SOME METABOLIC EFFECTS OF CORTICOSTERONE IN DIFFERENT TISSUES OF THE RAT AND THE SUBCELLULAR DISTRIBUTION OF (^{3}H) CORTICOSTERONE IN THESE TISSUES

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CHAPTER I

INTRODUCTION

Homeostatic conditions must be maintained by living organisms for survival. Homeostatic mechanisms must be able to alter the rate of production of various rate-limiting metabolites and enzymes to compensate for alteration in the external or internal environment.

Mammals have developed coordinated homeostatic systems to compensate for environmental changes. One of these systems involves stimulation and release of increased amounts of glucocorticoids from the adrenal cortex. These hormones aid the organism in maintaining an adequate blood glucose level when normal quantities of carbohydrates are not available to the cell. The importance of this increased release of glucocorticoids is indicated by the fact that blood glucose levels as low as 50 mg percent, for only a few minutes, leads to the loss of consciousness and if it persists death results.

Normal blood glucose level is maintained primarily by glycogenolysis and gluconeogenesis. Glucocorticoids are active in these processes. Changes in internal or external environmental conditions may stimulate the production and the release of ACTH from the anterior pituitary. ACTH in turn stimulates the release of glucocorticoids by the adrenal cortex. These hormones, by some unknown mechanism, reduce peripheral glucose utilization and cause the release of lactate, amino acids, and three-carbon precursors from extrahepatic tissues. Hepatic

gluconeogenic enzymes are increased and glucose production results from non-carbohydrate precursors. Glucocorticoids seem to affect almost all tissues, effects varying from catabolic in lymphatic and muscle tissues to anabolic in hepatic tissue.

Many investigators have studied the effects of cortisol, cortisone, and synthetic glucocorticoids on various metabolic activities in liver tissue of the rat. Since corticosterone is the natural glucocorticoid produced by the rat and glucocorticoids appear to affect almost all tissues, experiments were designed to study some of the metabolic effects of intramuscular administration of this hormone. Although corticosterone has been found to be only one third to one half as active as cortisol and cortisone in stimulating liver glycogen deposition and thymus involution, it was believed that a study of its effects on tissue metabolism deserved greater attention. It is possible that metabolic effects of corticosterone may differ from the effects of cortisol or cortisone in the rat.

A review of literature revealed that single injections of prednisolone and hydrocortisone increased oxygen uptake and caused other metabolic changes in rat liver homogenates. In order to study possible actions of corticosterone in other tissues, the first experiment was designed to measure metabolic activities in homogenates prepared from the heart, brain, diaphragm, liver, kidney, and testis taken from treated and non-treated animals. Criteria of metabolic activity investigated were changes in _in vitro_ oxygen uptake, lactic acid production, glycogen breakdown, protein levels, inorganic phosphate disappearance, and glucose utilization. After these biochemical changes

were measured, more basic experiments were performed.

A search of the literature further revealed that several investigators have suggested an effect of steroids on the structure and functions of mitochondria. The mitochondrial membrane contains lipid and protein substituents. Since steroids are lipid, they may change the membrane structure so that oxidation of substrate may be altered. In order to study further the effects of intramuscular injections of corticosterone on mitochondria, the second experiment was designed to measure the P/O ratio in mitochondria isolated from hepatic, cardiac, and brain tissues taken fron adrenalectomized corticosterone-treated and adrenalectomized non-treated controlled rats. NAD-dependent substrates and succinate were used. These substrates were selected since they are oxidized by different components of the electron transport chain. A steroid effect near the NAD-cytochrome reductase would be indicated by an effect on the P/O ratio with NAD-dependent substrate; however, no effect should be observed with succinate oxidation.

Various workers have reported that glucocorticoids stimulate de novo synthesis of many enzymes in the liver. Increased enzyme synthesis also occurs to some extent in extrahepatic tissues; a hormone-gene effect and a membrane effect have been suggested as primary sites of action of the glucocorticoid. However, at this time no real evidence has been presented in support of a hormone-gene or a membrane effect by glucocorticoids. If one assumes that these hormones act "in person" at their sites of action, localization of the hormone within the target cell would contribute greatly to the understanding of their mechanisms of action. Consequently the third experiment was designed to

localize (^{3}H) corticosterone within the subcellular fractions of the brain, the thymus, the heart, and the liver.

CHAPTER II

REVIEW OF LITERATURE

A complete review of the metabolic effects of glucocorticoids is beyond the scope of this treatise. Many recent reviews have appeared which cover the subject more thoroughly than it will be covered here (31, 36, 43, 93, 114). An attempt will be made to consider recent work in the following areas: (A) the effects of glucocorticoids on carbohydrates, lipids, proteins, and oxidative metabolism; (B) gene regulation of enzyme synthesis; and (C) subcellular distribution of glucocorticoids.

Carbohydrate Metabolism

Within the last few years abundant evidence has been published indicating a profound effect of glucocorticoids on carbohydrate metabolism. The best recognized effects are an increase in blood glucose and liver glycogen. Investigators generally agree that glucocorticoids accelerate hepatic gluconeogenesis, increase liver glycogen stores, and exert some action on peripheral glucose utilization (70). Whether the primary affect of glucocorticoids is upon hepatic or extrahepatic tissues is not known.

The well-known, early work of Long *et al.* (74) provided experimental evidence that adrenal cortex hormones have a direct and positive

role in the metabolism of carbohydrates and proteins. They found that adrenalectomized, fed rats maintained normal levels of liver and muscle glycogen. In adrenalectomized, fasted rats, a rapid decline in liver glycogen was observed followed by a depletion of muscle glycogen. Hypoglycemia occurred after a fast of 48 hours. A definite fall in the rate of protein catabolism, measured by urinary nitrogen excretion, was suggested by these workers to account for the decreased carbohydrate levels. Treatment of animals with adrenal cortex extracts reversed the above observations. These workers proposed that the cortical hormones increase the rate of protein catabolism in muscles, and by the process of gluconeogenesis, a normal blood glucose level was maintained at the expense of tissue protein.

In later work, Long et al. (75) suggested that the effects of corticosteroids on protein and carbohydrate metabolism are not always associated with each other. In fasted animals a quantitative relationship was observed between the protein breakdown and the carbohydrates deposited as liver glycogen, muscle glycogen, and as increased blood glucose. In fed animals alterations in the pattern of carbohydrate metabolism occurred without any significant change in protein catabolism.

Many other investigators have reported effects of glucocorticoids on blood glucose and liver glycogen stores since the original work by Long et al. (74). Some early work by Ingle et al. (52, 53, 54) demonstrated that prolonged glycosuria and hyperglycemia were produced in intact rats treated with adrenocorticotrophic hormone or with cortisone. Prolonged treatment of animals with glucocorticoids resulted

in a 20-30 fold increase of liver glycogen (62, 90, 117). Winternitz and Forest (128) reported that adrenalectomy in rats resulted in profound inhibition of glycogen storage when the rats were subjected to a carbohydrate load. These workers suggested that the excess carbohydrates were apparently disposed of by an over-all increased metabolic rate. Hockaday (49) found that the effect of epinephrine in causing a rise in blood glucose was further augmented within 15-20 minutes after hydrocortisone injection. Hydrocortisone may exert a synergistic action with epinephrine on carbohydrate metabolism.

Many investigators have postulated that glucocorticoids have a direct effect on extrahepatic tissues which could conceivably be the primary hormonal effect. The primary effect may be on decreased peripheral utilization of glucose rather than on gluconeogenesis. Glenn et al. (33) have shown that hydrocortisone administered to fasted, adrenalectomized rats resulted in early increases in plasma levels of lactate and glucose, whereas amino acid, nonesterified fatty acid, and protein levels in the plasma were not altered. These workers postulated that the rapid rise in blood glucose was due to inhibitory effects of hydrocortisone on glucose utilization in peripheral tissues and the primary effects were not on gluconeogenesis. Much evidence has accumulated supporting this viewpoint. Munck (84) reported a decreased rate of glucose uptake by isolated epididymal fat pads from rats injected with cortisol. Tissues removed from animals injected with glucocorticoids and studies in vitro using physiological concentrations of steroids suggest a decrease in glucose uptake by muscle (41), heart (56), thymus (83, 86), skin (91), and adipose tissue (69,

85). The rate of glucose utilization by the brain does not change during glucocorticoid administration (106). Ewing and Nobel (22) found that stress in rabbits, which presumably increased adrenal cortical hormone production, decreased glucose utilization by kidney and testis slices. The uptake of glucose by liver and muscle was not impaired following administration of adrenocortical hormones (88). Munck and Keritz (87) have shown that glucocorticoids enhanced slightly the incorporation of glucose by the isolated rat gastrocenemius. Fain et al. (23) reported that glucose uptake and its conversion to carbon dioxide and fatty acids were decreased by dexamethasone and corticosterone added in vitro to rat adipose tissue. Physiological concentrations (10^{-7}M) of cortisol inhibited glucose metabolism of free adipose cells during 4 hours of incubation according to Blecher (8). Furthermore, an intact cell boundary or membrane was required for the inhibitory action on glucose uptake by cortisol since phospholipase C prevented the action of cortisol.

In fresh rat thymus preparations, physiological concentrations of cortisol significantly inhibited glucose uptake according to Munck (86). Additional evidence indicated that in order to observe physiologically significant effects of glucocorticoids in vitro, the integrity of tissue cells must be preserved and steroids must be used at physiological concentrations; otherwise, physiological effects may be obscured by non-physiological effects.

Further evidence of an extrahepatic effect on carbohydrate metabolism has been reported by Lecocq et al. (70). These workers found that intravenous infusion of hydrocortisone into dogs produced an

immediate reduction in hepatic glucose output and a decrease in peripheral glucose utilization. These effects occurred within 20 minutes after the start of a continuous infusion of hydrocortisone at the rate of 1.3 mg per minute. The simultaneous inhibition of peripheral glucose utilization and the fall in hepatic glucose output resulted in an unchanged mean arterial concentration of glucose. These workers suggested that a decrease in hepatic phosphorylase or an increase in glycogen synthetase activity, or both, accounted for the reduced hepatic glucose output. This hypothesis is supported by Hilz et al. (48) who showed an increase in hepatic synthetase activity within 30 minutes after glucocorticoid administration in the rat. Holt et al. (51) have suggested that glucocorticoids enhance gluconeogenesis and glycogen synthesis and impair glycogen degradation. Goetsch and McDonald (35), Goetsch (34), and Geurkink (32) have shown that acute injections of glucocorticoids did not alter glycolytic rates in liver homogenates prepared from normal rats and normal goats.

A direct effect of glucocorticoids on carbohydrate metabolism in hepatic tissue was indicated by the observations of Eisenstein et al. (20). These workers observed that physiological amounts of glucocorticoids increased carbohydrate synthesis in liver slices of normal and adrenalectomized rats incubated with alamine. These observations support the hypothesis that glucocorticoids increase carbohydrate stores by promoting conversion of protein to carbohydrate.

A review of the literature indicated that many effects of glucocorticoids on carbohydrate metabolism have been reported. However the primary effects which result in the increased blood glucose and the

liver glycogen deposition still remain unknown. These effects could be the result of increased gluconeogenesis, the inhibition of peripheral utilization of glucose, or both. Whatever the mechanisms may be that influences these processes, they remain to be elucidated.

Lipid Metabolism

Many effects of glucocorticoids on lipid metabolism are well documented. Marked changes in fat distribution occur in the rabbit (81), the rat (47), and the human (108) after excessive treatment with glucocorticoids. Hill and Drake (47) reported the development of hyperlipemia and lipid deposition in the livers of rats which had been treated chronically with cortisone.

It is possible that the effects on glucose metabolism is secondary to the effect on fat metabolism since it has been found that hydrocortisone elevates the concentration of non-esterified fatty acids in venous blood 10 minutes after the start of a continuous, intra-arterial infusion into the human forearm (49). The rapid increase in plasma fatty acids was not observed by Maickel and Brodie (77) in adrenalectomized rats which were injected with cortisone; however, liver triglycerides rose much earlier and because of this they suggested a direct action of glucocorticoids on the liver.

Further studies by Hays and Hill (44), designed to determine whether or not cortisone acts directly upon the liver, resulted in the observation that ^{14}C-labeled palmitate was preferentially converted into the non-phospholipids in the liver of cortisone-treated rats. This suggested a specific intrahepatic effect of cortisone. Additional

work indicated an enzymatic basis for this preferential synthesis of neutral fat in the liver of cortisone-treated rats (44). This increased triglyceride-synthesizing activity was shown to be sensitive to actinomycin D and puromycin (44).

Many reports have appeared which indicate an *in vitro* effect of glucocorticoids on the release of free fatty acids from adipose tissue. Jeanrenaud and Renold (55) reported that cortisol or corticosterone failed to influence oxidation of glucose, pyruvate, or lipogenesis from glucose when they were added to rat adipose tissue *in vitro*. However, a significant increase in net release of free fatty acids from adipose tissue was observed. Fain *et al.* (23) found that the addition of physiological amounts of corticosterone increased the release of fatty acids by incubated parametrial and mesenteric adipose tissue. The onset of this action required about two hours of incubation.

Nejad and Chaikoff (89) found that adrenalectomy depressed the incorporation of glucose carbon into fatty acids of rat liver slices. Also, hypophysectomy resulted in a decreased incorporation of glucose carbon into fatty acids of liver slices. Daily injections of ACTH for 14 days brought about complete repair of the defective hepatic lipogenesis in the hypophysectomized rats, but not in hypophysectomized-adrenalectomized rats. These workers concluded that activation of the adrenal glands repaired the depressed hepatic lipogenesis.

Protein Metabolism

Profound effects of glucocorticoids upon protein metabolism are well established. However, it still remains to be elucidated whether

these effects are primary or secondary. Gluconeogenesis, one of the primary homeostatic mechanisms for maintaining normal blood glucose levels, is greatly stimulated by glucocorticoids. Enzymes involved in the breakdown of glucose are not increased. This has led to an intensive study of the effects of glucocorticoids on enzymes involved in gluconeogenesis.

Recent papers by Rosen (98) and Tomkins (111) provide excellent reviews of corticosteroid effects on tissue enzymes. There are four enzymes in the final pathway of gluconeogenesis which exert a rate-limiting role on the conversion of non-carbohydrate precursors to glucose (125). These are glucose-6-phosphatase, fructose 1,6-diphosphatase, phosphoenol pyruvate carboxykinase, and pyruvate carboxylase. These enzymes are designated as rate-limiting or key enzymes because they have low activity, they govern one-way reactions, and they are involved in overcoming thermodynamic barriers (125). These enzymes are chiefly localized in tissues capable of gluconeogenesis. They are decreased or absent in rapidly growing liver tumors (118, 119, 120, 123). These findings suggested that the failure of gluconeogenesis in liver tumors was due to the loss of the enzymes strategically located at the key steps of gluconeogenesis.

The key gluconeogenic enzymes are increased in the liver of rats treated with glucocorticoids (46, 48, 67, 79, 107, 122, 125) Weber et al. (122) studied glucose-6-phosphatase activity in the liver of rats treated chronically with cortisone. These investigators found that after five daily injections of cortisone there was an increased glucose-6-phosphatase activity in all particulate subcellular fractions of the

rat liver. The percentage distribution of glucose-6-phosphatase between the various subcellular fractions was not affected. Cortisone treatment produced heavy deposits of glycogen and fat in the rat liver. Body growth was inhibited and the liver weight to body weight ratio was increased. Later, Weber *et al.* (124, 125) found that glucose-6-phosphatase activity in the rat liver increased significantly within 4 hours after a single injection of triamcinolone, a synthetic glucocorticoid. Cortisol injections resulted in no increase in glucose-6-phosphatase activity in the rat liver during the first 24 hours. These investigators also found that the cortisol induced glucose-6-phosphatase activity was blocked by actinomycin, puromycin, or ethionine. The increased glucose-6-phosphatase activity was preceded by a rise in amino acid nitrogen and was followed by a rise in RNA. These workers proposed that the rapid rise in amino acid content enabled the liver to produce more glucose by saturating preexisting enzyme. However, synthesis of new enzyme occurred within a few hours thus indicating that *de novo* enzyme synthesis must play an important part in the early events of glucocorticoid action.

Yudoev and Levedeva (132) found that endogenous corticosterone produced during a state of stress resulted in increased rat hepatic glucose-6-phosphatase activity.

Mokrasch *et al.* (79) found that liver levels of fructose 1,6-diphosphatase were increased by feeding rabbits diets low in glucose but rich in protein or by chronic administration of corticosterone. It was suggested that the observed increase represented a physiological means of causing increased glucogenesis. Weber *et al.* (124, 125)

found that fructose 1,6-diphosphatase activity increased in the rat liver within two hours after cortisone or triamcinolone injections. This increase was blocked by actinomycin, puromycin, or ethionine. Underwood and Newsholme (116) have suggested that fructose 1,6-diphosphatase plays a key role as a pacemaker in gluconeogenesis. Properties of this enzyme which indicate its importance as a pacemaker are its very low Km and its inhibition by AMP and fructose 1,6-diphosphate.

Since an indirect reversal of the Embden-Meyerhof pathway is probably involved in gluconeogenesis, Sharago et al. (107) studied the effects of glucocorticoids on phosphoenolypyruvate carboxykinase and malic enzyme in the rat liver. The direct reversal of the Embden-Meyerhof pathway does not readily occur due to the strongly exergonic nature of glycolysis (66). Sharago et al. (107) found that phosphoenolpyruvate carboxykinase activity increased after chronic glucocorticoid treatment. This indicated a close correlation of this enzyme with carbohydrate formation especially since it was found to be present in sufficient amounts to account for phosphoenolpyruvate synthesis during glucogenesis. Malic enzyme must not be involved in glucocorticoid induced gluconeogenesis since its activity was not altered by glucocorticoid administration.

Henning et al. (46) found that cortisol increased pyruvate carboxylase activity in the rat liver.

Current evidence indicates that after glucocorticoid treatment glycogenic amino acids are subjected to transamination and pyruvate is formed. Pyruvate is carboxylated to oxalacetate by pyruvate carboxylase. Oxalacetate is converted to phosphoenolpyruvate by phos-

phoenolphyruvate carboxykinase. From this point the reversal of the Embden-Meyerhof pathway occurs during glucogenesis. Glucocorticoids increase selectively certain liver gluconeogenic enzymes, whereas other enzyme systems are not affected (121).

Several other liver enzymes that participate in amino acid degradation or in gluconeogenesis increase after glucocorticoid administration. These are tyrosine-ketoglutarate transaminase (60), glutamic-pyruvate transaminase (99), glycogen synthetase (48), and tryptophan pyrrolase (28).

Lin and Knox (73) found that tyrosine increased the tyrosine-α-ketoglutarate transaminase activity in the livers from intact rats but not in those from adrenalectomized rats. Kenney and Flora (60) found that tryptophan and suspensions of inorganic materials (celite) were as effective as tyrosine in inducing this increased activity within 5 hours after injection into intact rats, but not adrenalectomized rats. They also found that hydrocortisone was an effective inducer of this enzyme in both intact and adrenalectomized rats within 5 hours after its administration. These workers concluded that increased tyrosine-transaminase activity is mediated by adrenal hormones. Additional work by Kenney (61) has shown that glucocorticoids stimulate de novo synthesis of tyrosine-transaminase enzyme within 1 hour after injection in the rat.

Rosen et al. (99) have reported that treatment of rats for 1 week with glucocorticoids resulted in a 6 to 13 fold increase in liver glutamic-pyruvate transaminase activity. The high gluconeogenic potency of substrates of this enzyme and the fact that glucocorticoids

increase the activity of this enzyme led to the suggestion by Rosen et al. (100) that the control of this enzyme by glucocorticoids is related to the mechanism whereby glucocorticoids exert their action. Additional work by these investigators showed that glutamic-pyruvate transaminase activity also increased in the thymus gland and in Walker carcinoma 256 (99). Other conditions known to be associated with gluconeogenesis, eg., fasting, diabetes, and high protein diets, resulted in a 5 to 7 fold increase in rat liver glutamic-pyruvate transaminase activity (99). These workers pointed out that the activity of this enzyme may be rate-limiting in gluconeogenesis.

Hilz et al. (48) studied the effects of cortisol acetate injection on glucose polymerization in adrenalectomized rats. Thirty minutes after the injection, glucose-6-phosphate concentrations in rat liver began to rise and continued to do so for 6 hours. A 40 fold increase was observed at the end of 6 hours. Glucose-1-phosphate, glycogen levels, and glycogen synthetase activity were significantly increased, whereas the concentrations of UDPG-pyrophosphorylase, glycogen phosphorylase, and glucose-6-phosphatase increased very little. The increased glycogen synthetase activity, using saturated levels of glucose-6-phosphate, was not sufficient to account for the rapid glycogen formation that occurred. However, glucose-6-phosphate has been shown to be an activator of glycogen synthetase (71). Thus the combined stimulation of glycogen synthetase activity by increased concentrations of glucose-6-phosphate and the increase in the enzyme itself was sufficient to account for the increased glycogen synthesis. These workers concluded that cortisol primarily induces the formation of

glucose-6-phosphate and that the increased glycogen synthetase activity was a secondary effect.

Feigelson and Greengard (28) found that within 4 hours after a single intraperitoneal administration of tryptophan or cortisol to rats resulted in a 3 fold increase in the amount of tryptophan-pyrrolase enzyme protein in the liver. Unlike the substrate induction of tyrosine-α-ketoglutarate transaminase, substrate induction of tryptophan pyrrolase by tryptophan occurred in adrenalectomized animals as well (73). It was shown by immunochemical methods that cortisol and tryptophan stimulated de novo synthesis of enzyme protein (28). Tryptophan is also capable of activating existing tryptophan pyrrolase by the conversion of apoenzyme to holoenzyme (27). Therefore, the substrate-induced elevation of the enzyme activity consisted of an initial activation of the existing enzyme protein followed by an increase in enzyme protein. Cortisol had no effect on the existing enzyme; however, it stimulated de novo synthesis of tryptophan pyrrolase within 4 hours after injection in the rat (28).

Pitot et al. (92) suggested that corticosteroids, in very small amounts, are necessary to stimulate m-RNA synthesis in hepatic cells. Tryptophan was necessary to stabilize tryptophan pyrrolase and to prevent its breakdown. Acting in this manner the hormone stimulated m-RNA synthesis and de novo enzyme synthesis. The substrate permitted maximum activity and stabilized the enzyme.

