SOME EFFECTS OF GLUCOCORTICOIDS ON CARBOHYDRATE METABOLISM IN RATS

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CHAPTER I

INTRODUCTION

Glucocorticoids affect carbohydrate metabolism in three major areas: enhance the deposition of liver glycogen to a greater extent than muscle glycogen, disturb carbohydrate utilization, and promote gluconeogenesis. These effects have been well known for some twenty years; however, the literature reveals that there is yet, after two decades of research, no definite conclusion as to which of the above is the primary effect and what is the consequence of this initial event. Much of the more recent work in this area has been an attempt to elucidate the mechanism by which these steroids influence the metabolism of carbohydrate.

Conflicting reports have been published concerning the respiratory stimulating capacity of these glucocorticoids, both naturally occurring and synthetic. This conflict is not surprising in view of the various tissues used and differences in the duration of the treatment. At the present time there are only postulates to explain this respiratory stimulating property of the glucocorticoids. The research reported herein was carried out to either confirm or deny previous reports of increased respiration following glucocorticoid administration and to study further any effects which these hormones may have upon hepatic glycolysis in rat liver homogenates.

Prednisolone, a synthetic adrenal cortical hormone, was selected for use in this study because of its high glucocorticoid potency. Following the determination of the optimum dose and optimum time interval for prednisolone to stimulate respiration, an experiment was designed to study the liver reducing glycolytic intermediates and glycogen metabolism and respiration of intact, of adrenalectomized, and of fasted-adrenalectomized rats.

CHAPTER II

REVIEW OF LITERATURE

Deposition of Glycogen

In 1932, Britton and Silverte (14) demonstrated that adrenal cortical extracts caused an elevation of blood glucose and liver glycogen within one hour following injections into normal cats, rabbits, and rats. Their results showed that the storage of glycogen was enhanced three to five times above that found in controls. A study of the interrelation of the pituitary and the adrenal in the control of carbohydrate levels in the rat showed that the fasting muscle glycogen of adrenalectomized rats is maintained within normal limits by adrenal cortical extract and salt therapy (8). This same treatment had no effect on hypophysectomized rats although crude anterior pituitary extracts did maintain the fasting muscle glycogen (14, 71). Since then it has been shown that ACTH will restore to normal the decreased ability of hypophysectomized animals to synthesize glycogen from glucose (1, 21). Long and Lukens (58) noted that hypophysectomy or adrenalectomy alleviates diabetes resulting from pancreatomy in the cat as evidenced by a decrease in the excretion of glucose, nitrogen, and acetone bodies. In comparing the effects of growth hormone and ACTH on urinary glucose and nitrogen excretion by alloxan diabetic rats, Bennett (9) noted that only the adrenocorticotrophic hormone produced a typical exacerbation

of the diabetes with the enhancement of both glycosuria and nitrogen excretion. In another of the earlier studies on the relationship between the adrenal cortex and carbohydrate metabolism, Long and coworkers (57) demonstrated that the administration of cortical extracts or crystalline adrenal steroids to fasted normal or adrenalectomized rats and mice resulted in a large increase in liver glycogen and a slight hyperglycemia. Furthermore, the cortical extract decreased the proportion of glucose oxidized while there was an increase in the proportion deposited as liver glycogen.

Using $C^{14}O_2$ and glucose-6-C^{14} as labelled substrates, Ashmore et al. (6) studied the acute effects of hydrocortisone administration on liver glycogen deposition in fasted rats. Their results indicated that hydrocortisone increased the incorporation of the label from radioactive bicarbonate into liver glycogen but not into either blood glucose or liver dicarboxylic acids and increased the glycogen deposition from the labelled glucose. Recently Glenn et al. (27) demonstrated that in fasted-adrenalectomized rats, hydrocortisone influences the disposition of glucose or its immediate metabolites and that insofar as liver glycogen synthesis is concerned, fat, protein, and amino acids are relatively unimportant in this regard. These workers concluded that it is the availability of glucose, not the increased synthesis that determines the quantitative aspect of the glycogenic response to hydrocortisone.

Kostyo (42) reported that neither cortisol nor cortisone increased glycogen deposition in the uterus. It has been claimed (50) that hypophyseal hormones and insulin are both required in addition to the corticosteroids for deposition of liver glycogen. The results of Leonard (53) indicate that not all skeletal muscles respond

alike in deposition of glycogen under the influence of hormones. Cortisone acetate was injected into normal, gonadectomized, and hypophysectomized rats of both sexes to determine the effect on the glycogen levels in the following skeletal muscles; rectus femoris, abdominal muscles, cremaster of the male, and the diaphragm of the female. The hormone caused an increase in the glycogen content of the rectus femoris but not in the cremaster. A sexual difference was noted in that cortisone treatment caused more glycogen deposition in the muscles of the female than in the male.

It has been reported that hydrocortisone (compound F) (20) and cortisone (67) reduce carbohydrate tolerance in man, while fructose and galactose tolerances were found to be unchanged (67). Deposition of liver glycogen and decreased glucose tolerance can result from either decreased utilization or increased production of glucose or both.

Cortisone has been shown to inhibit oxidation of alpha-ketoglutarate by liver mitochondria even though the P/O ratios remained within normal ranges (16). This suggests the possibility of a partial block at some point in the Krebs cycle. This cortisone-induced block could explain the increased liver glycogen content in cortisone-treated animals.

Winteritz and associates (84) demonstrated that a subcutaneous injection of epinephrine into fasted-adrenalectomized rats promotes a decrease in muscle glycogen almost two times as great as intact animals, with practically no accumulation of liver glycogen. Injections of adrenalcortical hormones restored the normal pattern of response.

Kerpolla (39) demonstrated that cortisone retards skeletal

muscle phosphorylase activity in the rabbit, while Leonard (54) noted that cortisone had no effect on the phosphorylase activity of muscle and that both cortisone and cortisol increased muscle glycogen levels (59). A striking correlation between increased phosphorylase activity and liver glycogen deposition in fasted adrenalectomized rats receiving cortisone acetate was reported by Hess and coworkers (33). Adrenalectomy caused a significant decrease in the phosphorylase activity of fasted (33) and adrenalectomized rats (81).

Carbohydrate Utilization

Lacroix and Leusen (47) found that the utilization of glucose and pyruvate by surviving heart slices was markedly impaired by cortisone acetate administration to rats for 14 to 16 days before sacrifice. Alpha-ketoglutarate and succinate stimulated oxygen consumption by the heart slices to the same extent in the treated animals as in the control, suggesting a block in Krebs cycle somewhere between pyruvate and alpha-ketoglutarate.

It has been shown by Overell and associates (66) that near physiological doses of hydrocortisone, cortisone, prednisolone, and cortisone acetate inhibit glucose uptake in mouse skin. Herman and Ramey (31) noted that hydrocortisone inhibits glucose uptake by rat diaphragms incubated in Krebs-Ringer phosphate medium when added in vitro at a concentration of 40 ug./ml. or when injected into rats at a dose levels of 0.3 mg. daily for 7 days or 2 mg. six hours before sacrifice. Although a decrease in the production of lactic acid from pyruvate in the diaphragms of adrenalectomized rats was demonstrated by Villee et al. (76), the rate of conversion of pyruvate to CO_2 by this muscle remained normal. Cohn et al. (18) reported a decreased utilization of glucose in extra-hepatic tissues

of adrenalectomized dogs and an increased utilization in animals receiving cortisone. Cortisol restored to normal the decreased production of glucose by the liver in hypophysectomized dogs (2).

Glucose-6-phosphatase is the enzyme responsible for the last step in the conversion of glycogen to blood glucose, and glucose production by the liver seems to be correlated with this enzyme-activity (68). In the absence of glucose-6-phosphatase, an increase in liver glycogen would be expected. Cori and Cori (22) were the first to suggest that high glycogen levels in the liver may be due to the low activity of this enzyme. Nemeth (65) noted a striking correlation between glucose-6-phosphatase and glycogen content in the fetal guinea pig liver; glycogen in the fetal liver rose steadily during the last few days of gestation during which time glucose-6-phosphatase activity was virtually absent; at parturition the phosphatase activity was increased rapidly and liver glycogen levels fell off correspondingly.

Increased glucose-6-phosphatase activity has been reported in the livers of diabetic (4, 5, 25, 49, 55), of fasted (5, 25, 26), and of steroid treated (5, 45, 49, 55, 77, 78) rats. This in part explains the blood glucose elevation found in glucocorticoid treated animals and in metabolic conditions associated with an increased endogenous production of glucose. Glucose-6-phosphatase activity was also increased in the kidney of fasted or diabetic intact animals (25). Insulin reduces this increase in activity in steroid treated (55), diabetic (4, 55), and adrenalectomized-hydrocortisone treated (55) animals.

The administration of 17-OH corticosterone to adrenalectomized-diabetic rats stimulated an increased gluconeogenesis which

preceded by several hours the increase in liver glucose-6-phosphatase activity; the adrenal cortical hormones appear to act within 2 hours after injection to alter pyruvate metabolism in liver slices from adrenalectomized-diabetic rats (5). Weber et al. (77) demonstrated that the percentage intracellular distribution of glucose-6-phosphatase in liver homogenates was not affected by fasting nor by cortisone treatment.

Kvam and Parks (45) in an attempt to elucidate the mechanism by which hydrocortisone induced the changes in glucose-6-phosphatase and fructose diposphatase activity demonstrated that the increase was inhibited by the methionine antagonist, ethionine. The inhibition was reversed by the addition of methionine, suggesting that the increase in enzyme activity involves the synthesis of new enzyme protein rather than an activation mechanism since methionine had to be available before an increase in enzyme activity could occur.

One of the early suggestions as to the mechanism by which the adrenal cortical steroids inhibit carbohydrate utilization was made by Colowick, Cori, and Slein (19). These workers demonstrated that the hexokinase activity of muscle extracts from alloxan-diabetic rats could be inhibited by the addition of adrenal cortical extracts; whereas, hexokinase activity of extracts from normal muscle remained unchanged by treatment. An inhibition in hexokinase activity in the normal muscle was produced by the addition of a protein fraction from the anterior pituitary. It was concluded that the inhibitory effect of adrenal cortex extract depends on the presence of a factor of pituitary origin, probably somatotrophin. Bacila and Barron (7) suggested from their studies that

the adrenal hormones inhibit glycolysis by combining with the -SH groups of hexokinase. Kerpolla (40) proposed an impairment of the hexokinase function due to the uncoupling of oxidative phosphorylation by cortisone, resulting in depressed production of ATP. An overnight fast of rats led to decreased liver ATP content with corresponding increases in the ADP and AMP contents (10).

Belcher and White (11) determined the factors which limit the rates of anaerobic glycolysis of glucose, glucose-6-phosphate, fructose-6-phosphate, and fructose 1, 6-diphosphate in fortified cell-free homogenates of rat lymphosarcoma. These factors included a low level of tissue hexokinase, regulation of the activity of the hexokinase reaction by a limited glycolytic regeneration of ATP, and exceedingly active ATPase. This study demonstrates that cortisol inhibits anaerobic glycolysis of glucose at the hexokinase and ATPase for the supply of ATP.

The adrenal cortical steroids have been shown to affect the activity of other enzymes in the glycolytic pathway and in the Krebs cycle. Adrenalectomy reduced the activity of phosphoglucomutase and phosphoglucose isomerase (81). Cortisone, hydrocortisone, and corticosterone are all capable of maintaining an elevated level of phosphoglucose isomerase, whereas the two former hormones exert a similar effect upon phosphoglucomutase (81). Lee (52) observed that prednisolone inhibited pyruvic oxidase activity. Cortisone and ACTH decreased the rate of phosphoglyceromutase and enolase activity in both sexes of rats, thus depressing the formation of phosphoenol pyruvate from 3-phosphoglycerate (44). Kerppola and Pitkanen (41) found that the activities of glucose-6-phosphate

isomerase, aldolase, glycerophosphate kinase, and lactic dehydrogenase were unaltered in liver mitochondrial supernatants from rats previously treated with cortisone.

Grossfeld (30), using mouse fibroblast tissue cultures, noted that the addition to 150 ug. of hydrocortisone to the cell suspension in the Warburg apparatus inhibited respiration which was coupled with a significant increase of aerobic glycolysis. It was suggested that the increase of glycolysis may be due to inhibition of respiration in accordance the "Pasteur effect". Pasteur, in 1861, noted this effect in his studies on alcoholic fermentation; under anaerobic conditions much more sugar was taken up, per quantity of yeast present, than was consumed in the presence of air. Johnson (38) proposed that since in the absence of either inorganic phosphate or a phosphate acceptor neither glycolysis nor oxidation can proceed. The Pasteur effect could be due to cessation or reversal of glycolysis which occurs when inorganic phosphate and phosphate acceptors concentrations become low because of the phosphorylative oxidations which occur in the presence of oxygen. The stimulation of both respiration and aerobic glycolysis was demonstrated in tissue cultures to which 1.50 µg. of hydrocortisone was added at 96, 24, and 0 hours before the manometric experiments (30).

Chronic (7-17 days) administration of glucocorticoids has been shown to depress oxygen uptake of myocardial tissue (38, 46), kidney slices (38), and liver homogenates (28, 29). There are conflicting reports concerning the effect on oxygen consumption by the diaphragm; Kowalswski and Beksi (38) noted a significant decrease, while Lacroix and Leusen (46) demonstrated a pronounced increase in oxygen uptake by diaphragm muscle tissue. In both

instances male rats were given the steroid chronically. No significant change in oxygen uptake was demonstrated in the female rat diaphragm (46). Goetsch (28, 29) observed an increased oxygen uptake by liver homogenates following a single injection of prednisolone.

Cohen (17) recorded that glucose inhibited respiration in the postnatal rabbits retina prior to the formation of the sensory elements by as much as 40 percent.

An inhibition of the oxygen uptake by liver mitochondria from rats treated chronically with cortisone has been demonstrated (41); the decrease was noted at the cytochrome c oxidase portion of the cytochrome chain.

Respiratory quotients and $C^{14}O_2$ production from 2-C^{14} pyruvate were studied by Winternitz and Kline (83) in normal and adrenalectomized rats following the subcutaneous injection of epinephrine as a carbohydrate mobilizing agent. These results indicated an elevation in RQ values of greater magnitude and a greater expiration of $C^{14}O_2$ in adrenalectomized animals, pointing out that there is an increase in carbohydrate utilization in the adrenalectomized subject. In a later study Winternitz and Forrest (82) infused 2-C^{14} pyruvate for 90 minutes into normal and adrenalectomized rats. A large amount of highly active liver glycogen was formed in the normals but not in the adrenalectomized animals. It was suggested that the increased carbohydrate utilization following adrenalectomy may be secondary to the diminished glycogenesis and may occur over all available routes.

Goetsch (28) demonstrated that corticoid treatment did not alter the rate of glycolysis in fed intact rats as evidenced by the fact that carbohydrate utilization was similar in liver homogenates from treated and non-treated rats and by the fact that inorganic

phosphate levels were similar in treated and control liver homogenates incubated under nitrogen.

Inorganic phosphate level is often used as an indicator of glycolytic and respiratory rates. Mills and Thomas (60) noted that the inorganic phosphate concentrations in plasma and in whole blood are about the same and fall in a similar manner after the intravenous injection of cortisol. No consistent changes in glucose have been found to accompany the phosphate uptake (60).

