pancreatic output of insulin declined even when the blood glucose level continued to rise. This group failed to subtract the amount of insulin going into the pancreas from the amount of insulin leaving the pancreas and therefore over-estimated the pancreatic insulin output.

Using a slightly different approach, Hiebert et al. (75) attempted to estimate the insulin secretory rate in dogs subjected to hemorrhagic shock. Hiebert et al. (76) calculated an insulin secretory rate based on determinations of portal blood flow and the portal-arterial insulin gradient from the following formula:

$$ISR = Q_{hpv} (I_{hpv} - I_{art})$$

where:

ISR = insulin secretory rate (uU/min)

Q_{hpv} = portal plasma flow (ml/min)

I_{hpv} = portal vein IRI concentration (uU/ml)

I_{art} = arterial IRI concentration (uU/ml)

With this technique they demonstrated a fall in the

insulin secretory rate (ISR) of primates subjected to hemorrhage (74-77). When applied to dogs they reported a sixfold increase in ISR occurred in shocked dogs after glucose loading (75). From this they concluded that marked species differences exist in the insulin-glucose metabolic responses to shock.

Porte demonstrated that epinephrine exerts an inhibitory effect on insulin release which is mediated via alpha-adrenergic receptors in the islets (122; 123; 124). When injected after 1 hour of hemorrhagic shock, phentolamine elicits a rise in serum insulin with a subsequent decrease in glucose levels (26). Premedication with a ganglionic blocking agent or surgical denervation of the adrenal medulla prevents the hypoinsulinema of shock (74; 164; 68). The low insulin levels for the prevailing hyperglycemia observed after injury have been explained using this as a mechanism.

Epinephrine inhibition of insulin secretion does not seem to be the entire explanation for depressed insulin levels after injury. Epinephrine inhibition of insulin secretion is observed during acute but not chronic glucose stimulation (94). Epinephrine appears to inhibit selectively the rapid insulin response to glucose but not to

influence the insulin output stimulated by prolonged hyperglycemia (94; 95). This is probably why the insulin response to glucose is blunted in thermal injury (170). In light of the antagonism between epinephrine and glucose regarding insulin secretion, whether or not insulin is low in relation to the prevailing glucose concentrations will depend in part on the sensitivity of the β cells to adrenergic inhibition. Therefore suppression of insulin secretion is not a universal consequence of severe or even lethal injury (55).

F. Decreased Insulin Sensitivity After Injury.

Low levels of insulin in relation to the prevailing plasma glucose concentration and failure of insulin levels to rise in response to glucose stimulation cannot be the explanation for decreased glucose utilization in those cases of injury characterized by elevated insulin levels. In such cases a reduced rate of plasma glucose disposal is probably due, in part, to resistance to the hypoglycemic action of insulin.

Insulin resistance, or the failure of injected insulin to produce the expected decline in blood glucose has been reported to occur after injury. Howard (80)

observed this in his study on battlefield casualties. Bailey (6) reported hyperglycemia following thermal injury and cases in which 200 units of insulin per day failed to reduce the blood glucose. Harlow et al. (69) provided evidence that the mechanisms responsible for lowering blood glucose in response to low doses of insulin occur at a slower rate following injury. These abnormalities in the glucose response to injected insulin may persist for up to 5 days after injury. Olsen and Neutzel (122) also found resistance to the hypoglycemic action of exogenous insulin in patients with hepatic and infectious diseases.

Induction of peritonitis in pigs by ligating the cecum results in hyperinsulinemia. In spite of the quadrupling of the insulin levels, neither arterial glucose nor uptake of glucose by the hindlimb changed in the infected animals (121). As with pigs, peritonitis in rats results in hyperinsulinemia and euglycemia, suggesting resistance to the hypoglycemic action of insulin (137). Disturbances of glucose metabolism consequent to experimental peritonitis in rats were studied by measurement of insulin related metabolism of isolated tissues. It was found that adipose tissue glucose oxidation increased threefold, while the rate of conversion of U-^{14}C-glucose to $^{14}CO_2$ and labeled

glycogen by incubated hemidiaphragms was not significantly different in the septic animals when compared to the controls (137).

A number of investigators have documented insulin resistance following burn injury (2; 6). Similar methods of producing burn injury have been used by several investigators to study the development of insulin resistance after burn (54; 55; 57; 159; 179; 180). Frayn (54) investigated the tissue sensitivity to insulin by injecting insulin and measuring the blood glucose concentration over the first few minutes after injection. First-order kinetics were assumed and mean rate constants for glucose disappearance were calculated and expressed as K-values in a manner analogous to the intravenous glucose tolerance test. A first order rate system is defined by

$$dG/dt = -KG \tag{1}$$

whose solution is

$$G = G_o e^{-Kt} \tag{2}$$

where G_o is the value of G at time 0. By definition the distance between two parallel lines is independent of t. From equation 2, the equations for two parallel lines

would be

$$G_a = G_{ao}\, e^{-K_a t} \quad (3)$$

$$G_b = G_{bo}\, e^{-K_b t} \quad (4)$$

The vertical distance between these curves is C

$$C = G_{ao}\, e^{-K_a t} - G_{bo}\, e^{-K_b t} \quad (5)$$

This equation is dependent on t unless $G_{ao} = G_{bo}$ and $K_a = K_b$, in which case C = 0. Thus if two curves are parallel they cannot both describe first order rate systems. Nonetheless, if one persists in trying to analyze parallel curves on the assumption that both follow first order kinetics, then the K-values cannot be equal and the upper curve must have a smaller K-value (91).

When plotted semi-logarithmically, the plasma glucose decay curves for the control and scalded rats appear as parallel lines. The fact that the K-value for the scalded curve is less than the K-value for the control curve merely reflects the higher values of plasma glucose in the scalded animals and does not indicate a lower rate of glucose disappearance. Frayn (54) regards the rate

constant, K, as a rate of disappearance, utilization or assimilation.

In further experiments, Frayn (55; 57) concluded that scalding of mice or rats results in a considerable loss of sensitivity to insulin. The product of plasma glucose concentration and insulin concentration, which in a small animal, for a given rate of glucose turnover, may be taken as a measure of insulin resistance (71; 72; 98) was considerably elevated in these studies.

The effects of a constant infusion of insulin on glucose turnover was evaluated by Wolfe et al. (180) in normal and burned guinea pigs. Although the insulin infusion rate was the same in the controls and the burned animals, the plasma level of the exogenously-infused insulin was significantly higher in the burned animals. Nevertheless, the metabolic clearance rate in the burned animals given insulin was not increased above the value for the burned, untreated animals. This was interpreted as insulin resistance.

In scalded rates, the acute phase of glucose intolerance and inhibition of β cell secretion was followed by a much longer phase characterized by close to normal or normal glucose tolerance and hyperinsulinemia, which

indicated the presence of insulin resistance (159).

Ryan et al. (138; 139) made measurements of insulin related metabolism on samples of skeletal msucle and adipose tissue obtained sequentially for one month following hemorrhage and reinfusion in rabbits. As Ryan et al. failed to specify if the control tissue samples were obtained from the non-hemorrhaged animals only on the day of the hemorrhage or if they were obtained concurrently with the samples from the shocked animals, we must assume that the control values did not change over the month of observation. When compared with the controls, the production of CO_2 in the presence of insulin by both muscle and edipose tissue was significantly depressed for at least one month after hemorrhage. The conversion of glucose to neutral lipid by adipose tissue or to glycogen by muscle was also decreased.

The basal glucose uptake of soleus muscle isolated from rats at the end of a 1-½ hour period of hemorrhage hypotension was not different from that of control muscles; however, insulin failed to produce a stimulatory effect at concentrations less than 100 mU/ml, although maximal stimulation was seen at 200 mU/ml (32, 33). As these animals had been adrenalectomized four to five days prior

to the hemorrhagic episode, it was concluded that this alteration could not be due to increased plasma levels of steroid or epinephrine. A similar conclusion was reached by Ryan et al. (139) in their studies of the metabolic instability following hemorrhage in rhesus monkeys. Changes in plasma levels of adrenal steroids catecholamines, or circulating insulin levels were prevented by infusing cortisol acetate and insulin into adrenalectomized, streptozotocin diabetic rhesus monkeys. Tissue insulin resistance after hemorrhage was demonstrated by a depression of insulin stimulated conversion of glucose to CO_2 by rectus abdominus muscle obtained from intact rhesus monkeys before and sequentially after hemorrhage. Without presenting any data, they claim the effect of insulin was the same in the adrenalectomized diabetic group as in the intact group. The response of glycogen synthesis to insulin, expressed as percent of baseline by isolated skeletal muscle from intact and adrenalectonized-diabetic monkeys receiving hormone replacement showed no significant differences existed between the groups at any sampling time.

The maintenance in isolated tissues of the insulin resistance seen in vivo does not seem to occur following burn injury. Turinsky et al. (159) reported no decrease

in postburn responsiveness of diaphragms to insulin. Frayn (180) found no difference in either glycolysis or in glycogen synthesis between soleus muscles from control or scalded mice. Accumulation of (^{14}C)2-deoxyglucose was used as a measure of glucose transport and phosphorylation. Although basal transport was unchanged, insulin stimulated transport was actually significantly greater in muscles from scalded mice. This points to impairment of an intracellular metabolic step as the site of insulin resistance after injury (56).

G. Summary of Literature Review and Statement of Purpose and Objectives.

The acute metabolic response to injury, either trauma, burn, hemorrhage or infection, is characterized by profound disturbances in caloric homeostasis. Hyperglycemia is one change which occurs with great regularity in response to injury. The hyperglycemia following injury results mainly from the rapid mobilization of hepatic glycogen. The blood glucose concentration remains high for several hours after injury since the rate of peripheral glucose utilization fails to match the increased concentration. The association of a reduced rate of plasma

glucose disposal with a high plasma glucose concentration requires either that the release of insulin stimulated by hyperglycemia is inhibited or peripheral insulin sensitivity is impaired. Either mechanism could contribute to the glucose intolerance characteristic of the "diabetes of injury." It is therefore important to distinguish between these two responses to injury as ultimately treatment might be aimed at manipulation of one or the other effect.

The present study was planned in order:

(1) to standardize a model of injury which is not complicated by blood loss or infection--tumbling trauma in anesthetized male rates;

(2) to evaluate the temporal sequences of changes in plasma glucose and serum insulin following tumbling trauma;

(3) to determine if glucose-stimulated elevation of insulin levels is impaired following injury;

(4) to evaluate the effect of injury on the hypoglycemic response to insulin <u>in vivo</u>;

(5) to analyze the sensitivity of adipose tissue and skeletal muscle to insulin <u>in vitro</u>.

CHAPTER III

METHODS

A. Animals and Care.

Male rates (Holtzman Co., Madison, WI) between the ages of 30-40 days and weighing 115 $\pm$ 0.3 (2644) grams ($\bar{X}$ $\pm$ S.E.M. (N)) were used in this study. They were acclimated to the temperature (76 $\pm$ 2^{o}F) and the light period (0700 - 1900 Hrs.) in the animal quarters before use. Purina Laboratory Chow and tap water were provided ad libitum. Experiments were conducted on overnight fasted rats; this consisted of removing food, but not water, from the rats between 1500 - 1700 hours on the day preceding an experiment.

B. Traumatic Shock Model.

A graded, reproducible whole body injury without extensive hemorrhage or infection was produced with the apparatus described by Noble and Collip (117). The apparatus consisted of four metal drums (diameter 15", depth 8") constructed in such a manner that they were driven by

a single motor at 33 r.p.m. Two blunt triangular projections, 2" high and 3" on the base, were fastened on opposite sides of the inner circumference of each drum. The cover of the drum had a number of holes for ventilating the interior.

Rats were anesthetized with sodium pentobarbital (20 mg/kg i.m.) and either returned to their cages (controls) or subjected to traumatic injury. The limbs of the latter group were bound with adhesive tape before being placed in the drums. As the drums revolved at 33 r.p.m., the rats were carried part way up the inner circumference of the drum by the wedges until they slipped off and fell in front of the other wedge. Thus traumatic injury was due to the mechanical energy which acted on the rat during deceleration at every fall. The total number of revolutions was changed as necessary to produce about 50% mortality at 24 hours post-trauma.

C. Analysis of Plasma Glucose.

Sample of trunk blood from unanesthetized, decapitated rats were placed in ice-cold Beckman Microfuge tubes containing heparin and NaF. After 5 minutes of centrifugation in a Beckman 152 microfuge, the samples were frozen.

plasma glucose concentrations were made at a later date with a Yellow Springs Instrument Model 23A Glucose Analyzer.

D. Insulin Radioimmunoassay.

The Phadebas Insulin Test (Pharmacia) was used to assay the amount of immunoreactive insulin in rat serum. Blood from unanesthetized, decapitated rats was allowed to clot in 16 X 125 mm disposable glass culture tubes. Serum was prepared by centrifuging the clotted blood at $4^{o}C$. The serum was aspirated into 12 X 75 mm disposable glass culture tubes and stored frozen ($-20^{o}C$).

The exceptional reproducibility, sensitivity and reliability of the Phadebas Insulin Test have been described (160). This radioimmunoassay is based on the Sephadex Solid Phase Technique (171; 172) in which highly specific insulin antibodies raised in guinea pigs are covalently bound to Sephadex particles. The insulin in an unknown sample is allowed to compete with a fixed amount of ^{125}I-labelled insulin for the binding sites on the insulin antibodies. The concentration of insulin is then determined by comparing the competitive capacity of the serum sample to that of insulin standards of known concentration.

Using the lyophilized porcine insulin standard

supplied with each kit, seven insulin standards (3.2, 8, 16, 32, 80, 160 and 320 uU/ml) were prepared utilizing a combination of single and serial dilutions as described by the manufacturer. Serum samples were diluted 1:1 with the RIA Buffer solution. The Sephadex-Anti-Insulin complex and the lyophilized ^{125}I-insulin were reconstituted using the Buffer solution and transferred to a beaker containing a magnetic stirring bar. The suspension was stirred continuously on a magnetic stirrer while dispensing. A Hamilton Digital Dilutor (Hamilton 100200) was used to pick up 100 µl of the standards and 1:1 diluted samples and dispense 1.1 ml of the Sephadex-Anti-Insulin complex and ^{125}I-insulin mixture into 12 X 55 mm plastic tubes. The tubes were capped and incubated overnight on a horizontal shaker (Pharmacia). After incubation the capped tubes were centrifuged for two minutes at 1500 X G in an IEC Model PR-J refrigerated centrifuge in order to remove droplets from the caps. The caps were removed from the tubes and 1.5 ml of the Phadebas Decanting Aid Solution was added to the tubes. The tubes were centrifuged at 1500 X G for five minutes to sediment the Sephadex coupled anti-insulin and bound insulin. The supernatant was decanted by holding the tube upside down for a few seconds and touching the

rim of the tube to a piece of absorbent paper. Each tube was counted for 5 minutes in a Searle/Analytic Model 1185 Gamma Counter (Amersham/Searle, Arlington Heights, IL).