Conditions which lead to protein breakdown result in an increased activity of the urea cycle enzymes. Schimke (101) studied the effects of glucocorticoids on the urea cycle enzymes in the rat liver. It was

found that glucocorticoid effects on the urea cycle enzymes appear to be non-specific that is the enzyme levels increased due to the enhanced protein breakdown and the urea excretion. Intact adrenal glands were not necessary for the increased enzyme levels which were produced by a high protein diet.

Oxidative Metabolism

Many investigators have reported effects of glucocorticoids on oxidative metabolism (11, 31, 34, 35, 36, 41, 57, 63, 64, 70, 72, 110). These effects seem to vary, depending upon the tissue used and the length of treatment. Goetsch and McDonald (35) and Geurkink (32) have shown that single injections of prednisolone or hydrocortisone increased oxygen uptake in rat liver homogenates. Goetsch (34) has shown that prednisolone administered to normal goats 18 hours before surgical removal of a liver sample caused an increase in oxygen uptake by homogenates prepared from these liver samples. Repeated administration of glucocorticoids caused a decreased oxygen uptake by rat liver homogenates (35, 43). Roberts and Keller (97) found that 4 hours after cortisone was injected intravenously into rats, oxygen consumption of the anterior pituitary was reduced. At the same time an increase in the respiration and aerobic glycolysis of the posterior hypothalamus was observed. They suggested that cortisone activates the posterior hypothalamus. The posterior hypothalamus may initiate an inhibitory neurohumor which in turn inhibits activity in the anterior hypothalamus.

Clark and Pesch (11) found that chronically administered cortisone

lowered the oxygen uptake of normal rat liver mitochondria when α-keto glutarate was used as the substrate. No effect on oxidative phosphorylation was observed. They also found that the protein content and the relative weight of the liver increased in treated animals. Liver mitochondrial protein also increased.

The exact site of these effects on oxidative metabolism is unknown although much work has been done attempting to localize sites of action within the mitochondrion. Gordon et al. (36) have shown that the in vitro addition of adrenal cortical extracts and crystalline corticosterone depressed oxygen consumption by rat brain tissue in the presence of glucose. Methylene blue did not reverse the steroid-induced inhibition of oxygen consumption. This indicated a site of action preceeding the flavoproteins since methylene blue can act as a carrier between dehydrogenases and oxygen.

Most workers seem to agree that generally the inhibition of oxidative metabolism is limited to the activity of enzymes requiring pyridine nucleotides for electron transfer. The succinoxidase system is unaffected by steroids. Gallagher (31) provided evidence that hydrocortisone inhibited oxidative metabolism in rat liver mitochondria in vitro by destroying the selective semipermeability of mitochondrial membranes. This destruction of semipermeability resulted in a loss of soluble cofactors from the mitochondria. This was supported by the fact that hydrocortisone increased the uptake and reduction of NAD by mitochondria as measured spectrophotometrically at 340 mμ.

Jensen and Neuhard (57) found that the addition of steroids to a suspension of electron transport particles oxidizing NADH, spontane-

ously decreased the rate of oxidation. They found that steroids were concentrated in the electron transport particle phase and that the rate of inhibition was related to the solubility of the steroid in the particle more than the effect of the steroids upon the NADH oxidase enzyme activity. These workers (57) also found that corticosterone had an amytal-like effect upon the oxidation of NADH by heart sarcosome fragments. Succinate oxidation was not inhibited. They suggested that the site of steroid action in mitochondria is located near flavoprotein in the respiratory chain. Klingenberg and Hefen (65) found that corticosterone interfers with the H transfer between NAD and the succinate dehydrogenase region of the respiratory chain.

Bojesen and Jensen (9) reported that steroids had no effect on *in vitro* oxidation of NAD-dependent substrates in intact heart sarcosomes when measured by conventional Warburg manometry. However, when a Clark electrode was used to measure oxygen consumption during a short period of time, an inhibitory effect on oxygen consumption was observed on NADH and succinate oxidation. Hatefi *et al.* (42) suggested that the NADH oxidase system of beef heart mitochondria is very closely associated with a respiratory control mechanism and further suggested that the oxidation of succinate does not seem to be regulated by a respiratory control mechanism.

Hartman (41) found that mitochondria of the rat central nervous system become vacuolated after administration of cortisone. He proposed that the *in vivo* effects may be the same as the *in vitro* effects observed by Gallagher (31). Hartman (41) suggested that glucocorticoids may be a factor in governing the selective semi-permeability of

mitochondria *in vivo* and in regulating tissue metabolism.

In order to study further the possible effects of glucocorticoids on oxidative metabolism, Kerppola and Pitkanen (64) investigated the action of cortisone upon oxidative and glycolytic liver enzymes in the rat. They found that the activity of cytochrome oxidase was decreased in liver mitochondria isolated from normal rats chronically treated with cortisone. Decreased cytochrome oxidase was most obvious in old female rats. The activity of NADH-cytochrome c reductase was decreased only in old rats. The activity of succinate dehydrogenase was unchanged in animals of different age and sex. Cortisone treatment had no effect on glycolytic enzymes. These investigators suggested that mitochondrial functions are influenced by cortisone.

Kerppola (63) reported that chronic injections of cortisone into intact animals frequently inhibited oxidative phosphorylation in liver mitochondria from rats of all ages and of both sexes. The utilization of phosphorous in male rats was variably inhibited with succinate, malate, and pyruvate as substrate. Oxygen consumption was unchanged in young male rats when succinate or malate was used as substrate. With malate, a small decrease in the P/O ratio was obtained in male animals. With succinate as substrate, a decrease in P/O ratio was slight in young females, significant in old males, and even greater in old female rats.

Strickland (110) reported that elimination of endogenous corticosteroids failed to change the P/O values of rat liver mitochondria or homogenates. Respiratory rates based upon protein nitrogen in mitochondria and in homogenates were unaffected by adrenalectomy.

However, adrenalectomy was found to decrease liver homogenate respiratory rates based upon DNA levels. Since DNA is generally a measure of the number of liver cells, Strickland (110) postulated that a decrease in the number of mitochondria per liver cell may occur in adrenalectomized rats. This decrease may alter the maximum respiratory capacity even though the concentration of respiratory enzymes per milligram of mitochondrial protein remains unchanged.

Recent work by Liljeroot et al. (72) did not agree with the observations of Strickland (110). Liljeroot et al. (72) found that adrenalectomy caused a decreased P/O ratio in rat liver mitochondria. Addition of cortisone in vitro increased the mitochondrial P/O ratio. The impaired oxidative phosphorylation in adrenalectomized animals was the result of a decrease in adenosine triphosphate synthesis which was accompanied, in most cases, by an increased oxygen consumption. Cortisone added in vitro had no effect on the P/O ratio in liver mitochondria isolated from normal rats. These investigators suggested that the steroids may contribute to the integrity of the mitochondrial membranes and that steroids may enhance the efficiency of oxidative phosphorylation.

Gene Regulation of Enzyme Synthesis

Karlson (59) and Zalokar (133) suggested independently that several hormones may function by stimulating enzyme synthesis. This concept has been designated as the hormone-gene thesis. Hormones may exert their action by regulating genetic transcription at the chromosome or they may regulate translation at the ribosome. An action at

one or both of the above sites would be expressed in terms of enzyme formation followed by changes in cellular metabolism. Whatever the mechanism may be, there is considerable evidence that some of the biochemical effects elicited by the glucocorticoids involve the synthesis of enzymes.

The hormone-gene thesis is an attractive hypothesis for the mechanism of action of the glucocorticoids and there is considerable evidence supporting this hypothesis. A recent review by Hechter and Halkerston (45) provides an excellent essay on steroid hormone regulation of gene activity. They concluded that the action of steroid hormones at the cellular level does involve gene regulation; however, the exact site of the action and the primary effect are unknown.

Allosteric mechanisms and the operon theory for protein synthesis were suggested by Monod et al. (80). They suggest that many proteins possess two non-overlapping receptor sites. One, an active site, which binds the substrate; the other, an allosteric site, binds a metabolite or a hormone. The binding of an allosteric effector to the allosteric site results in an alteration of the protein structure so that the active site is altered resulting in the altered activity of allosteric enzymes.

The allosteric effects may be involved in the control of protein synthesis (80). Along the chromosomes, clusters of genes or units of DNA may be blocked by a repressor substance. If the repressor molecule is not present, the synthesis (transcription) of all types of RNA by the units of DNA proceeds. Messenger, ribosomal, and transfer RNA are synthesized by the units of DNA and they leave the nucleus and

enter the cytoplasm where they serve as templates for translation, or to transfer activated amino acids to the template. Hormones may be active in removing repressors from units of DNA.

There are many points in the sequence of protein synthesis that glucocorticoids may affect. They are known to affect the size of amino acid pools, energy supply, purine and pyrimidine biosynthesis, DNA-dependent RNA synthesis (transcription), and m-RNA directed assembly of amino acids (translation). Any one or several of the above points may be the primary site of glucocorticoid action.

Hechter (45) concluded that the action of the steroid hormones at the cellular level does involve gene regulation; however, there is no conclusive evidence for the direct action of glucocorticoids at the gene. Also, there is much evidence to indicate that some of the actions of glucocorticoids are not mediated through the genes.

In order to study the effects of glucocorticoids on amino acid pools, Betheil et al. (6) studied the differential effects of cortisone acetate on tissue and plasma amino acid levels in the rat. They found that 4 hours after cortisone acetate administration, liver and plasma tyrosine concentrations were depressed. This was probably due to the known induction of tyrosine-α-ketoglutarate transaminase in the liver. The principal amino acids formed by transamination namely glutamate, aspartate, and alanine were elevated. The concentration of numerous other amino acids in the liver was depressed due to the increased rate of protein synthesis. In thymus and muscle tissues, glutamate, aspartate, alanine and other amino acids rose, presumably due to depressed protein synthesis and involution (6). It was suggested that intra-

cellularly released amino acids may not be transported into the blood as rapidly as involution occurs. The alterations in amino acid concentrations may be due to the hormonal induced transaminase activity in the liver and in protein degredation in lymphoid and muscle tissues (6).

Rivlin et al. (96) recently reported that cortisone treatment depressed plasma tyrosine concentration in man. These workers suggested that cortisone depressed the concentration of tyrosine by increased hapatic tyrosine transaminase activity.

Recent work indicating an effect of glucorticoids on enzyme protein has led to many studies of glucocorticoid effects on RNA synthesis. Feigelson and Feigelson (26) found that four hours after cortisone-acetate injections into adrenalectomized rats, glycine-^{14}C incorporation into RNA of the liver increased, whereas in the spleen, incorporation of labelled glycine into RNA decreased. A similar but quantitatively smaller effect on protein metabolism was observed. Increased incorporation of glycine-2-^{14}C into rat liver RNA occurred in all subcellular fractions; however, quantitatively, the most rapid incorporation increase occurred in the nuclear fraction. Weber et al. (126) reported that triamcinolone and cortisone injections stimulated orotate incorporation into RNA. This reached a peak about 8 hours after the injection. In rats treated with triamcinolone, this increase was preceeded by a rise in amino acid nitrogen and fructose 1,6-diphosphatase activity. Glucose-6-phosphatase activity did not rise during the 24 hour study. Wick et al. (127) and Greenman et al. (38) recently reported that 2 hours after glucocorticoid treatment de novo synthesis

of all three major species of RNA was stimulated in the rat liver.

Since RNA and protein biosynthesis are endergonic reactions, it is possible that increased ATP production may stimulate the increased RNA and protein biosynthesis. Feigelson and Feigelson (25) found that adrenalectomy resulted in decreased hepatic ATP concentrations. Treatment of adrenalectomized animals with cortisol elevated these levels to near normal. Administration of glucose to adrenalectomized animals increased ATP levels more than cortisone administration; however, this had no effect on glycine-2-^{14}C incorporation into RNA or protein. They concluded that the primary action of glucocorticoids was not on ATP synthesis.

Feigelson and Feigelson (26) and Betheil (6) later observed that increased transaminase reactions following cortisone treatment in the rat formed glutamate, aspartate, and later glutamine which stimulate hepatic purine nucleotide and urea biosynthesis.

Currently there is no conclusive evidence indicating a direct effect of glucocorticoids at the gene locus; however, it still remains an attractive hypothesis. There is suggestive evidence that ecdysone, an insect hormone, and aldosterone, a mineralocorticoid, may act at the gene locus (3, 13, 15, 58).

Beermann (3) has presented evidence that the well defined bands on certain insect salivary chromosomes represent high concentrations of DNA. During metamorphosis or 15 to 30 minutes after ecdysone treatment, these bands exhibit a "puffing". The "puffs" are thought to represent sites of active RNA synthesis. Karlson (58) suggested that ecdysone acts directly on the gene. Later Karlson (59) found that

ecdysone injection led to puffing of a certain region on a specific salivary chromosome of Chironomus. The same locus undergoes puffing just before pupa formation. It was suggested that ecdysone may activate genes in some manner and thereby stimulate DNA-dependent synthesis of RNA; the RNA, in turn, carries the genetic information to the cytoplasm and directs the synthesis of specific proteins. Using tritium labeled ecdysone, Karlson (59) found a large proportion of the radioactivity in the nuclear fraction of the epidermis, the target organ. Incorporation of labeled precursors into RNA was enhanced by ecdysone. RNA produced in the epidermis of Colliphora, under the influence of ecdysone, stimulated incorporation of amino acids into protein and directed synthesis of dopa decarboxylase in an in vitro protein-synthesizing system from rat liver (103).

Aldosterone may exert it's effect on sodium transport at the gene. Sodium transport in mucosal cells of the toad bladder is blocked by actinomycin D (19). Edelman et al. (19) have shown by autoradiography that labeled aldosterone is concentrated in the nucleus or perinuclear region of the mucosal epithelium of the toad bladder.

Dahmus and Boomer (15) reported that hydrocortisone treatment 4 hours before sacrifice resulted in increased template activity for DNA-dependent RNA synthesis in chromatin isolated from the rat liver. The difference in template activity between chromatin extracted from treated rats and chromatin extracted from control rats was abolished by the removal of DNA-bound protein. These investigators concluded that hydrocortisone action appeared to be associated in some way with DNA-bound protein.

Sekeris and Lang (104) and Lang and Sekeris (68) have provided evidence that cortisol action in rat liver may act at the gene locus. Sekeris and Lang (104) reported direct evidence of cortisol stimulation of messenger RNA synthesis by the use of P^{32} turnover studies in rat liver nuclei and cytoplasm. Lang and Sekeris (68) stimulated protein synthesis in an *in vitro* amino acid incorporating system with nuclear RNA fractions isolated from normal and cortisol treated animals. RNA fractions from cortisol treated animals caused a higher incorporation of C^{14}-L-leucine into protein than the corresponding RNA fraction from normal animals.

As attractive as the hormone-gene thesis appears to be, some excellent work which indicates the primary effects of glucocorticoids are not on *de novo* enzyme synthesis has been presented; however, *de novo* enzyme synthesis appears to be necessary for complete expression of hormone activity. Ray *et al.* (94) studied the effect of hydrocortisone on blood glucose, hepatic glycogen, and hepatic phosphoenolpyruvate carboxykinase. Animals were treated with NaCl, actinomycin D, actinomycin D plus hydrocortisone and with hydrocortisone. The levels of glycogen and glucose were much higher in animals treated only with hydrocortisone. Actinomycin D blocked any rise in phosphoenolpyruvate carboxykinase in the above experiment. In the same experiment by Ray *et al.* (94), rats were similarly treated but also were given glucose intraperitoneally to insure a glucose load in all animals. Glycogen formation was stimulated by hydrocortisone plus actinomycin D as well as by hydrocortisone alone. Since actinomycin D blocks enzyme protein synthesis, these workers concluded that increased glycogen synthesis

after glucocorticoid treatment did not require de novo enzyme synthesis. This was also suggested earlier by Kvam and Parks (67).

Tomkins et al. (112) have recently suggested an important role of translation in enzyme synthesis. These workers found that tryptophan pyrrolase activity increased in the liver of adrenalectomized rats treated with hydrocortisone. This increase began 2 hours after treatment, peaked at 4 to 8 hours, and returned to normal 8 to 10 hours after treatment. This increase was completely blocked by previous treatment with actinomycin D. Although actinomycin D blocked the initial steroid-induced increase in tryptophan pyrrolase activity, later administration of the antibiotic caused an increase in trypotphan pyrrolase activity. These workers suggested that sufficient m-RNA was present for increased enzyme synthesis after tryptophan pyrrolase activity began to decrease; however, a cytoplasmic RNA-dependent repressor blocked further synthesis of the enzyme by m-RNA. Later administration of actinomycin D blocked the synthesis of the repressor and translation was permitted to continue. These investigators proposed that glucocorticoids may act on protein synthesis at some step beyond the gene transcription site.

The effects of glucocorticoids in the thymus, another target organ, are considerably different than those in the liver. In the thymus glucocorticoid-induced involution occurs by some unknown mechanism. Although there is considerable evidence that the glucocorticoids exert a hormone gene effect in the liver, existing evidence indicates that hormone effects in the thymus do not involve gene regulation.

Reduced rat thymic weights and K/Na ratios can be observed 6 hours after glucocorticoid treatment. Histologic studies by Stevens et al.

(109) 6 hours after cortisol treatment revealed depressed mitosis and the presences of a large number of shrunken pyknotic nuclei. Since there was no measurable change in the levels of nucleic acids, a preferential loss of water and cytoplasmic constituents probably occurred. DNA synthesis, measured by the rate of thymidine-2-^{14}C incorporation, decreased after cortisol administration.

Halkerston et al. (39) studied the effects of cortisol on gene regulation in the thymus. They found that cortisol treatment caused a similar reduction in both the thymus weight and K/Na ratio in both control and actinomycin D-treated rats. These workers also found that the thymus weights were not reduced in rats receiving actinomycin D alone. If involution was the result of catabolic enzyme synthesis, actinomycin D should block thymus involution after cortisol treatment. Alternatively, if cortisol represses the synthesis of enzymes necessary for anabolic processes, actinomycin D should exert a catabolic effect on the thymus also, by blocking the synthesis of anabolic enzymes. Since these effects were not observed, these workers concluded that cortisol action may not involve the gene locus in the thymus.

Subcellular Distribution of Glucocorticoids

Recently, several investigators have studied the subcellular distribution of labeled glucocorticoids in hepatic tissue. If one assumes that the hormone acts "in person" at it primary site of action, localization within the cell would provide some indication of its primary action. Rapid, preferential localization of tritiated aldosterone in the nuclear and perinuclear region of toad bladder mucosal cells has

provided suggestive evidence that this hormone may act at the gene locus (19). If it could be established that this radioactivity represents free aldosterone and not metabolic products, this would provide additional evidence for an effect at the gene locus.

Several investigators have studied the subcellular distribution of labeled glucocorticoids *in vivo* and *in vitro* (4, 5, 113). Ulrich (113) studied the subcellular distribution of (^{14}C) cortisol in rat hepatic tissue *in vitro*. He found 75 to 80% of the activity in the supernatant fraction, 18% in the microsomal fraction, 3% in the mitochondrial fraction, and 2.8% in the nuclear fraction. Incubation of isolated mitochondria with (^{14}C) cortisol demonstrated that the hormone entered the mitochondrion very rapidly by the process of diffusion. Two washings with sucrose removed 95% of the radioactive cortisol and its metabolites from the mitochondria, thus indicating very little or no binding of the steroid.

Bellamy *et al.* (5) studied the *in vivo* uptake of cortisol in rat tissues. It was noted that tissues of the rat differ greatly in their ability to concentrate (^{14}C) cortisol. Tissue/plasma ratios were found to be about 6 for liver, 5 for spleen, 3 for kidney, 3 for intestine, 1 for skeletal muscle, and less than one for nerve tissue. Much of the activity was probably due to metabolites of cortisol since a large percentage of the activity moved into the water phase when partitioned between chloroform and water. It was suggested that cortisol entered the cell by diffusion and that a favorable diffusion gradient was maintained by the intracellular adsorption of free cortisol. A barrier to the diffusion of cortisol into the brain seemed to exist since the

tissue plasma ratio was less than one.

Later Bellamy (4), in an *in vivo* study, found that (^{14}C) cortisol was not adsorbed to any type of rat liver cell particles. He found that about 14% of the total homogenate activity was present in the nuclear fraction, 6.6% in the mitochondrial fraction, 7.3% in the microsomal fraction, and 75% in the supernatant fraction. The concentration of ^{14}C counts/min/g. wet wt. was highest in the microsome fraction, and lowest in the nuclear fraction. Similar results were obtained with *in vitro* experiments. Most of the activity in the supernatant was due to polar metabolites of cortisol, whereas the non-polar compounds remained mostly in the particulate fractions. (^{14}C) Corticosterone was taken up by liver slices faster than cortisol; however, the subcellular distribution was similar. At low temperatures the adsorbed labeled steroid was not easily removed by washing. At higher temperatures it was readily removed. This indicated that metabolism of the steroid to more polar metabolites occurred and that the metabolites were readily leached out. Treatment of the particulate fraction with ribonuclease or removal of ribonucleic acids from tissues with hot trichloroacetic acid made no difference in the adsorption of corticosterone. It was suggested that the steroids were adsorbed to proteinaceous cell structures where they were relatively inaccessible to enzyme action. The tissue/plasma ratios were: brain 0.38, skeletal muscle 0.38, spleen 0.30, and liver 1.72.

The subcellular distribution of (^{14}C) cortisol and (^{14}C) corticosterone in rat liver and muscle has been studied *in vivo* and *in vitro* by De Venuto *et al.* (16). "Equilibrium fractionation" was used

during the separation by different fractions. This procedure provides for separation by differential centrifugation in such a way that the original suspending medium was utilized throughout the entire fractionation procedure, and therefore remained in equilibrium with all fractions. These workers compared the difference in the subcellular distribution of labeled steroids when prepared either by equilibrium fractionation or by the standard fractionation procedure. A difference was clearly shown. Standard fractionation procedures resulted in a higher percentage of the activity in the supernatant fraction and a lower percentage of the activity in the nuclear and mitochondrial fraction. The distribution pattern 5 minutes after the injection of cortisol is essentially the same as that of corticosterone, whereas *in vitro* the amount of corticosterone bound to the mitochondrial fraction is greater than the corresponding value for cortisol. The results are essentially the same in adrenalectomized rats or normal rats. The subcellular distribution of (^{14}C) corticosterone *in vivo* in adrenalectomized rats was as follows: nuclear fraction 13.4%, mitochondrial fraction 26.6%, microsomal 7.8%, and supernatant 51.1%. Equilibrium fractionation procedures were used to obtain the above fractions.