Ashmore et al. (3) observed that the adrenal cortical steroids had no effect on the phosphogluconate oxidation pathway.

GLUCONEOGENESIS

The conditions under which the corticosteroids exert their influence are largely those in which the animal is forced to form glucose from protein. An excess of these hormones always accelerates protein catabolism in such circumstances as fasting, insulin hypoglycemia and pancreatic diabetes (56). Long et al. (57) in 1940 suggested that one of the properties of the cortical hormones is the stimulation of protein catabolism and that the increase in carbohydrate levels and nitrogen excretion following their injection is an expression of this effect. Results of Ingle and coworkers (37) indicated that the effect of the C-11-17 oxgenated cortical steroids in stimulating protein catabolism was not prevented or significantly diminished by insulin.

Welt et al. (79) found that cortisone treatment caused a 7-fold increase in the production of glucose synthesized per hour from non-carbohydrate precursors over untreated controls and a 2-fold increase over normal in glucose production in alloxan diabetic rats. Administration of cortisone and glycine produced an increase in liver glycogen of fasted normal rats (32).

Rosen et al. (69), in an attempt to find an enzymatic basis for the gluconeogenic action of hydrocortisone demonstrated that chronic injections of 5 mg. of compound F daily for 7 days caused an increase of 500 percent in glutamic-pyruvic transaminase activity in rat livers. A slight increase in activity of glutamic-oxalacetic transaminase was also noted. These results strongly suggest that the glucocorticoids increase gluconeogenesis and impose a negative nitrogen balance by enhancing the transamination processes. Increased transaminase activity would be expected to alter the rate at which precursors of protein are interchanged with carbohydrate intermediates. These same workers made a further study of the increase in activity of glutamic-pyruvic transaminase in four conditions associated with gluconeogenesis (70). The conditions were the administration of corticosteroids, alloxan diabetes, fasting, and an elevated protein diet. In each instance there was a marked increase in the activity of liver glutamic-pyruvic transaminase gluconeogenic, suggesting an involvement of new enzyme synthesis rather than an activation of the enzyme. Brin and McKee (13) reported that cortisone treatment resulted in an increase and adrenalectomy in a decrease in transaminase activity in rat livers.

The effects of cortisol on liver glycogen deposition and glutamic-pyruvic transaminase gluconeogenic activity were studied in vitamin B_6 deficient and control rats by Einsenstein (24). It was found that the glucocorticoid action of cortisol is diminished in vitamin B_6 depleted animals as is the effect of the hormone on transaminase activity. Other parameters of cortisol action such as involution of lymphoid tissue were unaltered by the vitamin deficiency. It was concluded from this work that the gluconeogenic activity of the

adrenal corticoids depends not only on their molecular configuration but also upon the nutritional status of the animal.

The gluconeogenic activity of these steroid hormones has also been demonstrated in extrahepatic tissues. Landau (48) incubated pyruvate-2-C^{14} with rat kidney slices from normal, alloxan-diabetic, and cortisone-treated animals. This study showed: that pyruvate uptake and the incorporation of C^{14} into glucose and lactate were greater than normal in the diabetic and cortisone-treated groups; that incorporation of C^{14} into glycogen was increased above normal in the cortisone-treated animals; a several fold increase in glucose content in the diabetic and steroid treated slices; and that a large percentage of this newly formed glucose was synthesized from pyruvate carbon.

Segaloff and Many (72), using phloridizinized rats, showed that ACTH, 11-dehydrocorticosterone, corticosterone, cortisone, and hydrocortisone increased glycosuria and ketonuria in excess of what could be accounted for on the basis of the accompanying increase in nitrogen excretion; therefore, it was concluded that the rat is capable of deriving glucose from fat as well as protein. Cortisone has an antagonistic action on hepatic lipogenesis in intact animals (12). Chronic administration of ACTH or cortisone caused glycosuria and acetonuria in rats force fed on a high fat, low carbohydrate diet and cortisone induced glycosuria without acetonuria in rats on a medium carbohydrate diet (35, 36).

Mokrasch et al. (61) found an increase in liver fructose-1, 6-diphosphatase activity in rabbits fed diets low in glucose but rich in protein or in fructose. A large increase in

the enzyme content was obtained by the administration of cortisone. It was suggested that the observed increases represent a physiological means of causing increased gluconeogenesis in response to the stress imposed.

CHAPTER III

MATERIALS AND METHODS

Mature female albino rats (Sprague-Dawley strain), weighing 300 to 350 gm. were used as the experimental animals and were given free access to feed (Purina Rat Chow) and water both before and after treatment. They were housed at a temperature varying from 60°F to 75°F. Prednisolone ("Sterane", Pfizer) (Δ'F), a synthetic adrenalcortical hormone having strong glucocorticoid properties, was used in this study.

Immediately following sacrifice by decapitation the left half of the median lobe of the liver was removed and placed in 9 ml. of isotonic KCl cooled to 0 to 5°C. The isotonic KCl and liver sample were than transferred to an ice-chilled Potter-Elvehjem homogenizer and considered homogenized after 5 to 8 passes with the plunger. One and one-half ml. of the homogenate were pipetted into a Warburg flask which contained 1.5 ml. of phosphate buffer and 0.2 ml. of 25% KOH in the center well. A fluted piece of filter paper was placed in the center well of the Warburg flask to increase the absorption area for the carbon dioxide given off during incubation. A stock solution of the phosphate buffer was made by dissolving 9.92 gm. potassium chloride, 1.35 gm. magnesium chloride, 0.91 gm. potassium acid phosphate in 800 ml. of distilled water,

adjusting to pH 7.5 with potassium hydroxide and/or phosphoric acid and diluting to one liter (28). A 2 cc. syringe and 18 gauge needle were used for placing the KOH in the center well before putting the fluted filter paper in place. To further insure cold conditions, the Warburg flask was placed in a deep freeze for 15 minutes prior to the addition of the homogenate.

Following the addition of the homogenate to the Warburg flasks, they were placed on manometers and flushed with oxygen for five minutes. The manometers were then transferred to a constant temperature bath at 37°C and allowed to equilibrate for 8 minutes. Readings were taken thereafter every ten minutes for 30 minutes to determine oxygen uptake. Dry liver weight was determined by putting 1 ml. of the homogenate in a small tared container and drying in an oven for 12 to 24 hours at 150°C. After drying, the tared containers were weighed back on a Roller-Smith torsion balance.

In experiment I, stimulation of respiration was used as the criterion for determining the optimum dose of prednisolone. To each of three groups, Δ'F was randomly administered i.m., five hours before sacrifice, in quantities of 0.5 mg., 5.0 mg., and 20.0 mg. A fourth group served as a control and received 1 ml. of physiological saline (PSS) i.m. five hours before sacrifice. Oxygen uptake by the liver homogenate and the dry liver weight were determined as described above.

Experiment II was designed to learn the time interval following the steroid injection when maximum oxygen uptake occurred in incubating liver homogenates. Groups were assigned at random to receive 5.0 mg. of Δ'F at 8 hours, 4 hours, 2 hours,

and 1 hour prior to sacrifice. The control group was injected i.m. with 0.5 ml. of physiological saline 8 hours before sacrifice.

Experiment III was used to ascertain the effect of prednisolone on hepatic carbohydrate utilization and respiration in liver homogenates prepared from intact, from adrenalectomized, and from (24 hours) fasted-adrenalectomized rats. Four groups of intact animals (15 per group) were assigned at random to the following treatments: 0.5 ml. of physiological saline, 400 mg. of glucose, 5.0 mg. of Δ'F, and 400 mg. of glucose plus 5.0 mg. of Δ'F. The same sequence of treatments was repeated in four groups (15 rats per group) of adrenalectomized animals, and in four groups (10 rats per group) of fasted-adrenalectomized rats. The synthetic glucocorticoid and physiological saline injections were made intramuscularly while the glucose was administered intraperitoneally. Oxygen uptake was determined as previously described, and reducing glucolytic intermediates and liver glycogen were determined for each animal both before and after incubation. The term, reducing glycolytic intermediates, as used here includes glucose and any glycolytic intermediates that have a free or potentially free aldehyde group.

Adrenalectomies were performed five to seven days prior to use in these studies; the animals were maintained on one percent sodium chloride drinking water.

To determine reducing glycolytic intermediates (expressed as grams percent of glucose), a protein-free filtrate was first prepared from the liver homogenate by the addition of 1 ml. of 0.3N barium hydroxide to 1 ml. of homogenate. After a brownish color developed, 1 ml. of 10 percent zinc sulfate was added

followed by 7 ml. of distilled water. The mixture was then centrifuged at 3000 r.p.m. for 15 minutes, filtered, and stored in a deep freeze. Later the filtrate was thawed and 1 ml. of it was subjected to Nelson's (62) photometric adaptation of the Somogyi Method for determination of glucose. One ml. of the filtrate was pipetted into a Folin-Wu blood sugar tube graduated at 25 ml. To this was added 1 ml. of a mixture (prepared the day of use) of 25 parts of Nelson's reagent A to 1 part of Nelson's reagent B. Standards and 1 ml. of distilled water, which served as a blank, were set up in the same manner. The solutions were mixed and heated for 20 minutes in a boiling water bath. After cooling the tubes in a pan of running tap water, 1 ml. of the arsenomolybdate color reagent was added to each tube. The mixture was then diluted to the 25 ml. mark with distilled water and read at 540 mμ on a Coleman Jr. Spectrophotometer. The color which develops is very stable and can be read at convenience (62).

Liver glycogen isolation was accomplished by placing 1 ml. of liver homogenate and 1 ml. of 30 percent potassium hydroxide in a 12 or 15 ml. centrifuge tube and then placing the tube in a boiling water bath for 20 minutes. At the end of 20 minutes, 7 ml. of 95 percent ethyl alcohol was added and the tubes were returned to the water bath until the mixture just began to boil. Then the tubes were removed, allowed to cool at room temperature, and centrifuged at 3000 r.p.m. for 15 minutes. After the alcohol was decanted off, leaving only precipitated glycogen, the tubes were stored in a deep freeze. Later they were removed, thawed, the glycogen resuspended in 10 ml. of 1N sulfuric acid, and hydrolyzed in a boiling water bath for 3 to 4 hours. The hydrolysate, following

neutralization with 1N sodium hydroxide until a yellow color was obtained with phenol red indicator, was then analyzed for glucose by the Nelson method and liver glycogen values were expressed as grams percent of glucose.

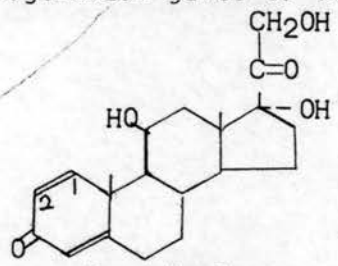
Chemicals used in the preparation of the Nelson's reagent were of the analytical grade. Copper reagent A was prepared by dissolving 25 gm. of anhydrous sodium carbonate, 25 gm. of Rochelle salt (potassium sodium tartarate), 20 gm. of sodium bicarbonate, and 200 gm. of anhydrous sodium sulfate in 800 ml. of water and diluted to one liter. The solution was stored where the temperature did not fall below 20°C. Copper reagent B was made from 15 percent cupric sulfate containing one or two drops of concentrated sulfuric acid per 100 ml. of water. The arsenomolybdate color reagent was prepared by dissolving 25 gm. of ammonium molybdate in 450 ml. of distilled water, to which was added 21 ml. of concentrated sulfuric acid and mixed, followed by the addition of 3 gm. of disodium arsenate dissolved in 25 ml. of water, mixed again and incubated at 37°C for 48 hours. This reagent was stored in a brown bottle in a dark place. The zinc sulfate and the barium hydroxide solutions were adjusted so that 5 ml. of zinc sulfate required between 4.7 ml. and 4.8 ml. of barium hydroxide to produce a definite pink to phenolphthalein. The zinc sulfate was diluted to 20 ml. with water and the barium hydroxide was added dropwise during the titration.

The student's "t" test was used to analyze the data in experiment I. An analysis of variance (AOV) was performed on all other data in these experiments and was followed by a Tukey's D test to locate the differences among the means.

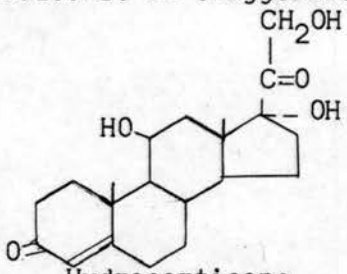
CHAPTER IV

RESULTS AND DISCUSSION

Prednisolone (△ 'F) differs structurally from the naturally occurring adrenal cortical hormone, hydrocortisone (compound F), in having an unsaturated bond between carbons 1 and 2. This dehydrogenation gives to the prednisolone molecule an exaggerated



Prednisolone



Hydrocortisone

glucocorticoid activity (64). The elevated glucocorticoid activity of prednisolone may also be attributed to the long plasma half-life, 204-241 minutes, as compared to that of hydrocortisone, 113 minutes (64). Apparently, because of this alteration in the molecular structure, prednisolone is metabolized much more slowly by the liver when compared to the naturally occurring hormone.

Microliters of oxygen taken up by the rat liver homogenates from experiment I are shown in Table I and illustrated graphically by Fig. 1. Intramuscular administrations of varying doses of prednisolone were made five hours prior to sacrifice to determine the optimum dose of the synthetic hormone. The control group received 1 ml. of PSS at the same time interval. Preliminary experiments indicated that sham injections of PSS did not affect

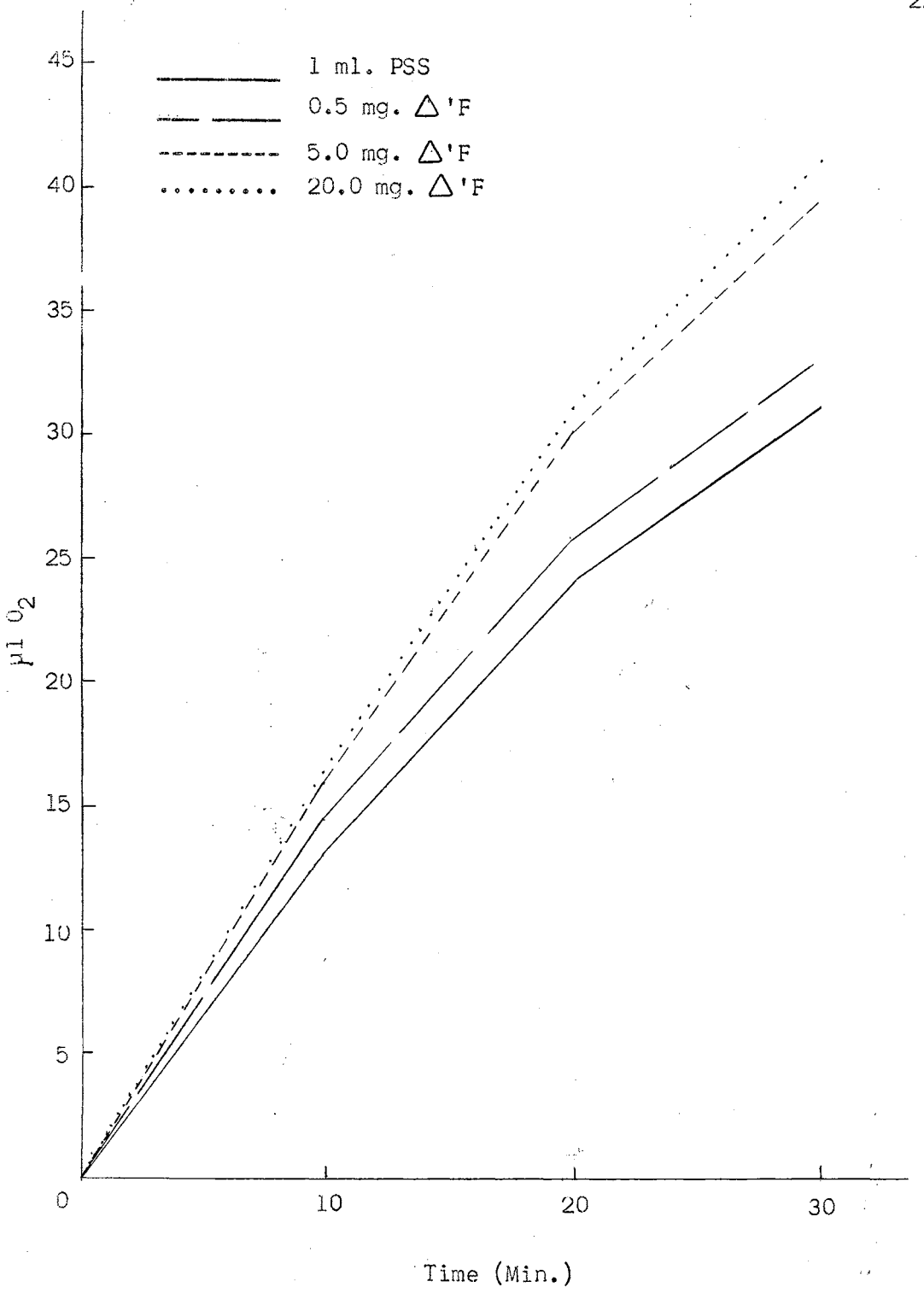


Figure 1. Average oxygen uptake (μl/mg. dry liver) by rat liver homogenates expressed as microliters O_2 taken up per mg. liver on a dry weight basis. Rats were treated i.m. with different levels of prednisolone 5 hours before sacrifice.