Each time the test was repeated, duplicates of the buffer blank, the seven insulin standards, a Pharmacia lyophilized pooled human serum, and pooled fed rat serum were run preceding the experimental samples. This permitted assessment of the inter-assay variation. Experimental samples were run in duplicate.

After subtracting background counts, the counts (B) for each of the standards and unknowns was expressed as a percentage of the mean counts of the zero standard (Bo). A standard curve was plotted and the concentration of insulin in uU/ml was read directly from the standard curve for each of the unknown samples.

E. Insulin and Glucose Following Traumatic Injury.

The temporal sequences of changes in plasma glucose and serum insulin following traumatic injury were determined by measuring these parameters at selected intervals. The measurements were compared with time matched controls.

F. Insulin Responsiveness In Vivo.

1. Insulin tolerance and glucose response.

The ability of exogenous insulin to lower blood glucose was assessed in both control rats and injured rats at either 3 hours or 1 day after injury. At these times the rates received 750 mU insulin/kg s.c. (Sigma, I-5500) or an equal volume of saline. At intervals from the time of injection, the rats were decapitated and blood was collected for plasma glucose and serum insulin determinations.

2. Intraperitoneal glucose tolerance and insulin response.

At either 3 hours or 1 day after injury, control and injured rats were given an i.p. injection of 1.0 g/kg D-glucose (in 15% w/v solution) or an equivalent volume of saline. At intervals from the time of injection, the rats were decapitated and blood was collected for plasma glucose and serum insulin determinations.

G. Insulin Responsiveness In Vitro.

1. The production of $^{14}CO_2$ from U-^{14}C-glucose by epididymal fat pads.

The conversion of glucose to CO_2 by epididymal fat

pads was measured as follows (49; 50; 130): The rats were decapitated and epididymal fat pads removed. The fat pads were rinsed in ice cold 0.9% saline for 15-30 minutes, blotted and weighed on a Roller-Smith torsion balance before being placed in metabolic flasks. Metabolic flasks containing the incubation medium were kept on ice throughout the period of tissue sample preparation.

The incubation medium was Krebs-Ringer bicarbonate buffer (Electrolyte composition in grams/liter: NaCl 6.9; KCl 0.35; $CaCl_2$ 0.28; KH_2PO_4 0.16; $MgSO_4$-$7H_2O$ 0.29 and $NaHCO_3$ 2.1). The medium also contained 1 mg/ml D-glucose and 0.5 uCi/ml U-^{14}C-glucose (Amersham/Searle). Five ml of incubation medium equilibrated with 95% O_2:5% CO_2 and pH 7.4 was added to 50 ml metabolic flasks (Kontes Glass Co., Vineland, NJ) shortly before the fat pad was added. One fat pad from each animal was incubated in the presence of bovine crystalline insulin (Sigma, I-5500) while the other fat pad was incubated in the absence of insulin.

After the fat pads were placed in the flasks and while still on ice, each flask was gassed for 60 seconds with 95% O_2:5% CO_2. The flasks were placed in a Dubnoff metabolic shaker bath at 37^{o}C and shaken at 100 cycles per minute. The expired CO_2 was collected by the method

of Saba and DiLuzio (140). At the end of the 60 minute incubation period, scintillation vials (Wheaton Scientific, Millville, NJ) with Teflon adaptors were attached to the side arms of the metabolic flasks. One ml of 62.5% citric acid was injected into each metabolic flask in order to liberate the CO_2. A 4" X 0.75" strip of Whatman #52 filter paper soaked with 0.3 ml of hyamine hydroxide (1 M of methanol, Amersham/Searle) and placed in each scintillation vial was used to trap the expired CO_2. Fifteen minutes after the citric acid addition, 15 ml of liquid scintillation fluor (1 L toluene:4g PPO:0.1 g POPOP) was added to each vial. The vials were counted for 20 minutes in a liquid scintillation counter (Isocap/300, Nuclear Chicago) after at least 1 hour of dark adaptation.

Data was expressed as DPM/hr/gram wet weight. Conversion of CPM to DPM was calculated for each sample and was based on a sample channels ratio vs. efficiency curve constructed from a ^{14}C standard quench set (Amersham/Searle). Most samples counted with an efficiency of 80%.

2. Incorporation of U-^{14}C-glucose into the glycogen of hemidiaphragms.

The incorporation of glucose into the glycogen

of hemidiaphragms was measured as follows (49): The rats were decapitated and hemidiaphragms were dissected free from the central tendon and chest wall. The hemidiaphragms were rinsed in iced 0.9% saline for 15-30 minutes (15; 18) and gently blotted before being placed in 25 ml Erlenmeyer flasks. The flasks containing the incubation medium were kept on ice throughout the period of tissue sample preparation.

The incubation medium was that described by Gey and Gey (62) (Electrolyte composition in grams/liter: NaCl 7.0; KCl 0.37; $CaCl_2$ 0.17; $MgCl_2$-$6H_2O$ 0.21; $MgSO_4$-$7H_2O$ 0.07; $NaHCO_3$ 2.27; NaH_2PO_4 0.12 and KH_2PO_4 0.03). The medium also contained 3 mg/ml D-glucose and 0.5 uCi/ml u-^{14}C-glucose (Amersham/Searle). Three ml of incubation medium equilibrated with 95% O_2:5% CO_2 and pH 7.4 was added to 25 ml Erlenmeyer flasks shortly before the muscle was added. One hemidiaphragm from each animal was incubated in the presence of bovine erystalline insulin (Sigma, I-5500), while the other was incubated in the absence of insulin.

Immediately prior to incubation, the flasks were gassed for 30 seconds with 95% O_2:5% CO_2 and sealed with rubber stoppers. The flasks were placed in a Dubnoff

metabolic shaker bath maintained at 37°C and shaken at 100 cycles per minute. At the end of the 90 minute incubation period, the flasks were placed on ice. The muscles were removed from the flasks, blotted and weighed on a Roller-Smith torsion balance.

Glycogen was extracted by placing the hemidiaphragms in 2.0 ml of 30% KOH in a boiling water bath (63). After alkaline digestion of the muscle, the glycogen was precipitated with 1 ml of 2% Na_2SO_4 and 2.5 ml of 95% ethanol (9; 183). The glycogen precipitate was washed twice with 2 ml 60% ethanol, dried and dissolved in 4 ml of water. One ml of the dissolved glycogen was added to a scintillation vial containing 9 ml PCS counting fluor. All counting was done in a liquid scintillation counter. Conversion of CPM to DPM was calculated as described above. The results were expressed as DPM/90 minutes/gram wet weight.

For chemical glycogen determinations, the remaining portion of the dissolved precipitate was hydrolyzed in 2 N HCl in a boiling water bath for 3 hours. The samples were neutralized (pH 6-8) with 5N NaOH and brought up to 10 ml with distilled water. An aliquot of the hydrolyzed glycogen was removed after neutralization and the glucose content of the solution was determined by an enzymatic method

(Glucostat, Worthington Biochemical Corp.; Glucose 510; Sigma Chemical Comp.). The results were expressed as mg glycogen/100 mg wet weight. This permitted calculation of the glycogen specific activity which was expressed as DPM/ mg glycogen.

H. Statistical Analysis.

The unpaired Student's t-test was used to test for significant differences between the injured and non-injured rats. A P value of less than 0.05 was considered to indicate a significant difference between the two groups. All results were expressed as means $\pm$ SEM (N).

CHAPTER IV

RESULTS

A. General Response to Injury.

Symptoms of severe injury were readily apparent in the rats following their removal from the Noble-Collip drum. The rats were comatose and there was an intense pallor in their ears and feet. In most cases a slight amount of blood was found around the margin of the mouth and nose. The intensity of the initial pallor decreased in those rats which did not immediately succumb to the effects of the injury. The rats became more responsive as the initial effects of the anesthesia decreased. They assumed a hunched-over posture. Piloerection was invariably present.

In those rats which died as a result of the trauma, unconsciousness was present as the terminal period approached. Respiratory arrest, preceded by convulsions marked the final stages of shock. Post-mortem findings showed slight vascular dilatation of the gastrointestinal tract. Engorgement and congestion was primarily limited to the distal portion of the ileum and the upper portion

of the colon and cecum. The major portion of the small bowel appeared normal in color. Varying amounts of free fluid containing blood were usually found in the abdomen. In the thorax, the heart appeared normal. The lungs were usually normal or slightly engorged, but in some cases the engorgement was more marked, with small petechial hemorrhages present.

It is known that after NCD trauma and during subsequent days, food intake is reduced and body weight is lost (81). In order to control for differences in food intake, the rats were not permitted access to food during the course of the experiment. After an overnight fast, the weight of the rats was 115 $\pm$0.8 (325) grams. On the day after injury, the weight of the survivors was 108 $\pm$1.1 (167) grams. Concurrent fasting controls weighed 106 $\pm$ 1.0 (158) grams. The difference between the two groups was not significant. Therefore, the metabolic changes between the injured and non-injured rats is not due to the influence of reduced food intake and loss of body weight.

B. Temporal Relations of Plasma Glucose and Serum Insulin Following Traumatic Injury.

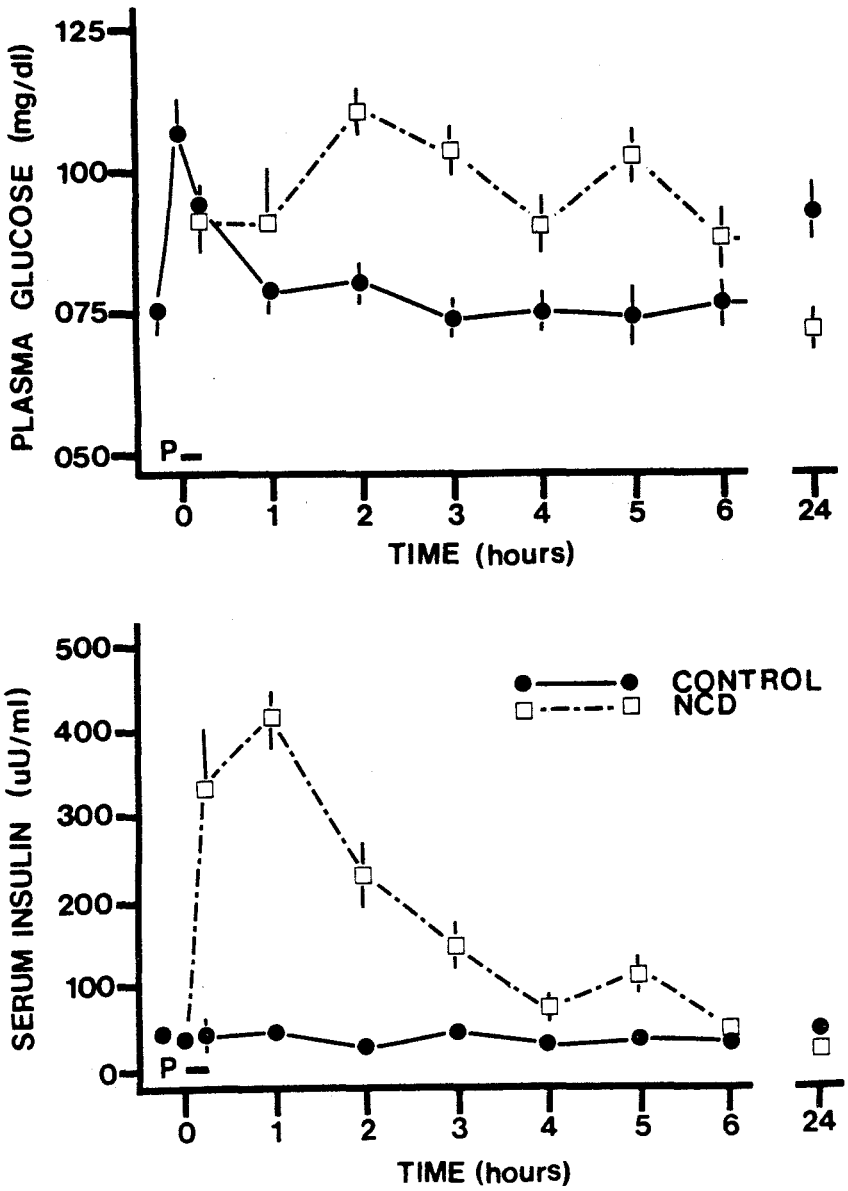
The time course of the changes that occur in the

plasma concentration of glucose and the serum immunoreactive insulin concentrations after Noble-Collip drum trauma are shown in Figure 1 and Table 1. Approximately 15 minutes before being placed in the Noble-Collip drum, overnight fasted rats were anesthetized with sodium pentobarbital (20 mg/kg i.m.). In response to the anesthesia, the plasma glucose rose from 76 $\pm$ 4.2 (10) mg/dl to 107 $\pm$ 6.4 (8) mg/dl at the time the trauma period would normally begin. At the end of a trauma period that produced 50% mortality at twenty-four hours with a survival time of less than 3 hours, the plasma glucose concentration of the injured rats was 91 $\pm$ 5.3 (11) mg/dl, which was not different ($P > 0.05$) from the 94 $\pm$ 3.7 (8) mg/dl present in non-injured rats.

While the plasma glucose concentration of the non-injured rats returned from the anesthesia induced hyperglycemia to control, fasting levels, the injured animals showed a prolonged phase of hyperglycemia. This reached a maximum concentration of 110 $\pm$ 3.6 (9) mg/dl at two hours after the initiation of the trauma period. On the day after injury, the plasma glucose concentration had fallen to 71 $\pm$ 3.2 (12) mg/dl in the injured animals. This was significantly lower than the plasma glucose concentration

FIGURE 1

PLASMA GLUCOSE AND SERUM INSULIN FOLLOWING



Overnight fasted, male rates were anesthetized with 20 mg/kg sodium pentobarbital (P) and then injured in the Noble-Collip drum (bar). Each value represents the mean ± SEM for 8-12 rats sacrificed at the indicated times.