Equilibrium dialysis experiments were prepared by placing the different fractions in dialysis bags and placing the bags and contents in wide mouth glass jars containing a known amount of radioactive steroid (16). After a predetermined shaking time the radioactivity of the solution inside the bags, outside of the bags, and the amount of radioactive material bound to the subcellular fractions was determined. The results of the dialysis studies were similar to the *in vivo*

studies. Far less steroid was bound to the nuclear and microsomal fractions than to the mitochondrial fraction.

The possibility of metabolic transformations of the steroids in vivo and in vitro was investigated by chromatography (16). It was found that the nuclei and supernatant fractions contained only original cortisol or corticosterone and no metabolites were detected. The radioactive material in the mitochondria and microsomes consisted of about 15 to 25 percent metabolic products. Previous work by De Venuto and Westphal (17) provided evidence that in the presence of a NADPH-regenerating system all fractions metabolized cortisol. In the absence of a NADPH-regenerating system microsomes and mitochondria metabolized cortisol rapidly, whereas nuclei and supernatant fractions did not metabolize the steroid.

Hanngren et al. (40) made whole body autoradiographs of mice injected with (^{14}C) labeled cortisone and cortisol Both were rapidly distributed to all tissue. Selective accumulation was observed in liver, kidney, intestines, bronchial mucosa, ductus epididymus, vas deferens, and interstitial cells of testicle and ovary. The brown fat in the neck region and the pituitary contained relatively high concentrations of the steroid. Radioactivity in joints, brain tissue, and cerebrospinal fluid was low. The radioactive steroids penetrated the placental barrier; however, uptake in the foetus was low.

Dingman and Sporn (18) studied in the intracellular binding in vivo of actinomycin D and hydrocortisone in rat liver. These workers found that DNA was the major binding site for labeled actinomycin D, whereas hydrocortisone did not bind with DNA. The activity in the

subcellular fractions was nuclei 5%, mitochondria 8%, microsomes 4%, and supernatant 85%. The possibility of binding sites in the supernatant fraction was investigated with equilibrium dialysis techniques. It was found that only 7-12% of the activity was non-dialyzable. Therefore a large number of binding sites probably do not exist in the soluble part of the cell. These workers concluded that the primary site of action of steroid hormones is not the genetic apparatus.

Bojesen and Jensen (9) studied the in vivo incorporation of (^{3}H) corticosterone in subcellular particles of heart and kidney. These workers reported that attempts to localize corticosterone in vivo in subcellular fractions of heart tissue indicated no localization within the particulate fraction. The major part of the activity was found in the supernatant fraction.

Additional attempts by these investigators were made to localize (^{3}H) corticosterone in order to gain clues concerning its site of action. Animals received a continuous infusion of (^{3}H) corticosterone and were sacrificed after 60 minutes of infusion. The heart and the kidney were quickly removed and homogenized in sucrose-mannitol solution containing (^{14}C) corticosterone. According to these investigators this permitted distinction between steroid incorporated into subcellular particles and steroid simply adsorbed or dissolved in these particles. This was done by determining the ^{3}H/^{14}C ratio in the supernatant and in the particulate fraction. The ^{3}H/^{14}C ratio in the supernatant fraction represents only steroid that is dissolved or adsorbed to particles. A large amount of incorporated steroid in a particulate fraction would result in a higher ^{3}H/^{14}C ratio than observed in the

supernatant fraction. One hour after starting a continuous infusion of (^{3}H) corticosterone the animal was sacrificed and the (^{3}H)/(^{14}C) was found to be 1.83 in the heart mitochondrial pellet and 1.04 in the heart supernatant solution. Reliable ratios were not found in the microsomal fraction. This indicated that within the mitochondrion the steroid is slightly incorporated and unexchangeable. Metabolism of the steroid in kidney tissue complicated the ratios being measured; however, it was found that a small amount of the steroid was bound in unexchangeable form to mitochondria and microsomes.

CHAPTER III

MATERIALS AND METHODS

Experiment I

Treatment of Animals: Young Holtzman strain male rats weighing 150 to 200 g were bilaterally adrenalectomized under ether anesthesia. Two separate incisions were made parallel to the last pair of ribs. The glands were removed and the abdominal opening was closed with two layers of sutures, a muscle-peritoneal layer and a skin layer. The rats were returned to cages and allowed to recover for three to five days. They were given a rat laboratory chow* and 1.0% salt water ad libitum.

The animals were fasted twelve hours before sacrifice. Five hours before sacrifice the treated animals were injected (IM) with five mg of corticosterone** suspended in a sterile aqueous vehicle.*** Control animals were injected with the carrier vehicle only.

Preparation of Homogenates and Warburg Flasks: Each animal was decapitated by a mechanical device. An incision was quickly made into the body and cranial cavities; about half of the cystic lobe of the liver, the right kidney, the right testis, the diaphragm, the

*Ralston-Purina Company, St. Louis.
**Nutritional Biochemical Corp., Cleveland, Ohio.
***The Upjohn Co., Kalamazoo, Michigan.

brain, and the heart were quickly removed and each placed in 9.0 ml of cold 0.154 M KCl. The entire brain was removed by unroofing the cranial vault. It was bisected sagittally and one half was used. Each tissue was cut into small pieces with scissors and homogenized for approximately thirty seconds in a chilled Potter-Elevehjem homogenizer. The diaphragm required an additional thirty seconds of homogenizing. Six ml of the homogenate were transferred to a large test tube containing three ml of cold 0.1 M phosphate buffer pH 7.4. Three ml of this mixture were transferred to a cold Warburg flask containing 0.2 ml of 25% KOH and a small piece of flutted, filter paper in the center well. One ml of the mixture was placed in a 10 ml tared beaker and dried for 12 hours in a 90° C. oven. The following aliquots were taken from the mixture for preincubation analysis: Two ml were deproteinized with 1.8% barium hydroxide and 2% zinc sulfate for glucose and lactic acid determinations, one tenth ml was removed for protein determination, one tenth ml was removed for inorganic phosphate determination, and 0.5 ml was removed for glycogen determination.

The Warburg flasks containing the tissues were placed on a Gilson respirometer and the tissues were equilibrated under air for 10 minutes at 37° C. The equilibration period was followed by a 30 minute incubation period during which oxygen uptake readings were taken at 10 minute intervals. At the end of the incubation period aliquots were removed from the flasks and used to determine protein, glucose, glycogen, inorganic phosphate, and lactic acid levels in a manner similar to the preincubation analyses noted above.

Protein was determined by the Biuret method (37). Glucose was

determined by the glucose oxidase method (24). Inorganic phosphate was determined according to the procedure of Lowery and Lopez (76). Lactic acid was determined according to the procedure of Barker and Summerson (1). Reagent grade chemicals were used in all the analytical procedures.

Experiment II

Treatment of Animals: The treated and control rats in this experiment were handled in an identical manner to treated and control rats in Experiment I.

Preparation of Mitochondria: Each animal was decapitated and homogenates were made of brain, heart, and liver tissue. The brain and heart were each homogenized in ice cold sucrose-EDTA solution (0.3 M sucrose; 0.005 M EDTA; 0.002 M tris buffer). The liver was homogenized in ice cold sucrose-EDTA solution (0.25 M sucrose; 0.005 M EDTA; 0.002 M tris buffer).

Brain mitochondria were isolated by a method previously described by Baylays (2). Heart mitochondria were isolated according to the procedure described by Cleland (12). Liver mitochondria were isolated according to the procedure described by Hogeboom (50).

Analysis: P/O ratios were measured in 15 ml Warburg vessels incubated for 15 minutes at 30° C. using a Gilson respirometer for oxygen uptake measurements.

The final volume of the reaction mixture was 3.0 ml. It contained: sucrose, 120 μ moles; tris buffer (pH 7.4), 200 μ moles; either succinate, 75 μ moles, 25 μ moles, or malate plus pyruvate, 20

μ moles of each; cytochrome c., 0.03 μ moles; phosphate, 40 μ moles; ATP, 3.0 μ moles; magnesium sulfate, 1.5 μ moles; and a mitochondrial suspension containing 4 to 10 mg of mitochondrial protein. The side arm contained 50 μ moles of glucose, 350 KM units of hexokinase, and 3.0 μ moles of ADP. Reagents were purchased from Sigma Chemical Company.* Duplicate flasks were prepared and were allowed to equilibrate under air for 10 minutes. The reaction was initiated by adding the hexokinase-glucose trap from the side arm. One flask of each pair was removed and immediately after adding the side arm contents, 0.2 ml was removed and placed in 0.8 ml of 5% TCA. This was analyzed for inorganic phosphate by the method of Lowery and Lopez (76). The other flasks were incubated for 15 minutes during which oxygen uptake was measured. At the end of 15 minutes the flask were removed and aliquots were taken for inorganic phosphate and protein determinations. The change in inorganic phosphate levels represented the amount bound as high energy phosphate. The P/O ratio was determined by comparing the decrease in inorganic phosphate, expressed in μ moles phosphate, to the μ atoms of oxygen taken up.

Experiment III

Treatment of Animals: Adrenalectomized rats weighing about 150 grams were anesthetized with sodium pentabarbital. The left femoral vein was exposed and each animal received 0.5 ml of solution containing 10 micrograms of "cold" corticosterone and 20 microcuries of (^{3}H)

*Sigma Chemical Co., St. Louis.

corticosterone* intravenously. The solution was prepared by evaporating to dryness 0.2 ml of benzene containing 0.2 millicuries of (^{3}H) corticosterone. The residue was dissolved in 0.2 ml of ethyl alcohol containing 0.10 mg of "cold" corticosterone. Saline was added to make a total volume of 5.0 ml. A (^{3}H) corticosterone with a specific activity of 33.9 curies per millimole was used without prior purification.

Fractionation of Tissues: Thirty minutes after injection of (^{3}H) corticosterone, one ml of blood was collected by cardiac puncture. The animals were immediately sacrificed by decapitation and the liver, heart, thymus, and brain were removed and rinsed twice in sucrose. The brain, thymus, and heart were each placed in 0.3 M sucrose, 0.005 M EDTA, and 0.003 M tris buffer, pH 7.5. The liver was placed in 0.25 M sucrose, 0.005 M EDTA and 0.003 M tris buffer, pH 7.5. Each tissue was cut into small pieces and homogenized in a Potter-Elvehjem homogenizer driven by an electric drill. Fifty µg of carrier corticosterone were added at this point. Aliquots of the homogenates were saved for extraction. The remaining homogenates were fractionated into nuclei (fraction 1), mitochondria (fraction 2), microsomes (fraction 3), and supernatant (fraction 4). Fraction 1 and fraction 2 of brain, heart, and liver were obtained as previously indicated in Experiment II. The thymus was fractionated by the same procedure used for the heart. The combined supernatant for each tissue was centrifuged at 105,000 x g for 60 minutes in a Beckman model L-2 ultracentrifuge in order to obtain fraction 3 and fraction 4.

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*New England Nuclear Corporation, Boston, Mass.

Microscopic examination of fraction 1 indicated the absence of intact cells in this fraction.

Electron micrographs of fraction 2 showed a very high concentration of mitochondria and very few contaminants in all preparations except from thymus. The thymus preparation was contaminated with other organelles and cell fragments to a greater extent than this fraction from the other tissues.

Extraction and Purification of Corticosterone: Each fraction was extracted with 15 volumes of hot acetone (54-60° C.) and filtered with #40 Whatman filter paper. The filtrate was evaporated to dryness using a Rotavapor and a water bath at 50° C. In order to remove acids, phenolic compounds, and conjugated steroids, 5 ml of water and 0.25 ml of 10% NaOH were added to the dry material and extracted 3 times with 10 ml diethyl ether. The ether extract was washed 3 times with 2 ml of water in order to remove all sodium hydroxide. The ether extract was evaporated to dryness under nitrogen. The material was reconstituted in 2 ml of 90% aqueous methanol and partitioned 3 times with 2 ml of ligroine in order to defat the extract. The partitioning was accomplished by placing three test tubes in a row, each containing 2 ml of 90% aqueous methanol. The first tube contained the reconstituted ether extract. Two ml of ligroine were added to the first tube and after thoroughly shaking with a vortex mixer, the tube was centrifuged briefly to break the emulsion and the ligroine was transferred with a pipet to the next tube containing 2 ml of 90% aqueous methanol. Fresh ligroine was added to the first tube and the above procedure was repeated 3 times. Each tube was shaken and transfers were made to the

next tube. The ligroine removed from the last tube in the series was discarded. The aqueous methanol in the 3 tubes was combined, placed in an evaporating flask and evaporated to dryness. Five tenths ml of water was added to each evaporating flask and extracted 4 times with diethyl ether. The ether was evaporated to dryness and the residue was reconstituted in 1 ml of chloroform-methanol (1:1). Fifty ug of carrier steroid were added at this point. One tenth ml of this mixture was taken and spotted on thin layer plates which were coated with silica gel (30 g Silicar* and 60 ml of H_2O) and chromatographed in a chloroform-methanol solvent (85:15). Spots corresponding to standard corticosterone were removed and placed in small test tubes. One tenth ml of water was added and the silica gel was extracted two times with 2 ml of benzene. The benzene was placed in a liquid scintillation vial and evaporated under nitrogen. Ten ml of scintillation fluid (4 g 2,5-Diphenyloxazole** and 0.3 g of 1,4-bis-2-4-Methyl-5-Phenyloxazolyl-Benzene** in 1 L of reagent toluene) were added and each vial was counted for 10 minutes in a Packard Tri Carb Scintillation Counter. The samples were prepared to give a counting rate at least twice the background count. The total counts were at least 400 for this counting time. It was possible to count ^3H with an efficiency of 13% using a voltage setting of 10-200 on channel A, and a voltage setting of 200-1000 on channel B.

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*Mallinckrodt, St. Louis.
**Packard Inst. Co., Downers Grove, Ill.

CHAPTER IV

RESULTS

The objectives of these studies were the following: first, to measure biochemical changes in different tissues of the rat after corticosterone treatment; second, to measure P/O ratios in mitochondria isolated from liver, heart, and brain tissues taken from adrenalectomized corticosterone-treated and adrenalectomized non-treated rats; and third, to localize (^{3}H) corticosterone within the subcellular fractions of the brain, the thymus, the heart and the liver. It is hoped that the results presented herein and the experiments suggested by this work will contribute to the elucidation of glucocorticoid action sites.

Experiment I. Biochemical Changes in Tissues

In order to make a comparison between glucocorticoid-induced changes in different tissues and metabolic alterations in these tissues, the first experiment was designed to measure the effects of acute administration of corticosterone on the metabolism of homogenates which were prepared from the brain, heart, diaphragm, liver, kidney, and testis. Preincubation levels and changes in the amount of oxygen uptake, lactic acid production, glycogen breakdown, protein changes, inorganic phosphate disappearance, and glucose utilization were measured during a 30 minute incubation period. Appropriate aliquots were removed from the

incubation mixture for preincubation determinations and after incubation similar aliquots were removed from the flask for postincubation determinations.

TABLE I

AVERAGE OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. NON-TREATED RATS RECEIVED CARRIER VEHICLE ONLY

Tissue Homogenate	Treatment	No. of Rats	Incubation Time (minutes) 10***	20***	30***
Brain	Treated	25	62 ±21	122 ±36	176 ± 50
Brain	Non-treated	25	58 ±17	113 ±32	155 ± 35
Heart	Treated	23	102 ±45	165 ±66	213 ± 77
Heart	Non-treated	23	119 ±41	180 ±58	226 ± 44
Diaphragm	Treated	23	43 ±34	70 ±41	98 ± 44
Diaphragm	Non-treated	22	41 ±30	59 ±38	79 ± 47
Liver	Treated	25	143 ±43	249* ±78	328** ±104
Liver	Non-treated	25	119 ±53	193 ±82	246 ± 96
Kidney	Treated	26	70 ±25	131 ±41	178 ± 51
Kidney	Non-treated	26	74 ±29	128 ±47	171 ± 61
Testis	Treated	25	27 ± 8	53 ±15	71 ± 26
Testis	Non-treated	25	30 ±16	51 ±21	69 ± 27

*P< = 0.05 **P< = 0.01. Treated vs non-treated.
***Values represent oxygen uptake during 10, 20, and 30 minutes of incubation.

The effect of acute corticosterone treatment on oxygen uptake by homogenates prepared from brain, diaphragm, liver, and testis are shown in Table I and illustrated graphically in Figures 1, 2, and 3. Only in the liver homogenate was there a significant treatment effect. The oxygen uptake by the liver homogenates which were prepared from corticosterone-treated animals was significantly greater after 20 minutes

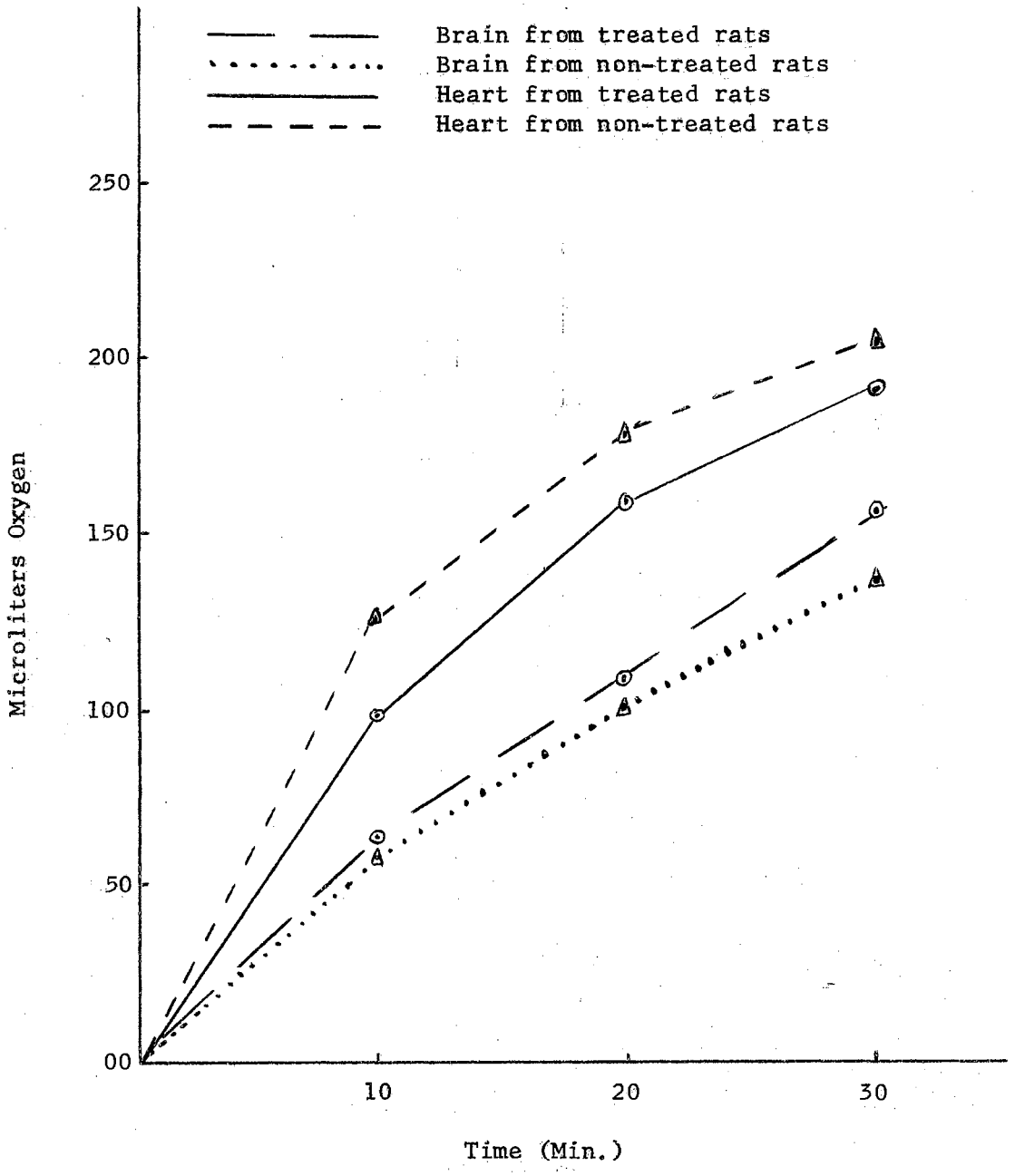


Figure 1. Average Oxygen Uptake (microliters/100 mg protein) by Brain and Heart Homogenates. Tissues prepared from fasted, adrenalectomized, corticosterone-treated rats and fasted, adrenalectomized, non-treated rats. Treated rats received 5.0 mg of corticosterone intramuscularily five hours before sacrifice.