TABLE I

AVERAGE OXYGEN UPTAKE BY RAT LIVER HOMOGENATES EXPRESSED AS MICRO-LITERS OF OXYGEN TAKEN UP PER MG. OF DRY LIVER. RATS WERE TREATED I.M. WITH DIFFERENT LEVELS OF PREDNISOLONE 5 HOURS BEFORE SACRIFICE

Group No.	Treatment	No. of Rats	Incubation Time (minutes)		
			10	20	30
1	1 ml. PSS	10	13.0	24.1	31.2
2	0.5 mg. △'F	10	14.6	25.8	33.3
3	5.0 mg. △'F	10	15.9	30.1	39.2
4	20.0 mg. △'F	10	16.1	31.1	41.2

Student's t-test: At 10 minutes, $P = 0.025$, $3 < 1$; at 20 minutes, $P = 0.05$, $3 < 1$; at 30 minutes, $P = 0.10$, $3 < 1$.

the respiration of rat liver homogenates. Five milligrams and 20.0 mg. of prednisolone caused a significant increase at each of the ten minutes intervals of incubation ($P = 0.025$ at 10 min., $P = 0.05$ at 20 min., $P = 0.10$ at 30 min.). The 20.0 mg. dose caused a slightly greater stimulation of respiration than did the 5.0 mg. dose; however, the difference was statistically significant. As the incubation time increased, the difference in oxygen uptake between the control and treated groups increased, but because of the wider sample variation at the end of 30 minutes, the differences were statistically less significant. It is apparent from these results that, under the conditions used in this experiment, 5.0 mg. of △'F is sufficient to stimulate near maximum respiration and that additional prednisolone will not further increase oxygen consumption to any great extent.

Once the optimum treatment level was determined, it seemed desirable to find out the time interval following the administration of prednisolone when maximum response occurs. The greatest stimulation

of respiration by liver homogenates from rats previously treated with 5.0 mg. Δ'F occurred four hours after the injection (Table II and Fig. 2). Oxygen uptake was significantly increased (P = 0.01) at both two and four hours after treatment. Respiration in the four hour group was also significantly greater than in the two hour group. Eight hours after treatment respiration was no longer stimulated to the extent noted in the two and four hour groups. This is further evidenced by oxygen uptake values not greatly increased over normal values. It is concluded from these findings that the biochemical reactions causing increased oxygen uptake are occurring to the greatest extent at approximately four hours after i.m. glucocorticoid administration and that the capacity of this synthetic steroid to stimulate respiration is practically non-existant eight hours after treatment of normal rats.

TABLE II

AVERAGE OXYGEN UPTAKE BY RAT LIVER HOMOGENATES EXPRESSED AS MICROLITERS OF OXYGEN TAKEN UP PER MG. OF DRY LIVER. RATS WERE TREATED I.M. WITH 5.0 MG. OF PREDNISOLONE AT VARIOUS TIME INTERVALS BEFORE SACRIFICE

Group No.	Hours After Treatment	No. of Rats	Incubation Time (Minutes)		
			10	20	30
1	0	10	9.9	17.5	21.4
2	1	10	13.6	23.2	29.0
3	2	10	15.1	27.3	36.5
4	4	10	16.1	29.8	40.2
5	8	10	13.1	23.7	30.9

AOV-Tukey's D test: at 10 and 20 minutes, P = .01; 4, 3$>$1; at 30 minutes P = 0.01; 3, 4$>$1; P = 0.05; 4$>$5.

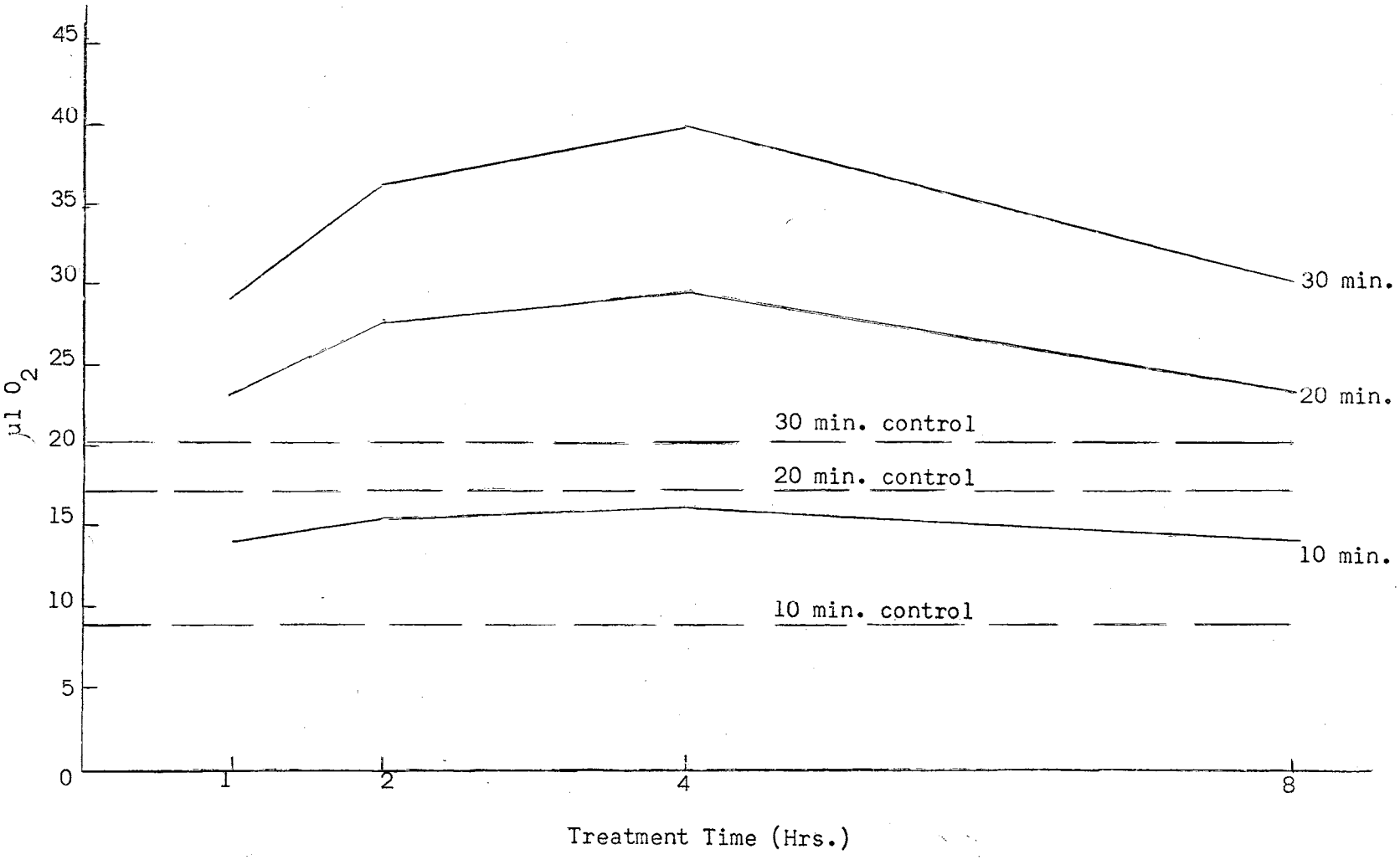


Figure 2. Average oxygen uptake (µl/mg. dry liver) by rat liver homogenates expressed as microliters O_2 taken up per mg. liver on a dry weight basis. Five mg. prednisolone administered i.m. at varying time intervals before sacrifice.

To ascertain the effect of prednisolone on hepatic carbohydrate utilization and respiration in liver homogenates prepared from intact, from adrenalectomized, and from (24 hrs.) fasted-adrenalectomized rats, 160 rats were assigned at random to one of the following treatments: 0.5 ml. of physiological saline, 400 mg. of glucose, 5.0 mg. △'F, or 400 mg. glucose plus 5.0 mg. △'F. This sequence of treatments was administered to intact, to adrenalectomized, and to fasted-adrenalectomized animals.

It is evident from Table III and Figures 3, 4, and 5, that these treatments resulted in some marked changes in the respiration of the liver homogenates. As in Experiments I and II, prednisolone caused a significant increase ($P = 0.05$) in oxygen consumption, during 30 minutes incubation, by liver homogenates prepared from rats with intact adrenals (Fig. 3). The singular administration of glucose to intact rats caused a slight depression of respiration.

Prednisolone and glucose, when given jointly to intact rats, caused a marked stimulation of oxygen consumption (Fig. 3.). When given alone or with glucose, prednisolone caused a significant increase ($P = 0.01$) in respiration of homogenates prepared from livers of adrenalectomized animals when compared to the adrenalectomized controls or intact controls. This can best be seen in groups 5 through 8 in Table III and in Fig. 4. Glucose by itself did not affect oxygen uptake in the adrenalectomized groups.

In the fasted-adrenalectomized rats, as in the adrenalectomized groups, prednisolone when given singularly or jointly with glucose significantly stimulated ($P = 0.01$) respiration by the liver homogenates (Table III and Fig. 5). However, it is interesting to note that when these two treatments were given together, the stimulation

TABLE III

AVERAGE OXYGEN UPTAKE BY RAT LIVER HOMOGENATES EXPRESSED AS MICRO-LITERS OF OXYGEN TAKEN UP PER MG. OF DRY LIVER. GROUPS 1-4 WERE INTACT, GROUPS 5-8 WERE ADRENALECTOMIZED, AND GROUPS 9-12 WERE FASTED-ADRENALECTOMIZED. TREATMENTS WERE ADMINISTERED 4 HOURS BEFORE SACRIFICE

Treatment	Group No.	No. of Rats	Incubation Time (minutes)		
			10	20	30
0.5 ml. PSS	1	15	14.3	26.1	32.9
400 mg. glucose	2	15	13.4	23.7	27.8
5.0 mg. △'F	3	15	15.4	30.8	40.9
glucose & △'F*	4	15	17.5	33.4	43.9
0.5 ml. PSS	5	15	13.2	23.2	28.9
400 mg. glucose	6	15	14.0	24.1	29.9
5.0 mg. △'F	7	15	17.6	31.4	40.1
glucose & △'F*	8	15	16.8	29.9	38.2
0.5 ml. PSS	9	10	10.0	16.0	21.4
400 mg. glucose	10	10	7.0	12.9	16.4
5.0 mg. △'F	11	10	16.0	28.8	36.1
glucose & △'F*	12	10	17.0	28.1	32.5

*400 mg. glucose and 5.0 mg. △'F

AOV-Tukey's D test: At 10 minutes; P = 0.01; 10 < all other groups, 9 < all other groups except 10; 5 < 4, 7, 8, 11, 12; 2, 6 < 4, 7, 8, 12; 1 < 4, 7, 12. P = 0.05; 1 < 8; 2 < 11. At 20 minutes: P = 0.01; 9, 10 < all other groups; 2, 5 < 3, 4, 7, 8, 11; 6 < 3, 4, 7, 8; 1 < 4, 7; 11, 12 < 4. P = 0.05; 2, 5 < 12; 6 < 11; 1 < 8. At 30 minutes; P = 0.01; 10 < 1, 2, 3, 4, 5, 6, 7, 8, 11, 12; 9 < 1, 2, 3, 4, 5, 6, 7, 8, 11, 12; 2, 5 < 3, 4, 7, 8, 11; 6 < 3, 4, 7, 8; 1 < 4, 7; 11, 12 < 4. P = 0.05; 2, 5 < 12; 1 < 3.