TABLE 1

TEMPORAL RELATIONS OF PLASMA GLUCOSE AND SERUM INSULIN FOLLOWING TRAUMATIC INJURY

GROUP	TIME (HOURS)[a] 0.25[b]	0[c]	+0.25[d]	1	2	3	4	5	6	24
	PLASMA GLUCOSE (MG/DL)[a]									
CONTROL	76±4.2[e] (10)	107±6.4 (8)	94±3.7 (8)	79±3.4 (8)	80±3.4 (8)	74±3.3 (12)	75±3.3 (8)	74±4.9 (10)	76±3.7 (12)	92±4.7 (12)
NCD			91±5.3 (11)	91±9.3 (11)	110±3.6 (9)	103±4.2 (12)	90±4.6 (11)	102±4.6 (12)	87±5.0 (12)	71±3.2 (12)
			P>0.05[f]	P>0.05	P<0.001	P<0.001	P<0.025	P<0.001	P>0.05	P<0.005
	SERUM INSULIN (μU/ML)[a]									
CONTROL	44±5.1 (10)	36±8.0 (8)	43±14.8 (8)	45±6.2 (8)	28±5.6 (8)	44±4.3 (12)	30±8.9 (8)	34±4.1 (10)	29±2.6 (12)	43±6.3 (12)
NCD			332±71.8 (3)	414±30.9 (11)	229±34.9 (9)	145±24.5 (12)	73±11.1 (11)	108±19.0 (12)	44±6.2 (12)	20±2.5 (12)
			P>0.05	P<0.001	P<0.001	P<0.005	P<0.05	P<0.005	P<0.05	P<0.005

[a]Overnight fasted, male rates were sacrificed at the indicated times. The plasma glucose and serum insulin determinations were made on the same blood samples.

TABLE 1, continued

[b]Unanesthetized rats.

[c]These rats were anesthetized with sodium pentobarbital (20 mg/kg i.m.) and sacrificed 15 minutes later. This corresponds to the time they would have otherwise been placed in the Noble Collip drum.

[d]This corresponds to the end of the trauma period.

[e]Each value represents the mean $\pm$ SEM (N).

[f]The P values are based on t-statistics for unpaired samples.

of 92 ± 4.7 (12) mg/dl ($P < 0.002$) found in the control animals.

The differences between the injured and non-injured animals are even more striking when their immunoreactive insulin concentrations are compared. The IRI concentration found in the rats fasted overnight was 44 ± 5.1 (10) μU/ml. At the beginning of the trauma period this was 36 ± 8.0 (8) μU/ml. No marked changes were observed in the IRI levels of the non-injured animals during the observation period. This is contrasted with the profound hyperinsulinemia found in the injured rats. The maximum IRI level of 414 ± 30.9 (11) μU/ml found at 1 hour after injury was significantly greater ($P < 0.001$) than the IRI level of 45 ± 6.2 (8) μU/ml in the control rats. By six hours after injury, the IRI concentration had decreased to 44 ± 6.2 (12) μU/ml, and although still significantly greater ($P < 0.05$) than the IRI concentration of 29 ± 2.6 (12) μU/ml found in time-matched controls, this is somewhat closer to normal concentrations. By one day after injury, the IRI concentration had declined to 20 ± 2.5 (12) μU/ml, significantly ($P < 0.005$) lower than the control level of 43 ± 6.3 (12) μU/ml at this time.

C. Glucose Tolerance and Insulin Response Following Noble-Collip Drum Injury.

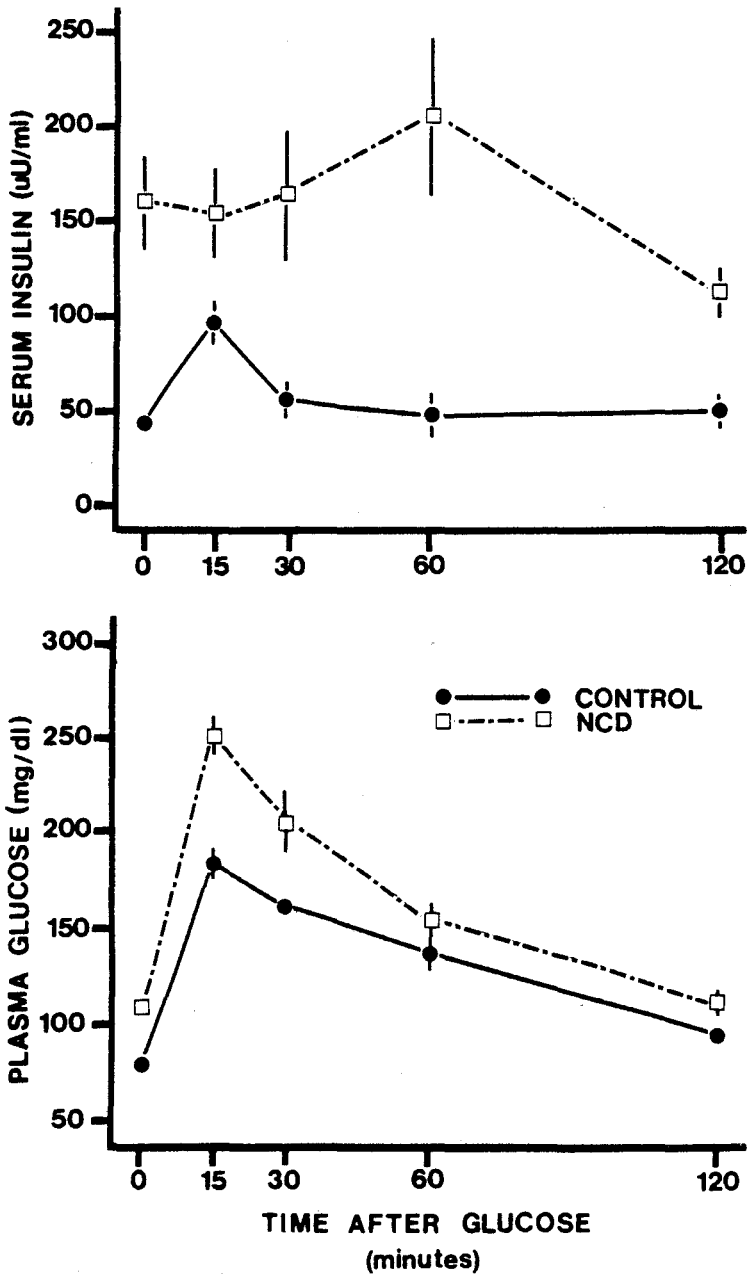
Plasma glucose and serum insulin concentrations before and after intraperitoneal injection of 1.0 g/kg D-glucose (in 15% w/v solution) are shown in Figures 2 and 3, as well as Tables 2 and 3. Preinjection glucose and insulin concentrations were significantly higher in the injured rats than in time-matched controls at three hours after injury (glucose, $P<0.001$; insulin, $P<0.001$). Treatment of the rats resulted in a prompt hyperglycemia which declined steadily during the next two hours. The injured rats responded to the glucose load with glucose intolerance; the plasma glucose concentration was significantly greater in the injured animals at all times following glucose administration.

Prior to the injection of glucose, the IRI concentration in the serum of the control animals was 43 ± 4.4 (14) μU/ml. In response to the hyperglycemic stimulus, the IRI concentration increased significantly to 97 ± 10.2 (8) μU/ml and then returned to control levels as the plasma glucose concentration fell. The injured rats did not respond to increased glucose levels with an increase in IRI levels.

FIGURE 2

GLUCOSE TOLERANCE AND INSULIN RESPONSE

AT 3 HOURS AFTER TRAUMATIC INJURY

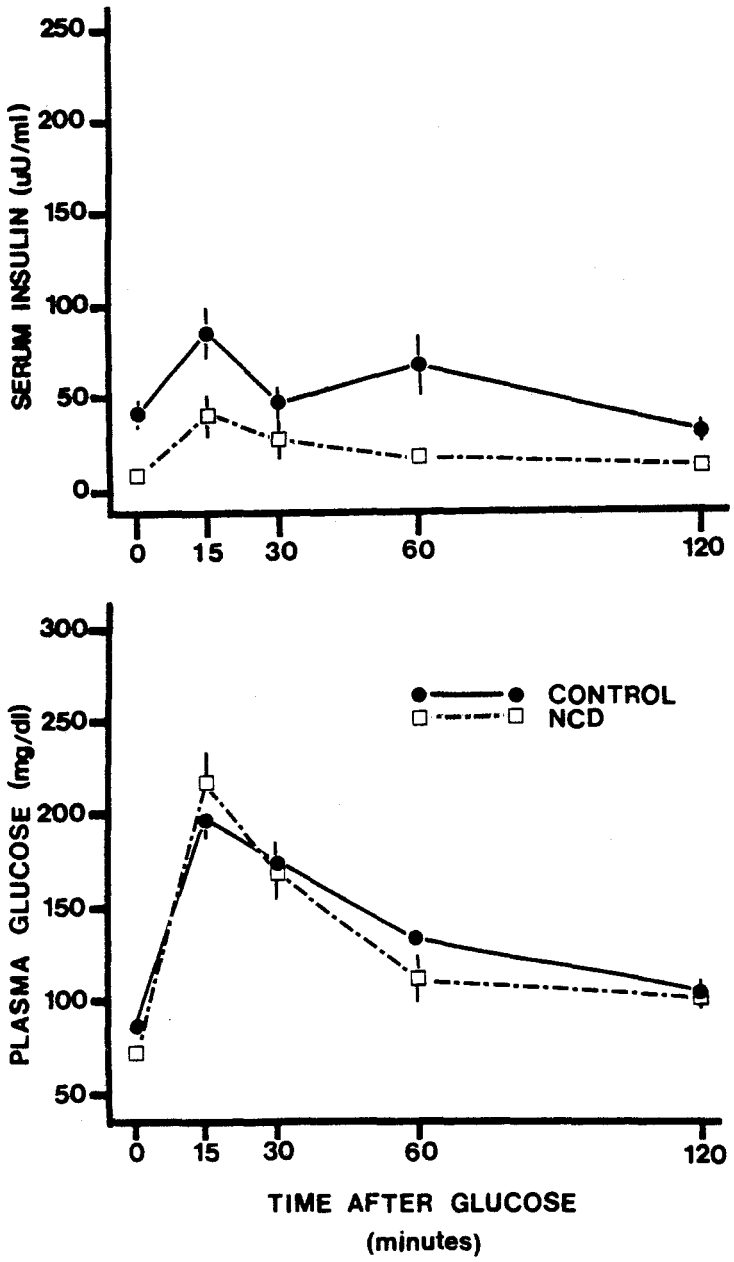


At 3 hours after injury (Time 0), rats were given 1 gm/kg glucose i.p. and sacrificed at the indicated times. Each value represents the mean ± SEM for 7-9 rats; 14 rats were used for the time 0 values.

FIGURE 3

GLUCOSE TOLERANCE AND INSULIN RESPONSE

AT 24 HOURS AFTER TRAUMATIC INJURY



On the day after injury (Time 0), rats were given 1 gm/kg glucose i.p. and sacrificed at the indicated times. Each value represents the mean ± SEM for 8-9 rats; 15 injured and 12 control rats were used for the Time 0 values.

TABLE 2

GLUCOSE TOLERANCE AND INSULIN RESPONSE AT

3 HOURS AFTER TRAUMATIC INJURY

	TIME (MINUTES)[a]				
	0	15	30	60	120
GROUP	PLASMA GLUCOSE (MG/DL)[b]				
CONTROL	78±3.5[c] (14)	184±6.2 (8)	161±3.1 (8)	136±6.9 (8)	93±2.2 (9)
NCD	109±2.5 (14)	252±8.9 (8)	205±14.9 (8)	154±7.2 (8)	111±4.9 (8)
	P<0.001[c]	P<0.001	P<0.025	P>0.05	P<0.01
	SERUM INSULIN (μU/ML)[b]				
CONTROL	43±4.4 (8)	97±10.3 (8)	57±9.2 (8)	48±10.2 (8)	50±7.3 (8)
NCD	161±23.3 (14)	154±22.6 (8)	164±32.8 (8)	206±39.5 (8)	112±11.6 (8)
	P<0.001	P<0.05	P<0.025	P<0.005	P<.001

[a]At three hours after injury (TIME 0) rats were given 1 gm/kg glucose i.p. and sacrificed at the indicated times.

[b]Plasma glucose and serum insulin determinations were made on the same blood samples.

[c]Each value represents the mean ± SEM (N).

[d]The P values are based on t statistics for unpaired samples.

TABLE 3

GLUCOSE TOLERANCE AND INSULIN RESPONSE

AT 24 HOURS AFTER TRAUMATIC INJURY

	TIME (MINUTES)[a]				
	0	15	30	60	120
GROUP	PLASMA GLUCOSE (MG/DL)[b]				
CONTROL	88±4.9[c] (12)	198±7.7 (10)	173±9.4 (8)	133±2.8 (8)	103±4.4 (8)
NCD	73±2.6 (15)	218±13.1 (8)	170±13.1 (9)	112±11.3 (8)	101±3.9 (8)
	P<0.05[d]	P>0.05	P>0.05	P>0.05	P>0.05
	SERUM INSULIN (μU/ML)[b]				
CONTROL	41±5.9 (12)	85±12.4 (10)	48±7.4 (8)	61±15.2 (8)	31±4.8 (8)
NCD	9±0.8 (15)	41±9.7 (8)	26±9.6 (9)	17±3.3 (8)	13±2.0 (6)
	P<0.001	P<0.025	P>0.05	P<0.025	P<0.01

[a]On the day after injury (TIME 0) rats were given 1 gm/kg glucose i.p. and sacrified at the indicated times.

[b]Plasma glucose and serum insulin determinations were made on the same blood samples.

[c]Each value represents the mean ± SEM (N).

[d]The P values are based on t-statistics for unpaired samples.

At one day after injury, the preinjection glucose concentration of the surviving injured rats was 72 ± 2.7 (15) mg/dl. This was significantly ($P<0.025$) lower than the 88±4.9 (12) mg/dl glucose concentration in the non-injured animals. The intraperitoneal injection of glucose resulted in a prompt hyperglycemia which steadily declined within two hours to a plateau of 100 mg/dl in both groups. At no time was there any significant difference in the plasma glucose concentration between the injured and non-injured rats.

The insulin response of the control rats to a glucose stimulus was the same at one day after injury as it three hours. In response to the elevated plasma glucose concentration, the IRI rose significantly ($P<0.01$) from a preinjection level of 41 ± 5.9 (12) μU/ml to 85 ± 12.4 (10) μU/ml. The basal immunoreactive insulin concentration of animals that had been subjected to Noble-Collip drum trauma was 9 ± 0.8 (12) μU/ml. This increased significantly ($P<0.025$) to 41 ± 9.7 (8) μU/ml after injection of glucose. The IRI concentration of the injured animals was lower than that of the non-injured controls at all times, except at the 30 minute sampling period.