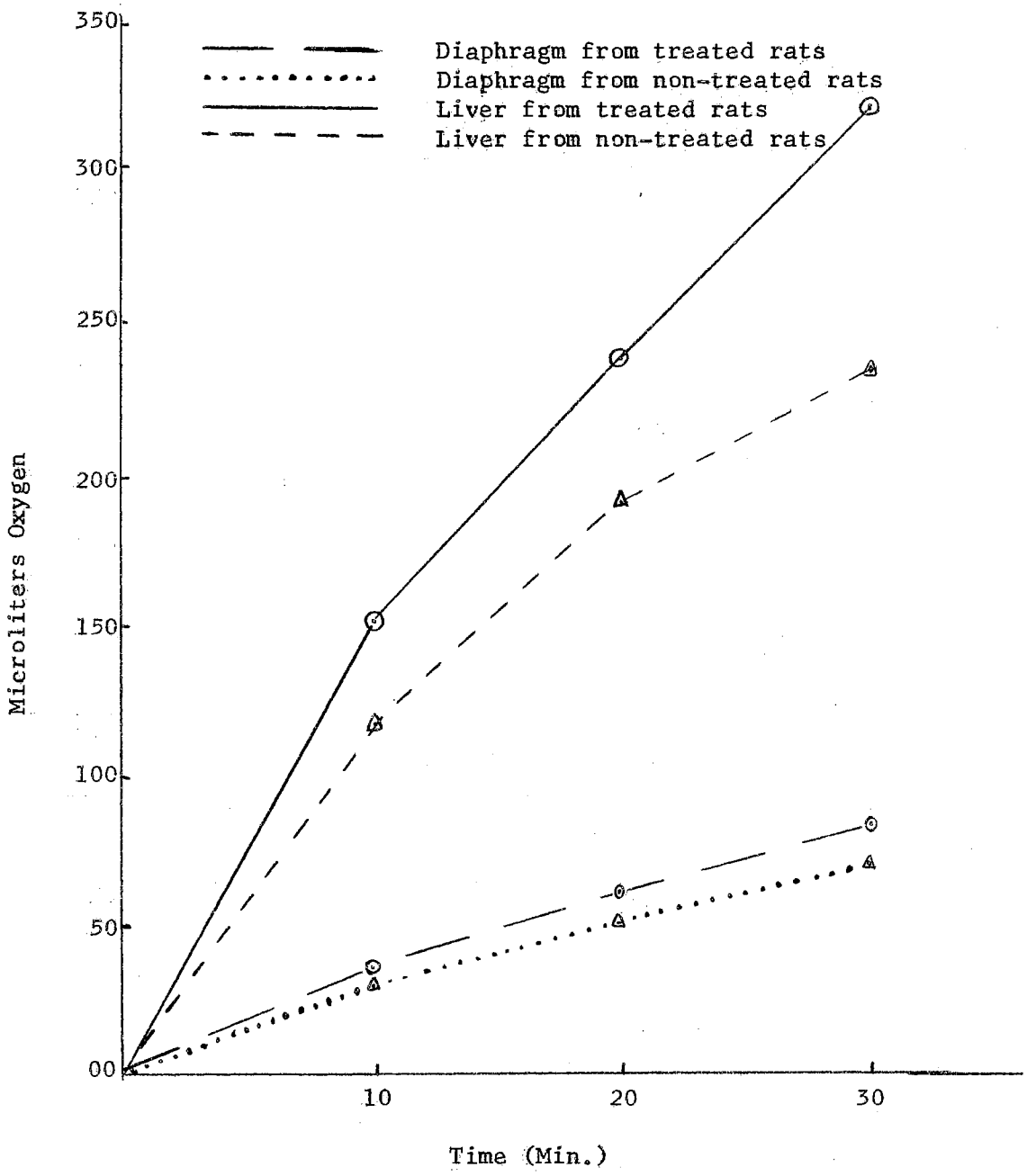


Figure 2. Average Oxygen Uptake (microliters/100 mg protein) by Diaphragm and Liver Homogenates. Tissue prepared from fasted, adrenalectomized, corticosterone-treated rats and fasted, adrenalectomized, non-treated rats. Treated rats received 5.0 mg of corticosterone intramuscularily five hours before sacrifice.

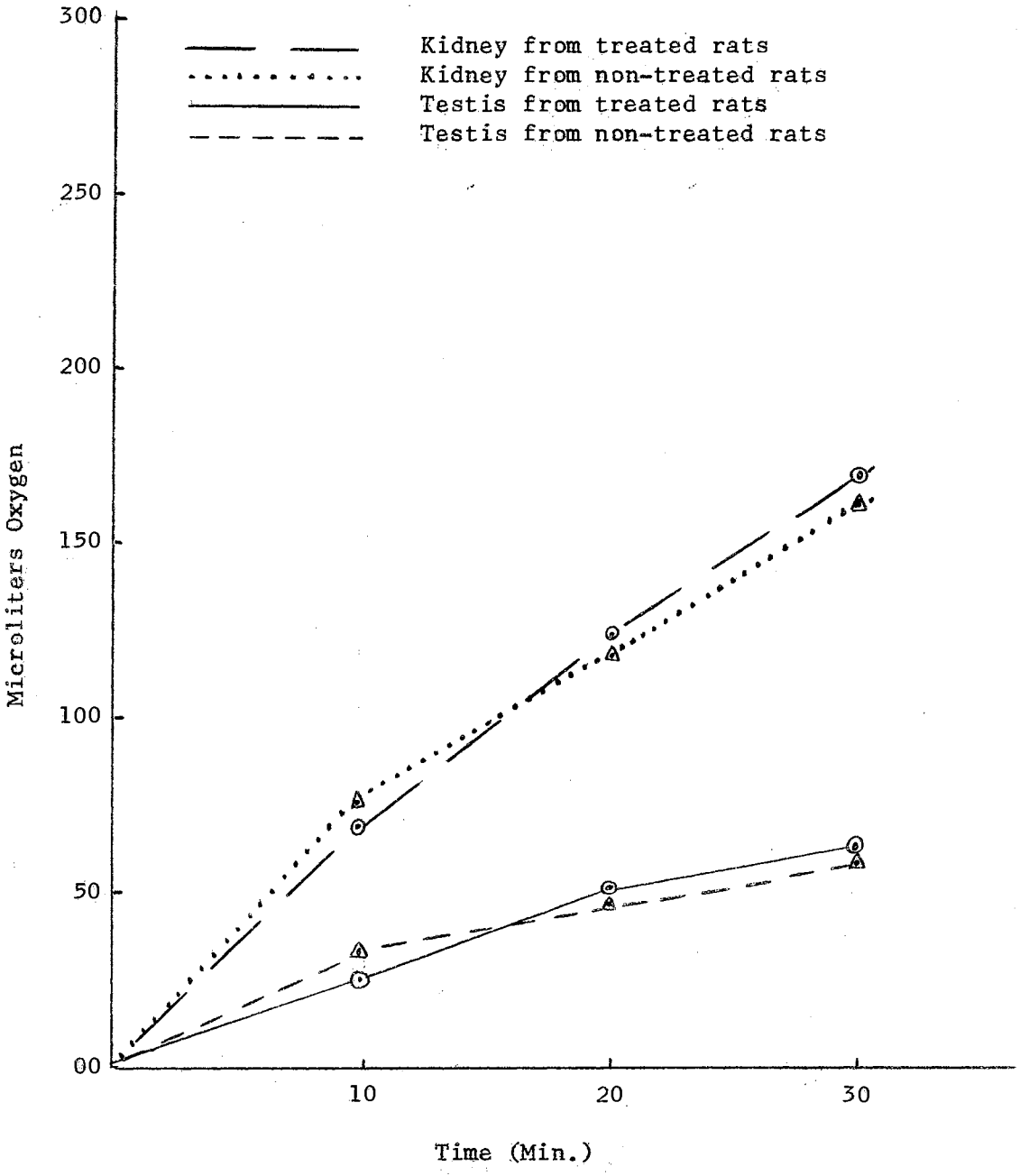


Figure 3. Average Oxygen Uptake (microliters/100 mg protein) by Kidney and Testis Homogenates. Tissue prepared from fasted, adrenalectomized, corticosterone-treated rats and fasted, adrenalectomized, non-treated rats. Treated rats received 5.0 mg of corticosterone intramuscularily five hours before sacrifice.

($P < 0.05$) and after 30 minutes ($P < 0.01$) of incubation when compared to the corresponding value for non-treated animals. The treatment produced no effect on oxygen uptake by homogenates prepared from the other tissues.

Protein levels in the different tissues taken from corticosterone-treated and non-treated rats measured before and after incubation in the Warburg apparatus are given in Table II. A treatment effect was

TABLE II

AVERAGE PROTEIN (MG/GM DRY TISSUE WT) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Preincubation	Postincubation	Difference
Brain	Treated	21	445 ± 88	436 ± 88	- 9
Brain	Non-treated	19	492 ±101	483 ± 89	- 9
Heart	Treated	24	405 ±123	364 ±138	-41
Heart	Non-treated	25	435 ±101	436 ±125	- 9
Diaphragm	Treated	20	431 ±140	417 ±156	-14
Diaphragm	Non-treated	21	452 ±152	430 ±163	-22
Liver	Treated	24	540 ±159	524 ±163	-16
Liver	Non-treated	25	550 ±151	553 ±171	3
Kidney	Treated	24	460* ± 51	443 ±108	-17
Kidney	Non-treated	22	529 ±119	498 ±124	-31
Testis	Treated	24	522 ±105	525 ±110	3
Testis	Non-treated	22	570 ±122	546 ±116	-24

*$P < 0.05$ Treated vs non-treated.

observed only in the kidney. In this tissue, the quantity of protein was significantly less ($P < 0.05$) in the kidney homogenates prepared from treated rats when compared to untreated rats. This is not sur-

prising since glucocorticoids are known to enhance protein degradation in extra hepatic tissue for the gluconeogenic process. There was no significant change between preincubation and postincubation protein levels in any tissue.

The effect of corticosterone treatment on glucose and glycogen levels in the different tissues and the changes that occurred during incubation were made in order to obtain some idea of substrate utilization. The results of these studies are shown in Tables II and IV.

TABLE III

AVERAGE GLYCOGEN (MG/100 G PROTEIN) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Preincubation	Postincubation	Difference
Brain	Treated	15	362 ± 272	269 ±155	- 93
Brain	Non-treated	15	288 ± 212	177 ±114	-111
Heart	Treated	14	436 ± 208	156 ±150	-291
Heart	Non-treated	15	361 ± 114	86 ± 52	-275
Diaphragm	Treated	14	305 ± 159	109 ± 98	- 96
Diaphragm	Non-treated	14	286 ± 149	121 ±107	-165
Liver	Treated	16	2200*** ±1420	241 ±224	-1959
Liver	Non-treated	14	89 ± 48	70 ± 46	- 19
Kidney	Treated	15	114 ± 63	84 ± 40	- 30
Kidney	Non-treated	14	88 ± 33	59 ± 36	- 29
Testis	Treated	14	76 ± 37	79 ± 41	3
Testis	Non-treated	14	69 ± 37	59 ± 33	- 10

*P < 0.05 **P < 0.001 ***P < 0.001 Treated vs Non-treated

Treatment produced a highly significant (P < 0.001) glycogen increase in

the liver (Table III). Although there was no significant glycogen increase in any other tissue, a numerical increase in glycogen levels was observed in all tissues of treated rats. A rapid breakdown of glycogen during incubation was observed in the brain, heart, diaphragm, and liver of both control and treated rats. A high standard deviation occurred in all tissues; however, this was expected since tissue glycogen levels are known to vary considerably in the rat.

The effects of treatment on tissue glucose levels and on glucose changes during incubation are shown in Table IV. Glucose levels were

TABLE IV

AVERAGE GLUCOSE (MG/100 G PROTEIN) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES EROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Preincubation	Postincubation	Difference
Brain	Treated	20	41 ± 25	28 ± 20	-13
Brain	Non-treated	22	54 ± 30	34 ± 29	-20
Heart	Treated	22	164 ± 81	183 ± 118	19
Heart	Non-treated	20	165 ± 91	167 ± 106	2
Diaphragm	Treated	20	157 ±108	193 ± 130	36
Diaphragm	Non-treated	19	143 ± 94	170 ± 103	27
Liver	Treated	21	784* ±318	2690 ±1228	1906*
Liver	Non-treated	20	361 ±184	745 ± 264	384*
Kidney	Treated	21	341 ±310	569 ± 280	228*
Kidney	Non-treated	20	329 ±171	593 ± 270	264*
Testis	Treated	14	30 ± 22	24 ± 21	-6
Testis	Non-treated	14	45 ± 53	40 ± 45	-5

*$P < 0.001$ Treated vs non-treated and preincubation vs postincubation

significantly higher ($P < 0.001$) in the livers of treated rats than in the livers of non-treated rats. Treatment produced no change in the glucose level in other tissues. During incubation of liver and kidney homogenates there was a highly significant glucose increase. The origin of this glucose was the breakdown of glycogen as indicated in Table III.

A further summary of corticosterone treatment effects on tissue carbohydrates is given in Table V. Since glycogen levels are expressed

TABLE V

AVERAGE TOTAL CARBOHYDRATE (MG/100 G PROTEIN) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Preincubation	Postincubation	Difference
Brain	Treated		403	297	-106
Brain	Non-treated		342	211	-131
Heart	Treated		600	328	-272
Heart	Non-treated		526	253	-273
Diaphragm	Treated		462	302	-160
Diaphragm	Non-treated		429	291	-138
Liver	Treated		2984	2931	- 53
Liver	Non-treated		450	815	365
Kidney	Treated		455	682	227
Kidney	Non-treated		417	652	235
Testis	Treated		106	103	- 3
Testis	Non-treated		114	99	- 15

as mg of glucose, the sum of the glycogen levels and glucose levels are given in this table. It can be seen that in almost all cases a dis-

appearance of total carbohydrate occurred during incubation. The carbohydrate decrease does not appear as lactic acid (Table VI). Since no exogenous substrate was added, the carbohydrate which disappeared was likely used as substrate for tissue metabolism. Total carbohydrate disappeared more rapidly in the liver homogenates from corticosterone-treated rats than from non-treated rats.

Lactic acid values for each tissue from treated and non-treated animals, before and after incubation, are summarized in Table VI. No

TABLE VI

AVERAGE LACTIC ACID (MG/100 G PROTEIN) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Preincubation	Postincubation	Difference
Brain	Treated	21	43 ±16	37 ±12	- 6
Brain	Non-treated	21	36 ±14	30 ±14	- 6
Heart	Treated	20	69 ±24	69 ±22	0
Heart	Non-treated	20	69 ±26	74 ±24	5
Diaphragm	Treated	20	100 ±69	88 ±52	-12
Diaphragm	Non-treated	20	82 ±31	69 ±32	-13
Liver	Treated	19	6 ± 4	14 ±13	8*
Liver	Non-treated	17	11 ± 9	12 ±11	1
Kidney	Treated	21	18 ±23	24 ±24	6
Kidney	Non-treated	20	21 ±16	14 ±11	- 7
Testis	Treated	19	19 ±24	22 ±43	3
Testis	Non-treated	19	12 ± 8	12 ± 9	0

*$P < 0.05$ Preincubation vs Postincubation.

significant treatment effects were observed on lactic acid levels in

the preincubation mixtures of any tissue. A significant lactic acid increase ($P < 0.05$) occurred in treated liver homogenates during incubation; however, only very small, nonsignificant changes occurred in other tissues during incubation.

The changes in inorganic phosphate levels during incubation are shown in Table VII. Since the homogenates were buffered in phosphate

TABLE VII

CHANGES IN INORGANIC PHOSPHATE (MICROMOLES/G PROTEIN) IN BRAIN, HEART, DIAPHRAGM, LIVER, KIDNEY, AND TESTIS HOMOGENATES FROM FASTED, ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND FASTED, ADRENALECTOMIZED, NON-TREATED RATS. TREATED RATS RECEIVED 5.0 MG OF CORTICOSTERONE IM 5 HOURS BEFORE SACRIFICE. SAMPLES WERE TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR.

Tissue Homogenate	Type of Rats	No. of Rats	Difference between Preincubation and Postincubation
Brain	Treated	22	71.6
Brain	Non-treated	20	53.5
Heart	Treated	21	78.1
Heart	Non-treated	23	68.7
Diaphragm	Treated	20	-19.0
Diaphragm	Non-treated	17	- 2.4
Liver	Treated	23	11.7
Liver	Non-treated	25	31.2
Kidney	Treated	19	48.0
Kidney	Non-treated	21	25.7
Testis	Treated	19	26.3
Testis	Non-treated	19	45.6

buffer, initial levels of phosphate in the tissue are not given. Only the changes that occurred during incubation are reported in Table VII. These data indicate no significant treatment effect on inorganic phosphate levels in any tissue. Although not significant, the increase

of inorganic phosphate during incubation of liver homogenates prepared from corticosterone-treated rats was less than that observed for non-treated rats.

Experiment II. Mitochondrial P/O Ratios.

In order to study further the effect of glucocorticoid treatment on oxidative metabolism, a second group of experiments was designed to measure the effect of corticosterone treatment on phosphorous/oxygen utilization ratio. Animals were treated as previously indicated (p 37), and immediately after sacrifice the brain, heart, and liver were removed and homogenized in sucrose-EDTA solution. A mitochondrial pellet from each tissue was isolated by differential centrifugation as previously indicated (p 39). P/O ratios were determined using succinate, malate, and malate+pyruvate as substrate. These substrates were selected because they enter the electron transport chain at different points and a steroid effect at one certain locus might have an effect on the oxidation of one substrate and not on the other.

Electron micrographs were made from sample pellets of each tissue. All pellets examined were found to contain highly concentrated intact mitochondria. The results are shown in Figure 4 and Figure 5.

The effects of corticosterone treatment on oxygen consumption, ATP formation, and P/O ratios by rat brain mitochondria are given in Table VIII. Regardless of substrate, corticosterone treatment had no significant effect on oxygen consumption or ATP formation in rat brain mitochondria. A slightly increased P/O ratio ($P < 0.2$) was observed using malate as substrate. This change was due to a slight decrease in oxygen

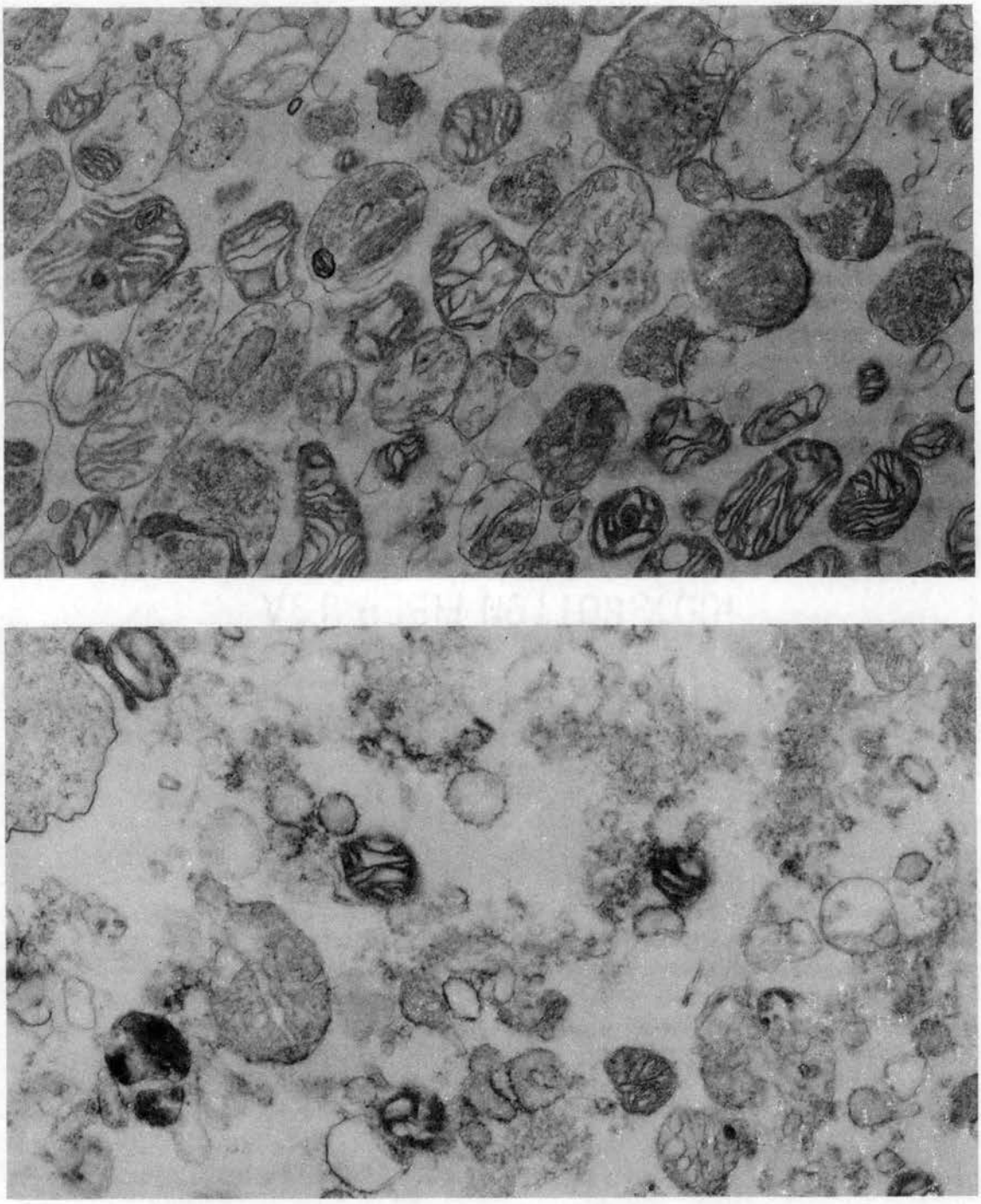


Figure 4. (X35,000) Electron Micrographs of Brain (upper) and Thymus (lower) Mitochondria Isolated from Adrenalectomized Rats. A few cell processes and synaptic vesicles are apparent in the brain mitochondria. A lymphocyte, ribosomes, and portions of the cytoplasmic matrix are present in the thymus mitochondrial pellet. Some dehydration of the mitochondrial matrix occurred. Stained in lead citrate, embedded in epoxy resin and sectioned 0.1 micron.