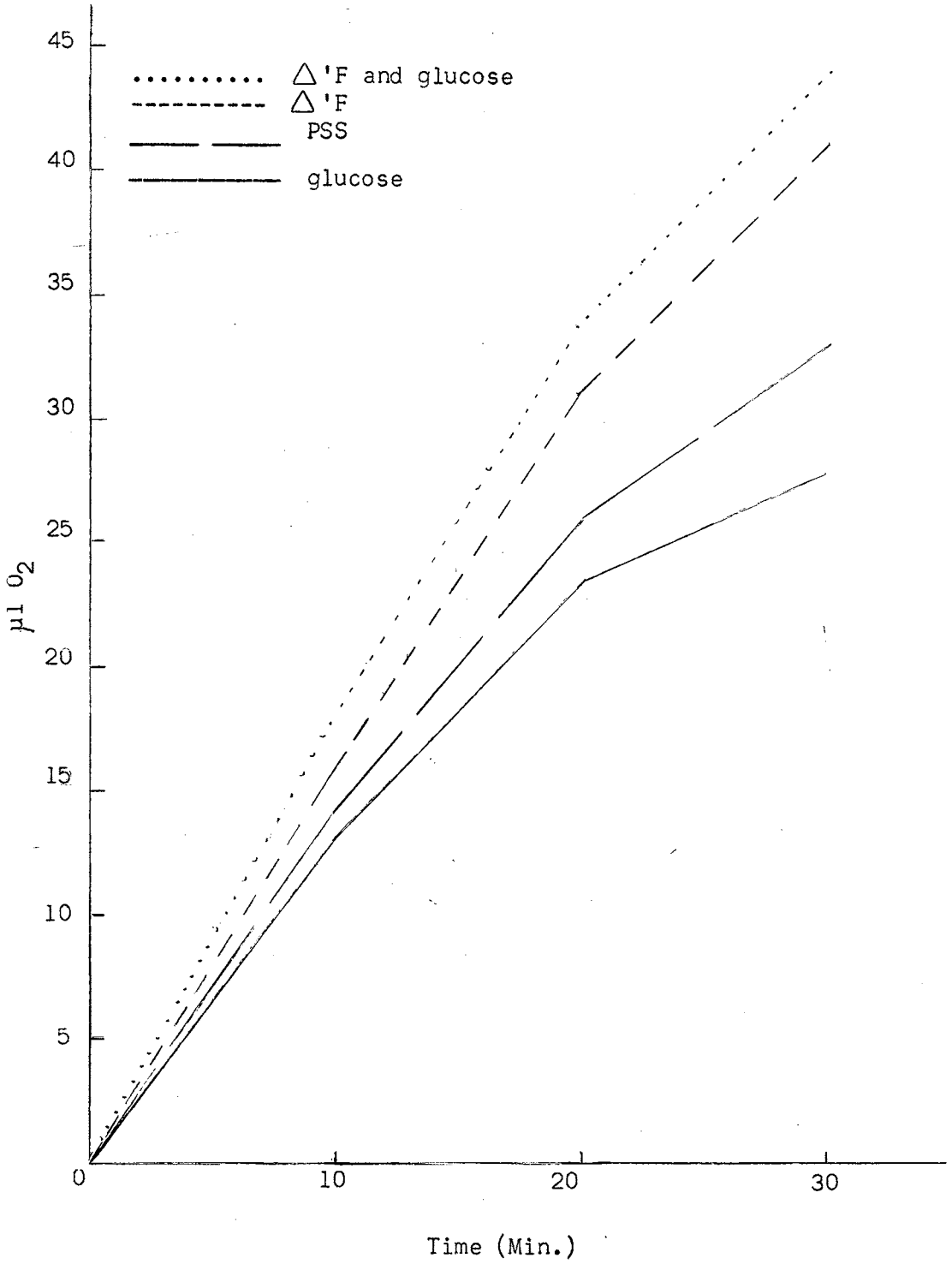


Figure 3. Average oxygen uptake (µl/mg. dry liver) by liver homogenates prepared from intact rats expressed as microliters O_2 taken up per mg. liver on a dry weight basis.

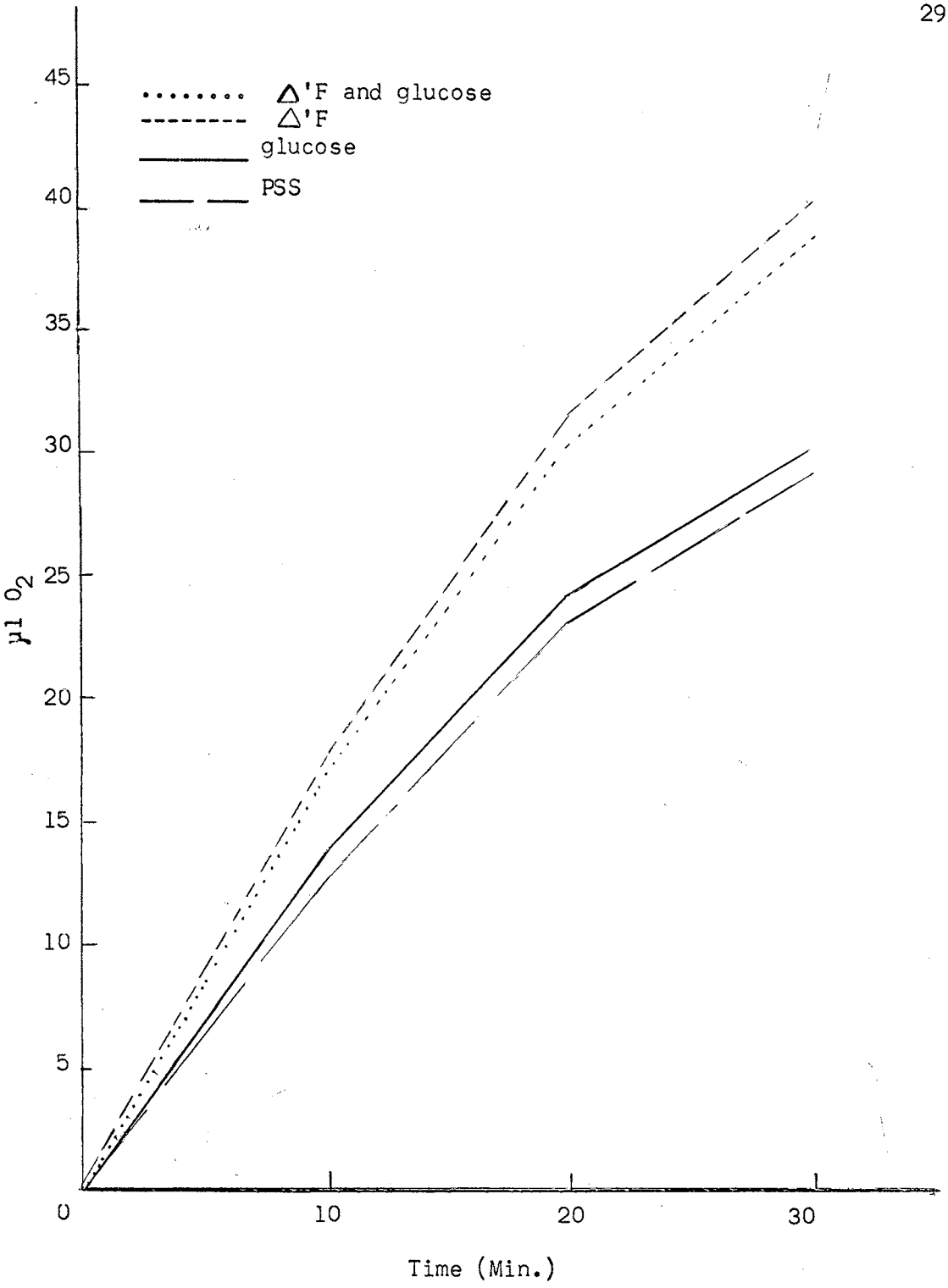


Figure 4. Average oxygen uptake (µl/mg. dry liver) by liver homogenates prepared from adrenalectomized rats expressed as microliters O_2 taken up per ml. liver on a dry weight basis.

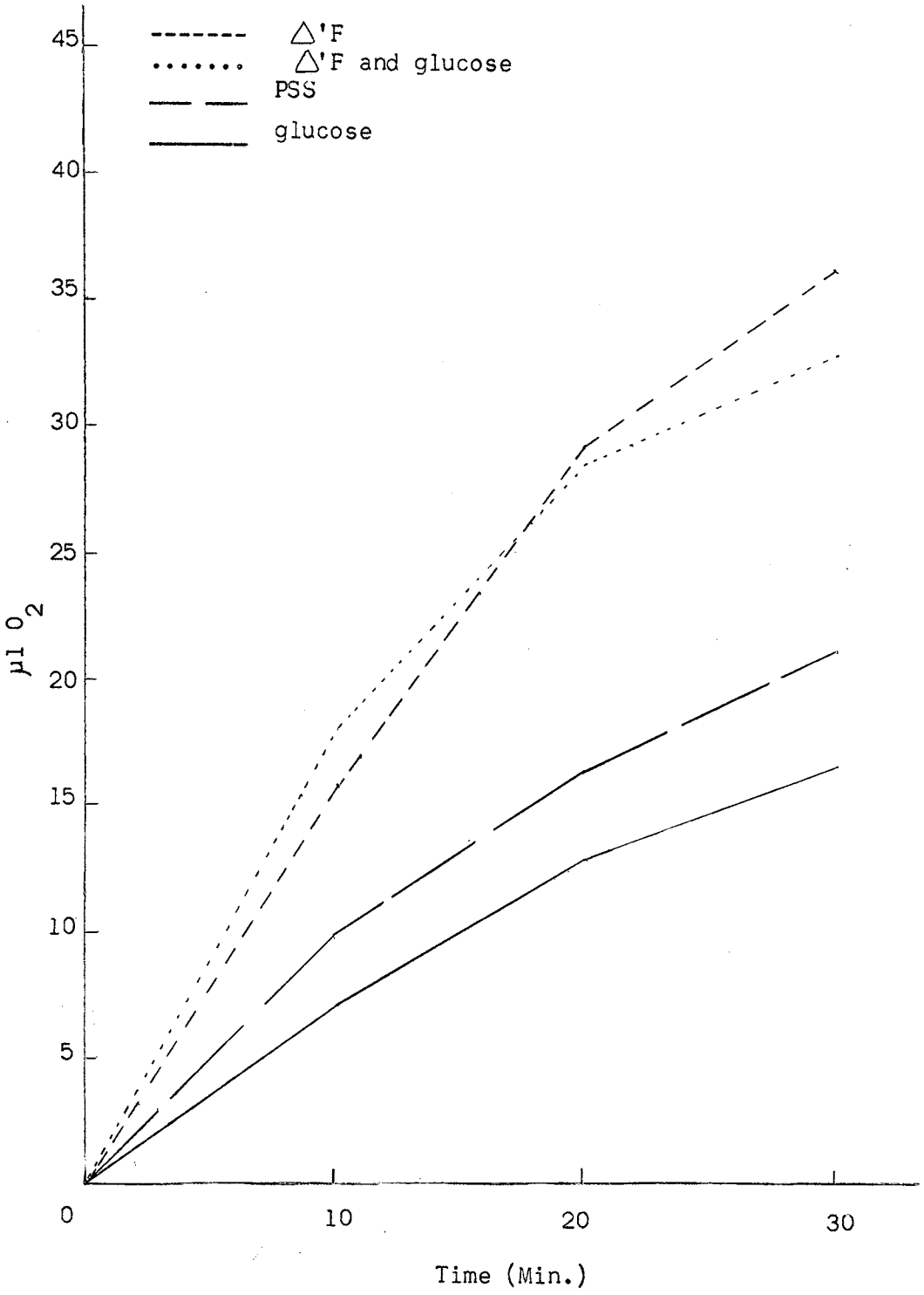


Figure 5. Average oxygen uptake (µl/mg. dry liver) by liver homogenates prepared from fasted-adrenalectomized (24 hrs.), rats expressed as microliters O_2 taken up per mg. liver on a dry weight basis.

of respiration was slightly less than when prednisolone was administered singly.

Glucose injections into fasted-adrenalectomized rats resulted in a significant decrease ($P = 0.01$) in respiration as compared to the intact or adrenalectomized controls and to intact or adrenalectomized groups to which glucose was administered (Table III). The apparent decrease in oxygen consumption observed between the fasted-adrenalectomized controls and the fasted-adrenalectomized animals receiving glucose was not statistically significant. It is, therefore, concluded that the marked depression observed in the glucose-treated, fasted-adrenalectomized animals is an expression of the fasting state. However, the possibility remains that glucose treatment may have further increased the depression in respiration.

Consideration of all twelve groups in Table III and Figures 3, 4, and 5 reveal that ablation of the adrenal had little if any effect on respiration of the rat liver homogenates. Furthermore, prednisolone when administered singly or in conjunction with 400 mg. of glucose elicited approximately the same respiratory response in adrenalectomized or intact rats. A comparison of all twelve groups also indicates that the 24 hour fast resulted in a significant reduction ($P = 0.01$) of respiration and that prednisolone can restore respiration in fasted-adrenalectomized rats to approximately the values observed in intact or adrenalectomized animals treated similarly.

Previous workers (28, 29, 30, 41) have reported conflicting results concerning the respiratory-stimulating property of the glucocorticoids. The disagreement is resolved if the duration of the treatment is taken into consideration. The results observed herein do not agree with those reported by Kerpolla and Pitkanen (41), who

demonstrated an inhibition of oxygen uptake by liver mitochondria from rats treated chronically with cortisone; the decrease was noted at the cytochrome c oxidase portion of the respiratory chain. The results reported herein do agree with the findings of Goetsch (28,29) and Grossfeld (30). A single injection of prednisolone five hours previous to sacrifice stimulated respiration of rat liver homogenates (28, 29). The stimulation of oxygen consumption was demonstrated by Grossfeld (30) in mouse fibroblast tissue cultures to which 1.50 ug. of hydrocortisone was added at 96, 24, and 0 hours before the manometric experiments.

Concurrently with the determination of oxygen uptake, values for the reducing glycolytic intermediates (Table IV) and glycogen (Table V) were determined both before and after the thirty minutes incubation period. Total carbohydrate values (Table VI) were obtained by adding the values for the reducing glycolytic intermediates to the values for glycogen both before and after incubation. This was done to learn the effect of prednisolone on liver carbohydrate utilization and to investigate the possibility that there might be some relationship between carbohydrate disappearce or utilization in the liver and the enhanced homogenate respiration noted following prednisolone treatment.

The reducing glycolytic intermediates (Table IV) include glucose and any other glycolytic intermediate with a free or potentially free aldehyde group and were determined to obtain, when added to glycogen determinations, total carbohydrate values. No treatment effects were noted on the preincubation values for reducing glycolytic intermediates in adrenalectomized groups. A significant decrease ($P = 0.01$) in these values for homogenates from fasted-adrenalectomized

TABLE IV

SUMMARY OF AVERAGE REDUCING GLYCOLYTIC INTERMEDIATE VALUES (EXPRESSED AS GM.% OF GLUCOSE) IN EXPERIMENT III. GROUPS 1-4 WERE INTACT, GROUPS 5-8 WERE ADRENALECTOMIZED, AND GROUPS 9-12 WERE FASTED-ADRENALECTOMIZED. RATS WERE TREATED 4 HOURS BEFORE SACRIFICE. DETERMINATIONS WERE MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN

Group No.	Treatment	No. of Rats	Preincubation	Postincubation	Difference
1	0.5 ml. PSS	15	2.00	4.44	2.44
2	400 mg. glucose	15	2.07	4.17	2.10
3	5.0 mg. △'F	15	1.97	5.00	3.03
4	glucose and △'F*	15	2.29	4.78	2.49
5	0.5 ml. PSS	15	2.01	4.04	2.03
6	400 mg. glucose	15	1.87	3.92	2.05
7	5.0 mg. △'F	15	2.12	4.73	2.61
8	glucose and △'F*	15	2.00	4.55	2.55
9	0.5 ml. PSS	10	0.82	1.39	0.57
10	400 mg. glucose	10	0.88	1.56	0.68
11	5.0 mg. △'F	10	1.06	1.85	0.79
12	glucose and △'F*	10	1.15	2.73	1.58

* 400 mg. glucose and 5.0 mg. △'F.

AOV-Tukey's D test:

Preincubation: $P = 0.01$; 9, 10, 11, 12 < 1, 2, 3, 4, 5, 6, 7, 8; 6 < 4.

Difference: $P = 0.01$: 9, 10, 11 < 1, 2, 3, 4, 5, 6, 7, 8, 12; 2, 5, 6 < 3; 12 < 1, 3, 4, 7, 8; $P = 0.05$: 1, 4 < 3; 5, 6 < 7.

rats prior to incubation was observed; this undoubtedly was due to the fasting condition. Prednisolone singly or with glucose enhanced the appearance of reducing glycolytic intermediates during incubation in all three types of rats.