D. Insulin Tolerance and Glucose Response Following Noble-Collip Drum Injury.

The hypoglycemic action of crystalline bovine insulin was tested in rats at either three hours or one day after injury in the Noble-Collip drum. The plasma glucose response of the injured rats to the subcutaneous administration of insulin (750 mU/kg) was compared with that of time-matched, non-injured rats. The plasma glucose and serum IRI concentrations in blood collected from rats decapitated at 15 minute intervals after insulin injection are shown in Figures 4 and 5, as well as Tables 4 and 5.

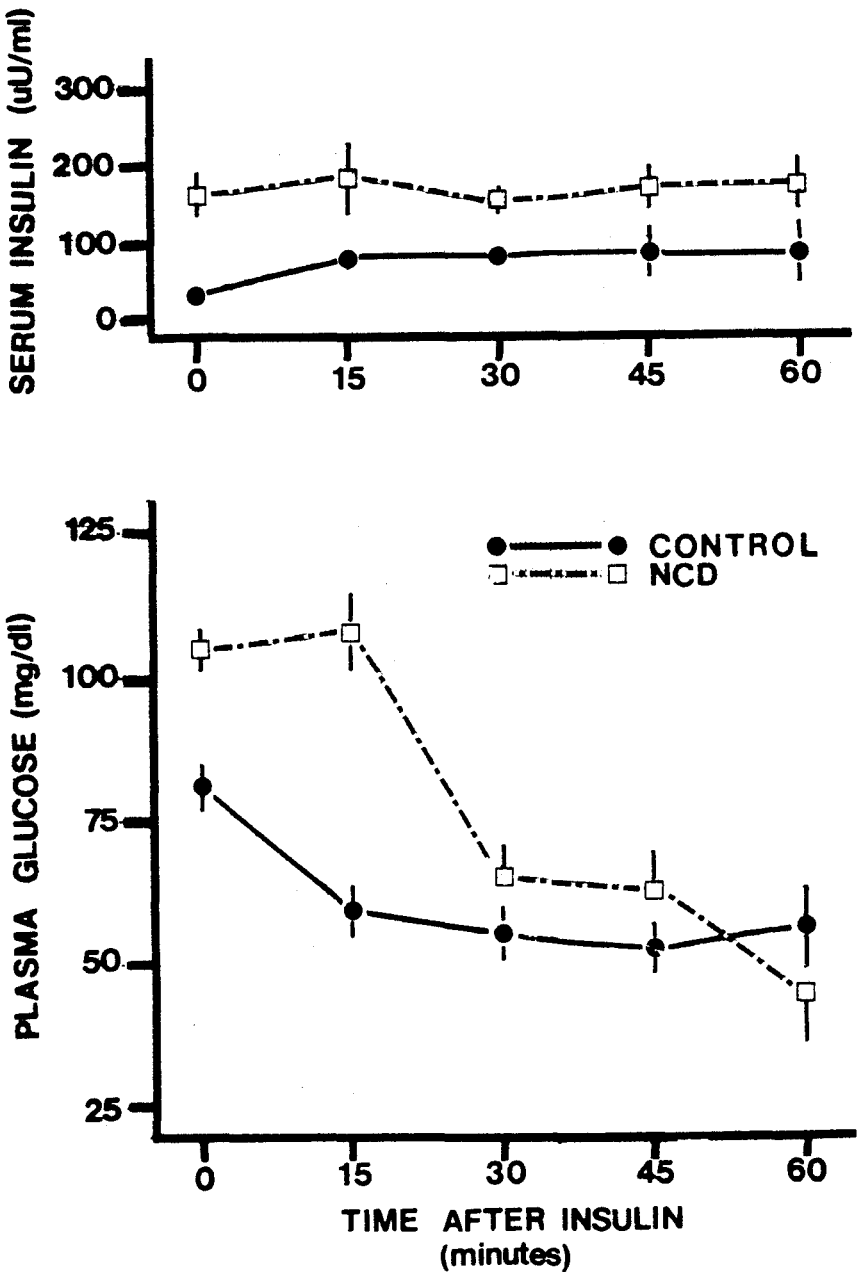
Pre-injection (Time 0) glucose and insulin concentrations were significantly higher in the injured rats than in time-matched controls at three hours after injury (glucose, $P<0.001$; insulin, $P<.001$). The insulin injection increased ($P<0.01$) the IRI concentration of the non-injured rats from 31 $\pm$ 3.2 (13) µU/ml to 80 $\pm$ 14.0 (10) µU/ml within 15 minutes. This increase in insulin caused a prompt fall in plasma glucose. The preinjection concentration was 81 $\pm$ 4.0 (14) mg/dl. This fell significantly ($P<0.002$) to 59 $\pm$ 4.1 (10) mg/dl by the first sampling period.

The onset of the hypoglycemic action of insulin

FIGURE 4

GLUCOSE RESPONSE TO INSULIN AT 3 HOURS

AFTER TRAUMATIC INJURY

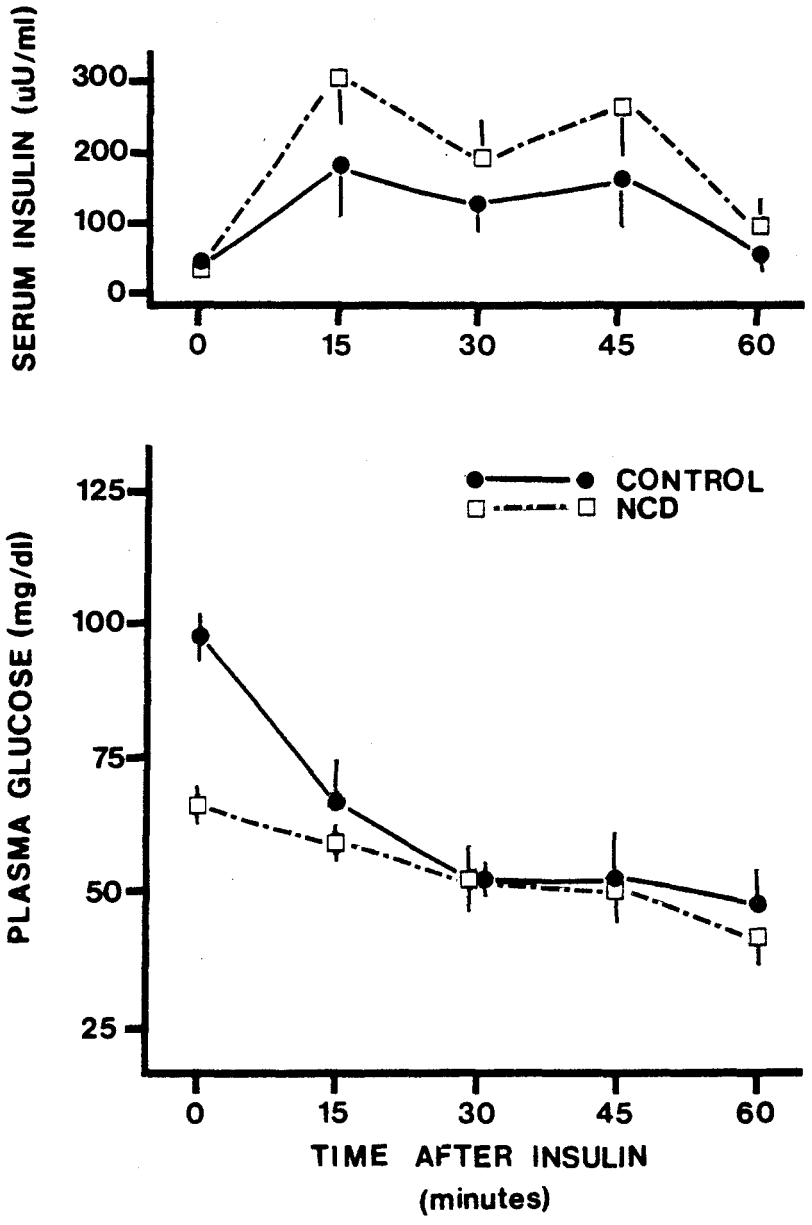


At 3 hours after injury (Time 0), rats were given 750 mU/kg insulin s.c. and sacrificed at the indicated times. Each value represents the mean ± SEM for 7-14 rats.

FIGURE 5

GLUCOSE RESPONSE TO INSULIN AT 24 HOURS

AFTER TRAUMATIC INJURY



On the day after injury (Time 0), rats were given 750 mU/kg insulin s.c. and sacrificed at the indicated times. Each value represents the mean $\pm$ SEM for 7-11 rats.

TABLE 4

GLUCOSE RESPONSE TO INSULIN AT 3 HOURS

AFTER TRAUMATIC INJURY

	TIME (MINUTES)[a]				
	0	15	30	45	60
GROUP	PLASMA GLUCOSE (MG/DL)[b]				
CONTROL	84±4.0[c] (14)	59±4.1 (10)	55±4.4 (8)	52±4.1 (7)	55±7.0 (8)
NCD	105±3.5 (13)	108±6.6 (9)	65±5.7 (11)	62±7.0 (9)	44±3.5 (9)
	P<0.001[d]	P<0.001	P>0.05	P>0.05	P>0.05
	SERUM INSULIN (μU/ML)[b]				
CONTROL	31±3.3 (13)	80±14.0 (10)	79±13.7 (8)	87±32.6 (7)	86±41.0 (8)
NCD	163±28.0 (13)	186±45.2 (9)	154±14.8 (11)	170±27.3 (9)	174±53.6 (9)
	P<0.001	P<0.05	P<0.005	P>0.05	P>0.05

[a]At three hours after injury (Time 0) rats were given 750 mU/kg insulin s.c. and sacrificed at the indicated times.

[b]Plasma glucose and serum insulin determinations were made on the same blood samples.

[c]Each value represents the mean ± SEM (N).

[d]The P values are based on t-statistics for unpaired samples.

TABLE 5

GLUCOSE RESPONSE TO INSULIN AT 24 HOURS

AFTER TRAUMATIC INJURY

	TIME (MINUTES)[a]				
	0	15	30	45	60
GROUP	PLASMA GLUCOSE (MG/DL)[b]				
CONTROL	100±4.5[c] (7)	69±8.0 (8)	55±2.6 (10)	55±8.4 (9)	50±6.3 (8)
NCD	68±3.4 (8)	61±3.2 (9)	55±6.1 (8)	53±5.7 (9)	44±5.5 (9)
	P<0.001[d]	P>0.05	P>0.05	P>0.05	P>0.05
	SERUM INSULIN (μU/ML)[b]				
CONTROL	37±8.1 (7)	174±69.7 (8)	123±35.3 (10)	162±65.6 (9)	59±21.1 (8)
NCD	22±4.4 (8)	297±66.5 (8)	188±55.3 (7)	266±74.0 (9)	99±40.5 (9)
	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

[a]On the day after injury (Time 0) rats were given 750 mU/kg insulin s.c. and sacrificed at the indicated times.

[b]Plasma glucose and serum insulin determinations were made on the same blood samples.

[c]Each value represents the mean ± SEM (N).

[d]The P values are based on t-statistics for unpaired samples.

was delayed in the injured rats. Rats which had been injured 3 hours prior to the insulin injection did not show a significant decrease in plasma glucose until the 30 minute sampling period when the plasma glucose had fallen to 65 $\pm$ 5.7 (11) mg/dl from an initial level of 105 $\pm$ 3.5 (13) mg/dl ($P<0.001$).

At one day after injury, the preinjection glucose concentration of the surviving rats was 68 $\pm$ 3.4 (8) mg/dl. The plasma glucose concentration of the non-injured control rats was 100 $\pm$ 4.5 (7) mg/dl (7). The difference between the two groups was significantly different. As exogenous insulin was absorbed, the IRI concentration increased from 37 $\pm$ 8.1 (7) µU/ml to 174 $\pm$ 69.7 (8) µU/ml ($P>0.05$) in the control rats. This resulted in a significant ($P<0.01$) fall in plasma glucose from the preinjection level of 100 $\pm$ 4.5 (7) mg/dl to 69 $\pm$ 8.0 (8) mg/dl by the first sampling period. Although the IRI concentration in the surviving rats increased from a preinjection concentration of 22 $\pm$ 4.4 (8) µU/ml to 297 $\pm$ 66.5 (8) µU/ml ($P>0.05$), the plasma glucose did not fall. Forty-five minutes were required before the plasma glucose fell from 68 $\pm$ 3.4 (8) mg/dl to 53 $\pm$ 5.7 (8) mg/dl ($P<0.05$).

E. Insulin Sensitivity of Skeletal Muscle and Adipose Tissue In Vitro.

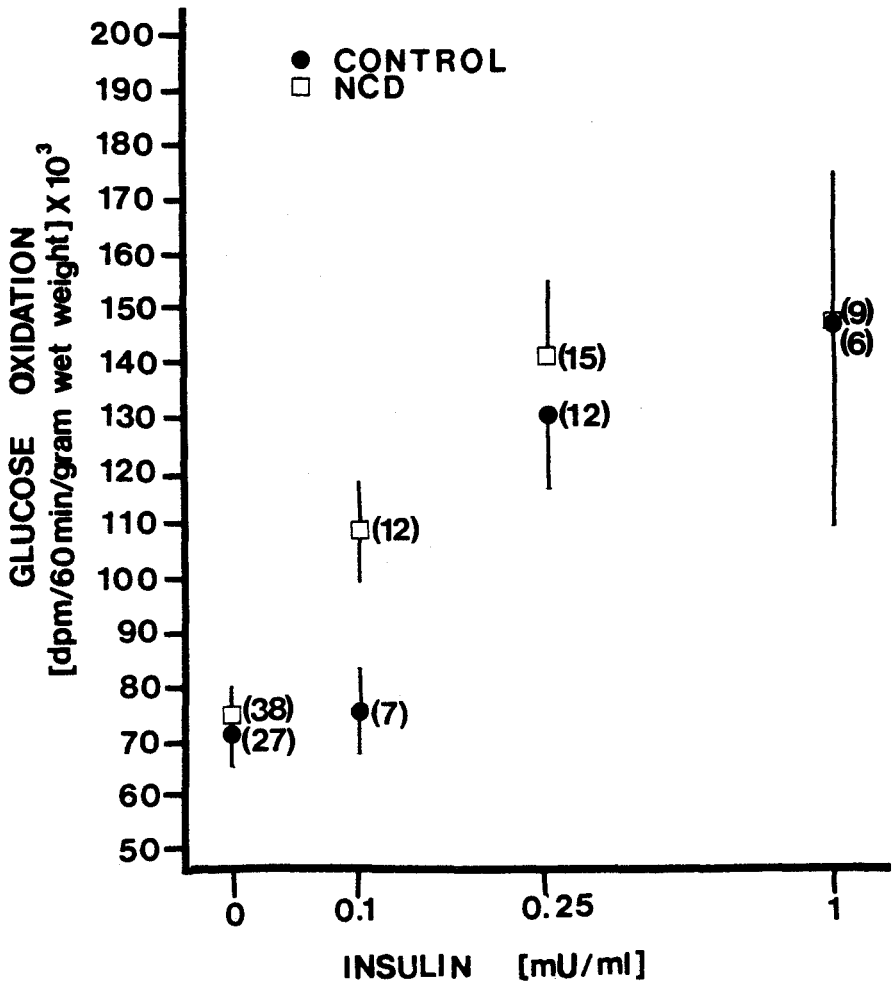
The sensitivity and response of skeletal muscle and adipose tissue to bovine crystalline insulin was assessed by testing in vitro the effect of insulin on the incorporation of radioactive glucose into glycogen by hemidiaphragms and by measuring the production of $^{14}CO_2$ from radioactive glucose by episidymal fat pads incubated in the presence or absence of insulin. The results of these experiments are depicted in Figures 6, 7, 8 and 9, as well as Tables 6, 7, and 8.