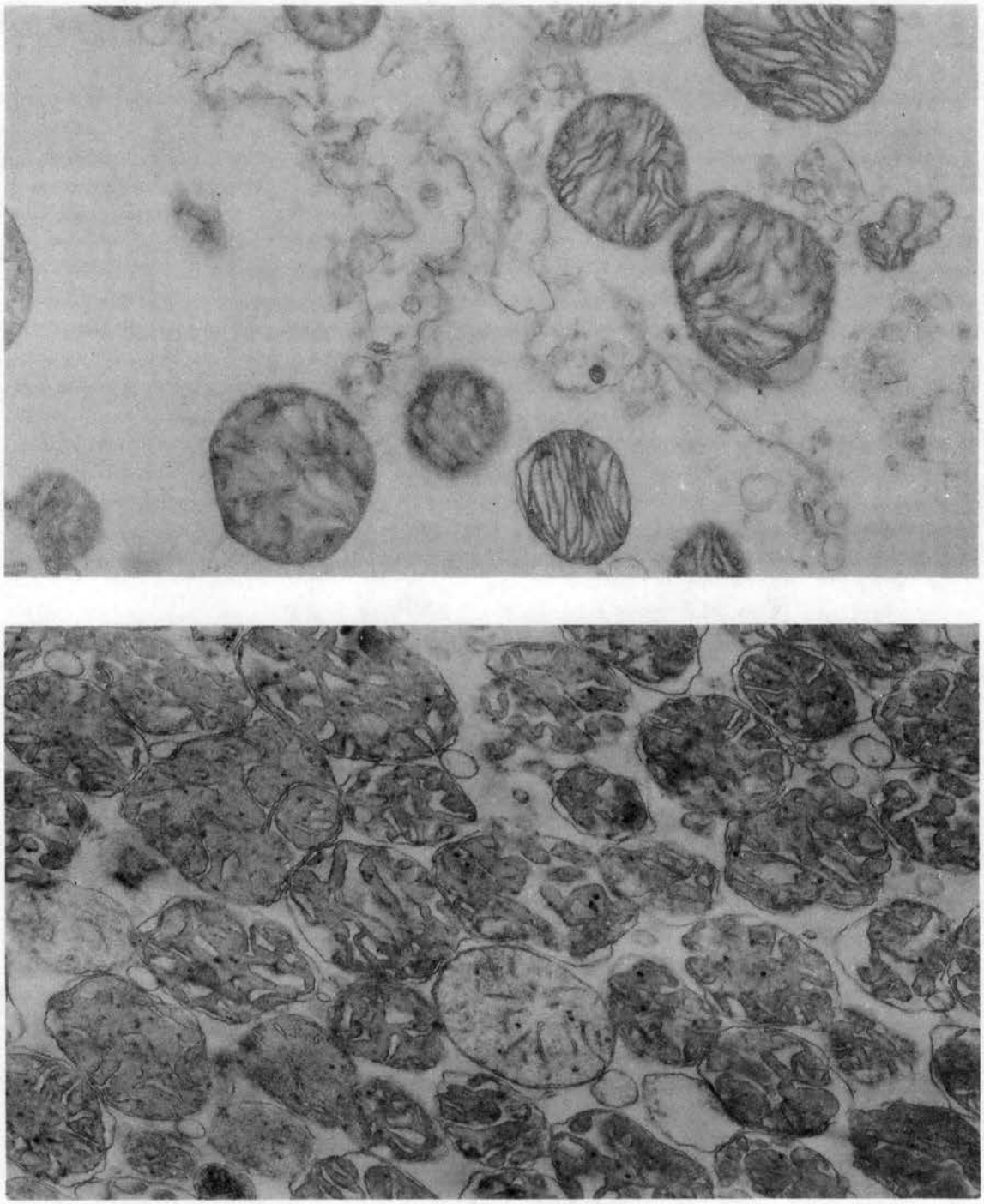


Figure 5. (X35,000) Electron Micrographs of Heart (upper) and Liver (lower) Mitochondria Isolated from Adrenalectomized Rats. Portions of the sarcoplasmic reticulum are apparent in the heart pellet. Some dehydration of the mitochondrial matrix occurred. Stained in lead citrate, embedded in epoxy resin and sectioned 0.1 micron.

TABLE VIII

OXYGEN CONSUMPTION, ATP FORMATION, AND P/O RATIOS IN BRAIN MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED, NON-TREATED CONTROL RATS.

Substrate	Type of Animals	No. of Animals	O_2** Uptake	ATP*** Formed	P:O
Succinate	Treated	10	60.5 ±28.0	69.8 ±29	1.17 ±0.08
Succinate	Non-treated	10	64.4 ±19.9	76.3 ±29	1.18 ±0.25
Malate	Treated	12	22.2 ± 4.3	32.9 ±85	1.53*±0.5
Malate	Non-treated	9	24.6 ± 3.9	29.6 ± 7.5	1.24 ±0.41
Malate+ Pyruvate	Treated	6	38.7 ± 4.3	88.5 ±17.8	2.29 ±0.45
Malate+ Pyruvate	Non-treated	6	36.9 ± 8.5	87.2 ±24	2.37 ±0.49

*$P < 0.2$ Treated vs Non-treated.
**Microatoms/g. mitochondrial protein/min.
***Micromoles of inorganic phosphate disappearing/g of mitochondrial protein/min.

consumption and a slight increase in ATP production.

The effects of corticosterone treatment on oxygen uptake, ATP formation, and P/O ratios by mitochondria isolated from the rat heart are reported in Table IX. There was no treatment effect on oxygen uptake, ATP formation, or P/O ratios using succinate or malate+pyruvate as substrates. When malate was used as substrate, corticosterone treatment caused a decreased oxygen uptake ($P < 0.1$) and an increased P/O ratio ($P < 0.1$).

Corticosterone treatment had a more profound effect on liver mitochondria than on mitochondria isolated from other tissues; however, this is not surprising in view of the well known fact that the liver is a target organ of the glucocorticoids. The effects of corticosterone

TABLE IX

OXYGEN CONSUMPTION, ATP FORMATION, AND P/O RATIOS IN HEART MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED, NON-TREATED CONTROL RATS.

Substrate	Type of Animals	No. of Animals	O_2** Uptake	ATP*** Formed	P:O
Succinate	Treated	7	129.3 ±26	64.3 ±27.8	0.5 ±0.18
Succinate	Non-treated	8	116.1 ±28.9	67.4 ±30.6	0.57 ±0.2
Malate	Treated	9	25.8*± 5.4	59.7 ±22.3	2.25*±1.0
Malate	Non-treated	12	33.2 ±10.7	58.1 ±29.6	1.72 ±0.5
Malate+ Pyruvate	Treated	6	59.9 ±13.1	126.1 ±49.3	2.06 ±0.55
Malate+ Pyruvate	Non-treated	5	53.16±24.0	119.8 ±13.74	2.14 ±0.51

* $P < 0.1$ Treated vs Non-treated.
** Microatoms/g. mitochondrial protein/min.
*** Micromoles of inorganic phosphate disappearing/g of mitochondrial protein/min.

treatment on liver mitochondrial oxidative metabolism is given in Table X. A significant treatment effect was found on both the quantity of ATP formed and the P/O ratios in liver mitochondria oxidizing malate ($P < 0.01$) and malate+pyruvate ($P < 0.05$) as substrate. Corticosterone treatment increased ATP formation and increased the P/O ratios in mitochondria oxidizing either malate or malate+pyruvate. No treatment effect was observed when succinate was used as the substrate.

Experiment III. Subcellular Distribution of (^{3}H) Corticosterone.

The third experiment was designed to localize (^{3}H) corticosterone within the cells of the brain, heart, thymus, and liver. Thirty minutes after an IV injection of 10 micrograms of corticosterone and 20 micro-

TABLE X

OXYGEN CONSUMPTION, ATP FORMATION, AND P/O RATIOS IN LIVER MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED, CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED, NON-TREATED CONTROL RATS.

Substrate	Type of Animals	No. of Animals	O_2** Uptake	ATP*** Formed	P:O
Succinate	Treated	9	75.8 ±22.8	74.9 ±27.4	0.98 ±0.13
Succinate	Non-treated	9	68.5 ±18.2	64.2 ±20.6	0.95 ±0.23
Malate	Treated	14	19.07± 3.9	36.2*± 9.95	1.91*±0.41
Malate	Non-treated	14	19.23± 4.5	26.9 ±11.9	1.33 ±0.4
Malate+ Pyruvate	Treated	6	25.18± 3.3	52.1 ±12.2	2.04*±0.3
Malate+ Pyruvate	Non-treated	6	22.3 ± 2.4	32.3 ±13.1	1.47 ±0.5

* $P < 0.05$, ** $P < 0.01$ Treated vs Non-treated.
** Microatoms/g mitochondrial protein/min.
*** Micromoles of inorganic phosphate disappearing/g of mitochondrial protein/min.

curies of (^3H) corticosterone, these organs were removed, homogenized, and fractionated into nuclei (fraction 1), mitochondria (fraction 2), microsomes (fraction 3), and supernatant (fraction 4). The disintegrations per minute of (^3H) corticosterone in each subcellular fraction expressed as percent of total disintegrations per minute contained in the homogenate are given in Table XI and shown graphically in Figure 6. In each tissue fraction 4 contained significantly more activity than fraction 1, 2 or 3. This difference was highly significant in all cases except the liver. In the liver, there was a highly significant difference between the supernatant and the mitochondrial fractions, and a significant difference ($P < 0.02$) between the supernatant and the other fractions. Fraction 1 of the brain, heart and thymus contained

significantly more activity than fraction 3. Fraction 1 of each tissue contained significantly more activity than fraction 2.

TABLE XI

MEAN SUBCELLULAR DISTRIBUTION OF (^{3}H) CORTICOSTERONE IN RAT BRAIN, THYMUS, HEART, AND LIVER 30 MINUTES AFTER AN IV INJECTION OF 20 MICROCURIES OF (^{3}H) CORTICOSTERONE. ACTIVITY EXPRESSED AS PERCENT OF TOTAL ACTIVITY CONTAINED IN THE HOMOGENATE.

	Brain	Thymus	Heart	Liver
Number of Rats	3	4	4	4
Nuclei*	14.4	13.9	15.2	23.8
Mitochondria	6.4	6.8	3.1	6.5
Microsomes	4.6	4.0	6.1	14.4
Supernatant	69.0	76.5	75.7	48.0
Recovery	94.4	101.1	100.1	92.7

* The possibility of cross contamination cannot be completely excluded.

A comparison of one tissue fraction with the corresponding fraction of other tissues is given in Figure 7. It was found that fraction 4 of the thymus and the heart was significantly greater ($P < 0.05$) than fraction 4 of the liver. A difference ($P < 0.1$) was observed between fraction 4 of the brain and fraction 4 of the liver. Fraction 3 of the liver was significantly ($P < 0.05$) greater than fraction 3 of the brain, thymus, and heart. Fraction 2 of the liver was significantly greater than fraction 2 of the heart.

The tissue/blood ratio was determined by collecting one ml of blood by cardiac puncture just before sacrificing the rat. The specific gravity of whole blood and the disintegrations per minute per ml of blood were used in order to calculate the disintegrations per gram of blood. The wet weight of each tissue sample was determined and the disinte-

grations per gram wet weight of each tissue were calculated. From this, the tissue/blood ratio was calculated. It represents the disintegrations per minute per gram wet tissue weight compared to the disintegrations per minute per gram of blood. The average results of these determinations are shown in Figure 7. The tissue/blood ratios are brain 0.57, thymus 0.42, heart 0.59, and liver 1.51.

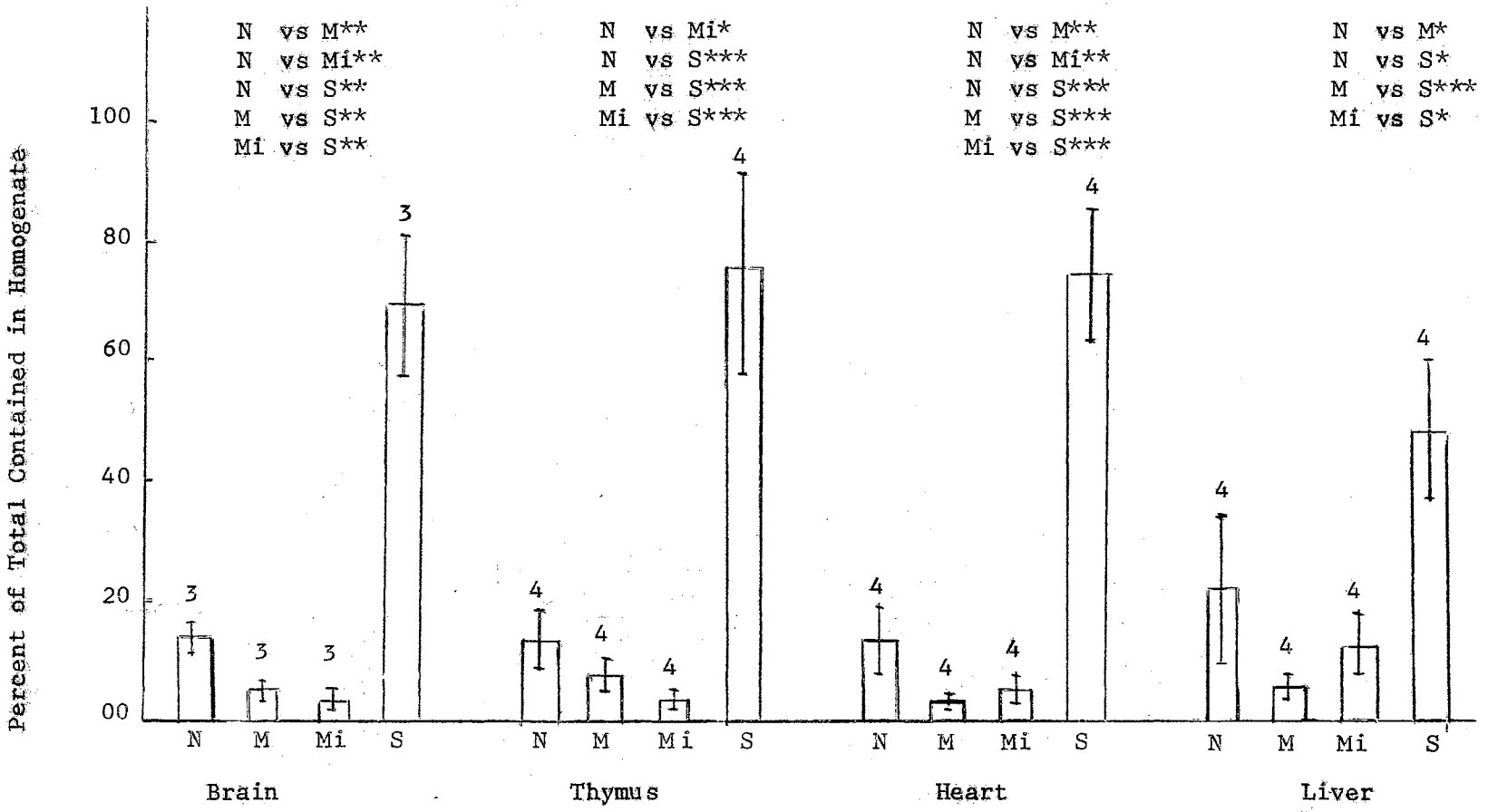


Figure 6. Uptake of (^{3}H) Corticosterone by Various Cell Fractions. Activity expressed in percent of the total (^{3}H) corticosterone in the initial homogenate. Thirty minutes after IV injection of (^{3}H) corticosterone the tissues were removed, homogenized, fractionated, and extracted. Standard deviations are represented by the vertical lines. The number of rats contributing to each value is given above the standard deviation. Symbols used are nuclei (N), mitochondria (M), microsomes (Mi), and supernatant (S).

* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

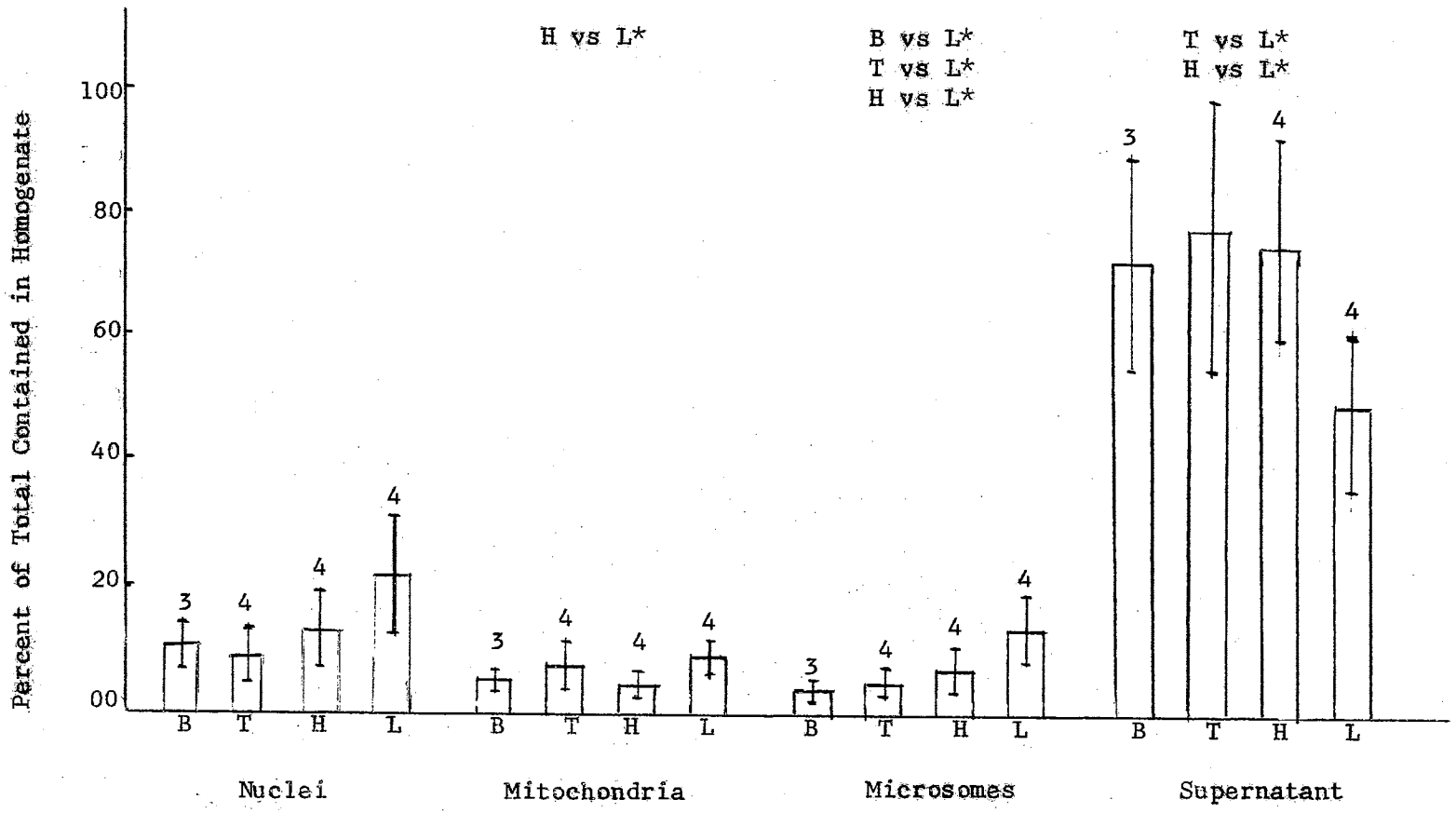


Figure 7. A Comparison of the (^{3}H) Corticosterone Uptake by One Tissue Fraction with the Corresponding Fraction of Other Tissues. Activity in each fraction is expressed as percent of the total (^{3}H) corticosterone in the initial homogenate. Thirty minutes after IV injection of (^{3}H) corticosterone the tissues were removed, homogenized, fractionated, and extracted. Standard deviations are represented by the vertical lines. The number of rats contributing to each value is given above the standard deviation. Symbols used are brain (B), thymus (T), heart (H), and liver (L).

* $P < 0.05$

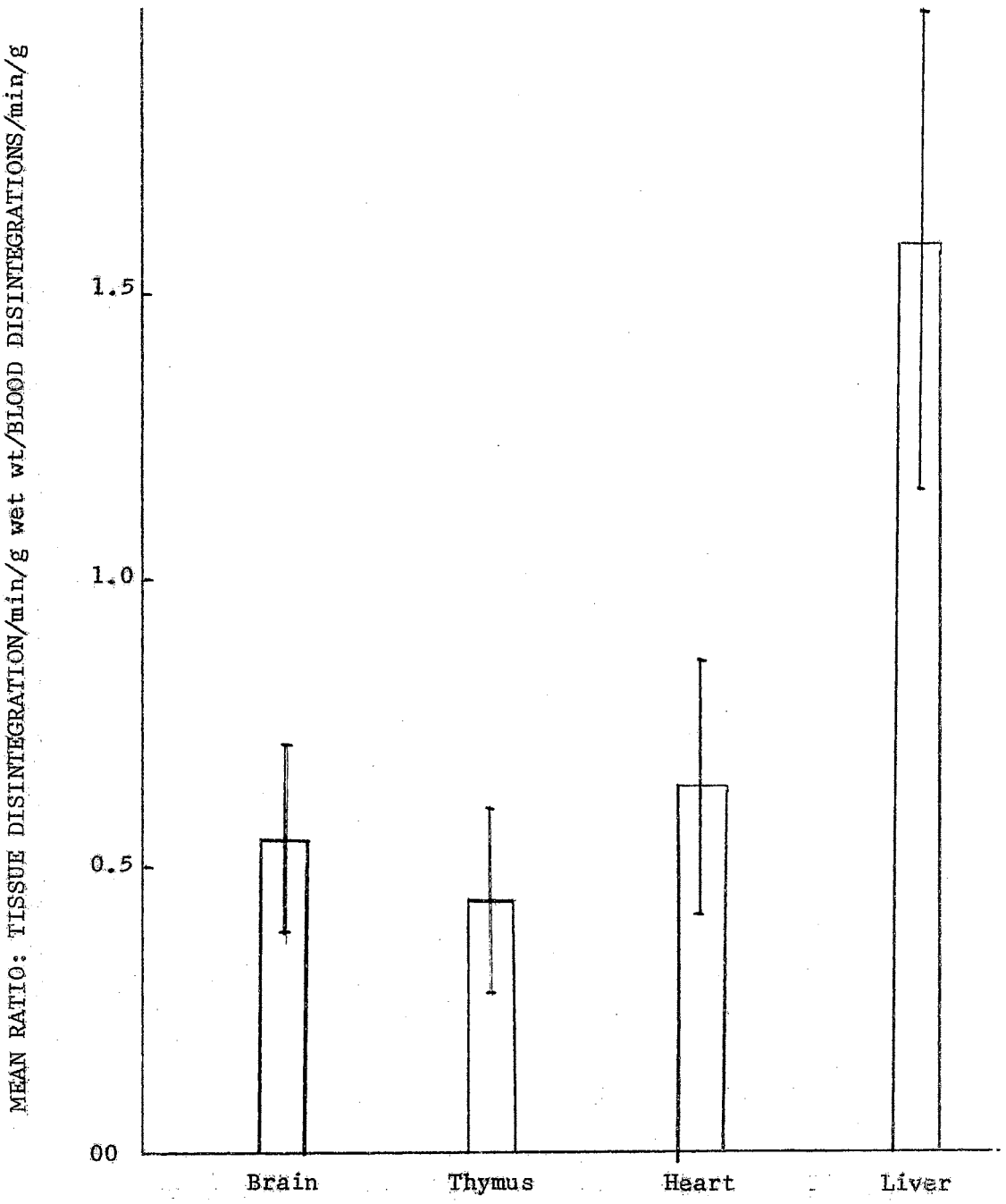


Figure 8. Mean Ratio of Radioactivity (dpm/min/g wet wt.) Between the Various Tissues and the Blood. Tissues were removed 30 minutes after intravenous injection of 20 microcuries of (^{3}H) corticosterone.