There were no statistically significant differences in the preincubation glycogen values within the intact groups except that prednisolone treatment increased liver glycogen ($P = 0.05$) over the glucose treated group (Table V). The lack of statistical significance is probably due to the wide variation in liver glycogen values of the individual rats. This is not surprising in view of the well known

TABLE V

SUMMARY OF AVERAGE LIVER GLYCOGEN VALUES (EXPRESSED AS GM.% OF GLUCOSE) IN EXPERIMENT III. GROUPS 1-4 WERE INTACT, GROUPS 5-8 WERE ADRENALECTOMIZED, AND GROUPS 9-12 WERE FASTED-ADRENALECTOMIZED. TREATMENTS WERE ADMINISTERED 4 HOURS BEFORE SACRIFICE. DETERMINATIONS WERE MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN

Group No.	Treatment	No. of Rats	Preincubation	Postincubation	Difference
1	0.5 ml. PSS	15	9.86	7.52	2.34
2	400 mg. glucose	15	7.17	4.16	3.01
3	5.0 mg. △'F	15	10.90	7.26	3.64
4	glucose and △'F*	15	8.94	7.04	1.90
5	0.5 ml. PSS	15	9.41	6.34	3.07
6	400 mg. glucose	15	8.02	6.35	1.66
7	5.0 mg. △'F	15	9.26	7.68	1.57
8	glucose and △'F*	15	11.05	9.34	1.71
9	0.5 ml. PSS	10	1.65	1.62	0.03
10	400 mg. glucose	10	1.74	1.6	0.08
11	5.0 mg. △'F	10	3.14	1.79	1.35
12	glucose and △'F*	10	4.68	2.68	2.00

*400 mg. glucose and 5.0 mg. △'F
AOV-Tukey's D Test:
Preincubation: P = 0.01: 9, 10, 11, 12 < 1, 3, 4, 5, 7, 8; 9, 10, 11 < 2, 6. P = 0.05: 2 < 3, 8.

fact that liver glycogen fluctuates greatly over a 24 hour period. However, it is of interest to note that prednisolone did increase the glycogen levels in intact animals. This is in agreement with numerous reports stating that the glucocorticoids increase liver glycogen deposition (6, 14, 16, 33, 50, 57, 84). Clark and Pesch (16) explained the increased glycogen levels in cortisone treated animals on the basis of a partial block at some point in the Krebs cycle. This was concluded from the observation that cortisone inhibited the oxidation of alpha-ketoglutarate by liver mitochondria even though the P/O ratios remained within normal ranges.

TABLE VI

SUMMARY OF AVERAGE TOTAL CARBOHYDRATE VALUES (EXPRESSED AS GM.% OF GLUCOSE) IN EXPERIMENT III. GROUPS 1-4 WERE INTACT, GROUPS 5-8 WERE ADRENALECTOMIZED, AND GROUPS 9-12 WERE FASTED-ADRENALECTOMIZED. TREATMENTS WERE ADMINISTERED 4 HOURS BEFORE SACRIFICE. DETERMINATIONS WERE MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN

Group No.	Treatment	No. of Rats	Preincubation	Postincubation	Difference
1	0.5 ml. PSS	15	11.85	11.62	0.23
2	400 mg. glucose	15	9.24	8.33	0.91
3	5.0 mg. △'F	15	12.87	12.27	0.60
4	glucose and △'F*	15	11.23	11.81	-0.59
5	0.5 ml. PSS	15	11.40	10.25	1.15
6	400 mg. glucose	15	9.89	10.27	-0.38
7	5.0 mg. △'F	15	11.37	12.41	-1.04
8	glucose and △'F*	15	13.06	13.89	-0.83
9	0.5 ml. PSS	10	2.47	3.01	-0.54
10	400 mg. glucose	10	2.62	3.22	0.60
11	5.0 mg. △'F	10	4.20	3.64	0.56
12	glucose and △'F*	10	5.82	5.33	0.49

*400 mg. glucose and 5.0 mg. △'F
AOV-Tukey's D test:
Preincubation: $P = 0.01$: 9, 10, 11 < 1, 2, 3, 4, 5, 6, 7, 8; 12 < 1, 3, 4, 5, 6, 7, 8; $P = 0.05$: 2 < 3, 8; 12 < 2.

Within the adrenalectomized groups there was also a lack of statistically significant differences in the preincubation glycogen values (Table V). Prednisolone when given with glucose did cause a noticeable increase in liver glycogen before incubation in the adrenalectomized and the fasted-adrenalectomized rats. The results suggest that adrenalectomy tended to decrease glycogen deposition when adrenalectomized controls are compared to intact controls. This observation is in accord with the well known fact that the glucocorticoids enhance liver glycogen deposition. Equally apparent is the fact that the administration of prednisolone in addition to glucose tends to restore liver glycogen deposition in adrenalectomized rats.

In fasted-adrenalectomized animals one would expect to find less variation in the liver glycogen levels and thus a more valid evaluation of the effects of prednisolone and/or glucose administration should be obtained from these animals. As would be expected, fasting resulted in a marked drop ($P = 0.01$) in liver glycogen values in all four fasted-adrenalectomized groups when compared with intact or adrenalectomized animals.

Treatment effects on liver glycogen deposition were not significant within the fasted-adrenalectomized groups; however, glucose, prednisolone, or prednisolone and glucose treatments caused an elevation of liver glycogen in that order.

No statistically significant treatment effects were obtained between any of the twelve groups in rate at which glycogen disappeared during 30 minutes of incubation.

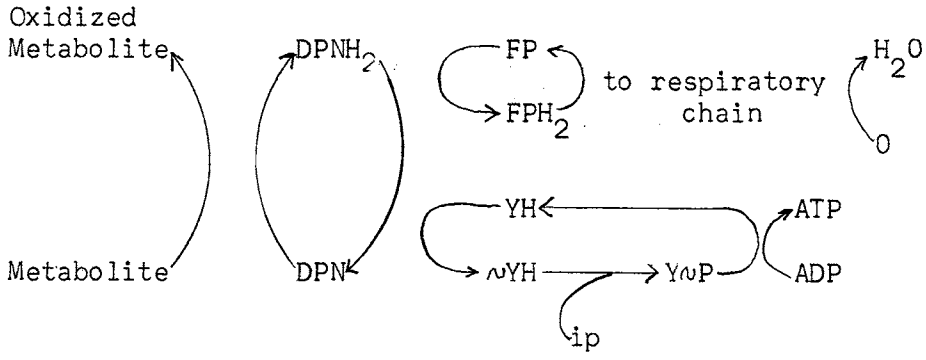
To obtain the overall picture of carbohydrate utilization during incubation, total carbohydrate values were computed by adding the values for the reducing glycolytic intermediates and glycogen together both before and after incubation. The difference between preincubation and postincubation values were then recorded and appear in Table VI. It is apparent that fasting markedly decreased ($P = 0.01$) the total liver carbohydrate in the fasted-adrenalectomized groups prior to incubation. Similar trends are seen in the preincubation values for total carbohydrate (Table VI) of the intact and adrenalectomized groups. There were no statistically significant treatment effects on the rate at which the total carbohydrate was utilized in the liver homogenates during incubation.

The trend towards carbohydrate synthesis during incubation in groups 4, 6, 7, 8, 9 and 10 are difficult to explain; statistically they are not different from the groups showing utilization. In groups 4, 7, 8, all of which received prednisolone, this increase in total carbohydrate could conceiveably represent gluconeogenesis.

The trend towards increased carbohydrate utilization in the adrenalectomized control rats compared with the intact controls (Table VI) tends to confirm the results of Winternitz and Kline (83) who studied respiratory quotients and $C^{14}O_2$ production from 2-C^{14}-pyruvate in adrenalectomized animals and reported an increase in carbohydrate utilization in the adrenalectomized subject.

Since there were no treatment effects of prednisolone on total carbohydrate utilization to accompany the respiratory-stimulating property, the cause of the enhanced respiration apparently does not lie in the area of carbohydrate metabolism studied in this experiment. This confirms earlier reports by Goetsch (28).

An explanation for the increased respiration following the administration of glucocorticoids may well lie in the process called respiratory chain phosphorylation or oxidative phosphorylation which may be depicted as follows (80).



In this process, three moles of ATP are formed as each pair of electrons are passed from the metabolite up the respiratory chain to molecular oxygen. The phosphorylation of ADP to ATP is thus coupled through some unknown compound to the respiratory chain; thus, in a steady state condition inorganic phosphate (iP) and/or ADP may exert a regulatory influence on the respiration process (51).

Chronic treatment (7 days) with cortisone has been shown to uncouple oxidative phosphorylation in rat liver mitochondria (40). This could have conceively also occurred in the rat liver homogenates of this experiment following a single injection of prednisolone. This uncoupling would have released the regulatory influence of ADP and inorganic phosphate and allowed respiration to proceed at an increased rate. It has been shown that the rate of oxidation of ferrocytochrome c is increased in the presence of some uncoupling agents (63). A large proportion of tissue ATP is produced through oxidative phosphorylation; uncoupling of oxidative phosphorylation by the glucocorticoids could inhibit the production of ATP which may in turn result in an impairment of the hexokinase function, and/or the mobilization of glycogen from the tissues because of an inhibition of the activation of phosphorylase b to phosphorylase a.

Whether the single injection of prednisolone uncoupled oxidative phosphorylation can be neither confirmed nor denied from the results reported herein since no concurrent studies were made on the oxidative phosphorylation process. The hypothesis is offered only as a possible explanation.

CHAPTER V

SUMMARY AND CONCLUSIONS

The conflicting reports which have been published concerning the respiratory stimulating property of both naturally occurring and synthetic glucocorticoids can probably be best accounted for on the basis of the variety of tissues used and the differences in the duration of the treatment. This study was carried out to further clarify the respiratory stimulating property of glucocorticoids and to determine any effects which these hormones may have upon hepatic glycolysis in rat liver homogenates.

Mature female albino rats were used as the experimental animals. Prednisolone, a synthetic adrenal cortical hormone similar to naturally occurring hydrococtisone, was used in this study because of its exaggerated glucocorticoid properties.

In experiment I, stimulation of respiration was used as the criterion for determining the optimum dose of prednisolone. Different doses of the synthetic hormone were administered i.m. at random to three groups of rats; a fourth group received i.m. injections of PSS and served as the control. It was apparent from the results obtained under the conditions of this experiment that 5.0 mg. of Δ'F is sufficient to stimulate near maximum respiration and that additional prednisolone will not significantly increase oxygen consumption.

Experiment II was designed to learn the time interval following the steroid injection when maximum oxygen uptake by the incubating liver homogenates occurred. Groups were assigned at random to receive 5.0 mg. of Δ'F at varying periods of time prior to sacrifice. Oxygen uptake was significantly increased ($P = 0.01$) at both two and four hours following prednisolone treatment, the greatest stimulation being at a four hour interval. Prednisolone failed to stimulate respiration at 8 hours post-treatment. It was concluded from these findings that the biochemical reactions causing increased oxygen uptake are occurring to the greatest extent about four hours after glucocorticoid administration and that the capacity of this synthetic steroid to stimulate respiration is practically non-existant eight hours after treatment in normal rats.

Experiment III ascertained the effect of prednisolone on hepatic carbohydrate utilization and respiration in liver homogenates prepared from intact, from adrenalectomized, and from (24 hr.) fasted-adrenalectomized rats. One hundred sixty rats were assigned at random to one of the following parenteral treatments: 0.5 ml of physiological saline, 400 mg. of glucose, 5.0 mg. of Δ'F, and 400 mg. of glucose plus 5.0 mg. of Δ'F. This sequence of treatments was in turn given to intact, to adrenalectomized, and to fasted-adrenalectomized rats. Ablation of the adrenal had little, if any, effect on respiration of the rat liver homogenates. Prednisolone, when administered singly or in conjunction with glucose, elicited approximately the same increase ($P = 0.01$) in respiration in all three types of animals. The 24 hour fast resulted in a significant reduction ($P = 0.01$) of respiration; however, prednisolone treatment restored homogenate respiration to values similar to those observed

for intact and adrenalectomized animals, treated similarly with prednisolone.

No treatment effects were noted on the preincubation values for reducing glycolytic intermediates in intact or in adrenalectomized groups. A significant decrease ($P = 0.01$) in the reducing glycolytic intermediates of the fasted-adrenalectomized rats prior to incubation of the homogenate was observed; this undoubtedly was due to the fasting condition. Prednisolone singly or with glucose enhanced the appearance of reducing glycolytic intermediates during incubation.

There was no significant difference in preincubation liver glycogen values between intact or adrenalectomized groups treated similarly. The fasted-adrenalectomized groups exhibited a significant decrease ($P = 0.01$) in preincubation glycogen when compared to intact and adrenalectomized animals.

A study of the total carbohydrate utilized during incubation revealed that the treatments given had no statistically significant effects. Therefore, the respiratory stimulating property of the glucocorticoids does not appear to lie in this area of metabolism. It is suggested from the results of other workers that the glucocorticoids may uncouple oxidative phosphorylation, which in turn may release the regulatory influence of inorganic phosphate and/or ADP, thus permitting respiration to proceed at a more rapid rate.

BIBLIOGRAPHY

1. Abood, L. G. and J. J. Kocsis. Effect of ACTH on Glycogenesis and Glycolysis in Hypophysectomized Rats, Proc. Soc. Exptl. Biol. Med., 75:55 (1950).

2. Altszuler, N., R. Steele, J. S. Wall, and R. C. deBodo. Effects of Adrenocortical Steroids on Carbohydrate Metabolism in Hypophysectomized Dogs; Studies with C^{14}-labeled Glucose, Am. J. Physiol., 191:29 (1957).

3. Ashmore, J., G. F. Cahill, Jr., R. Hillman, and A. E. Renold. Adrenal Cortical Regulation of Hepatic Glucose Metabolism, Endocrinol., 62:621 (1958).

4. Ashmore, J., A. B. Hastings, and F. B. Nesbett. The Effect of Diabetes and Fasting on Liver Glucose-6-Phosphatase, Proc. Nat. Acad. Sci., 40:673 (1954).

5. Ashmore, J., A. B. Hastings, F. B. Nesbett, and A. E. Renold. Studies on Carbohydrate Metabolism in Rat Liver Slices VI. Hormonal Factors Influencing Glucose-6-Phosphatase, J. Biol. Chem., 218:77 (1956)

6. Ashmore, J., F. Stricker, W. C. Love, and G. Kilskeines. Cortisol Stimulation of Glycogen Synthesis in Fasted Rats, Endocrinol., 68:599 (1961)

7. Bacila, M. and E. S. G. Barron. The Effect of Adrenal Cortical Hormones on the Anaerobic Glycolysis and Hexokinase Activity, Endocrinol., 54:591 (1954).

8. Bennett, L. I. The Interrelation of Pituitary and Adrenal in the Control of Carbohydrate Levels in The Rat, Endocrinol., 22:193 (1938).

9. Bennett, L. I. and C. H. Li. The Effects of Growth Hormone and Adrenocorticotrophic Hormone on the Urinary Glucose and Nitrogen Excretion of Diabetic Rats, Endocrinol., 39:63 (1956).

10. Blaylock, B. A., E. Rothrock, and J. Slacks. Alloxan Diabetes Insulin and Adrenosine Phosphates of Liver, Am. J. Physiol., 198:1063 (1960).

11. Blecher, M. and A. White. Loci of Action of Adrenal Cortical Steroids in Anaerobic Glycolysis by Cell-free Preparations of Rat Lymphosarcoma, J. Biol. Chem., 235:282. (1960).