At 3 hours after injury in the Noble-Collip drum, the epididymal fat pads from injured rats produced $^{14}CO_2$ at a rate of 74, 679 $\pm$ 4766 (38) DPM/hr/gram wet weight when incubated in the absence of insulin. This was not different ($P>0.05$) from the 70, 891 $\pm$ 5950 (27) DPM/hr/gram wet weight produced by fat pads from control rats. When insulin was included in the incubation medium, fat pads from both injured and non-injured rats increased their production of CO_2. At an insulin concentration of 0.1 mU/ml, the glucose oxidation of fat pads from injured rats was 108, 395 $\pm$ 31, 174 (12) DPM/hr/gram wet weight. This was significantly greater ($P<0.025$) than the

FIGURE 6

INSULIN SENSITIVITY OF ADIPOSE TISSUE

3 HOURS AFTER TRAUMATIC INJURY

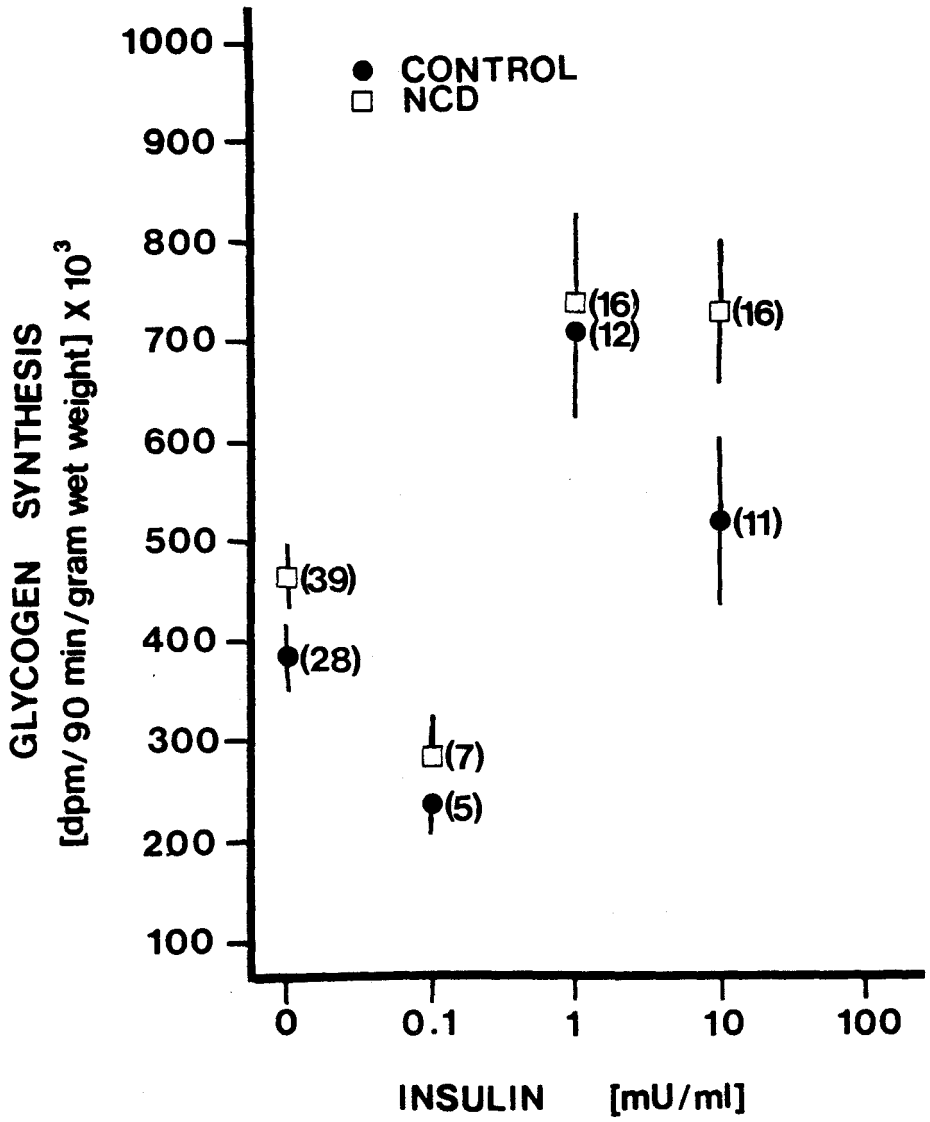


Epididymal fat pads were removed from rats at 3 hours after injury and incubated *in vitro* at the indicated insulin concentrations. The production of $^{14}CO_2$ from ^{14}C-U-glucose was measured as described in the Methods section. Each value represents the mean ± SEM (N).

FIGURE 7

INSULIN SENSITIVITY OF SKELETAL MUSCLE

3 HOURS AFTER TRAUMATIC INJURY

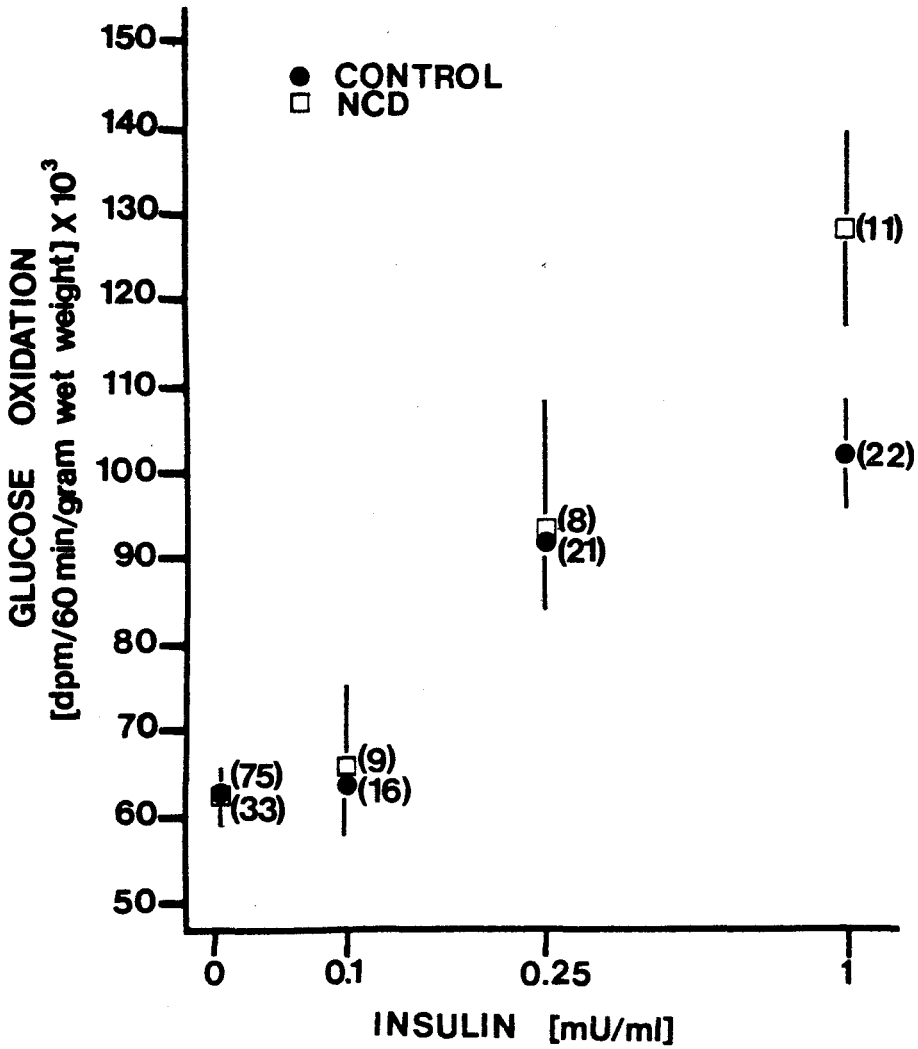


Hemidiaphragms were removed from rats at 3 hours after injury and incubated in vitro at the indicated insulin concentrations. The incorporation of ^{14}C into glycogen was measured as described in the Methods section. Each value represents the mean ± SEM (N).

FIGURE 8

INSULIN SENSITIVITY OF ADIPOSE TISSUE

24 HOURS AFTER TRAUMATIC INJURY

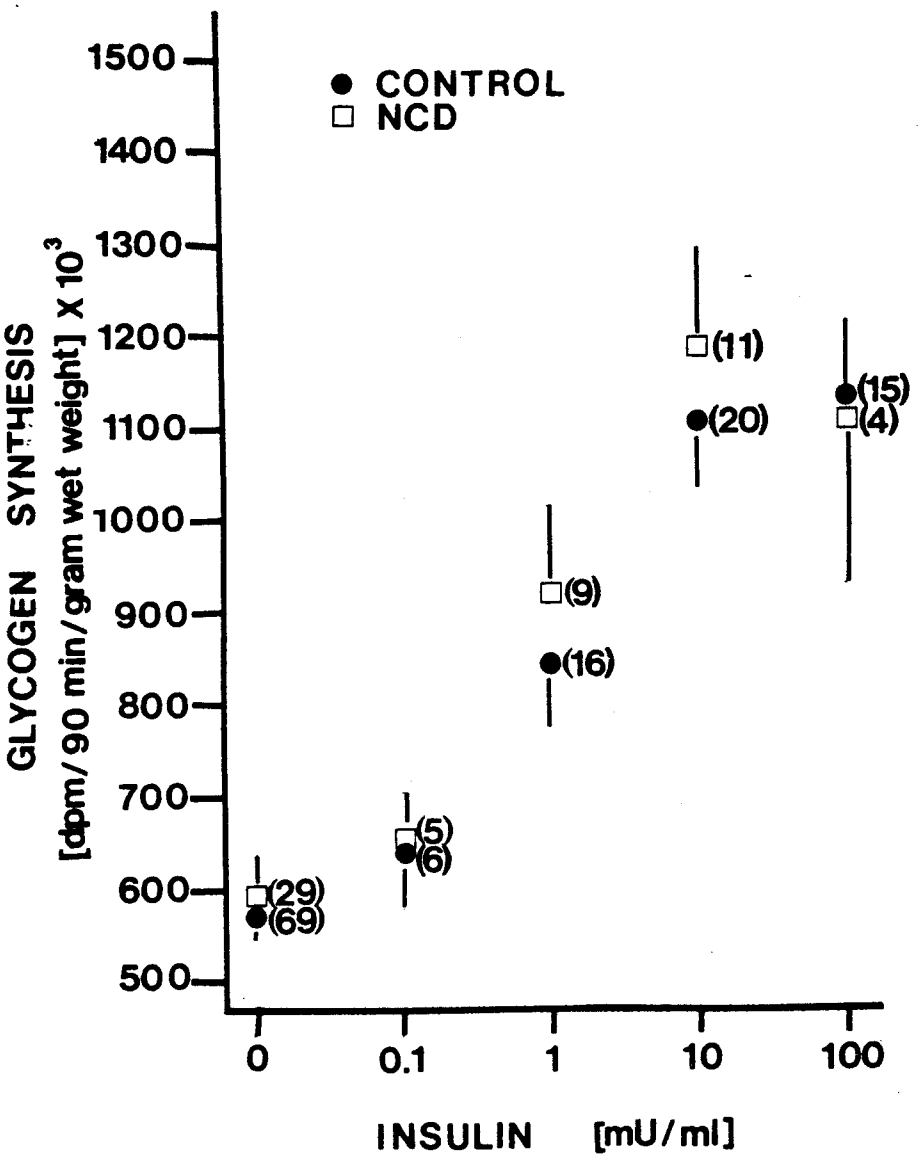


Epididymal fat pads were removed from rats on the day after injury and incubated *in vitro* at the indicated insulin concentrations. The production of $^{14}CO_2$ from ^{14}C-U-glucose was measured as described in the Methods section. Each value represents the mean ± SEM (N).

FIGURE 9

INSULIN SENSITIVITY OF SKELETAL MUSCLE

24 HOURS AFTER TRAUMATIC INJURY



Hemidiaphragms were removed from rats on the day after injury and incubated in vitro at the indicated insulin concentrations. The incorporation of ^{14}C into glycogen was measured as described in the Methods section. Each value represents the mean ± SEM (N).

TABLE 6

INSULIN SENSITIVITY OF ADIPOSE TISSUE AT 3 HOURS AND 24 HOURS AFTER INJURY IN THE NOBLE-COLLIP DRUM

GROUP	INSULIN CONCENTRATION			
	0mU/ml	0.1mU/ml	0.25mU/ml	1mU/ml
	CONVERSION OF U-^{14}C-GLUCOSE TO $^{14}CO_2$			
	DPM $^{14}CO_2$/HOUR/GRAM WET WEIGHT			
Control at 3 Hours	70891 ±5950 (27)	74919 ±7338 (7)	130013 ±13149 (12)	146652 ±34198 (6)
NCD at 3 Hours	74679 ±4766 (38)	108395 ±8999 (12)	140706 ±13132 (15)	147672 ±25355 (9)
	NS	P<0.025	NS	NS
Control at 24 Hours	63072 ±2621 (75)	63057 ±5720 (16)	91048 ±7720 (21)	101364 ±5909 (22)
NCD at 24 Hours	62637 ±3361 (33)	65072 ±9466 (9)	92686 ±13884 (8)	127568 ±10497 (11)
	NS	NS	NS	P<0.05

Epididymal fat pads were removed from control and injured (NCD) rats at either 3 hours or 24 hours after injury in the Noble Collip drum and incubated at the indicated insulin concentrations. The production of $^{14}CO_2$ from ^{14}C-U-glucose was measured as described in the Methods. Each value represents the mean ± SEM (N). P values were determined by unpaired t-tests between each group; NS indicates $P > 0.05$.