CHAPTER V

DISCUSSION

Experiment I. Biochemical Changes in Tissues

The fact that acute corticosterone treatment increased oxygen uptake by liver homogenates is indicated in Table I and Figure 2. No significant effect was observed on homogenates prepared from the brain, heart, diaphragm, kidney, or testis. A trend toward a decreased oxygen uptake by heart homogenates prepared from corticosterone-treated rats was observed; however, it was not found to be significant. The increased oxygen uptake by glucocorticoid-treated liver homogenates reported here and by others (34, 35) may be necessary for the enhanced oxidative phosphorylation observed by other investigators. Adrenalectomy is known to decrease oxidative phosphorylation (72) and ATP levels within the rat liver (25). Treatment with glucocorticoids enhances oxidative phosphorylation and ATP levels (25). There is ample evidence to indicate that a decreased glucose utilization occurs after glucocorticoid treatment in many extra hepatic tissues (69, 82, 83, 84, 85, 86, 91, 95). It is possible that the decreased glucose uptake by extra hepatic tissues might be accompanied by a decreased oxygen uptake by these tissues; however, the results reported here do not indicate any significant decrease in oxygen uptake by brain, heart, diaphragm, or kidney tissues of treated rats.

The increased oxygen uptake by liver homogenates of corticosterone-treated rats reported in Table I may be due to alterations in metabolic pools within the cell or mitochondrial alterations that affect oxidative metabolism. The results of studies on mitochondrial oxidation of various substrates support the hypothesis of altered mitochondrial oxidation rates (Tables VIII, IX and X). These data, although not significant, indicate that corticosterone treatment may decrease the oxidation rate of NAD-dependent substrate and increase the rate of succinate oxidation. The increased rate of oxygen uptake may be due to altered mitochondrial conditions which permit increased oxidation of succinate.

The results reported here agree with those reported by Goetsch and McDonald (35) and Geurkink (32) on the effect of acute injections of prednisolone and hydrocortisone. They found that acute treatment with these hormones resulted in increased oxygen uptake in liver homogenates. Chronic treatment resulted in a decreased oxygen uptake. The above investigators reported a much greater effect than reported here; however, prednisolone and hydrocortisone are known to have a much higher glucocorticoid activity than corticosterone as indicated by greater liver glycogen deposition. Additional work by Goetsch (34) has shown that prednisolone treatment stimulated oxygen uptake by homogenates prepared from the liver of goats. The results of this experiment provides additional evidence for an acute effect of glucocorticoids on oxygen uptake in rat liver.

Gordan et al. (36) reported that glucocorticoids in vitro depressed oxygen consumption by the rat brain. The results reported in

Figure 1 do not indicate such an effect on brain tissue taken from corticosterone-treated rats. These differences are not alarming since pharmacological amounts of glucocorticoids were used in the in vitro studies; therefore, the results may represent nonphysiological conditions and, in fact, they may not even be related to in vivo effects. Roberts and Keller (97) found that cortisone treatment produced enhanced oxygen uptake in the posterior hypothalamus, whereas cortisone treatment reduced oxygen uptake in the anterior pituitary. The results reported in Table I and Figure 1 do not indicate any significant effect of glucocorticoid treatment on homogenates prepared from the entire brain; however, a trend was observed. The results reported herein represent the effects on the entire brain rather than on a particular part. Additional studies on certain portions of the brain might reveal local effects.

The results reported here do not indicate any effect of acute glucocorticoid treatment on oxygen uptake by kidney or testis. Ewing and Nobel (22) found that stress for 5 days produced an increase in oxygen uptake by rabbit testis and kidney slices in intact animals. There was no significant increase by day 3 and by day 10 a decrease was observed. Stressful conditions result in a variety of responses by an organism to compensate for alterations that occur in the external or internal environment. Selye (105) has shown that glucocorticoids and epinephrine are released in greater amounts by the adrenal cortex and the adrenal medulla during stress. Five hours after treatment, results reported here do not indicate any effect of corticosterone treatment on oxygen uptake by kidney or testis homogenates (Figure 3).

Continued treatment for 5 days may have produced an increased oxygen uptake by kidney and testis similar to that observed by Ewing and Nobel (22); however, it is known that stress causes an increased release of epinephrine as well as glucocorticoids. The increased oxygen uptake observed by Ewing and Nobel (22) may have been due to the effect of epinephrine rather than an effect of glucocorticoids. There are indications that acute injections of glucocorticoids stimulate an initial rise in oxygen uptake (32, 34, 35), whereas chronic treatment results in decreased oxygen uptake by liver tissue (22, 35, 106).

The data reported herein indicate that only in the kidney was there any measureable effect of corticoid treatment on protein content of tissues. There was no observed effect of treatment on protein changes during incubation in any tissue. It is known that glucocorticoids stimulate catabolism in extrahepatic tissue and anabolism in hepatic tissue. These effects are not apparent after a single treatment. If anabolic or catabolic effects occurred in tissues other than the kidney, the turn over rate was not sufficient to be detected by the procedures used in this study. There is ample evidence for de novo enzyme synthesis in hepatic tissue after glucocorticoid treatment (46, 48, 67, 79, 99, 107, 122). The increased enzyme concentrations have been detected by increased enzyme activity, immunochemical titrations, and radioactive precursor incorporation into enzyme protein. These procedures measure much smaller changes than the total protein determination procedure used in this study.

Clark and Pesch (11) reported that chronically administered corticosterone increased protein content per kg. wet liver weight and

relative weight of the rat liver. The results of this experiment did not indicate any increase in liver protein based upon dry weight (Table II). The explanation of these different observations probably lies in the length of treatment. Data reported here are the results of a single injection of corticosterone, whereas data reported by Clark and Pesch (11) are the results of chronically treated animals.

The glycogen levels in all tissues taken from corticosterone-treated animals appeared higher than the glycogen levels in tissues taken from non-treated animals. The increased glycogen level was highly significant in the liver of treated animals when it was compared with the liver glycogen in control animals (Table III). Increased glycogen levels in the liver of glucocorticoid-treated animals was first reported by Long et al. (74, 75) as early as 1940. It was suggested that the source of this new glycogen was the degradation of protein.

It is likely that the small glycogen increase observed in the extrahepatic tissues after corticosterone treatment resulted from increased blood glucose levels and decreased rates of glucose utilization by these tissue. Corticosterone may have reduced the rate of glycogen breakdown and maintained glycogen reserves in extrahepatic tissues.

In all tissues of both treated and non-treated animals the difference between preincubation and postincubation indicated glycogen breakdown. In most cases the glycogen breakdown during incubation can be accounted for as increased glucose formed during incubation (Table III and Table IV). These data agree with those reported by Goetsch and McDonald (35) and Geurkink (32).

It is apparent that some glucose is unaccounted for especially in

the brain and the heart tissue. Glucose is the principal substrate for the brain; therefore, it is likely that the lost glucose may be accounted for over normal metabolic pathways.

Heart glycogen disappeared rapidly during incubation; however, there was only a small increase in glucose or lactic acid during incubation (Table IV and Table VI). It is possible that some of the glycogen which was broken down resulted in lactate formation. The lactate could have then been used as substrate by the heart; thus lactate would show very little change during incubation.

The glucose levels in the liver of corticosterone-treated animals was significantly higher than those in the liver of non-treated animals (Table IV). This is likely due to increased gluconeogenesis within the hepatic tissue.

The increased glucose levels in the kidney homogenates after incubation cannot be completely due to the breakdown of glycogen since the total carbohydrate after incubation was greater than the total carbohydrate before incubation (Table V). Undoubtedly some gluconeogenesis occurred within the kidney homogenate during incubation. There was no difference between treated and non-treated animals, with respect to kidney glucose changes during incubation. Both glycogen and glucose levels in the testis are very low (Table III and Table VI). These findings are supported by other workers who have found low glycogen levels in testis tissue (78).

A further summary of corticosterone treatment effects on tissue carbohydrates is shown in Table V. Since glycogen levels are expressed as mg of glucose, the sum of the glycogen levels and glucose levels

are given. It can be seen that in almost all cases a disappearance of total carbohydrate occurred during incubation.

The disappearance of total carbohydrate did not appear as lactic acid (Table VI). Since no exogenous substrate was added, the carbohydrate was likely used as substrate for the metabolism in the different tissues.

Total carbohydrate disappeared more rapidly in the liver homogenate of corticosterone-treated animals than in that from non-treated animals (Table V). Part of the carbohydrate loss appeared as lactic acid (Table VI). The increased respiration rate of liver homogenates from treated animals also required additional substrate and this may account for at least part of the carbohydrate loss in the liver homogenates of treated animals. These data do not agree with those reported by Goetsch and McDonald (35), and Geurkink (32); however, differences in techniques used for the analysis of glucose may account for the different observations. The results reported here indicate that the enhanced respiration and carbohydrate utilization are related. It is possible that the enhanced respiratory rate in liver homogenates prepared from rats receiving an acute injection of glucocorticoids may be due to the increased substrate pools present in the cell; however, Goetsch and McDonald (35) provided evidence that the mere presence of additional glucose which is present in the livers from corticosterone-treated rats (Table III and Table IV) does not, in itself, stimulate respiration.

Corticosterone treatment produced no effect on lactic acid levels in the preincubation mixtures of any tissue which was studied. A

significant increase of lactic acid occurred during incubation of treated liver homogenates. This accompanied a rapid decrease of total carbohydrate. Similar results were reported by Goetsch and McDonald (35) after treatment with prednisolone. They also reported a similar increase in lactic acid for control animals, an observation not made in this experiment. This discrepancy may be due to the fact that normal animals were used in their work and adrenalectomized animals were used in the experiment reported here. The presence of small amounts of endogenous hormone in normal animals may have prevented the observation of this difference.

The increased lactic acid production during incubation of liver homogenates from treated animals indicates that corticosterone treatment enhances the rate of aerobic glycolysis. There are increased amounts of glucose and glycogen in the liver (Table III and Table IV) and, according to the "Crabtree effect", this should enhance glycolysis and reduce respiration (14). These data indicate not only an increased rate of glycolysis (Table VI) but also an increased rate of respiration in liver homogenates (Table I). It is possible that glucocorticoids enhanced lactate formation as well as increasing respiration. The marked increase in glucose levels in the liver homogenates of treated animals (Table IV) may have enhanced glycolysis (14). Simultaneously induced effects at the mitochondrion or altered levels of other metabolites or cofactors may have enhanced respiration. The data reported in Table X indicate that the steroid effect on liver mitochondria metabolizing malate did not result in an increased respiration but rather an enhanced phosphorylation. It is possible that

mitochondrial alterations resulted in enhanced oxidation of substrates other than those requiring NAD-dependent enzymes for oxidation. Although it is not statistically significant, there is some evidence (Tables IX and X) that treatment enhances the oxidation of succinate by liver and heart mitochondria, whereas treatment decreased the rate of malate oxidation by heart mitochondria (Table IX). In view of the results observed here it seems possible that corticosterone treatment enhances both glycolysis and respiration. Glycolysis may have been enhanced due to the large concentration of glucose in the liver homogenates of treated animals. Respiration may have been increased due to mitochondrial alterations that enhanced oxidation of substrates not requiring NAD-dependent enzymes for oxidation.

Another possibility for the increased lactate formed during incubation may be the deamination or transamination of certain amino acids which produce pyruvate. Reduction of pyruvate by NADH to lactate may have occurred. This would require a source of NADH which could be supplied by the oxidation of glyceraldehyde-3-phosphate. It is also possible that NADH was released more readily from the mitochondria of corticosterone treated animals. Gallagher (30) found that hydrocortisone destroyed the selective semipermeability of mitochondrial membranes and increased the movement of NAD across the membrane. Corticosterone treatment may destroy the selective semipermeability of the membrane and loose NAD and NADH which would be available for the oxidation of glyceraldehyde-3-phosphate and the reduction of pyruvate to lactate.

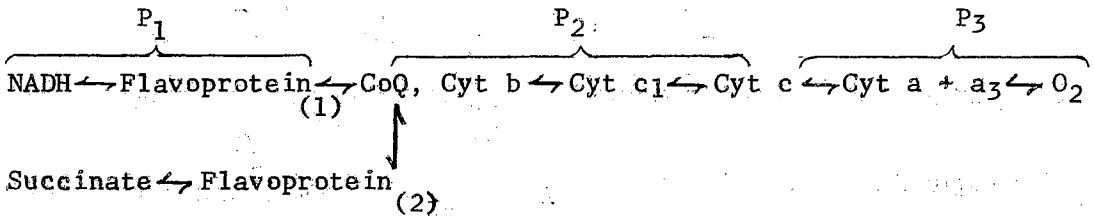
Treatment produced no significant effect on inorganic phosphate levels during incubation of any tissue homogenate. Apparently a break-

down of ATP, presumably by ATPase action, occurred at a more rapid rate than ATP synthesis in all tissue homogenates except the diaphragm. In the diaphragm indications are that synthesis occurred at a greater rate than breakdown since inorganic phosphate decreased during incubation. A rupturing of cells during homogenization may release large amounts of ATPase enzyme; therefore, any new ATP formed or present may have been cleaved by ATPase action releasing inorganic phosphate.

Goetsch and McDonald (35) reported no increase of inorganic phosphate during incubation of liver homogenates prepared from prednisolone; however, a highly significant rise occurred in control rats. They suggested that the glucocorticoids in some way enhance phosphorylation. Data reported in Table VII support their suggestion. The fact that prednisolone was used in their experiment, and its glucocorticoid actions are many times greater than corticosterone, used in this experiment, may account for the greater efficiency of phosphorylation observed in their work.

Experiment II. Mitochondrial P/O Ratios.

In order to study further the effect of glucocorticoid treatment on oxidative metabolism, P/O ratios were determined in mitochondria isolated from the liver, heart, and brain. Succinate, malate, or malate+pyruvate were used separately as substrates for determining P/O ratios. These substrates were selected because they are metabolized differently by the mitochondrion. A steroid effect at one certain locus might have an effect on the oxidation of one substrate and not on the other. This may be indicated by the following schema of electron transport (21).



A steroid effect at some point along the chain near the formation of the first high energy phosphate (P_1) would have an effect on the P/O ratios of NAD-dependent substrate, whereas no effect on the P/O ratios should be observed using succinate as substrate; therefore, the objective of this experiment was to determine if corticosterone treatment had any effect on the oxidation of NAD-dependent substrate or succinate.

Many investigators have reported effects of glucocorticoids on mitochondrial oxidation (29, 31, 36, 57, 82, 106). Whether these are primary or secondary effects remain to elucidated. There has been suggestive evidence that the glucocorticoids may have an effect near the NADH Flavoprotein portion of the electron transport chain. This was first suggested by Gordan et al. (36) after their observation that methylene blue did not reverse the steroid-induced inhibition of oxygen consumption in rat brain tissue. This indicated a site of action preceeding the flavoproteins since methylene blue can act as a carrier between dehydrogenases and oxygen. Gallagher (31) suggested that cortisone destroyed the selective semipermeability of mitochondrial membranes and a loss of NAD occurred. Jensen and Neuhard (57) found that steroids are concentrated in electron transport particles in vitro. They also reported that corticosterone had an amytal-like effect upon oxidation of NADH by heart sarcosome fragments. Bojesen and Jensen (9) reported that steroids had no effect in vitro on oxidation of NAD-

dependent substrates in intact heart sarcosomes when measured by conventional Warburg monometry; however, if a Clark electrode were used to measure oxygen consumption for a few minutes an inhibitory effect on oxygen consumption was observed. This inhibition was observed on both NADH and succinate oxidation.

There was no significant effect of corticosterone treatment on brain mitochondrial metabolism. There was a very slight trend toward an increased P/O ratio ($P < 0.2$) in brain mitochondria from treated rats using malate as substrate. This slight change was apparently due to a slightly decreased oxygen consumption and a very slightly increased ATP production. Hartman (41) has suggested that cortisone affects the selective permeability of brain mitochondria and it may help regulate metabolism in nerve tissue. The data reported here do not clearly support this suggestion.

No treatment effect was observed on oxygen uptake, ATP formation, or P/O ratios on heart mitochondria using succinate or malate+pyruvate as substrate. A slight effect on oxygen uptake and P/O ratios was observed using malate as substrate. Treatment caused a decrease in oxygen uptake ($P < 0.1$). Results reported here agree with those reported by Jensen and Neuhard (57) that steroids, added *in vitro* to electron transport particles oxidizing NADH decrease the rate of oxidation. They also reported that corticosterone had an amytal-like effect upon the oxidation of NADH by heart sarcosome fragments. The results reported here do not agree with those reported by Bojeson and Jensen (9) that steroids *in vitro* had no effect on oxidation by NAD-dependent substrates in intact heart sarcosomes when measured by con-

ventional Warburg monometry. The seeming difference in the data reported here and those reported by Bojesen and Jensen (9) may represent effects *in vitro* that are different from effects *in vivo*.

Corticosterone treatment had a more profound effect on liver mitochondria than on mitochondria isolated from other tissues. This is not surprising in view of the well known fact that the liver is a target organ of the glucocorticoids. A significant treatment effect was found upon the quantity of ATP formed and the P/O ratios in liver mitochondria oxidizing both malate and malate+pyruvate as substrate. Corticosterone treatment increased ATP formation and the P/O ratios in mitochondria oxidizing either malate or malate+pyruvate. No treatment effect was observed with succinate as substrate.

The data reported here are somewhat difficult to compare to those reported by other investigators because of differences in the treatment of animals, the type of animals used, and the type of substrate used. In this experiment adrenalectomized animals received one injection of corticosterone 5 hours before sacrifice. Non-treated adrenalectomized animals were injected with carrier vehicle only. The substrates used are indicated in Table X. Other investigators have used normal animals chronic injections, or different substrates to study mitochondrial oxidative metabolism.

Clark and Pesch (11) found that chronically administered cortisone lowered oxygen uptake of normal rat liver mitochondria and no effect was observed on oxidative phosphorylation using α-keto glutarate as substrate. The results reported here do not agree with the above observations; however, it should be pointed out that in the work reported

here adrenalectomized animals were treated with a single injection of corticosterone 5 hours before sacrifice. Differences between acute and chronic injections have been reported by Goetsch and McDonald (35).

Glucocorticoid effects on mitochondria were first suggested by Gordan et al. (36) after showing that adrenal cortical extract or corticosterone depressed oxygen consumption by rat brain tissue and methylene blue did not reverse the steroid-induced inhibition. Gallagher (31) later reported that steroids altered the semipermeability of the mitochondrial membrane and NADH was rapidly lost from rat liver mitochondria. Jensen and Neuhard (57) reported that the addition of steroids to a suspension of electron transport particles oxidizing NADH decreased the rate of oxidation. Kerppola and Pitkanen (64) reported that cortisone reduced NADH-cytochrome c reductase activity in the livers of old rats.

Several investigators have reported an effect of glucocorticoids on P/O ratios in liver mitochondria. Kerppola (63) reported that chronic injections of cortisone into normal rats inhibited oxidative phosphorylation by liver mitochondria prepared from these animals. The experiment reported here indicated treatment enhanced oxidative phosphorylation; however, adrenalectomized animals were used and only a single injection of corticosterone was made. These data support the recent work reported by Liljeroot et al. (72) that adrenalectomy resulted in a decreased P/O ratio in rat liver mitochondria and the addition of cortisone in vitro increased P/O ratios.

The results of the experiment reported herein indicate that corticosterone treatment altered mitochondrial oxidation and ATP formation. Whether this is a primary or a secondary effect remains to be resolved.

This effect was most apparent in liver mitochondria; however, there was also evidence of an effect in heart mitochondria. This effect was most apparent on mitochondria oxidizing NAD-dependent substrate. The observed effect appeared to be due to altered rates of substrate metabolism and a tighter coupling of oxidative phosphorylation.

These data support the hypothesis that the glucocorticoids affect mitochondrial oxidation at some point near the NADH$\longleftrightarrow$flavoprotein site. The fact that corticosterone treatment had an effect on ATP formation and P/O ratios with malate as substrate but had no effect when succinate was used as the substrate indicated a glucocorticoid effect near the NADH$\longleftrightarrow$flavoprotein part of the electron transport chain.

The effect of corticosterone treatment on oxidative metabolism may be due to the association of the steroid molecule with the mitochondrial membranes or elementary particles, altering the structure slightly so that altered oxidative phosphorylation occurred. Evidence presented in Table XI and Figure 6 and by other investigators indicates that the steroid is present and weakly bound within the mitochondrion (16).

Blair et al. (7) found that submitochondrial particle contains all the fixed components of the electron transfer system. It has been suggested that this elementary particle is composed of several complexes, each complex containing certain components of the electron transport chain. Electrons from substrates may enter the chain by one of two complexes. After entry the electrons travel identical paths until they are picked up by molecular oxygen. If the electrons come from NADH, they enter at one point of the chain but if the electrons are donated by succinate, they enter at another point. It has been suggested pre-

viously that a steroid effect at some point along the chain near the formation of the first high energy phosphate (~P) would have an effect on the P/O ratios of NAD-dependent substrate, whereas no effect on the P/O ratio should be observed using succinate as substrate. The results reported here indicate that corticosterone treatment enhanced ATP formation and the P/O ratio by isolated liver and heart mitochondria oxidizing malate, whereas no significant effect was observed using succinate as substrate. It is therefore suggested that corticosterone treatment has an effect on liver and heart mitochondrial oxidation. These data indicate that the site of action may be near the NADH-flavoprotein portion of the elementary particle within the mitochondrion. This hypothesis is even more attractive in view of the fact that Hatefi et al. (42) have provided evidence that the NADH-oxidase system of beef heart mitochondria appears to be very closely associated with a respiratory control mechanism.

Experiment III. Subcellular Distribution (^{3}H) Corticosterone

This experiment was designed to localize (^{3}H) corticosterone within the cells of known target tissues as well as other tissues not recognized as target tissues of these hormones. Thirty minutes after an IV injection of 10 micrograms of corticosterone and 20 microcuries of (^{3}H) corticosterone the rats were sacrificed and the various tissues removed, homogenized, fractionated, and extracted. The liver was not perfused in situ after sacrifice to remove red blood cells since Bellamy (4) reported that perfusion made no difference to either the amount of particulate-bound corticosterone or the distribution pattern.