12. Brady, R. O., F. D. W. Lukens, and S. Gurin. Synthesis of Radioactive Fatty Acids in Vitro, and Its Hormonal Control, J. Biol. Chem., 193:459 (1951).

13. Brin, M. and R. W. McKee. Effects of X-Irradiation, Nitrogen Mustard, Fasting, Cortisone, and Adrenalectomy on Transaminase Activity in the Rat, Arch. Biochem. Biophys., 61:384 (1956).

14. Britton, S. W. and H. Silverte. Effects of Cortico Adrenal Extract on Carbohydrate Metabolism in Normal Animals, Am. J. Physiol., 100:693 (1932).

15. Cahill, C. A., J. Ashmore, S. Zotter, and A. B. Hastings. Studies on Carbohydrate Metabolism in Rat Liver Slices: IX. Ionic and Hormonal Effects on Phosphorylase and Glycogen, J. Biol. Chem., 224:237 (1957).

16. Clark, J. H. and L. A. Pesch. Effects of Cortisone Upon Liver Enzymes and Protein Synthesis, J. Pharmacol. Expt. Ther., 117:202 (1956).

17. Cohen, L. H. Glucose Inhibition of Respiration in the Developing Retina, Fed. Proc., 16:165 (1957).

18. Cohn, C., B. Katz, B. Huddlestein, M. Kilinsky, and R. Levine. Utilization of Carbohydrate by Extrahepatic tissues of the Adrenalectomized Dog, Am. J. Physiol, 170:87 (1952).

19. Colowick, S. P., G. T. Cori, and M. W. Slein. The Effect of Adrenal Cortex and Anterior Pituitary Extracts and Insulin on the Hexokinase Reaction, J. Biol. Chem., 168:583 (1947).

20. Conn, J. W., L. H. Louis, and S. S. Fajans. The Probability that Compound F (17-hydroxycorticorterone) is the Hormone Produced by the Normal Human Adrenal Cortex, Science, 113:713 (1951).

21. Constantinides, P., C. Fortier, and F. R. Skeleton. The Effect of Glucose on the Adrenal Response to ACTH in Hypophysectomized Rats, Endocrinol., 47:351 (1950).

22. Cori, G. T. and C. F. Cori. Glucose-6-Phosphatase of the Liver in Glycogen Storage Disease, J. Biol. Chem., 199:661 (1952).

23. Crabtree, H. G. Observations on Carbohydrate Metabolism of Tumors, Biochem. J., 23:536 (1929).

24. Eisenstein, A. B. Relationship of Vitamin B_6 to Gluconeogenic Action of Cortisol, Endocrinol., 67:97 (1960).

25. Froesch, E. R., J. Ashmore, and A. E. Renold. Comparison of Renal and Hepatic Effects of Fasting, Cortisone Administration and Glucose Infusion in Normal and Adrenalectomized and Glucose Infusion in Normal and Adrenalectomized Rats, Endocrinol., 62:614 (1958).

26. Foresch, E. R., A. I. Winegrad, A. E. Renold, and G. W. Thorn. Mechanism of the Glucosuria Produced by the Administration of Steroids with Glucocorticoid Activity, J. Clin, Invest., 37:524 (1958).

27. Glenn, E. M., B. J. Bowman, R. B. Bayer, and C. E. Meyer. Hydrocortisone and Some of Its Effects on Intermediary Metabolism, Endocrinol., 69:386 (1961).

28. Goetsch, D. D. Some Metabolic Effects of Glucocorticoids in Cattle and Rats. Doctor's Thesis, Oklahoma State University, (1961).

29. Goetsch, D. D. and L. E. McDonald. Glucocorticoid Effects on Respiration and Metabolism of Rat Liver Homogenates, Am. J. Physiol., 202:343 (1962).

30. Grossfeld, H. A. Action of Hydrocortisone on Respiration and Aerobic Glycolysis of Cultured Cells, Science, 127:148 (1958).

31. Herman, M. S. and E. R. Ramey. Effect of Hydrocortisone and Epinephrine on Glucose Uptake by the Rat Diaphragm, Endocrinol. 67:650 (1960).

32. Hess, W. C. and I. P. Shaffran. Effect of Cortisone upon Formation of Liver Glycogen from Glycine, Proc. Soc. Exptl. Biol. Med., 83:804 (1953).

33. Hess, W. C., I. P. Shaffran, and E. L. Everitt. Effect of Cortisone on Liver Phosphorylase Activity, Proc. Soc. Exptl. Biol. Med., 103:695 (1960).

34. Ingle, D. J. and R. C. Meeks. Comparison of Some Metabolic and Morphologic Effects of Cortisone and Hydrocortisone Given by Continuous Injection to Rats, Am. J. Physiol., 170:77 (1952).

35. Ingle, D. J., M. C. Prestrud, and C. H. Li. Effects of Administering Adrenocorticotrophic Hormone by Continuous Injection to Normal Rats, Am. J. Physiol., 166:165. (1951).

36. Ingle, D. J., M. C. Prestrud, and J. A. Negamis. Effects of Administering Large Doses of Cortisone Acetate to Normal Rats, Am. J. Physiol., 166:171 (1951).

37. Ingle, D. J., R. Sheppard, J. S. Evans, and M. H. Kuizenga. A Comparison of Adrenal Steroid Diabetes and Pancreatic Diabetes in the Rat, Endocrinol., 37:341 (1945).

38. Johnson, M. J. The Role of Aerobic Phosphorylation in the Pasteur Effect, Science, 94:200 (1941).

39. Kerppola, W. Inhibition of Phosphorylase with Cortisone and Its Activation with Adrenaline in the Rabbit, Endocrinol., 51:192 (1952).

40. Kerppola, W. Inhibition of Oxidative Phosphorylation with Cortisone in Liver Mitochondria, Endocrinol., 67:252 (1960).

41. Kerppola, W. and E. Pitkanen. The Action of Cortisone on Oxidative and Glycolytic Enzyme Activities in Rats of Different Age and Sex, Endocrinol., 67:162 (1960).

42. Kostyo, J. L. Effect of Some Steroids on Glycogen Metabolism in Uterus and Skeletal Muscle, Proc. Soc. Exptl. Biol. Med., 97:405 (1958).

43. Kowalewski, K. and G. Belesi. Tissue Oxygen Consumption in Rats Treated with Cortisone and with An Anabolic Androgen, Proc. Soc. Exptl. Biol. Med., 106:300 (1961).

44. Kun, E. and D. R. McCurley. Physiological Regulation of Phosphopyruvate Formation from 3-Phosphoglycerate in Rat Tissues, Proc. Soc. Exptl. Biol. Med., 75:797 (1950).

45. Kvam, D. C, and R. E. Parks. Hydrocortisone-Induced Changes in Hepatic Glucose-6-Phosphatase and Fructose Diphosphatase Activities, Am. J. Physiol., 198:21 (1960).

46. Lacroix, E. and I. Leusen. The Influence of Cortisone on the Oxygen Consumption of Myocardium and Diaphragm Slices of the Rat, Arch. Internationales de Pharm. Ther., 114:103 (1958).

47. Lacroix, E. and I. Leusen. The Influence of Cortisone on Substrate Utilization by Rat Heart Slices, Acta. Endocrinol., 31:324 (1959).

48. Landau, B. R. Gluconeogenesis and Pyruvate Metabolism in Rat Kidney, In Vitro, Endocrinol., 67:744 (1960).

49. Langdon, R. G. and D. R. Weakly. The Influence of Hormonal Factors and of Diet Upon Hepatic Glucose-6-Phosphatase Activity, J. Biol. Chem., 214:167 (1955).

50. Langley, L. L. and C. H. Guenthorpe. Adrenal, Hypophyseal and Pancreatic Hormones in the Liver Glycogen Response to Low Atmospheric Pressure, Am. J. Physiol., 191:342 (1957).

51. Lardy, H. A. and H. Wellman. Oxidative Phosphorylation: Role of Inorganic Phosphate and Acceptor Systems in Control of Metabolic Rates, J. Biol. Chem., 195:215 (1952).

52. Lee, L. E. Effect of Steroid Hormones on Rat Liver Pyruvic Oxidase, Fed. Proc., 17:262 (1958).

53. Leonard, S. L. Glycogen Deposition in Different Skeletal Muscles Induced by Cortisone and Estradiol, Endocrinol., 53:226 (1953).

54. Leonard, S. L. The Effect of Hormones on Phosphorylase Activity in Skeletal Muscle, Endocrinol., 60:619 (1957).

55. Linke, A. Experimental Study of the Significance of Liver Glucose-6-Phosphatase for Regulation of Carbohydrate Metabolism, Med. Welt., 46:2475 (1960).

56. Long, C. N. H. A Discussion of the Mechanism of Action of Adrenal Cortical Hormones on Carbohydrate and Protein Metabolism, Endocrinol., 30:970 (1943).

57. Long, C. N. H., B. Katzin, and E. Fry. The Adrenal Cortex and Carbohydrate Metabolism, Endocrinol., 26:309 (1940).

58. Long, C. N. Hand, F. D. W. Lukens. The Effects of Adrenalectomy and Hypophysectomy upon Experimental Diabetes in the Cat, J. Exptl. Med., 63:465 (1936).

59. Mills, J. N. and S. Thomas. The Influence of Hydrocortisone upon Phosphate Metabolism in Muscle, J. Physiol., 142:506 (1958).

60. Mills, J. N. and S. Thomas. The Influence of Adrenal Corticoids on Phosphate and Glucose Exchange in Muscle and Liver of Man, J. Physiol., 148:227 (1959).

61. Mokrasch, L. C., W. D. Davidson, and R. W. McGilvery. The Response to Gluconeogenic Stress of Fructose-1-6-Diphosphatase in Rabbit Liver, J. Biol. Chem., 222:179 (1956).

62. Nelson, N. A Photometric Adaptation of the Somogyi Method for the Determination of Glucose, J. Biol. Chem., 153:375 (1944).

63. Nielsen, B. O. and A. L. Lehninger. Phosphorylation Coupled to the Oxidation of Ferrocytochrome c, J. Biol. Chem., 215:555 (1955).

64. Nugent, C. A., K. Eik-Nes, and F. H. Tyler. A Comparative Study of the Metabolism of Hydrocortisone and Prednisolone, J. Clin. Endocrinol. and Metab., 19:526 (1959).

65. Nemeth, A. M. Glucose-6-Phosphatase in the Liver of the Fetal Guinea Pig, J. Biol. Chem., 209:773. (1954).

66. Overell, B. G., S. E. Condon, and V. Petrow. The Effects of Hormones and Their Analogues Upon the Uptake of Glucose by Mouse Skin In Vitro, J. Pharm. and Pharmacol., 12:150 (1960).

67. Papper, S., L. Saxon, and H. C. Alpert. The Effects of Cortisone on the Galactose Tolerance Tests of Normal Men, J. Lab. Clin. Med., 50:394 (1957).

68. Renold, A. E., C. T. Teng, F. B. Nesbett, and A. B. Hastings. Studies on Carbohydrate Metabolism in Rat Liver. II. The Effect of Fasting and of Hormonal Deficiencies, J. Biol. Chem., 204:533 (1953).

69. Rosen, F., M. R. Roberts, L. E. Budnick, and C. A. Nichol. An Enzymatic Basis for the Gluconeogenic Action of Hydrocortisone, Science, 127:287 (1958).

70. Rosen, F., M. R. Roberts, and C. A. Nichol. Glucocorticoids and Transaminase Activity. I. Increased Activity of Glutamic-pyruvic Transaminase in Four Conditions Associated with Gluconeogenesis, J. Biol. Chem., 234:476 (1959).

71. Russell, J. A. and L. L. Bennett. Maintenance of Carbohydrate Levels in Fasted Hypophysectomized Rats Treated with Anterior Pituitary Extracts, Proc. Soc. Exptl. Biol. Med., 34:406 (1936).

72. Segaloff, A. and A. S. Many. Proc. 2nd Clin. ACTH Conf., I, 301 (1951), As cited in Rev. Physiol., 14:466 (1952).

73. Topper, Y. J. and L. A. Persch. Galactose: Its In Vitro Oxidation as Influenced by Steroids, Fed., Proc., 17:323 (1958).

74. Turner, C. D. General Endocrinology. Philadelphia W. B. Saunders Company, 1960.

75. Umbreit, W. W., R. H. Burris, and J. F. Stauffer. Manometric Techniques. Minneapolis: Burgess Publishing Company, 1957.

76. Villee, C. A., V. K. White, and A. B. Hastings. Metabolism of C^{14}-labeled Glucose and Pyruvate by Rat Diaphragm Muscle in Vitro, J. Biol. Chem., 195:287 (1952).

77. Weber, G., C. Allard, C. deLamirande, and A. Cantero. Liver Glucose-6-Phosphatase Activity and Intracellular Distribution After Cortisone Administration, Endocrinol., 58:40 (1956).

78. Weber, G., C. Allard, G. deLamirande, and A. Cantero. Increased Liver Glucose-6-Phosphatase Activity After Cortisone Administration, Biochem. Biophys. Acta, 16:613 (1955).

79. Welt, I. D., D. Stetten, D. J. Ingle, and E. H. Morley. Effect of Cortisone Upon Rates of Glucose Production and Oxidation in the Rat, J. Biol. Chem., 197:57 (1952).

80. West, K. M. Comparison of the Hyperglycenic Effects of Glucocorticoids in Human Beings, Diabetes, 6:168 (1957).

81. Willmer, J. S. Changes in Hepatic Enzyme Levels After Adrenalectomy. I. Phosphorylase, Phosphoglucomutase, and Phosphoglucose Isomerase, Canad. J. Biochem., 38:1095 (1961).

82. Winternitz, W. W. and W. G. Forrest. Metabolism of 2-C^{14}-Pyruvate After Adrenalectomy, Am. J. Physiol., 199:1054 (1960).

83. Winternitz, W. W. and D. L. Kline. Respiratory Studies on the Utilization of Carbohydrates After Adrenalectomy, Am. J. Physiol., 192:506 (1958).

84. Winternitz, W. W., R. Pintzis, and C. N. H. Long. Further Studies on the Adrenal Cortex and Carbohydrate Metabolism, Endocrinol., 61:724 (1957).