TABLE 7

INSULIN SENSITIVITY OF SKELETAL MUSCLE IN VITRO AT 3 HOURS AFTER INJURY IN THE NOBLE-COLLIP DRUM

GROUP	INSULIN CONCENTRATION			
	0mU/ml	0.1mU/ml	1.0mU/ml	10mU/ml
	GLYCOGEN SYNTHESIS DPM/90 MIN/GRAM WET WEIGHT			
Control	383480 ±32375 (28)	237930 ±27993 (5)	708260 ±78741 (12)	520160 ±80923 (11)
NCD	462870 ±31106 (39)	284410 ±37968 (7)	737660 ±86711 (16)	729840 ±70167 (16)
	NS	NS	NS	NS
	GLYCOGEN CONTENT Mg GLYCOGEN/100MG WET WEIGHT			
Control	0.21 ±.017 (28)	0.14 ±.292 (5)	0.35 ±.026 (12)	0.23 ±.031 (10)
NCD	0.18 ±.013 (39)	0.23 ±.117 (7)	0.32 ±.024 (16)	0.22 ±.027 (16)
	NS	NS	NS	NS
	GLYCOGEN SPECIFIC ACTIVITY DPM/MG GLYCOGEN			
Control	200660 ±16334 (28)	204750 ±43553 (5)	197860 ±8095 (12)	225430 ±17454 (10)

Continued

Table 7, continued

	OmU/ml	0.1mU/ml	1.0mU/ml	10mU/ml
NCD	266870	240480	231920	354630
	±9.67	±40415	±17056	±18304
	(39)	(7)	(16)	(16)
	P<0.001	NS	NS	P<0.001

Hemidiaphragms were removed from control and injured (NCD) rats at 3 hours after injury in the Noble Collip drum and incubated at the indicated insulin concentrations. Glycogen synthesis was measured by determining the incorporation of ^{14}C-glucose into glycogen as described in the Methods. Total glycogen was determined and the glycogen specific activity calculated. Each value represents the mean ± SEM (N). P values were determined by unpaired t-tests; NS indicates P>0.05.

TABLE 8

INSULIN SENSITIVITY OF SKELETAL MUSCLE IN VITRO AT 24 HOURS AFTER INJURY IN THE NOBLE-COLLIP DRUM

GROUP	INSULIN CONCENTRATION				
	0mU/ml	0.1mU/ml	1.0mU/ml	10mU/ml	100mU/ml
	GLYCOGEN SYNTHESIS DPM/90 MIN/GRAM WET WEIGHT				
Control	569830 ±23230 (69)	636970 ±56498 (6)	841480 ±67426 (16)	1104028 ±68593 (20)	1131715 ±75096 (15)
NCD	591390 ±41006 (29)	651260 ±44160 (5)	917780 ±93183 (9)	1184884 ±100126 (11)	1106178 ±155270 (4)
	NS	NS	NS	NS	NS
	GLYCOGEN CONTENT MG GLYCOGEN/100 MG WET WEIGHT				
Control	0.26 ±.012 (69)	0.30 ±0.17 (6)	0.33 ±.035 (16)	0.43 ±.030 (20)	0.47 ±.044 (15)
NCD	0.37 ±.023 (29)	0.35 ±.033 (5)	0.50 ±.046 (9)	0.62 ±.044 (11)	0.59 ±.091 (4)
	P<0.001	NS	P<0.01	P<0.005	NS
	GLYCOGEN SPECIFIC ACTIVITY DPM/MG GLYCOGEN				
Control	288050 ±20562 (69)	212270 ±11246 (6)	299740 ±43416 (16)	283500 ±51083 (20)	264000 ±23042 (15)

Continued

Table 8, continued

	0mU/ml	0.1mU/ml	1.0mU/ml	10mU/ml	100mU/ml
NCD	170570 ±13999 (29)	191200 ±10438 (5)	181650 ±11191 (9)	196340 ±16535 (11)	189340 ±3408 (4)
	P<0.001	NS	P<0.025	P<0.025	P<0.025

Hemidiaphragms were removed from control and injured rats (NCD) at 24 hours after injury in the Noble Collip drum and incubated at the indicated insulin concentrations. Glycogen synthesis was measured by determining the incorporation of ^{14}C-glucose into glycogen as described in the Methods. Total glycogen was determined and the glycogen specific activity calculated. Each value represents the mean ± SEM (N). P values were determined by unpaired t-tests; NS indicates P>0.05.

74,919 ± 7,338 (7) DPM/hr/gram wet weight produced by the fat pads from control animals.

Glycogen synthesis was assessed by measuring the incorporation of U-^{14}C-glucose into glycogen. The response of the hemidiaphragms to increasing amounts of insulin was not particularly striking in this series of experiments, primarily because of day-to-day variation in the response of the diaphragms to the incubation. What is immediately apparent though, is that diaphragms from both injured and control rats are able to synthesize glycogen at the same rate. There was no significant difference ($P > 0.05$) between the two groups of rats at any concentration of insulin.

In a separate series of experiments, the *in vitro* sensitivity of muscle and adipose tissue was tested at 24 hours after injury in the Noble-Collip drum. The epididymal fat pads from non-injured rats produced $^{14}CO_2$ at a rate of 63,072 ± 2621 (75) DPM/hr/gram wet weight. This was not different ($P<0.05$) from the 62,637 ± 3361 (33) DPM/hr/gram wet weight produced by fat pads from the injured rats. When insulin was included in the incubation medium, fat pads from both injured and non-injured rats increased their production of CO_2. At an insulin

concentration of 1.0 mU/ml, the glucose oxidation of fat pads from injured rats was 127,568 ± 10,497 (11) DPM/hr/gram wet weight. This was significantly greater ($P<0.05$) than the 101,364 ± 5909 (22) DPM/hr/gram wet weight produced by the fat pads from the control animals.

Glycogen synthesis by hemidiaphragms from both injured and control animals showed an increase in a manner dependent on the concentration of insulin in the incubation medium. The rate of glycogen synthesis was not significantly different ($P>0.05$) in both groups at any concentration of insulin.

The above experiments were not performed simultaneously. This does not permit valid comparisons to be made between the results of both control and injured groups obtained at 3 hours after injury with those obtained at 1 day after injury. Certain portions of the above experiments were repeated in an attempt to confirm the reproducibility of the results as well as to permit comparisons between the two time periods. The results of these experiments are shown in Tables 9, 10, and 11. In addition to measuring the incorporation of glucose into glycogen by hemidiaphragms, the response of soleus muscles to insulin and different concentrations of glucose was also tested.

TABLE 9

INSULIN SENSITIVITY OF ADIPOSE TISSUE AT DIFFERENT GLUCOSE CONCENTRATIONS

GROUP	GLUCOSE CONCENTRATION 1mg/ml		2mg/ml		3mg/ml	
	INSULIN CONCENTRATION 0mU/ml	1mU/ml	0mU/ml	1mU/ml	0mU/ml	1mU/ml
	GLUCOSE OXIDATION µmoles Glucose Converted to CO_2/Hour/Gram Wet Weight					
Control at 3 Hours	0.030 ±.0060 (8)	0.089 ±.0210 (8)	0.066 ±.0079 (8)	0.191 ±.0244 (8)	0.092 ±.0092 (8)	0.180 ±.0103 (8)
NCD at 3 Hours	0.039 ±.0087 (9)	0.092 ±.0149 (9)	0.066 ±.0078 (8)	0.137 ±.0167 (8)	0.084 ±.0066 (9)	0.189 ±.0251 (9)
	NS	NS	NS	NS	NS	NS
Control at 24 Hours	0.031 ±.0021 (8)	0.067 ±.0042 (8)	0.038 ±.0027 (8)	0.082 ±.0081 (8)	0.048 ±.0052 (8)	0.092 ±.0088 (8)
NCD at 24 hours	0.040 ±.0039 (9)	0.077 ±.0038 (9)	0.043 ±.0076 (9)	0.105 ±.0100 (9)	0.047 ±.0096 (9)	0.0892 ±.0062 (9)

Table 9, continued

	0mU/ml	1mU/ml	0mU/ml	1mU/ml	0mU/ml	1mU/ml
	NS	NS	NS	NS	NS	NS

Epididymal fat pads were removed from control and injured (NCD) rats at either 3 hours or 24 hours after injury in the Noble Collip drum and incubated *in vitro*. The glucose concentration and insulin concentration of the medium were varied independently. In all cases the medium contained 0.5μC/ml ^{14}C-U-glucose. Each value represents the mean ± SEM (N). P values were determined from unpaired t-tests; NS indicates P>0.05.

TABLE 10

INSULIN SENSITIVITY OF DIAPHRAGMS AT DIFFERENT GLUCOSE CONCENTRATIONS

GROUP	GLUCOSE CONCENTRATION 1mg/ml		2mg/ml		3mg/ml	
	INSULIN CONCENTRATION 0mU/ml	10mU/ml	0mU/ml	10mU/ml	0mU/ml	10mU/ml
	GLYCOGEN SYNTHESIS DPM/90 Min/Gram Wet Weight					
Control at 3 Hours	340605 ±21088 (8)	560924 ±69168 (8)	510299 ±23064 (8)	746686 ±110817 (8)	616078 ±43031 (8)	911788 ±106807 (8)
NCD at 3 Hours	324179 ±59055 (9)	593504 ±16989 (9)	625651 ±27932 (8)	743072 ±60755 (8)	738328 ±32394 (9)	942533 ±65490 (9)
	NS	NS	P<0.01	NS	P<0.05	NS
Control at 24 Hours	271702 ±53871 (8)	618437 ±129994 (8)	442367 ±76547 (8)	772639 ±128261 (8)	623839 ±38041 (8)	950009 ±120161 (8)
NCD at 24 Hours	344052 ±40371 (9)	500877 ±72347 (9)	526311 ±21419 (9)	823408 ±74348 (9)	617673 ±79617 (9)	788664 ±105395 (9)

Table 10, continued

	0mU/ml	10mU/ml	0mU/ml	10mU/ml	0mU/ml	10mU/ml
	NS	NS	NS	NS	NS	NS

Hemidiaphragms were removed from control and injured (NCD) rats at eigher 3 hours or 24 hours after injury in the Noble Collip drum and incubated in vitro. The glucose concentration and insulin concentration of the medium were varied independently. The specific activity of the glucose was 0.166 uC ^{14}C-U-glucose/mg D-glucose. Each value represents the mean ± SEM (N). P values were determined from unpaired t-tests; NS indicates P>0.05.

TABLE 11

INSULIN SENSITIVITY OF SOLEUS MUSCLE AT DIFFERENT GLUCOSE CONCENTRATIONS

GROUP	GLUCOSE CONCENTRATION 1mg/ml		2mg/ml		3mg/ml	
	INSULIN CONCENTRATION 0mU/ml	10mU/ml	0mU/ml	10mU/ml	0mU/ml	10mU/ml
	GLYCOGEN SYNTHESIS DPM/90 Min/Gram Wet Weight					
Control at 3 Hours	135332 ±17227 (8)	366247 ±37399 (8)	188595 ±28747 (8)	430948 ±98003 (8)	143306 ±27148 (8)	565373 ±70575 (8)
NCD at 3 Hours	120418 ±21503 (9)	365791 ±54718 (9)	145254 ±25229 (8)	360317 ±74708 (8)	178188 ±19925 (9)	484109 ±48535 (9)
	NS	NS	NS	NS	NS	NS
Control at 24 Hours	135786 ±12520 (8)	298848 ±48797 (8)	203726 ±21164 (8)	444515 ±62118 (8)	207181 ±19644 (8)	513489 ±77931 (8)
NCD at 24 Hours	134356 ±24360 (9)	331262 ±23723 (9)	250857 ±47259 (9)	392817 ±76441 (9)	288226 ±34028 (9)	646743 ±64318 (8)

Table 11, continued

	0mU/ml	10mU/ml	0mU/ml	10mU/ml	0mU/ml	10mU/ml
	NS	NS	NS	NS	NS	NS

Soleus muscles were removed from control and injured (NCD) rats at either 3 hour or 24 hours after injury in the Noble Collip drum and incubated *in vitro*. The glucose concentration and insulin concentration of the medium were varied independently. The specific activity of the glucose was 0.166 μC ^{14}C-U-glucose/mg D-glucose. Each value represents the mean ± SEM (N). P values were determined from unpaired t-tests; NS indicates $P>0.05$.

These experiments showed that the conversion of glucose to CO_2 by adipose tissue and the synthesis of glycogen by muscle increased with increasing amounts of glucose in the medium. Tissues from injured animals functioned in the same fashion as tissues from non-injured animals.

CHAPTER V

DISCUSSION AND CONCLUSIONS

The present studies were undertaken to assess the role played by insulin and the sensitivity of the peripheral tissues to insulin in the regulation of plasma glucose after injury. The experiments conducted were designed to provide answers to the following questions:

1. Is the effectiveness of insulin in lowering plasma glucose decreased following injury?

2. Do adipose tissue and skeletal muscle show a decreased sensitivity to the effects of insulin *in vitro* after injury?

The ensuing discussion will relate the findings of this study to the results of previous investigations and will focus on providing an answer to each of the primary questions.

A. General Response to Injury.

The injuries sustained by anesthetized male rates are qualitatively similar to those described by other

investigators utilizing the Noble-Collip drum (28; 118; 129; 133). In unanesthetized animals, Noble and Collip (118) reported free hemorrhage present in less than 1% of the cases. When anesthetized with sodium pentobarbital (19-26 mg/kg) half the rats showed intra-abdominal hemorrhage. In the present study 38% of the rats showed evidence of intra-abdominal hemorrhage if they died in less than two hours. The amount free fluid in the abdomen varied from a thin film covering the organs to large amounts. By using an amount of NCD trauma that produced 50% mortality and waiting for three hours, by which time the injured animals would be considered survivors, the role of hemorrhage was minimized.

B. Plasma Glucose Levels After Injury.

The data depicted in Figure 1 demonstrated that induction of anesthesia with sodium pentobarbital produced hyperglycemia (5; 58). The immunoreactive insulin levels did not rise in response to the glucose stimulus. Aynsley-Green, et al. (5) reported a 40% fall in insulin during induction of anesthesia with sodium pentobarbital. The failure of insulin levels to increase in response to the glycemic stimulus was probably the result of barbiturate

depression of transmission in parasympathetic ganglia (145; 181).