The disintegrations per minute of (^{3}H) corticosterone in each fraction were determined. The tissue/blood ratio was determined and it was found that only the liver was capable of concentrating (^{3}H) corticosterone from the blood. The tissue/blood ratio may be about 15 percent higher than tissue/plasma ratios. Studies on the binding of corticosterone to red blood cells have not been reported; however, about 25 percent of hydrocortisone in a given volume of blood (hematocrit, 40 percent) was found in the red blood cells (10). The ability of the liver to concentrate corticosterone is not surprising in view of the fact that the liver is a target organ of the glucocorticoids and it is also the major site of conjugation and degradation of the steroids into normal excretory forms. Since cortisol enters the liver by diffusion, a favorable diffusion gradient appears to be maintained by the intracellular adsorption of free cortisol (5). The data reported in Table XI indicate that the nuclear and microsomal fractions of the liver may in some way bind corticosterone more than these fractions in other tissues and provide a favorable diffusion gradient.

Bellamy et al. (5) reported liver/plasma ratios of about 6 for (^{14}C) cortisol in rats. This is considerably higher than the 1.51 liver/blood ratio reported here. The difference may be even greater since the tissue/blood ratio is about 15 percent greater than the corresponding tissue/plasma ratio (10). Factors responsible for the observed difference may be due to extraction and purification procedures. The procedures followed in this experiment were designed to eliminate conjugated steroids and polar metabolites of corticosterone. Since these are produced in the liver, it is very likely that the

lower liver/blood ratio reported here does not include many of the metabolites that were present in the work reported by Bellamy et al. (5). In fact, these investigators reported that about 75% of the activity in the liver passed into the water phase when partitioned between an organic solvent and water. The data reported here do agree with those later reported by Bellamy (4) in which he reported a liver/-plasma ratio of 1.72.

Tissue/blood ratios of less than one are reported for tissues other than the liver in Figure 8. Apparently corticosterone was not concentrated by the brain, thymus, or heart. In fact, a barrier may be present that prevents the uptake of the hormone by these tissues. If the tissue/blood ratio for corticosterone is about 15 percent higher than the corresponding tissue/plasma ratio, the data reported here agree with those reported by Bellamy (4) in which he reported a tissue/plasma ratio of 0.38 for brain, 0.38 for skeletal muscle, and 0.30 for spleen. The low ratio for the thymus reported in Figure 8 and the low activity for spleen (4) was somewhat surprising since it is known that at least the thymus is a major target organ of glucocorticoids. It is possible that the low concentration is sufficient to initiate any direct effect this hormone might have upon the thymus. Low tissue/blood ratios for the heart and brain are reported in Figure 8 and by Bellamy (4). Again only relative low concentrations of the hormone may be necessary to initiate any direct action these hormones may have upon these tissues. At this time there is little convincing evidence that the glucocorticoids have a direct effect on the heart and brain. The tissue/blood ratio for the thymus is about the same

as for heart and brain. Therefore it is possible that concentration greater than that found in the blood are not necessary for physiological actions. This steroid may be concentrated only in organs involved in the metabolism of the steroid.

It is well known that adrenal cortex steroids enhance nerve transmission. An insufficiency of adrenal cortex steroids results in a decreased ability to support reflex activity. It has been suggested by Woodbury (129) that the defect may be in the supply of essential substrate to the nerve tissue rather than a direct effect of the hormone.

Comparing the distribution in the various subcellular fractions, it was found that in each tissue fraction 4 contained significantly more activity than fraction 1, 2, or 3. This is given in Table XI and shown graphically in Figure 7. These data indicate that most of the activity within the cells of these tissues was not firmly bound to any particulate fraction. Standard fractionation procedures were used to obtain the subcellular fractions, i.e., fresh sucrose solution was used to resuspend each pellet for the next centrifugation. All supernatant fluids were then combined for the final supernatant. This procedure enhanced the removal of most of the free unbound corticosterone from the particulate fraction leaving only corticosterone that was bound in some manner to the particulate fraction. The procedure used here differs from the "equilibrium fractionation" used by De Venuto et al. (16) for fractionation of rat liver and rat muscle tissue. Using an "equilibrium fractionation" procedure, the supernatant was used each time to resuspend the particulate fraction and no new sucrose was added. Each fraction remained in equilibrium with the initial sucrose

solution; therefore, the dissolved steroid as well as bound steroid remained in each particulate fraction.

One should not overlook the fact that binding sites may be present within the supernatant. Evidence that this is the case has been presented by Dingman and Sporn (18). They reported that 7 to 12 percent of the activity in the supernatant were nondialyzable. It should be realized that the relative volumes occupied by each of these subcellular fractions varies considerably within the cell. The volume occupied by the supernatant part of the cell is probably many times greater than the volume occupied by the nucleus, mitochondria, or microsomes; therefore, a particulate fraction may have a much higher concentration of the steroid than the supernatant, yet when a comparison is made of the percentage of total activity in the various fractions the supernatant appears to contain by far the most activity. Because of this it cannot be concluded that the activity was definitely not concentrated in some particulate fraction. If the relative volumes of the various particulate fractions were known, a more accurate estimate of relative concentrations could be made.

Table XI and Figure 6 show that fraction 1 of brain, heart, and thymus contained more activity than fraction 3. This was not the case in the liver. Fraction 1 of all tissues contained more activity than fraction 2. A comparison of fraction 4 of the different tissues is depicted in Figure 7. The percentage of activity in fraction 4 of the thymus and heart was significantly greater than the corresponding fraction of the liver. Fraction 4 of the brain also appeared to be greater than fraction 4 of the liver although the difference was not

significant ($P < 0.1$). It can be seen in Figure 6 that the subcellular distributions within the brain, heart, and thymus are similar, whereas within the liver the pattern of subcellular distribution differs from the other tissues. It is suggested that the subcellular distribution may be related to the different effects observed in different tissues.

The percent of the activity in the microsomal fraction of the liver was much greater than the activity in the microsomal fractions of the other tissues (Figure 7). These data suggest that corticosterone may have a relatively active role in the liver microsomes but not in the microsomes of the other tissues studied. Although not significant, the nuclear fraction of the liver contained more activity than the nuclear fractions of the other tissues. These differences may be related to the known different effects of glucocorticoids in different tissues of the body. The high percent of activity in the nuclear and microsomal fractions of the liver is compatible with the concept that glucocorticoids have a role at the transcription site (nuclei) and translation site (microsomes) of protein synthesis. Due to the very low activity in the microsomal fraction of the other tissues it is suggested that corticosterone may have less effect at the translation site in brain, heart, and thymus. The nuclear effect may also be less in these tissues. It is possible that certain units of DNA on the chromosomes are permanently repressed in these tissues while only temporarily repressed in the liver. Corticosterone may combine with a repressor in the liver and derepress the units of DNA so that transcription and the production of RNA can continue. The corresponding units of DNA in the other tissues may be permanently repressed so that corticosterone

cannot combine with the repressor.

Little difference was observed in the mitochondrial fractions of the different tissues. Figure 7 indicates that a significant difference occurred only between the heart and liver mitochondria. The relatively constant percentage of activity in this fraction of these tissues suggests that if there is an active site within the mitochondrion, it may be the same in all tissues. This suggestion is supported by the results obtained in Experiment II. Corticosterone appears to have some effects on mitochondria isolated from brain, heart, and liver; however, it is greater in the liver than other tissues.

Some differences exist between the data reported in Table XI, Figure 6, and Figure 7 compared to those reported by other workers; however, it should be pointed out that a great deal of difference in procedures used by various investigators may account for part of this discrepancy. De Venuto et al. (16) studied the subcellular distribution of (^{14}C) corticosterone using equilibrium fraction, a procedure not used in the studies reported here. Centrifugation speeds for fractionation, time after injection before sacrifice, amount of hormone injected, and the use of adrenalectomized animals in some experiments may all contribute to these differences. The degree of purification of the extracted steroid before counting may have caused large differences in the observed results. The data reported here do not include the more polar metabolites and conjugated steroids that passed into the water phase during the partitioning step.

Table XI shows that the average liver nuclear fraction in this study contained 23.8% of the total activity in the homogenate. This

corresponds to 13.4 reported by De Venuto et al. (16). Calculations from the figures reported by Bellamy (4) indicated that he recovered about 14.7% in the nuclear fraction of rats injected with (^{14}C) cortisol. Dingman and Sporn (18) found 7% in the nuclear fraction of rats injected with cortisol. A possible explanation for this difference may be the purity of the pellet or a difference in the labeled substance counted. Purification procedures used for the work reported here eliminated conjugated steroids and more polar metabolites of corticosterone. These were not removed in the procedures reported by the above investigators. Although it cannot be said that the activity reported here represents only (^{3}H) corticosterone, many labeled metabolites were removed by the purification procedure used.

According to the results reported here, the liver mitochondrial pellet contained 6.5% of the total activity present in the homogenate. De Venuto et al. (16) reported 26.6% in the mitochondrial pellet. Calculation from Bellamy (4) indicated about 6.6% in this fraction. Dingman and Sporn (18) reported 8% in the mitochondrial pellet. These differences are difficult to explain. Electron micrographs (Figure 4 and Figure 5) indicated a relatively pure mitochondrial pellet was extracted in this experiment. De Venuto et al. (16) reported that about 25% of the activity in their mitochondrial pellet was due to metabolites of the steroid. This, plus the fact that other fractions also contained metabolites, may account for a large part of these differences. Fractionation procedures used may also account for these differences. Equilibrium fractionation was used by De Venuto et al. (16). Standard fractionation procedures were used for the work reported here

and the work reported by Bellamy (4) and Dingman and Sporn (18). Results obtained by standard fractionation procedures agree very well and they all differ from the values reported by De Venuto et al. (16) who used equilibrium fractionation. Ulrich (113) reported that (^{14}C) cortisol enters the mitochondrion by diffusion and is leached out by washing. The equilibrium fractionation procedures used by De Venuto et al. (16) did not remove the steroid that diffused into the mitochondrion, therefore higher concentrations were reported. Apparently the (^{3}H) corticosterone is not leached out of the nuclear or microsomal fractions or, at least, it is more tightly bound than in the mitochondrion.

The microsome fraction of the liver contained 14.4% of the total activity contained in the homogenate. De Venuto (16) reported 7.8% for this fraction. The results reported by Bellamy (4) indicate about 7.3% for this fraction. Dingman and Sporn reported about 4% for this fraction. It appears here as before that differences in fractionation procedures, centrifugation speeds, extraction procedures, and purification procedures may account for the different results observed.

The data reported here indicate that in the liver about 48% of the (^{3}H) corticosterone activity in the homogenate was in the supernatant fraction. De Venuto et al. (16) reported 51.1% of the homogenate corticosterone activity present in the supernatant fraction. Bellamy (4) reported about 75% of the (^{14}C) cortisol radioactivity of the homogenate was found in the supernatant solution. Dingman and Sporn (18) reported about 80% of cortisol in the supernatant fraction. This indicates that the subcellular distribution of corticosterone may be different than cortisol in adrenalectomized rats in vivo. This

should be further investigated since it is also indicated in Table IV of the work reported by De Venuto et al. (16) and Figure I of the work reported by Bellamy (4).

A review of the data reported here and that reported by other investigators indicates that some important differences may occur in the subcellular distribution of labeled corticosterone and cortisol in rat tissues. The data reported in Figure 6 indicated a difference in the subcellular distribution of (^{3}H) corticosterone in the tissues studied. This difference is of interest because of the wide range of effects that results after glucocorticoid treatment. It is unknown if the accumulation of corticosterone or cortisol is in any way associated with a metabolic effect, or if it is dependent only upon physiochemical properties.

At the present time it seems very unlikely that the numerous effects of glucocorticoids can be the result of one site of action between the steroid molecule and a single receptor molecule in one or two target tissues. The wide distribution of the hormone within the body indicates that it may act at a number of different sites. Some of these sites may be vulnerable in some tissues and not in others. This may result in an over-all effect in one organ much different from the over-all effect in another organ. For example, glucocorticoids may have a minor effect on cell membranes, mitochondria, nuclei (transcription site for proteins synthesis), microsomes (translation site for protein synthesis), and allosteric effects upon enzymes in the soluble portion of the cell. All of these sites may be available in the liver but only one or two may be available in the thymus. This could result in completely different

over-all effects in the different organs. The hormone-gene hypothesis is a very attractive hypothesis to explain some of the glucocorticoid effects; however, some effects seem to occur that do not require enzyme-protein synthesis. It is possible that the glucocorticoids have an effect of derepression of repressed genes within cells. This may readily occur in the liver by a reaction of the hormone in some way with a gene repressor and, as a result, the gene is no longer repressed and RNA synthesis occurs. This same gene in the thymus may be permanently repressed and the hormone may be unable to derepress the gene. Under this hypothesis protein synthesis would occur in the liver but not in the thymus. This same type of reasoning could be extended to mitochondria, microsomes, and enzymes within the soluble part of the cell. This hypothesis seems more attractive in view of the fact that many investigators have reported numerous effects of glucocorticoids *in vivo* and *in vitro*. It seems reasonable that the glucocorticoids may influence not only one site in one or two target tissues, but they may act at a number of sites within the cells. The availability of these sites within the cells may vary from one tissue to another. The glucocorticoid effects in one tissue may depend upon the number of site available in that tissue and some effects may occur in all tissues of the body. The data reported here does not in any way present any clues as to the type of steroid receptors; however, these data do indicate that the steroid is present in all subcellular fractions of the tissues studied. It conceivably may be present in all body cells and exert some action in each cell.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Three experiments were designed to study effects of single injections of corticosterone on tissue metabolism in the rat and provide additional information concerning the sites of action of this hormone. The first experiment was designed to study possible metabolic effects in brain, heart, diaphragm, liver, kidney, and testis tissues. Criteria of metabolic activity investigated were oxygen uptake, lactic acid production, glycogen deposition, glucose levels, protein concentrations, and inorganic phosphate changes. The second experiment was designed to measure the P/O ratios in mitochondria isolated from the brain, heart, and liver taken from adrenalectomized-treated and adrenalectomized-control rats using succinate, malate, or malate+pyruvate as substrate. The third experiment was designed to localize (^{3}H) corticosterone within the subcellular fraction of the brain, thymus, heart, and liver 30 minutes after IV injection.

The first experiment revealed that corticosterone treatment produced an increased oxygen uptake by liver homogenates ($P < 0.01$). The increased oxygen uptake was accompanied by increased carbohydrate utilization and simultaneous increases on mitochondria ATP formation ($P < 0.05$) and P/O ratios ($P < 0.01$). Glycogen and glucose levels were increased in the livers of treated animals ($P < 0.001$). Lactate increased during in-

cubation of liver homogenates of treated animals indicating increased glycolysis as well as increased respiration.

The experiment designed to measure P/O ratios revealed that treatment significantly increased ATP formation ($P < 0.05$) and the P/O ratio ($P < 0.01$) in liver mitochondria when malate or malate+pyruvate were used as substrates. There was also evidence of a treatment induced increase in P/O ratios in brain ($P < 0.2$) and heart ($P < 0.1$) mitochondria. These data support the hypothesis that glucocorticoids affect mitochondrial oxidation at some point near the NADH$\leftrightarrow$flavoprotein site since corticosterone treatment enhanced ATP formation and P/O ratios by mitochondria oxidizing malate, whereas no significant effect was observed using succinate as substrate. Whether this is a primary or a secondary effect remains to be resolved.

The results of the third experiment indicate differences in the subcellular distribution of labeled corticosterone in the tissues studied. Only the liver was capable of concentrating (^3H) corticosterone from the blood. The steroid was present in all subcellular fractions of the tissues studied. The supernatant fraction of each tissue contained more activity than nuclei, mitochondria, or microsomes. The nuclear fraction of each tissue contained more activity than the mitochondrial fraction. Comparing one tissue fraction with the corresponding fraction of other tissues it was found that the percent of total activity in the supernatant fraction of the thymus ($P < 0.05$) and the heart ($P < 0.05$) was greater than the percent of total activity in the supernatant fraction of the liver. The microsomal fraction of the liver was greater than the microsomal fraction

of the other tissues studied ($P < 0.05$). The distribution pattern of the liver was different from that observed in the other tissues. The nuclear and microsomal fractions of the liver may in some way bind more corticosterone than the corresponding fractions in other tissues. It is unknown if the accumulation of corticosterone is in any way associated with its effects. It is suggested that the subcellular distribution may be related to the different effects observed in different tissues.

These experiments were not designed to study the types of steroid receptors which may exist at the cellular level; however, the steroid was present in the subcellular fraction of all tissues studied. It may be present in all body cells and exert some effect, but not necessarily the same effect, in each cell.

A SELECTED BIBLIOGRAPHY

(1) Barker, B. S., and W. H. Summerson. "The Colorimetric Determination of Lactic Acid in Biological Material." J. Biol. Chem. Vol. 138. (1941) 535-554.

(2) Baylazs, R. "Control of Glutamate Oxidation in Brain and Liver Mitochondrial Systems." Biochem. J. Vol. 95. (1965) 497-508.

(3) Beermann, W. "Cytological Aspects of Information Transfer in Cellular Differentiation." Am. Zoologist. Vol. 3 (1963) 23-32.

(4) Bellamy, D. "The Adsorption of Corticosteroids to Particulate Preparations of Rat Liver." Biochem. J. Vol. 87. (1963) 334-339.

(5) Bellamy, D., J. G. Phillips, I. C. Jones, and Ruth Leonard. "The Uptake of Cortisol by Rat Tissues." Biochem. J. Vol. 85. (1962) 537-545.

(6) Betheil, J., M. Feigelson and P. Fiegelson. "The Differential Effects of Glucocorticoid on Tissue and Plasma Amino Acid Levels." Biochem. et Biophys Acta. Vol. 104. (1965) 92-97.

(7) Blair, P., T. Oda, D. Green, and H. Fernandez-Moran. "Studies on the Electron Transfer System. LIV. Isolation of the Unit of Electron Transfer." Biochem. Vol. 2 (1963) 756-764.

(8) Bleecher, Melvin, "Phospholipase C and Mechanism of Action of Insulin and Cortisol on Glucose Entry into Free Adipose Cells." Biochem. and Biophys. Res. Comm. Vol. 21. (1965) 202-209.

(9) Bojesen, E. and P. K. Jensen. "In Vivo Incorporation of Corticosterone in Subcellular Particles of Heart and Kidney Cortex." in Structure and Metabolism of Corticosteroids Ed by J. R. Pasqualini and M. F. Jayle (1964) 31-41.

(10) Braunsberg, H. and V. H. James. "The Determination of Cortisol and Corticosterone in Blood: A Review." J. Clin. Endocrinol and Metab. Vol. 21. (1961) 1146-1188.

(11) Clark, J. H. and L. Pesch. "Effects of Cortisone Upon Liver

Enzymes and Protein Synthesis." J. Pharmacol. Expt. Ther. Vol. 117. (1956) 202-207.

(12) Cleland, K. W. and E. C. Slater. "Respiratory Granules of Heart Muscle." Biochem. J. Vol. 53 (1953) 547-556.

(13) Clever, U. and P. Karlson. "Induktion Von Puff-Veranderungen in Den Speicheldrusen-chromosomen Von Chironomus Tentans Durch Edyson." Experimental Cell Research. Vol. 20. (1960) 623-626.

(14) Crabtree, H. G. "Observations on the Carbohydrate Metabolism of Tumors." Biochem. J. Vol. 23. (1929) 536-545.

(15) Dahmus, M. and J. Bonner. "Increased Template Activity of Liver Chromatin, A Result of Hydrocortisone Administration." Proc. Natl. Acad. Sci. Vol. 54. (1965) 1370-1375.

(16) De Venuto, F., P. C. Kelleher and U. Westphal. "Interactions Between Corticosteroids and Fractions of Rat Liver and Muscle Cells as Determined by "Equilibrium Fractionation" and Equilibrium Dialysis." Biochimica et Biophysics Acta. Vol. 63. (1962) 434-452.

(17) De Venuto, F. and U. Westphal. "Metabolism of Cortisol by Subcellular Fractions of the Rat Liver." Biochimica et Biophysica Acta Vol. 54. (1961) 294-303.

(18) Dingman, C. W. and M. B. Sporn. "Actinomycin D and Hydrocortisone Intracellular Binding in Rat Liver." Science. Vol. 149. (1965) 1251-1254.

(19) Edelman, I. S., Rita Bogoroch, and G. A. Porter. "On the Mechanism of Action of Aldosterone on Sodium Transport: The Role of Protein Synthesis." Proc. Natl. Acad. Sci. U. S. Vol. 50. (1963) 1169-1183.

(20) Eisenstein, A. B., E. Berg, D. Goldenberg and B. Jensen. "In Vitro Effect of Adrenal Steroids on Carbohydrate Synthesis in Liver." Endocrinol. Vol. 74. (1964) 123-127.

(21) Ernster, L. "Control of Cell Metabolism at the Mitochondriol Level." Federation Proceedings. Vol. 24. (1965) 1222-1236.

(22) Ewing, L. E., D. Noble, and K. Ebner. "The Effect of Epinephrine and Competition Between Males on The in Vitro Metabolism of Rabbit Testis." Canadian J. Physiol and Pharmacol. Vol. 42. (1964) 527-532.

(23) Fain, J., R. Scow and S. Chernick. "Effects of Glucocorticoids on Metabolism of Adipose Tissue in Vitro." J. Biol. Chem. Vol. 238. (1963) 54-58.

(24) Fales, F., J. Russell, and J. Fain. "Some Applications and Limitations of Enzymic, Reduction (Somogyi) and Anthrone Methods for Estimating Sugars." Clin. Chem. Vol. 7 (1961) 389-403.

(25) Feigelson, P. and M. Feigelson. "Studies on the Mechanism of Regulation by Cortisone of the Metabolism of Liver Purine and Ribonucleic Acid." J. Biol. Chem. Vol. 238. (1963) 1073-1077.

(26) Feigelson, P. and M. Feigelson. "Studies on the Mechanism of Cortisone Action." in Actions of Hormones on Molecular Processes. Edited by G. Litwack and D. Kritchevsky. John Wiley & Sons Inc. N.Y. (1964) 218-233.

(27) Feigelson, P. and O. Greengard. "A Microsomal Iron-Porphyrin Activator of Rat Liver Tryptophan Pyrrolase." J. Biol. Chem. Vol. 236. (1961) 153-157.

(28) Feigelson, P. and O. Greengard. "Immunochemical Evidence for Increased Titers of Liver Tryptophan Pyrrolase During Substrate and Hormonal Enzyme Induction." J. Biol. Chem. Vol. 237 (1962) 3714-3717.