APPENDIX

EXPERIMENT I

OXYGEN UPTAKE (μL/MG. LIVER) AT 10, 20, AND 30 MINUTES OF INCUBATION BY RAT LIVER HOMOGENATES. TREATMENTS ADMINISTERED I.M. 5 HOURS BEFORE SACRIFICE. GROUP 1: 1.0 ML. PSS; GROUP 2: 0.5 MG. PREDNISOLONE; GROUP 3: 5.0 MG. PREDNISOLONE; GROUP 4: 20.0 MG. PREDNISOLONE

Group 1			Group 2		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
13.5	25.0	38.3	16.0	27.0	34.7
14.1	25.9	38.1	15.6	24.0	37.4
10.9	23.1	25.1	12.7	24.4	26.9
7.8	10.2	12.4	12.5	20.2	24.2
11.4	12.8	21.5	13.7	24.4	31.2
13.7	29.8	36.5	16.6	29.6	39.7
16.4	31.3	44.9	16.9	29.4	37.0
14.0	25.3	30.5	14.4	27.1	35.6
15.0	27.2	34.9	13.9	24.5	29.2
13.1	25.1	30.0	14.1	26.9	36.6
Mean:					
13.0	24.1	31.2	14.6	25.8	33.3
S.D.					
2.4	6.1	9.5	1.6	2.8	3.9
Group 3			Group 4		
18.7	35.6	43.8	19.2	37.4	41.2
18.8	38.3	54.4	20.4	38.3	53.9
11.7	21.0	23.3	15.0	28.6	37.0
16.6	29.7	41.9	12.5	24.4	33.8
18.4	32.7	39.8	17.1	32.8	44.7
15.1	28.2	35.7	15.5	31.2	43.5
17.3	32.4	43.0	14.8	28.2	37.6
16.0	30.8	40.7	14.3	27.4	37.3
12.6	23.5	28.8	16.8	32.8	44.5
14.2	29.0	40.2	15.1	29.4	38.2
Mean:					
15.9	30.1	39.2	16.1	31.1	41.2
S.D.					
2.5	5.2	7.3	2.4	4.4	5.8

EXPERIMENT II

OXYGEN UPTAKE (μL/MG. LIVER) AT 10, 20, AND 30 MINUTES OF INCUBATION BY RAT LIVER HOMOGENATES. 5.0 MG. PREDNISOLONE ADMINISTERED I.M. AT DIFFERENT TIME INTERVALS BEFORE SACRIFICE. GROUP 1: 0.5 ML. PSS AT 8 HOURS; GROUP 2 AT 1 HOUR: GROUP 3 AT 2 HOURS; GROUP 4 AT 4 HOURS; GROUP 5 AT 8 HOURS

Group 1			Group 2		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	
13.7	19.8	20.4	16.7	27.7	32.9
11.3	21.7	26.7	12.0	22.8	31.8
10.7	19.3	23.8	14.0	21.0	24.8
11.5	20.8	28.3	16.1	30.3	41.3
7.8	11.9	14.5	13.4	22.9	29.1
7.7	13.8	16.7	14.5	22.2	26.3
15.1	26.7	30.7	10.5	20.6	24.4
0.4	5.3	8.6	17.3	30.1	36.9
11.1	17.6	22.5	8.5	10.9	13.1
Mean:					
9.9	17.5	21.4	13.6	23.2	29.0
S.D.					
4.1	6.0	6.7	2.8	5.6	7.7

Group 3			Group 4		
18.3	31.7	43.1	17.2	29.7	41.1
14.5	26.0	35.2	15.1	28.9	40.5
16.3	29.0	40.1	15.2	26.4	35.8
13.2	25.9	35.7	13.7	28.1	38.4
16.2	28.4	38.7	16.5	30.2	41.6
15.1	25.5	37.3	14.2	25.9	37.0
12.4	26.0	35.4	14.0	28.6	43.3
16.7	31.4	39.7	19.7	35.1	44.7
12.3	19.5	23.1	17.2	31.5	38.3
15.9	30.0	37.0	17.9	33.8	41.6
Mean:					
15.1	27.3	36.4	16.1	29.8	40.2
S.D.					
1.9	4.9	5.3	1.9	2.9	2.8

EXPERIMENT II (Cont'd)

Group 5		
Incubation Time (Minutes)		
10	20	30
18.4	31.3	39.3
14.6	27.2	37.5
13.6	23.5	29.6
14.6	26.0	35.1
8.9	18.0	23.8
17.5	33.1	41.9
7.4	9.8	11.8
11.3	22.0	31.7
14.3	25.6	30.0
Mean:		
13.1	23.7	30.9
S.D.		
3.6	6.7	8.7

EXPERIMENT III

OXYGEN UPTAKE (μL/MG. LIVER) AT 10, 20, AND 30 MINUTES OF INCUBATION BY RAT LIVER HOMOGENATES. TREATMENTS ADMINISTRATED 4 HOURS BEFORE SACRIFICE. GROUP 1: INTACT, 0.5 ML. PSS I.M.; GROUP 2: INTACT, 400 MG. GLUCOSE I.P.; GROUP 3: INTACT, 5.0 MG. PREDNISOLONE I.M.

(Incubation Time)			(Incubation Time)			(Incubation Time)		
10	20	30	10	20	30	10	20	30
13.8	20.6	24.1	4.3	13.2	14.5	22.1	39.2	54.6
12.6	19.5	22.2	12.1	19.7	23.5	13.9	20.6	25.0
15.9	29.6	36.7	13.4	22.5	27.6	13.7	19.4	23.6
15.1	22.4	28.2	13.3	18.2	21.2	14.6	28.0	37.6
12.5	26.1	33.0	10.4	18.3	20.9	12.9	25.3	32.3
10.9	19.9	23.7	10.3	17.1	20.9	15.9	32.1	45.8
14.4	29.5	37.3	13.7	26.9	33.6	15.3	30.9	37.4
16.9	30.1	36.6	15.7	26.1	30.4	16.3	30.0	43.0
16.4	31.4	41.0	14.8	26.2	32.5	20.4	39.6	53.1
13.2	18.9	22.8	14.8	28.7	35.1	18.9	35.2	49.2
13.1	25.2	36.5	12.8	22.5	29.4	13.1	27.4	37.0
16.4	33.9	46.9	15.7	31.4	43.3	13.2	26.6	36.3
11.9	25.5	30.7	16.1	30.2	36.9	16.0	29.4	39.8
13.9	27.6	38.5	11.9	21.0	26.2	18.6	34.3	40.7
17.9	31.7	35.2	17.4	32.8	41.2	22.0	43.9	57.4
Mean:								
14.3	26.1	32.9	13.4	23.7	27.8	15.4	30.8	40.9
S.D.								
2.03	4.9	7.4	2.3	5.8	10.9	5.3	6.9	10.0

EXPERIMENT III

OXYGEN UPTAKE (μL/MG. LIVER) AT 10, 20, AND 30 MINUTES OF INCUBATION BY RAT LIVER HOMOGENATES. TREATMENTS ADMINISTERED 4 HOURS BEFORE SACRIFICE. GROUP 4: INTACT, 400 MG. GLUCOSE I.P. AND 5.0 MG. PREDNISOLONE I.M.; GROUP 5: ADRENALECTOMIZED, 0.5 ML. PSS I.M. GROUP 6: ADRENALECTOMIZED, 400 MG. GLUCOSE I.P.

Group 4 (Incubation Time)			Group 5 (Incubation Time)			Group 6 (Incubation Time)		
10	20	30	10	20	30	10	20	30
15.0	30.8	39.7	7.4	9.9	12.6	10.3	15.0	17.6
19.0	36.8	51.0	13.7	20.0	22.8	12.2	20.5	24.8
20.7	36.0	44.2	16.5	27.6	32.9	17.9	26.5	32.2
16.7	31.1	36.7	16.1	24.4	30.0	10.1	14.9	19.2
12.3	26.5	34.6	11.5	18.4	22.4	10.8	19.3	23.3
17.6	30.7	35.7	9.6	16.2	19.2	13.5	25.8	31.1
18.6	38.6	49.9	14.1	23.7	26.9	15.2	24.8	29.5
19.2	35.5	49.7	7.7	19.2	24.8	14.0	19.0	22.8
17.2	33.6	45.7	14.4	28.0	38.0	15.6	28.8	35.8
17.6	32.7	47.0	18.0	31.3	43.1	15.3	28.6	38.7
17.3	34.5	51.0	13.3	25.8	33.7	14.2	26.6	34.4
12.7	26.4	37.5	12.1	23.9	30.2	12.1	19.9	23.2
21.2	39.8	55.9	15.7	29.0	38.6	21.3	39.9	54.3
21.7	40.1	48.6	13.4	20.3	22.7	14.8	26.2	31.7
16.2	28.3	31.8	15.2	30.5	36.2	12.9	25.0	30.5
Mean:								
17.5	33.4	43.9	13.2	23.2	28.9	14.0	24.1	29.9
S.D.								
2.8	4.5	7.4	3.1	5.9	8.2	2.9	6.3	9.1

EXPERIMENT III

OXYGEN UPTAKE (uL/MG. LIVER) AT 10, 20, AND 30 MINUTES OF UNCUBATION BY RAT LIVER HOMOGENATES. TREATMENTS ADMINISTERED 4 HOURS BEFORE SACRIFICE. GROUP 7: ADRENALECTOMIZED, 5 MG. PREDNISOLONE I.M.; GROUP 8: ADRENALECTOMIZED, 5 MG. PREDNISOLONE I.M. AND 400 MG. GLUCOSE I.P.; GROUP 9: FASTED-ADRENALECTOMIZED, 0.5 ML. PSS I.M.

Group 7 (Incubation Time)			Group 8 (Incubation Time)			Group 9 (Incubation Time)		
10	20	30	10	20	30	10	20	30
15.1	27.6	33.7	12.5	24.7	31.6	10.5	16.0	22.3
18.4	36.0	48.4	19.7	36.1	49.6	4.0	10.1	16.3
23.7	40.7	54.8	24.7	38.4	49.8	5.0	12.9	18.4
13.6	20.7	24.0	14.1	27.8	37.7	7.7	12.0	15.1
17.0	27.4	32.8	10.9	16.0	19.7	18.4	25.7	30.7
18.6	32.4	37.9	16.1	29.0	35.9	12.6	16.2	21.2
14.9	22.4	26.6	14.4	22.2	26.1	7.8	12.1	15.6
18.1	31.5	38.8	19.1	31.8	37.3	10.2	15.8	18.9
14.8	27.2	35.7	18.5	32.3	38.1	14.2	22.5	26.1
16.1	29.0	39.0	17.2	30.5	42.0	9.6	16.5	19.2
15.6	31.8	45.0	14.9	27.6	33.0			
18.4	34.6	45.4	15.8	31.3	42.0			
19.2	36.4	50.5	25.1	47.0	65.2			
18.2	36.0	46.4	19.2	33.5	41.5			
22.6	37.6	44.1	15.1	21.0	23.6			
Mean:								
17.6	31.4	40.1	16.8	29.9	38.2	10.0	16.0	21.4
S.D.								
2.8	5.7	8.7	4.4	7.5	11.4	4.3	4.9	5.0

EXPERIMENT III

OXYGEN UPTAKE (uL/MG. LIVER) AT 10, 20 AND 30 MINUTES INCUBATION BY RAT LIVER HOMOGENATES. TREATMENTS ADMINISTERED 4 HOURS BEFORE SACRIFICE. GROUP 10: FASTED-ADRENALECTOMIZED, 400 MG. GLUCOSE I.P.; GROUP 11: FASTED-ADRENALECTOMIZED, 5 MG. PREDNISOLONE I.M.; GROUP 12: FASTED-ADRENALECTOMIZED, 5 MG. PREDNISOLONE I.M., 400 MG. GLUCOSE I.P.

Group 10 (Incubation Time)			Group 11 (Incubation Time)			Group 12 (Incubation Time)		
10	20	30	10	20	30	10	20	30
6.7	13.4	17.0	28.8	58.3	82.9	9.6	15.3	17.4
4.6	14.3	21.1	12.0	20.8	25.7	15.6	23.4	25.3
3.0	9.3	11.3	3.7	7.8	11.1	32.4	51.9	59.2
10.4	15.3	19.8	13.0	25.4	30.1	16.4	27.1	30.5
5.1	8.7	12.3	20.1	39.6	47.1	8.0	13.8	16.3
6.7	13.4	17.2	15.8	27.6	35.5	16.8	32.9	39.7
5.2	7.8	9.9	20.9	33.7	37.9	15.9	22.5	26.0
7.1	13.3	16.7	18.8	36.2	45.3	12.0	22.4	28.1
9.5	16.5	19.9	5.7	8.7	9.7	17.0	27.7	32.5
12.0	16.9	18.9	20.8	30.2	35.9	26.3	43.5	49.8
Mean:								
7.0	12.9	16.4	16.0	28.8	36.1	17.0	28.1	32.5
S.D.								
2.8	3.2	3.9	7.6	18.2	20.7	7.3	11.9	13.4

EXPERIMENT III - GROUP I

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. INTACT CONTROL RATS RECEIVED 0.5 ML. PSS I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
3.32	6.08	2.76	6.64	1.11	5.53	9.96	7.19	2.77
3.24	5.43	2.19	6.27	12.53	-6.26	9.51	12.96	-3.45
1.44	4.87	3.43	6.52	10.06	-3.54	7.96	14.93	-6.97
2.07	2.67	0.60	0.75	0.19	0.56	2.75	2.86	-0.11
2.37	4.32	1.95	15.86	3.02	12.84	18.23	7.34	10.89
2.72	4.76	2.04	12.53	8.52	4.01	15.25	13.28	1.97
1.98	4.25	2.27	6.23	2.15	4.08	8.21	6.40	1.81
2.79	4.97	2.18	9.34	9.22	0.12	12.13	14.19	-2.06
1.19	4.22	3.03	8.07	21.09	-13.02	9.26	25.31	-16.05
1.72	4.30	2.58	9.35	5.69	3.66	11.07	9.99	1.08
1.31	4.07	2.76	27.31	12.63	14.68	28.62	16.70	11.92
1.48	4.15	2.67	25.07	18.66	6.41	26.55	22.81	3.74
1.07	3.55	2.48	4.91	3.86	1.05	5.98	7.41	-1.43
1.14	3.44	2.30	0.54	1.40	-0.86	1.68	4.84	-3.16
2.20	5.49	3.29	8.46	2.63	5.83	10.66	8.12	2.54
Mean:								
2.00	4.44	2.44	9.86	7.52	2.34	11.85	11.62	0.23
S.D.								
0.75	0.87	0.67	7.68	6.52	6.93	7.64	6.44	6.71

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 2

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. INTACT RATS RECEIVED 400 MG. GLUCOSE I.P. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
2.97	5.34	2.37	3.96	2.28	1.68	6.93	7.62	-0.69
3.76	4.95	1.19	3.54	4.76	-1.22	7.30	9.71	-2.41
1.79	5.37	3.58	6.09	6.27	-0.18	7.88	11.64	-3.76
1.88	1.71	-0.17	2.73	0.34	2.39	4.61	2.05	2.56
2.12	3.78	1.64	6.52	2.99	3.53	8.64	6.75	1.89
2.85	3.93	1.08	6.02	3.59	2.43	8.87	7.52	1.35
2.08	4.61	2.53	5.04	3.76	1.28	7.12	8.37	-1.25
2.92	5.60	2.68	5.01	2.13	1.88	7.63	8.73	-0.80
1.68	3.96	2.28	4.65	2.57	2.08	6.33	6.53	-0.20
1.65	4.05	2.40	10.03	5.74	4.29	11.68	9.79	1.89
1.59	1.63	0.04	18.55	14.67	3.88	20.14	16.30	3.84
1.68	5.61	3.93	22.88	0.75	22.13	24.56	6.36	18.20
1.00	3.78	2.78	4.57	9.45	-4.88	5.57	13.23	-7.66
1.00	2.93	1.93	1.64	1.64	0.0	2.64	4.57	-1.93
2.15	5.37	3.22	6.27	0.45	5.82	8.42	5.82	2.60
Mean:								
2.07	4.17	2.10	7.17	4.16	3.01	9.24	8.33	0.91
S.D.								
0.76	1.30	1.18	5.88	3.79	5.87	5.76	3.52	5.69