The results of this study indicated that immediately after tumbling injury in the Noble-Collip drum, the plasma glucose concentration of the injured animals was not different from control animals. There did not appear to be any increase of glucose utilization while the animals were actually being injured. The changes in plasma glucose levels following injury are dependent on the feeding state of the animals. Fasted animals usually respond to an injury with hypoglycemia (96; 163). The use of pentobarbital as an anesthetic also exerts an influence on the changes in plasma glucose found after injury. Vigas and Nemeth (162) reported the plasma glucose level decrease significantly in fasted, conscious traumatized animals, but remained unchanged in animals anesthetized with pentobarbital.

The injured rats were capable of producing a significant elevation of plasma glucose. The slow onset of the hyperglycemia as well as the known depletion of hepatic glycogen in overnight fasted rats (78; 104) suggests that the hyperglycemia was probably due to enhanced gluconeogenesis or depressed glucose utilization.

Vigas, et al. (167) measured the amounts and radioactivity of hepatic glycogen one hour after the administration of a mixture of unidentified, uniformally labeled ^{14}C amino acids. The little data they present does not permit any valid conclusion to be reached about the capability of rats to form glycogen from amino acids at 90 minutes after injury. Using a comparable severity of NCD injury, Filkins et al. (51) reported a diminished capacity of traumatized animals to conduct gluconeogenesis from alanine, lactate, pyruvate, or fructose.

The period immediately following Noble-Collip drum trauma is characterized by a depression of energy production. There is a consistent drop in temperature (28; 118) and oxygen consumption (103). Koltun and Gray (88) studied the effect of NCD trauma on the ability of individual organs to convert acetate-2-^{14}C to nonvolatile metabolites. They concluded that the ability of the liver, kidney, and muscle to metabolize acetate was impaired. Using Noble-Collip drum traumatized rats, Buchanan (19) obtained data demonstrating depression of the conversion of a tracer dose of U-^{14}C-glucose to $^{14}CO_2$. Therefore the increased plasma levels of glucose which occur in response to tumbling injury in the Noble-Collip drum are at least partly due to

depressed glucose utilization.

C. Serum Insulin Levels After NCD Injury.

The present study revealed a striking elevation of insulin levels in the injured rats. The insulin levels began to increase while the rats were actually being injured and continued to do so for at least one hour. The elevated insulin levels found in this study contrast with the reports of Vigas, Nemeth, and Jurcovicova (163, 164). Utilizing fed animals, they reported insulin levels of 30 to 40 μU/ml during the first 90 minutes of the post-injury hyperglycemic reaction. This group routinely rotated the drums used in their laboratory at 60 rpm. The total dose of trauma used was 400 revolutions, which does not substantially harm the animals (114; 166). The time for which the animals were exposed to the influence of tumbling was less than half the time used in the present study. As pentobarbital anesthesia substantially influences the metabolic response to Noble-Collip drum trauma, their experiments were conducted on conscious animals (162). The hyperinsulinemia found in the present study was probably a reflection of the different severity of injury used in this study.

It is not likely that the insulin levels of 30 to 40 μU/ml reported by Vigas et al. (165) is an artifact of the method they used to measure insulin. The method of insulin radioimmunoassay used in the present study was essentially that of Velasco et al. (160) and employed anti-insulin-antibody covalently coupled to Sephadex as originally developed by Wide and coworkers (171; 172). The major source of imprecision in radioimmunoassay arises from the inability of most separation procedures to separate completely bound and free labelled antigen. With respect to precision and freedom from separation-stage artifact, the solid coupled antibody system offers the great advantages of simplicity, speed and completeness of separation. Separation can be achieved with only a few minutes of centrifugation compared with 18 hours of incubation and subsequent centrifugation for the double-antibody system. Bolton and Hunter (17) evaluated this technique and compared it with others and suggested it to be the method of choice for a number of radioimmunoassay procedures.

The consistency of this technique was evident in the observation that the standard curves were nearly identical. The possibility that the elevated levels of immunoreactive insulin found in the injured rats were an

artifact of the assay system appeared remote for several reasons: (a) dilution of serum from injured rats showed superimposable curves for standards and for pooled serum taken from rats at 1 hour or 3 hours after tumbling trauma, and (b) after alkaline treatment of the above serum pools using the modification of the method of Grodsky and Forsham (64) as describedby Velasco et al. (160), no insulin could be detected in samples that had previously contained that hormone

D. Insulin Sensitivity of Peripheral Tissues In Vivo.

The hyperglycemia and concomitant hyperinsulinemia that characterized the acute response to Noble-Collip drum injury suggested that insulin was ineffective in lowering plasma glucose. The ability of insulin to lower plasma glucose was tested by menas of glucose tolerance and insulin tolerance tests. These procedures were performed at three hours after the initiation of injury, or on the day after traumatic injury in the Noble-Collip drum (Figures 2-5; Tables 2-5).

The administration of glucose to rats at three hours after injury resulted in a prompt hyperglycemia which declined steadily over two hours. The injured rats

demonstrated glucose intolerance; the glucose tolerance curve of the injured rats was elevated above the control curve at all times during the course of the glucose tolerance test. The injured rats had insulin levels that were significantly greater than what was measured in the control rats. The control rats were able to respond to the hyperglycemia with an increase in the concentration of insulin. At the time of the apogee of the glucose curve, the insulin levels measured in the injured rats were not increased above preinjection values. This may be due to the possibility that the β cells of the pancreas could not respond to the glucose stimulus because they were already maximally secreting insulin.

The administration of insulin raised the insulin concentration of the control animals and as a result there was an immediate fall in the plasma glucose concentration. An immediate hypoglycemic effect of insulin was not observed in the injured rats. The delay in insulin action did not appear to be the result of impaired absorption.

By one day after injury the ability of the injured animals to lower glucose was not different from control animals. Fifteen minutes after glucose administration, the plasma glucose concentration of both injured and

non-injured rats had risen to 200 mg/dl. This hyperglycemia caused a rise in the IRI concentration of both the control and injured animals. The glucose tolerance and the insulin response of the control animals was the same at one day after injury as it had been at the three hour time period. The insulin concentration of the injured animals was less than in control animals throughout the period of induced hyperglycemia.

The ability of injured rats to dispose of glucose at lower insulin concentrations than in control rats suggested that the injured rats are more sensitive to insulin than are the non-injured rats. The sensitivity of the injured rats to insulin was tested by assessing the ability of exogenous insulin to lower plasma glucose. When insulin was injected into control animals, there was a prompt hypoglycemia. The same amount of insulin was unable to lower the plasma glucose concentration of the injured animals. This suggests that insulin was not entirely responsible for lowering the plasma glucose during the glucose tolerance test.

E. Insulin Sensitivity of Skeletal Muscle and Adipose Tissue *In Vitro*.

Adipose tissue represents one of the major targets for insulin action in many species. The adipose tissue in rat is extremely active metabolically and also particularly sensitive to the action of insulin. It is currently well established that the transport of glucose across the plasma membrane of fat cells is normally the major rate-limiting step in the utilization of glucose by adipose tissue and that the major action of insulin on carbohydrate metabolism in this tissue is to accelerate this translocation process (86).

In addition to stimulating glucose uptake (89), insulin exerts a number of metabolic effects in adipose tissue. It stimulates glycogen synthesis (22), glucose oxidation, and lipid synthesis (22; 176). According to Jeanrenaud and Renold (83) glucose oxidation and the incorporation of glucose carbon into fatty acids gives the most favorable dose-activity relation. Since, however, carbon dioxide production from radioactively labelled glucose is easily detected, that technique was used to assess the insulin sensitivity of epididymal fat pads.

The symmetry, delicacy and convenient anatomical

location of the epididymal fat pads were taken advantage of in a procedure which measures the basal activity of the tissue as well as the magnitude of any insulin effect. The stromal cells of adipose tissue contribute very little to the tissues overall glucose metabolism despite their large number (132); therefore, the conversion of glucose to carbon dioxide and the stimulatory action of insulin on that process represents the metabolism of adipocytes and not that of the structural elements of the tissue. The results of the experiments in which either the glucose concentration or the insulin concentration of the incubation medium was changed, indicate that epididymal fat pads from injured rats are able to convert glucose to carbon dioxide as well as adipose tissue from non-injured rats.

The prompt hypoglycemic effect of insulin is accounted for by a marked stimulation of glucose uptake in certain tissues, (97) primarily by skeletal muscle which constitutes the largest single volume of insulin sensitive cells in the body. Gemmill (59; 60) and Gemmill and Hamman (61) made the important observation that insulin stimulates glucose uptake and brings about a net increase in the total glycogen content of diaphragm muscle. The increase in glycogen content with insulin was shown to be

due to an activation of glycogen synthesis; insulin had no effect on the degradation of glycogen (38; 59).

Insulin stimulation of glycogen synthesis is an observation that has been confirmed by many investigators (18; 119; 126; 151), among them Villee and Hastings (169) who were the first to use U-^{14}C-glucose and follow the fate of the ^{14}C-labelled carbon in various fractions. They were unable to account for a large proportion of the glucose which disappeared from the medium. Their observations were extended by the experiments of Chain (27), who identified the metabolites formed from glucose as carbon dioxide, lactate, phosphate esters, maltose, and oligosaccharides. Only glycogen, the oligosaccharides and maltose were increased in the presence of insulin; there was no apparent effect of insulin on CO_2 production or on the lactic acid, phosphate esters and glucose present in the tissue.

The glycogen present in muscle at the end of an incubation period is the sum of the glycogen present initially plus the net amount of glycogen synthesized during the incubation period. Chemical analyses show (Table 12) that muscle incubated in a glucose-salt medium in the absence of insulin was able to synthesize small amounts of glycogen, so that usually there was a slight increase in

TABLE 12

GLYCOGEN CONTENT OF DIAPHRAGMS

Group	Mg Glycogen / 100 Mg Wet Weight			Mg Wet Weight / Mg O.D.W.		
	Before	After		Before	After	
Control @ 3 Hours	0.07±.016 (8)	0.11±.009 (8)	NS	4.0±.27 (8)	4.8±.28 (8)	NS
NCD @ 3 Hours	0.05±.004 (8)	0.11±.020 (8)	P<0.05	4.5±.25 (8)	5.3±.16 (8)	P<0.05
	NS	NS		NS	NS	
Control @ 24 Hours	0.09±.023 (8)	0.13±.023 (8)	NS	4.5±.24 (8)	4.4±.30 (8)	NS
NCD	0.11±.018 (8)	0.18±.015 (8)	P<0.025	4.6±.09 (8)	4.7±.12 (8)	NS
	NS	NS		NS	NS	

The glycogen content or wet weight/oven dry weight (O.D.W.) was determined for hemidiaphragms either before or after the 90 minute incubation period. No insulin was included in the incubation medium. Each value represents the mean ± SEM (N). The P values are based on t-statistics for unpaired samples.

the total amount of glycogen present at the end of the incubation period over that at the beginning. The method employed in which ^{14}C-labelled glycogen was extracted from muscle by alkaline hydrolysis followed by precipitation with ethanol measured glycogen and oligosaccharides not contaminated by ^{14}C-glucose or ^{14}C-products soluble in 60% ethanol (119). Recovery of ^{14}C-labelled glycogen from muscles incubated in the absence of insulin also demonstrated their ability to synthesize glycogen. Measurement of the amount of ^{14}C-glucose incorporated into glycogen undoubtedly yields a more sensitive means of evaluating changes in the rate of glycogen synthesis than measurement of the increase in glycogen content.

When radioactively labelled glucose was included in the glucose-salt incubation medium, there was an incorporation of the radio labelled glucose into glycogen. If two groups of muscle with different initial glycogen contents synthesize glycogen from glucose at the same rate, then the specific activity of the total glycogen at the end of the incubation period will be different in the two groups of muscle. Those muscles with the higher initial (and higher final) glycogen content will have a lower specific activity. On the other hand, if two groups of

muscles have different concentrations of glycogen at the end of the incubation period but had equivalent concentrations of glycogen prior to incubation, then the muscles with the higher final glycogen content will have a greater incorporation of radioactive glucose into glycogen (DPM/UNIT TIME/UNIT MUSCLE MASS) and consequently a larger specific activity. With this in mind, the conclusion to be drawn from the data in Tables 7, 8 and 12 is that at 3 hours after injury in the Noble-Collip drum, the muscles have lost an insignificant amount of glycogen. These muscles incorporate glucose into glycogen at the same rate as do the controls. By the day after injury, the muscles from the injured animals have greater glycogen content at the end of the incubation period than do the non-injured muscles. Because the rate of glycogen synthesis is the same in both groups of animals and the specific activity of the final glycogen is lower in the injured muscles, it is very likely that the same difference in glycogen content found at the end of the incubation period existed prior to incubating the tissues.

The single metabolic process in muscle cells which is most markedly stimulated by insulin is the synthesis of glycogen. Glycogen synthesis is a metabolic process

specifically stimulated by insulin (109; 168), whereas glucose uptake is affected by the osmolarity of the medium (90) and by certain amino acids and hormones (127). The results presented in Tables 7, 8, 10 and 11 demonstrate that insulin brought about an increase in the total glycogen content of diaphragm muscle (59; 126; 151). When insulin was added to the incubation medium, the recovery of ^{14}C-labelled glycogen also increased. The increase in both glycogen content and ^{14}C-labelled glycogen resulted in a constant value for the specific activity of the glycogen present at the end of the incubation period. (Tables 7 and 8.)

The use of the isolated rat diaphragm for in vitro study of metabolic processes was originally prepared by Meyerhof and Himwich (108). Since first being used by Gemmill (59; 60) and Gemmill and Hammon (61) to demonstrate that insulin stimulates glycogen deposition in muscle, it has become the classical choice for in vitro studies of insulin action. The response of the diaphragm to insulin has been shown to decrease in proportion to the number of fibers which have been cut (87; 99). With the purpose of preserving the integrity of the muscle fibers, various modifications have been devised, in which the muscle is

incubated with its attachments to the rib cage intact (87). Another limitation of the diaphragm preparation is that the muscle is contracting rhythmically up to the moment of its isolation. Because most skeletal muscles contain two or more fiber types, which may differ considerably with respect to structure (113) and metabolism (16), it was desirable to supplement the information gained from the diaphragm studies with evidence from another skeletal muscle preparation, the isolated soleus muscle (29). The results of the experiments in which skeletal muscle, either hemidiaphragms or soleus muscle, were incubated in a bicarbonate buffered medium containing different concentrations of glucose or insulin indicate that skeletal muscle from injured rats are able to synthesize glycogen as well as muscle from non-injured rats.

F. Evidence of Insulin Resistance in Response to Noble-Collip Drum Injury.

Insulin resistance may be defined as a requirement for greater-than-normal amounts of insulin in order to elicit a quantitatively normal response (13). Insulin resistance may be the manifestation of an inherent end organ unresponsiveness due to a receptor dysfunction.

Macaron and Famuyiwa (102) recently reviewed the subject of receptor dysfunction and hormone resistance in human diseases. Insulin resistance can be due to a partial or complete absence of receptor, as in obesity. It may also be due to a defect in receptor affinity, such as occurs in diabetic ketoacidosis and congenital generalized lipodystrophy. Insulin resistance also may be the result of the action of some substance that prevents the hormone from exerting its usual action. The possible mechanisms by which the latter result may be brought about fall into the two broad classifications of insulin opposition and insulin antagonism.

Insulin opposition may be defined as a state in which an effect opposite to that usually produced by insulin is brought about by mechanisms which in themselves do not affect insulin or its actions on tissues. For example, the hyperglycemia produced by glucagon through its glycogenolytic effect should be regarded as insulin opposition rather than direct antagonism of the action of insulin on muscle or fat.

Insulin antagonism refers to the alteration of insulin to a biologically less active form or to the interference with its action on tissues directly or through

some effect on the ability of the tissue to respond. Insulin antagonism can be brought about through binding of insulin to a plasma or tissue protein or other substance so as to prevent access of insulin to active sites on or within insulin sensitive cells; or antagonism at the cellular level with the response of the cell to insulin.

The rate of glucose uptake by insulin sensitive tissues is proportional to the concentration of glucose and insulin at the cell surface, which are closely proportional to their plasma concentrations (82; 147). Under these conditions the rate of glucose utilization (R) is given by (71; 72):

$$R=K(\text{glucose})_p = K'(\text{insulin})_p(\text{glucose})_p$$

where K is a measure of the capacity of the rat to take up glucose into its cells. K consists of the product of $(\text{insulin})_p$ and K' which can be considered a measure of the overall sensitivity of the tissues to insulin. Differences in K are not accounted for by differences in $(\text{insulin})_p$ but rather with K'. Considering that both plasma glucose and insulin jointly operate to increase glucose utilization, Levine and Haft (98) proposed the usefulness of the $(\text{glucose})_p$ X $(\text{insulin})_p$ product as an index of the degree

of insulin resistance. For a given rate of utilization, the higher the product, the greater the loss of effectiveness of insulin.

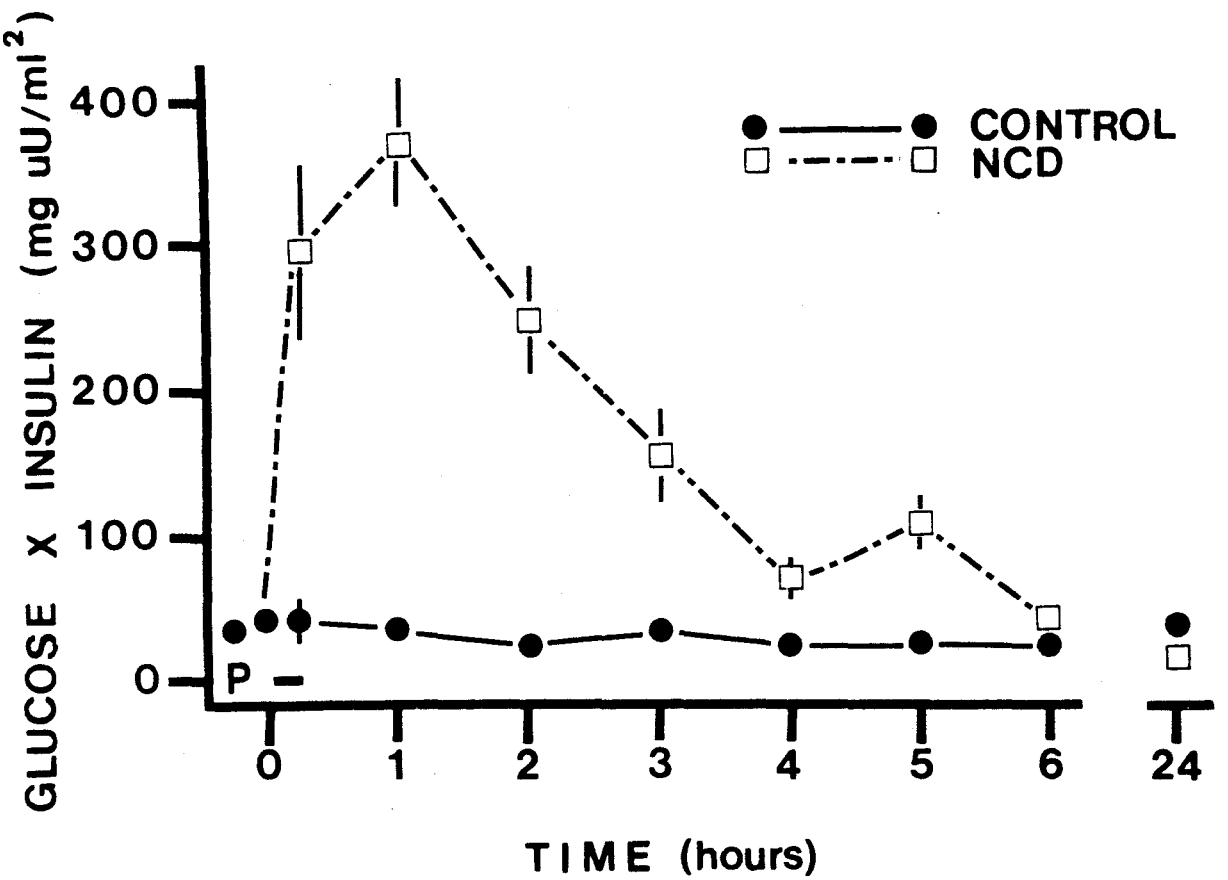
Insulin is ineffective in lowering plasma glucose after injury, especially during the period of depressed energy production. In response to Noble-Collip drum injury, there is a period which is characterized by both hyperglycemia and by hyperinsulinemia. The dual observations of hyperglycemia and hyperinsulienmia indicate an insulin resistant state. The product of plasma glucose and insulin concentrations, which in the small animal, for a given rate of glucose utilization, may be taken as a measure of insulin resistance was considerably elevated (Figure 10). Because the rate of glucose utilization was depressed in the injured animals, the overall sensitivity of the tissues to insulin was overestimated by the $(\text{glucose})_p \times (\text{insulin})_p$ product. These animals also showed glucose intolerance and insulin intolerance, confirming the presence of insulin resistance as an acute response to injury.

Although reports of glucose intolerance, glycosuria and diabetes of injury have appeared in the literature since at least the time of Claude Bernard, it is only recently that systematic in vitro investigations have been

FIGURE 10

PLASMA GLUCOSE X SERUM INSULIN PRODUCT

FOLLOWING TRAUMATIC INJURY



The plasma glucose x serum insulin product (mg $\mu U/ml^2$) was computed for the rats in Figure 1 and Table 1.

conducted with the aim of elucidating the mechanism of insulin resistance after injury. Ryan et al. (137) studied the metabolism of hemidiaphragms and epididymal fat pads from rats after 72 hours of fasting and sepsis. The septic animals had hyperinsulinemia and euglycemia suggesting the presence of insulin resistance. Since insulin was not included in the incubation medium, the authors did not test directly the insulin sensitivity of these two tissues. Ryan and his colleagues (138; 139) also conducted in vitro experiments on sequential samples of skeletal muscle and adipose tissue obtained from rabbits and rhesus monkeys for up to four weeks after hemorrhage and reinfusion. The tissues exhibited, on a percent basis, a decreased effect of insulin on glucose utilization. No attempt was made to elicit a quantitatively normal response by using greater-than-normal amounts of insulin in the incubation medium.

Chaudry, Sayeed and Baue (32; 33; 34) studied isolated rat soleus muscle from animals subjected to severe hemorrhagic shock and determined the effect of insulin on glucose uptake. The insulin concentrations ranged from 0 to 300 mU/ml; although basal glucose uptake in control and "shock" muscles was the same, the control but not the "shock" muscles responded to insulin concentrations below

100 mU/ml. Maximal stimulation of glucose uptake in "shock" muscles was observed at an insulin concentration of 200 mU/ml.

Since similar results were obtained in adrenalectomized rats and rats with intact adrenals, Chaudry and his colleagues concluded that the altered responsiveness of the soleus muscle to insulin could not be due to increased steroid or epinephrine output during shock. Ryan and his group also concluded that the decreased effect of insulin on glucose utilization was not dependent on concurrent changes in the plasma levels of adrenal steroids, catecholamines or circulating insulin levels (139).

The critical role of ATP in carbohydrate metabolism and tissue respiration as an energy supplier for various intracellular reactions is well recognized. Potter and his school (93; 105) sought to explain the ultimate collapse and death of animals in various models of shock in terms of the depletion of tissue energy stores below a certain critical level. In a comprehensive study of tissue metabolites during shock from hemorrhage, tourniquets, and tumbling trauma in the Noble-Collip drum, analyses were made of brain, skeletal muscle, liver, kidney and heart for their content of high energy phosphate compounds. In

general there was a fall in the tissue concentration of ATP, ADP and creatinine phosphate with an accumulation of inorganic phosphate. Chaudry et al. (31) have also reported a significant decrease in ATP levels in rat soleus muscle during late hemorrhagic shock. The insulin resistance observed by this group was reversed by addition of 1mM ATP-$MgCl_2$ to the in vitro incubation medium (34) but this was without effect on the intracellular ATP content of control or shock muscles. Reversal of insulin resistance was also obtained by the infusion of ATP-$MgCl_2$ at the end of the shock period (35).

Diaphragms isolated from rats (159) and soleus muscles taken from mice after scalding (57) showed no decrease in responsiveness to insulin. The results of the present study show that in addition to skeletal muscle, adipose tissue also retains its normal sensitivity to insulin. The lack of maintenance in isolated tissues of the insulin resistance seen in vivo contrasts with the findings of Chaudry, Sayeed and Baue (32; 33; 34) and of Ryan and his coworkers (138; 139). The difference probably reflects the differences between these forms of injury. Immediately following trauma in the Noble-Collip drum, arterial pressures or heart rates were not different from control

values (133). Respiratory changes preceded the abrupt fall of arterial pressure to hypotensive levels. When T-1824 dye was injected intravenously before trauma, Chambers et al. (28) observed evidence of considerable loss of dye into the gastro-intestinal tract and over bony prominences. When a volume of blood equivalent to the amount found in the engorged gastro-intestinal circulation was removed by hemorrhage, no deaths resulted (120). The conclusion reached by North and Levy (120) was that the amount of fluid loss and pooling in the splanchnic circulation was insufficient to cause death.

In the case of injury in the Noble-Collip drum, it seems probable from the present results that the insulin resistance seen in vivo was not the result of an inherent end organ unresponsiveness, and presumably was the result of the action of some substance that prevented insulin from exerting its usual action. No hormones other than insulin were measured in the present studies.

Clowes et al. (40) have postulated the existence of an "active agent" in the blood capable of altering the metabolism of muscle and adipose tissue following trauma or sepsis such that muscle becomes resistant to the action of insulin while adipose tissue retains its normal

sensitivity to insulin (137). Steroids and catecholamines have been implicated. Although both exert effects in opposition to that of insulin, the recent findings reported by Ryan et al. (139), demonstrate that adrenal steroids or catecholamines do not have a causal role in the development of insulin resistance in skeletal muscle after hemorrhage. Clowes refers, in an anecdotic fashion, to experiments suggesting the presence of a substance in the plasma from injured patients which produces a decrease in glucose uptake, an increase in lactate production and no change in oxygen consumption or FFA uptake by the hindquarters of anesthetized rabbits (40). It has not been reported if insulin can overcome the opposition of this "active agent" in either an in vivo or in vitro test situation or whether it causes a differential tissue insensitivity to insulin.

Rats injured by tumbling in a Noble Collip drum demonstrated the existence of insulin resistance in vivo. The lack of maintenance in isolated tissues of the insulin resistance seen in vivo was of importance for several reasons. First, it suggested that insulin resistance may be the result of the action of some substance that prevented the hormone from exerting its usual action. When the insulin sensitivity of adipose tissue and skeletal muscle

was tested under *in vitro* conditions, this substance could no longer influence insulin action and the isolated tissues responded normally to insulin. The significance of this is that further investigations of insulin resistance after traumatic injury will have to be conducted *in vivo*.

Secondly, it has been generally believed that insulin resistance is an integral part of the composite picture of the metabolic alterations produced by traumatic injury. As long ago as 1794, John Hunter wrote with a rare perception, "There is a circumstance attending accidental injury which does not belong to disease--namely that the injury done has in all cases a tendency to produce both the disposition and the means of cure." It is not known whether insulin resistance contributes to the eventual survival of the animal or if it is part of the processes that eventually lead to the death of the rat. The model of injury developed by Noble and Collip is particularly suited to investigate this problem. It is possible, by gradually increasing the dose of "drumming," to make the animal capable of withstanding an amount of "drumming" which would be certainly fatal in an unconditioned animal. Assessment of the overall sensitivity of the tissues to insulin with *in vivo* techniques, especially in trauma-resistant rats, will

then make it possible to determine if insulin resistance contributes to the survival of the injured animal.

CHAPTER VI

SUMMARY

After trauma in the Noble-Collip drum, injured rats are capable of producing an elevation of plasma glucose. The rise in glucose concentration occurred in spite of a concomitant hyperinsulinemia. The dual observations of hyperglycemia and hyperinsulinemia indicate a state of insulin resistance. The ability of insulin to lower plasma glucose was evaluated by means of glucose tolerance and insulin tolerance tests. The injured rats showed a biphasic response to exogenous glucose. They showed glucose intolerance and hyperinsulinemia shortly after injury. On the day after injury, the glucose tolerance was normal and hypoinsulinemia was present. At both of these times, exogenous insulin was ineffective in lowering the concentration of plasma glucose, thus confirming the presence of insulin resistance in vivo as a response to injury.

Adipose tissue and skeletal muscle represent two major targets for insulin action and collectively comprise a large volume of the insulin sensitive cells in the body. When representative samples of adipose tissue and skeletal

muscle were removed from the hormonal milieu of the injured rat, they retained a normal sensitivity and responsivity to insulin. The lack of maintenance in isolated tissues of the insulin resistance seen *in vivo* suggests that insulin resistance may be the result of the action of some substance that prevents the hormone from exerting its usual action, rather than an inherent end organ unresponsiveness.

CHAPTER VII

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

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

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