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 3

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. INTACT RATS RECEIVED 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
4.40	6.51	2.11	7.06	12.65	-5.59	11.46	19.16	-7.70
0.54	7.52	6.98	9.49	9.84	-0.36	10.03	17.37	-7.34
2.12	4.43	2.31	7.10	1.39	5.71	9.22	5.82	3.40
1.86	4.30	2.44	10.03	4.48	5.55	11.89	8.88	3.01
2.96	5.35	2.39	5.43	4.43	1.00	8.39	9.78	-1.39
1.87	3.88	2.01	7.22	3.25	3.97	9.09	7.13	1.96
2.77	5.11	2.34	16.82	9.30	7.62	10.59	14.41	5.18
1.29	4.75	3.46	21.78	5.46	16.32	23.07	10.21	12.86
2.33	5.34	3.01	9.58	6.45	3.13	11.91	11.79	0.12
2.16	4.86	2.70	22.74	14.40	8.34	24.90	19.26	5.64
1.54	4.86	2.70	22.82	13.12	9.70	24.36	17.51	6.85
1.46	6.23	4.77	4.83	0.86	3.97	6.29	7.02	-0.08
1.07	2.97	1.90	11.39	17.73	-6.34	12.46	20.70	-8.24
1.39	4.40	3.01	1.65	2.84	-1.19	3.04	7.24	-4.20
1.84	5.03	3.19	5.55	2.69	2.86	7.39	7.72	-0.33
Mean:								
1.97	5.00	3.03	10.90	7.26	3.64	12.87	12.27	0.60
S.D.								
0.92	1.11	1.30	6.90	5.26	6.32	6.84	5.27	6.04

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 4

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. INTACT RATS RECEIVED 400 MG. GLUCOSE I.P. AND 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrates		
Before	After	Difference	Before	After	Difference	Before	After	Difference
3.43	4.09	0.66	4.01	3.27	0.74	7.44	7.36	0.08
2.11	6.47	4.36	12.48	8.72	3.76	14.59	15.19	-0.60
2.23	4.83	2.60	9.98	5.37	4.57	12.17	10.20	1.97
2.85	5.28	2.43	4.82	5.69	-0.87	7.67	10.97	-3.30
2.70	4.82	2.12	14.56	6.56	8.00	17.26	11.38	5.88
2.07	4.68	2.61	10.44	4.18	6.26	12.51	8.86	3.65
2.94	5.75	2.81	4.61	11.52	-6.91	7.66	17.27	-9.72
1.38	4.25	2.87	7.93	15.34	-7.41	9.31	10.59	-10.28
2.21	4.49	2.28	8.89	3.12	5.77	11.10	7.61	3.49
2.26	4.94	2.68	18.90	12.53	6.37	21.16	17.47	3.69
2.04	4.91	2.87	14.29	14.20	0.09	16.33	19.11	-2.78
1.37	3.39	2.02	2.22	0.29	1.93	3.59	3.68	-0.09
1.63	4.76	3.13	6.06	6.27	-0.21	7.69	11.03	-3.34
1.77	4.38	2.61	1.55	1.66	-0.11	3.32	6.04	-2.72
Mean:								
2.29	4.78	2.49	8.94	7.04	1.90	11.23	11.81	-0.59
S.D.								
0.65	0.71	0.83	5.09	4.56	4.69	5.23	4.90	4.91

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 5

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. ADRENALECTOMIZED CONTROL RATS RECEIVED 0.5 ML. PSS.I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
2.83	4.55	1.72	4.37	0.26	4.11	7.20	4.81	2.39
3.61	4.79	1.18	1.69	1.88	-0.19	5.30	6.67	-1.37
2.33	4.83	2.50	7.16	7.34	-0.18	9.49	12.17	-2.68
1.98	4.71	2.73	12.86	7.26	5.24	14.48	12.33	2.15
2.35	4.23	1.88	9.70	5.05	4.65	12.05	9.28	2.77
2.41	3.96	1.55	3.36	3.36	0.0	5.77	5.32	0.45
1.96	4.79	2.83	7.24	2.73	4.51	9.20	7.52	1.68
2.39	3.99	1.60	6.72	7.63	-0.91	9.11	11.62	-2.51
1.33	3.36	2.03	9.99	5.54	4.45	11.32	8.90	2.42
2.15	3.82	1.67	17.76	12.53	5.23	19.81	16.35	3.56
1.42	3.72	2.30	33.42	23.81	9.61	34.84	27.53	7.31
1.43	3.12	1.69	4.05	0.96	0.09	5.48	4.08	1.40
1.11	3.89	2.78	17.49	13.65	3.84	18.60	17.54	1.06
1.11	2.92	1.81	2.51	2.61	-0.10	3.62	5.53	-1.91
1.79	3.97	2.18	2.86	0.09	2.77	4.65	4.06	0.59
Mean:								
2.01	4.04	2.03	9.41	6.34	3.07	11.40	10.25	1.15
S.D.								
0.69	0.61	0.60	8.37	6.35	2.88	8.13	6.41	2.61

* Reducing Glycolytic Intermediates.

EXPERIMENT III - GROUP 6

LIVER REDUCING LYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. ADRENALECTOMIZED RATS RECEIVED 400 MG. GLUCOSE I.P. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
2.58	2.46	-0.12	3.42	0.34	3.08	6.00	2.80	3.20
2.76	7.02	4.26	10.86	11.45	-0.59	13.62	18.47	-4.85
2.57	5.56	2.99	19.00	4.45	14.55	21.57	10.01	11.56
2.34	4.05	1.99	7.62	4.49	3.13	9.96	8.54	1.42
2.16	3.43	1.27	3.48	2.48	1.00	5.64	5.91	-0.27
1.95	4.26	2.31	7.71	4.92	2.79	9.66	9.18	0.48
2.13	3.80	1.67	3.12	0.86	2.26	5.25	4.66	0.59
1.73	4.27	2.54	3.65	0.33	3.32	5.38	4.60	0.78
1.50	3.18	1.68	3.22	15.04	-11.82	4.72	18.22	-13.50
1.46	1.67	0.21	20.37	17.96	2.41	21.83	19.63	2.20
1.85	4.34	2.59	15.35	16.53	-1.18	17.20	20.87	-3.67
1.50	2.90	1.40	2.10	1.05	1.05	3.60	3.95	-0.35
0.95	4.66	3.71	8.95	12.78	-3.83	9.90	17.44	-7.54
1.04	3.67	2.63	6.36	1.52	4.84	7.40	5.19	2.21
1.59	3.46	1.87	5.08	1.12	3.96	6.67	4.58	2.09
Mean:								
1.87	3.92	2.05	8.02	6.35	1.66	9.89	10.27	-0.39
S.D.								
0.55	1.27	1.15	5.92	6.47	5.45	6.00	6.67	5.54

* Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 7

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. ADRENALECTOMIZED RATS RECEIVED 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
3.22	3.48	0.26	3.01	5.05	-2.04	6.23	8.53	-2.30
3.76	7.95	4.19	5.69	12.89	-7.20	9.45	20.84	-11.39
2.43	6.17	3.74	14.93	11.06	3.87	17.36	17.23	0.13
2.35	4.47	2.12	7.37	11.13	-3.76	9.72	15.60	-5.88
2.43	4.47	2.04	13.56	8.41	5.15	15.99	12.88	3.11
2.58	9.34	6.76	6.15	1.48	4.67	8.73	10.82	-2.09
2.75	4.79	2.04	10.77	12.39	-1.62	13.52	17.18	-3.66
1.60	3.81	2.21	2.39	2.62	-0.23	3.99	6.43	-2.44
1.76	3.05	1.29	9.24	5.91	3.33	11.00	8.96	2.04
1.96	3.91	1.95	21.62	16.0	5.62	23.58	19.91	3.67
1.33	4.13	2.80	20.63	15.90	4.73	21.96	20.03	1.96
1.57	3.79	2.22	3.06	3.59	-0.53	4.63	7.38	-2.75
1.05	3.92	2.87	12.37	6.93	5.44	13.42	10.85	2.57
1.31	3.84	2.53	4.40	1.36	3.04	5.71	5.20	0.51
1.67	3.88	2.21	3.64	0.49	3.15	5.31	4.37	0.94
Mean:								
2.12	4.73	2.61	9.26	7.69	1.57	11.37	12.41	-1.04
S.D.								
0.76	3.08	1.46	6.23	5.30	3.95	6.19	5.68	3.97

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 8

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. ADRENALECTOMIZED RATS RECEIVED 400 MG. GLUCOSE I.P. AND 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
3.45	4.11	0.66	4.98	8.94	3.96	8.43	13.05	-4.62
2.42	6.80	4.38	6.65	8.77	-2.12	9.07	15.57	-6.50
2.16	5.45	3.29	33.84	28.20	5.64	36.00	33.65	2.35
2.22	4.76	2.54	8.19	3.76	4.43	10.41	8.52	1.89
2.69	4.66	1.97	8.33	4.74	3.59	11.02	9.40	1.62
1.59	4.08	2.49	18.71	16.11	2.60	20.30	20.19	0.11
2.36	4.73	2.37	14.72	10.53	4.19	17.08	15.26	1.82
1.63	4.33	2.70	5.64	5.37	0.27	7.27	9.70	-2.43
2.05	3.28	1.23	8.42	4.81	3.61	10.47	8.09	2.38
1.74	3.86	2.12	18.15	13.48	4.67	19.89	17.34	2.55
2.00	4.17	2.17	7.31	12.57	-5.26	9.31	16.74	-7.43
1.40	3.87	2.47	2.11	3.01	-0.90	3.51	6.88	-3.37
1.21	4.95	3.74	15.98	8.36	7.62	17.19	13.31	3.88
1.17	3.93	2.76	2.45	5.42	-2.97	3.62	9.35	-5.73
1.94	5.28	3.34	10.34	6.02	4.32	12.28	11.30	0.98
Mean:								
2.00	4.55	2.55	11.05	9.34	1.71	13.06	13.89	-0.83
S.D.								
0.60	0.94	0.93	8.20	6.48	3.95	8.20	6.72	3.80

*Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 9

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. FASTED-ADRENALECTOMIZED CONTROL RATS RECEIVED 0.5 ML. PSS I.M. 4 HOURS PRIOR TO SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
0.93	1.62	0.69	1.74	1.16	0.58	2.67	2.78	-0.11
1.17	1.62	0.45	1.50	0.56	0.94	2.67	2.18	0.49
1.31	2.03	0.72	2.62	1.63	0.99	3.93	3.66	0.27
0.66	1.26	0.60	1.50	1.96	-0.46	2.16	3.26	-1.06
0.96	1.50	0.54	0.90	0.60	0.30	1.86	2.10	-0.24
0.87	1.39	0.52	1.45	0.14	1.31	2.32	1.53	0.79
0.58	0.64	0.06	1.53	2.51	-0.98	2.11	3.15	-1.04
0.52	1.07	0.55	1.48	1.25	0.23	2.00	2.32	-0.32
0.73	1.38	0.65	1.09	2.43	-1.34	1.82	3.81	-1.89
0.42	1.34	0.92	2.72	3.97	-1.25	3.14	5.31	-2.17
Mean:								
0.82	1.39	0.57	1.65	1.62	0.03	2.47	3.01	-0.54
S.D.								
0.29	0.37	0.22	0.58	1.15	0.97	0.66	1.09	0.94

* Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 10

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. FASTED-ADRENALECTOMIZED RATS RECEIVED 400 MG. GLUCOSE I.P. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
0.81	1.29	0.49	1.75	1.21	0.54	2.57	2.50	0.07
1.67	3.12	1.45	3.76	2.95	0.81	5.43	6.07	-0.64
0.57	1.41	0.84	1.05	0.45	0.60	1.62	1.86	-0.24
0.69	2.29	1.60	1.80	1.31	0.49	2.49	3.60	-1.11
1.05	1.54	0.49	1.14	1.14	0.0	2.19	2.68	-0.49
0.79	1.03	0.24	1.58	2.18	-0.60	2.37	3.21	-0.84
0.71	1.83	1.12	1.06	2.35	-1.29	1.77	4.18	-2.41
0.66	0.66	0.0	1.06	2.35	-1.29	1.72	3.01	-1.29
0.75	0.60	-0.15	1.07	1.07	0.0	1.82	1.67	0.15
1.13	1.80	0.67	3.09	1.61	1.48	4.22	3.41	0.81
Mean:								
0.88	1.56	0.68	1.74	1.66	0.08	2.62	3.22	-0.60
S.D.								
0.32	0.76	0.56	0.93	0.76	1.09	1.24	1.26	2.82

* Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 11

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. FASTED-ADRENALECTOMIZED RATS RECEIVED 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
1.91	3.76	1.85	6.45	2.28	4.17	8.36	6.04	2.32
1.41	2.65	1.24	3.01	1.80	1.21	4.42	4.45	-0.03
1.03	2.05	1.02	1.20	2.56	-1.36	2.23	4.61	-2.38
0.75	1.59	0.84	1.30	1.16	0.14	2.05	2.75	-0.70
1.09	1.75	0.66	4.36	1.70	2.66	5.45	3.45	2.00
1.05	1.14	0.09	2.73	1.82	0.91	3.78	2.96	0.82
0.86	1.40	0.54	1.61	1.48	0.13	2.47	2.88	-0.41
0.82	1.44	0.62	2.32	1.55	0.77	3.14	2.99	0.15
0.66	0.66	0.0	2.26	1.50	0.76	2.92	2.16	0.76
1.03	2.04	1.01	6.11	2.04	4.07	7.14	4.08	3.06
Mean:								
1.06	1.85	0.79	3.14	1.79	1.35	4.20	3.74	0.56
S.D.								
0.36	0.86	0.54	1.89	0.41	0.45	2.16	1.15	1.61

* Reducing Glycolytic Intermediates

EXPERIMENT III - GROUP 12

LIVER REDUCING GLYCOLYTIC INTERMEDIATES, GLYCOGEN, AND TOTAL CARBOHYDRATE (EACH EXPRESSED AS GM.% OF GLUCOSE) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. FASTED-ADRENALECTOMIZED RATS RECEIVED 400 MG. GLUCOSE I.P. AND 5.0 MG. PREDNISOLONE I.M. 4 HOURS BEFORE SACRIFICE

RGI*			Glycogen			Total Carbohydrate		
Before	After	Difference	Before	After	Difference	Before	After	Difference
1.45	3.12	1.67	2.60	2.02	0.58	4.05	5.14	-1.09
1.44	3.76	2.32	9.02	5.87	3.15	10.46	9.63	0.83
1.75	4.12	2.37	6.27	3.34	2.93	8.02	7.46	0.56
0.75	2.52	1.77	3.76	0.81	2.95	4.51	3.33	1.18
0.82	1.70	0.88	0.82	0.98	-0.16	1.64	2.68	-1.04
0.78	2.40	1.62	2.31	1.88	0.43	3.09	3.48	-0.39
1.26	2.43	1.17	6.30	2.99	3.31	7.56	5.42	2.14
1.02	1.26	0.24	3.03	3.03	0.0	4.05	4.29	-0.24
0.92	2.51	1.59	5.26	3.01	2.25	6.18	5.52	0.66
1.26	3.49	2.23	7.39	2.82	4.57	8.64	6.31	2.33
Mean:								
1.15	2.73	1.58	4.63	2.68	2.00	5.82	5.33	0.49
S.D.								
0.34	0.89	1.18	2.58	1.43	1.65	2.80	2.10	1.19

* Reducing Glycolytic Intermediates

VITA

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Master of Science

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