(29) Fortier, Claude. "Adrenohypophysis and Adrenal Cortex." Annual Review of Physiology. Vol. 24. (1962) 223-258.

(30) Gallagher, C. H. "Effect of Hydrocortisone on Mitochondrial Membrane Permeability." Nature. Vol. 182. (1958) 1315-1316.

(31) Gallagher, C. H. "The Mechanism of Action of Hydrocortisone on Mitochondrial Metabolism." Bio. Chemical J. Vol. 74. (1959) 38-43.

(32) Geurkink, J. W. "Some Effects of Glucocorticoids on Carbohydrate Metabolism in Rats." (unpub. M.S. thesis, Okla. State Univ., 1962).

(33) Glenn. E. M., Barbara Bowman, R. Bayer, and C. Meyer. "Hydrocortisone and Some of its Effects on Intermediary Metabolism." Endrocrinol. Vol. 68. (1961) 386-410.

(34) Goetsch, D. D. "Glucocorticoid Effects on Respiration and Metabolism of Goat Liver Homogenates." Am. J. Vet. Res. Vol. 24. (1963) 867-870.

(35) Goetsch, D. D. and L. E. McDonald. "Glucocorticoid Effects in Respiration and Metabolism by Rat Liver Homogenates." Am J. Physiol. Vol. 202 (1962) 343-346.

(36) Gordan, G. S., R. Bentinck and E. Eisenberg. "The Influence of Steroids on Cerebral Metabolism." Annals of the N. Y. Academy of Science. Vol. 54. (1951) 575-607.

(37) Gornall, A. B., C. J. Bardawill and M. M. David. "Determination of Serum Protein by Means of the Biuret Reaction." J. Biol. Chem. Vol. 177 (1949) 751-766.

(38) Greenman, D. L., W. Wicks, and F. Kenney. "Stimulation of Ribonucleic Acid Synthesis by Steroid Hormones II High Molecular Weight Components." J. Biol. Chem. Vol. 240 (1965) 4420-4426.

(39) Halkerston, I. D., E. Scully, M. Feinstein, and O. Hechter. "Cortisol Action in Thymus in Relation to Gene Regulation." Life Science. Vol. 4 (1965) 1473-1479.

(40) Hanngren, A., E. Hansson, S. E. Sjostrand and S. Ullberg. "Autoradiographic Distribution Studies with ^{14}C-Corticosterone and ^{14}C-Cortisol." Acta Endocrinol. Vol. 47. (1964) 95-104.

(41) Hartman, J. F. "Electron Microscope of Mitochondria in the Central Nervous System." J. Biophys. Biochem. Cytol. Vol. 2 Supplement (1956) 375-378.

(42) Hatefi, Y., P. Jurtshuk and A. G. Haavik. "Studies on the Electron Transport System XXXII. Respiratory Control in Beef Heart Mitochondria." Archives of Biochem. and Biophy. Vol. 94. (1961) 148-155.

(43) Hayano, M. and R. I. Dorfman. "Studies on the Inhibition of Various Enzymes by Steroids." Annals of the N. Y. Academy of Science. Vol. 54. (1951) 608-618.

(44) Hays, A. P. and R. Hill, Jr.. "Enzymes of Lipid Synthesis in the Liver of the Cortisone-Treated Rat. Biochimica et Biophysica Acta. Vol. 98. (1965) 646-648.

(45) Hechter, O. and I. O. Halkerston. "Effects of Steroid Hormones on Gene Regulation and Cell Metabolism." Ann. Rev. Physiol. Vol. 27. (1965) 133-162.

(46) Henning, H. V., I. Sieffert, and W. Scubert. "Cortisol Induzierter Anstieg der Pyruvatcarboxylast-aktivitat in der Rattenleber." Biochim. et Biophys. Acta. Vol. 77. (1963) 345-348.

(47) Hill, R. B., Jr. and W. Droke. "Production of Fatty Liver in the Rat by Cortisone." Proc. Soc. Exptl. Biol. Med., Vol. 114. (1963) 766-769.

(48) Hilz, H., W. Tarnowski and P. Arend. "Glucose Polymerisation and Cortisol." Biochem. Biophys. Res. Commun. Vol. 10 (1963) 492-497.

(49) Hockaday, T. D., "Rapid Action of Hydrocortisone on the Increase in Blood Glucose After Adrenaline." Nature. Vol. 203. (1964) 1-5.

(50) Hogeboom, G. "Fractionation of Cell Components of Animal Tissue." Methods in Enzymology. Vol. V. (1955) 16-19.

(51) Holt, V. C. and J. Fisler, "The Effects of Cortisol on Synthesis and Degradation of Liver Glycogen." Biochimica et Biophysica Acta. Vol. 90. (1964) 232-238.

(52) Ingle, D. J. "The Production of Glycosuria in the Normal Rat by Means of 17-Hydroxy-11-Dehydrocorticosterone." Endocrinol. Vol. 29, (1941) 649-652.

(53) Ingle, D. J., C. H. Li and H. M. Evans. "The Effect of Adrenocorticotrophic Hormone on the Urinary Excretion of Sodium, Chloride, Potassium, Nitrogen and Glucose in Normal Rats." Endocrinol. Vol. 39, (1946) 32-42.

(54) Ingle, D. J., R. Sheppard, J. Evans and M. Kuizenga. "A Comparison of Adrenal Steroid Diabetes and Pancreatic Diabetes in the Rat." Endocrinol. Vol. 37, (1945) 341-350.

(55) Jeanrenaud, B. and A. Renold. "Studies on Rat Adipase Tissue in Vitro." J. Biol. Chem. Vol. 238. (1960) 2217-2223.

(56) Jensen, P. K. "Corticosterone Inhibition of Pyridine Nucleotide Oxidase from Heart Sarcosomes." Nature. Vol. 184. (1959) 451-452.

(57) Jensen, P. K. and J. Neuhard. "Steroid Inhibition of Reduced Diphosphopyridine Nucleotide-Oxidase Activity in Electron-Transport Particles." Biochimica et Biophysica Acta. Vol. 52. (1961) 97-105.

(58) Karlson, P. "New Concepts on the Mode of Action of Hormones." Perspectives in Biol. and Med. Vol. 6 (1963) 203-213.

(59) Karlson, P. "Biochemical Studies of Ecdysone Control of Chromosomal Activity." J. Cellular and Comparative Physiol. Vol. 66. (1965) 69-75.

(60) Kenney, F. T., and R. Flora. "Induction of Tyrosine-α-Ketoglutarate Transaminase in Rat Liver." J. Biol. Chem. Vol. 236. (1961) 2699-2702.

(61) Kenney, F. T. "Induction of Tyresine-α-Ketoglutarate Transaminase in Rat Liver. Evidence For an Increase in the Rate of Enzyme Synthesis." J. Biol. Chem. Vol. 237. (1962) 3495-3498.

(62) Kerppola, W. "Inhibition of Phosphorylase with Cortisone and Its Activation with Adrenaline in the Rabbit." Endocrinol. Vol. 51. (1952) 192-202.

(63) Kerppola, W. "Uncoupling of the Oxidative Phosphorylation with Cortisone in Liver Mitochondria." Endocrinol. Vol. 67. (1960) 252-263.

(64) Kerppola, W. and E. Pitkanen. "The Action of Cortisone on Oxidation and Glycolytic Liver Enzyme Activities in Rats of Different Age and Sex." Endocrinol. Vol. 67. (1960) 162-165.

(65) Klingenbert, M. and Helga Hafen. "Wege des Wasserstaffs in Mitochondrion." Biochemische Zeitschrift. Vol. 337. (1963) 120-145.

(66) Krebs, H. A., Bull. Johns Hopkins Hosp. Vol. 95. (1954) 95.

(67) Kvam, D. C. and R. Parks, Jr.. "Hydrocortisone-Induced Changes in Hepatic Glucose-6-Phosphatase and Fructose Diphosphatase Activity." Am. J. Physiol. Vol. 198. (1960) 21-24.

(68) Lang, N. and C. E. Sekeris. "Stimulation of RNA-Polymerase Activity in Rat Liver by Cortisol." Life Science. Vol. 3. (1964) 391-393.

(69) Leboeuf, B., A. E. Renold, and G. F. Cahill, Jr.. "Studies on Rat Adipose Tissue In Vitro. IX. Further Effects of Cortisol on Glucose Metabolism." J. Biol. Chem. Vol 237. (1962) 988-991.

(70) Lecocq, F. R., D. Mebane, and L. Madison. "The Acute Effect of Hydrocortisone on Hepatic Glucose Output and Peripheral Glucose Utilization." J. of Clin. Invest. Vol. 43. (1964) 237-246.

(71) Leloir, L. F., J. Olavarra, Sara Goldenberg, and H. Carminatti. "Biosynthesis of Glycogen from Uridine Diphosphate Glucose." Archives of Biochemistry and Biophysics. Vol. 81. (1959) 508-520.

(72) Liljeroot, B. S. and J. C. Hall. "Oxidative Phosphorylation in Live Mitochondria from Adrenalectomized Rats and the Response to Hormones Added in vitro." J. Biol. Chem. Vol. 240. (1965) 1446-1452.

(73) Lin, E. C. and W. E. Knox. "Adaptation of the Rat Liver Tyrosine-α-Ketoglutarate Transaminase." Biochimica et Biophysica ACTA. Vol. 26. (1957) 85-88.

(74) Long, C. N., B. Katzin and E. Fry. "The Adrenal Cortex and Carbohydrate Metabolism." Endocrinol. Vol. 26. (1940) 309-344.

(75) Long, C. N., O. Smith and E. Fry. "Metabolic Effects of Adrenal Hormones." G. E. Wolstenholme and M. O'Connor, Eds., Ciba Foundation Study Group No. 6, J. & A. Churchill, London. (1960).

(76) Lowery, O. H. and J. Lopez. "The Determination of Inorganic Phosphate in the Presence of Labile Phosphate Esters." J. Biol. Chem. Vol. 162. (1946) 421-428.

(77) Maickel, R. P. and B. Brodie. "Interaction of Drugs With the Pituitary-Adrenocorticol System in the Production of the Fatty Liver." Annals N.Y. Academy Science. Vol. 104. (1963) 1059-1064.

(78) Means, A. R. "Biochemical Changes in Scrotal and Cryptorchid Rat Testis During Postnatal Maturation." (unpub. M.S. thesis, Okla. State University, 1963).

(79) Mokrasch, L. C., W. Davidson, and R. McGilbery. "The Response to Glucogenic Stress of Fructose-1,6-Diphosphatase in Rabbit Liver." J. Biol. Chem. Vol. 222. (1956) 179-184.

(80) Monod, J., J. P. Changeux, and F. Jacob. "Allosteric Proteins and Cellular Control Systems." J. Mol. Biol. Vol. 6. (1963) 306-329.

(81) Moran, T. J. "Cortisone-Induced Alterations in Lipid Metabolism." Arch. Path. Vol. 73. (1962) 300-312.

(82) Morgan, H. E., M. J. Henderson, D. M. Regen, and C. R. Park. "Regulation of Glucose Uptake in Heart Muscle from Normal and Alloxan-Diabetic Rats: The Effect of Insulin Growth Hormone, Cortisone, and Anoxia." Ann. N.Y. Acad. Sci. Vol. 82. (1959) 387-402.

(83) Morita, Y. and A. Munck. "Effects of Glucocorticoids In Vivo on Net Glucose Uptake and Amino Acid Incorporation by Rat-Thymus Cells." Biochimica et Biophysica Acta. Vol. 93. (1964) 150-157.

(84) Munck, Allen. "The Effect of Cortisol on Glucose Uptake by Rat Epididymal Fat Pads." Endocrinol. Vol. 68. (1961) 178-180.

(85) Munck, Allen. "Studies on the Mode of Action of Glucocorticoids In Rats." Biochimica et Biophysica Acta. Vol. 57 (1962) 318-326.

(86) Munck, Allen. "Steroid Concentration and Tissue Integrity as Factors Determining the Physiological Significance of Effects of Adrenal Steroids in Vitro." Endocrinol. Vol. 77. (1965) 356-360.

(87) Munck, A., and S. Koritz. "Some Early Effects of Hydrocortisone on Carbohydrate Metabolism in Adrenalectomized Rats." Acta Endocrinologica, Supplement No. 51. (1960) 821.

(88) Munck, A. and S. Koritz. "Studies on the Mode of Action of Glucocorticoids in Rats. I Early Effects of Cortisol on Blood Glucose and on Glucose Entry into Muscle, Liver and Adipose Tissue." Biochimica et Biophysica Acta. Vol. 57. (1962) 310-317.

(89) Nejad. N. S. and I. L. Chaikoff. "Effect of Adrenocorticotropic Hormone on Incorporation of Glucose Carbon Into Fatty Acids by Livers of Hypophysectomized and Hypophysectomized-Adrenalectomized Rats." Endocrinol. Vol. 75. (1964) 923-936.

(90) Olsen, R. E., S. A. Thayer and L. J. Kapp. "The Glycogenic Activity of Certain Crystalline Steroids of the Adrenal Cortex When Administered Singly and With Cortical Extract to Fasted, Normal and Adrenalectomized Rats." Endocrinol. Vol. 35. (1944) 464-472.

(91) Overell, B. G. "The Effect of Hormones and Their Analogues Upon the Uptake of Glucose by Mouse Skin In Vitro." J. of Pharm. Pharmacol. Vol. 12. (1960) 150-153.

(92) Pitot, H., Y. Sang Cho, C. Lamar, Jr., and C. Peraino. "The Interaction of External and Internal Controls on Enzyme Levels in Liver and Hepatoma." J. Cellular and Comparative Physiol. Vol. 66. (1965) 163-174.

(93) Randle, P. J. "Endocrine Control of Metabolism." Annual Review of Physiology. Vol. 25. (1963) 291-324.

(94) Ray, P. D., D. D. Foster, and H. A. Lardy. "Mode of Action of Glucocorticoids I. Stimulation of Gluconeogenesis Independent of Synthesis De Novo of Enzymes." J. Biol. Chem. Vol. 239. (1964) 3396-3400.

(95) Riddick, F. A. Jr., R. Rusler and D. Kipnis. "The Sugar Transport System in Striated Muscle: Effect of Growth Hormone, Hydrocortisone and Alloxan Diabetes." Diabetes. Vol. 11. (1962) 171.

(96) Rivlin, R. and K. Melmon. "Cortisone-Provoked Depression of Plasma Tyrosine Concentration: Relation to Enzyme Induction in Man." J. Clin. Invest. Vol. 44. (1965) 1690-1698.

(97) Roberts, S. and Martha Keller. "Influence of Epinephrine and Cortisone on the Metabolism of the Hypophysis and Hypothalamus of the Rat." Endocrinol. Vol. 57. (1955) 64-69.

(98) Rosen, F. "Enzymes in Tissues Responsive to Corticosteroids." Cancer Research. Vol. 23. (1963) 1447-1458.

(99) Rosen, F., Nira 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. Vol. 234. (1959) 476-480.

(100) Rosen, F., Nira Roberts, L. Budnick and C. Nichol. "An Enzymatic Basis for the Gluconeogenic Action of Hydrocortisone." Science. Vol. 127. (1958) 287-288.

(101) Schimke, R. T. "Studies on Factors Affecting the Levels of Urea Cycle Enzymes in Rat Liver." J. Biol. Chem. Vol. 238. (1963) 1012-1018.

(102) Seifter, S., S. Dayton, B. Novic, and E. Muntwyler. "The Estimation of Glycogen with the Anthrone Reagent." Arch. Bio. Chem. Vol. 25. (1950) 191-198.

(103) Sekeris, C. E., "Action of Ecdysone on RNA and Protein Metabolism in the Blowfly, Calliphora Erythrocephala." In Mechanism of Hormone Action. ed., P. Karlson, Georg Thieme Verlag, Stuttgart, and Academic Press, New York. (1965) 149-167.

(104) Sekeris, C. E. and N. Lang. "Stimulation of Messenger RNA Synthesis in Rat Liver by Cortisol." Life Science. Vol. 3. (1964) 169-173.

(105) Selye, H. "The General Adaption Syndrome and the Diseases of Adaptation." J. Clin. Endrocinol. Vol. 6. (1946) 117-230.

(106) Sensenbach, W., L. Madison and L. Ochs. "The Effect of ACTH and Cortisone on Cerebral Blood Flow and Metabolism." J. Clin. Invest. Vol. 32. (1953) 372-380.

(107) Shrago, E., H. A. Lardy, R. Nordlie, and D. L. Foster. "Metabolic and Hormonal Control of Phosphoenolphruvate Carboxykinase and Malic Enzyme in Rat Liver." J. Biol. Chem. Vol. 238. (1963) 3188-3192.

(108) Skanse, B., W. von Studnitz, Nancy Skoog. "The Effect of Corticotrophin and Cortisone on Serum Lipids and Lipoproteins." Acta Endocrinol. Vol. 31. (1959) 442-450.

(109) Stevens, W., C. Colessides, and T. F. Dougherty. "Effects of Cortisol on the Incorporation of Thymidine-2-^{14}C into Nucleic Acids of Lymphatic Tissue from Adrenalectomized

CBA Mice." Endocrinol. Vol. 76. (1965) 1100-1108.

(110) Strickland, Hardin. "Respiration and Phosphorylation in Liver Mitochondria and Homogenates from Adrenalectomized Rats." Archieves of Bio. Chem. & Bio. Physics. Vol. 100. (1962) 110-118.

(111) Tomkins. G. M. and Elizabeth Maxwell. "Some Aspects of Steroid Hormone Action." Ann. Rev. Biochem. Vol. 32, (1963) 677-708.

(112) Tomkins, G. M., L. Garren, R. Howell, and B. Peterkofsky. "The Regulation of Enzyme Synthesis by Steroid Hormones: The Role of Translation." J. Cell. and Comp. Physiol. Supplement I. Vol. 66. (1965) 137-151.

(113) Ulrich, F. "Uptake of Cortisol in Vitro by Rat Liver Mitochondria." Am. J. Physiol. Vol. 196. (1959) 572-578.

(114) Umbreit, W. W. "The Influence of Adrenalectomy and Cortisone Treatment on Enzymatic Reactions in Rat Tissues." Annals of the N.Y. Academy of Science. Vol. 54. (1951) 569-574.

(115) Umbreit, W. W., R. Burris, and J. Stauffer. Manometric Techniques, 4th ed. Burgess Publishing Co., Minneapolis 15, Minnesota. (1964).

(116) Underwood, A. H. and E. A. Newsholme. "Some Properties of Fructose-1,6-Diphosphatase of Rat Liver and Their Relation to the Control of Gluconeogenesis." Biochem. J. Vol. 95, (1965) 767-774.

(117) Venning, E. H., V. Kazmin and J. C. Bell. "Biological Assay of Adrenal Corticoids." Endocrinol. Vol. 38. (1946) 79-89.

(118) Wagle, S. R., H. P. Morris and G. Weber. "Phosphopyruvate Carboxylase in Liver Tumors of Different Growth Rates." Biochim. et Biophys. Acta. Vol. 78. (1963) 783-785.

(119) Weber, G. A. and A. Cantero. "Glucose-6-phosphatase Activity in Normal Precancerous, and Neoplastic Tissues." Cancer Research. Vol. 15. (1955) 105-108.

(120) Weber, G. A. and A. Cantero. "Fructose-1,6-Diphosphatase and Lactic Dehydrogenase Activity in Hepatoma and in Control Human and Animal Tissues." Cancer Research. Vol. 19. (1959) 763-768.

(121) Weber, G. A., G. Banerjee, and S. Bronstein. "Role of Enzymes in Homeostasis. III. Selective Induction of Increases of Liver Enzymes Involved in Carbohydrate Metabolism." J.

Biol. Chem. Vol. 236. (1961) 3106-3111.

(122) Weber, G. A., G. De Lamirande and A. Cantero. "Liver Glucose-6-Phosphatase Activity and Intracellular Distribution After Cortisone Administration." Endocrinol. Vol. 58. (1956) 40-50.

(123) Weber, G. A., and H. Morris. "Comparative Biochemistry of Hepatomas III. Carbohydrate Enzymes in Liver Tumors of Different Growth Rates." Cancer Research. Vol. 23. (1963) 987-994.

(124) Weber, G. A. and R. Singhol. "Role of Enzymes in Homeostasis IV. Effects of Triamcinolone and Other Steroids on Enzymes Involved in Gluconeogenesis." Biochem. Pharmacol. Vol. 13. (1964) 1173-1187.

(125) Weber, G. A., R. Singhal and S. Srivastava. "Effect of Nutritional State on Hormonal Regulation of Liver Enzymes." Canadian J. Biochem. Vol. 43. (1965) 1549-1563.

(126) Weber, G. A., S. Srivastava and R. L. Singhal. "Role of Enzymes in Homeostasis VIII. Early Effects of Corticosteroid Hormones on Hepatic Gluconeogenic Enzymes, Ribonucleic Acid Metabolism, and Amino Acid Level." J. Biol. Chem. Vol. 240. (1965) 750-756.

(127) Wicks, W. D., D. L. Greenman, and F. T. Kenney. "Stimulation of Ribonecleic Acid Synthesis by Steroid Hormones." J. Biol. Chem. Vol. 240 (1965) 4414-4419.

(128) Winternitz, W. W. and W. Forrest. "Metabolism of 2-^{14}C Pyruvate after Adrenalectomy." Am. J. Physiol. Vol. 199 (1960) 1059-1063.

(129) Woodbury, D. M. "Relation Between the Adrenal Cortex and the Central Nervous System." Pharmacol. Rev. Vol. 10. (1958) 275-357.

(130) Yielding, K. L. and G. M. Tomkins. "Inhibition of the Enzymic Oxidation of DPNH by Steroid Hormones." Proc. Natl. Acad. Sci. U.S. Vol. 45. (1959) 1730-1739.

(131) Yielding, K. L., G. M. Tomkins, Janet Munday, and I. J. Cowley. "The Effects of Steroids on Electron Transport." J. Biol. Chem. Vol. 235. (1960) 3413-3416.

(132) Yudaeu, N. and Lebedeva, M. "Role of Adrenal Cortex in Glucose-6-Phosphatase Adaptation in Rat Liver." Fed. Proc. (Transl. Suppl.). Vol. 23. (1964) 496-498.

(133) Zalokar, M. Control Mechanisms in Cellular Processes. Ronald Press, New York. (1961) 87-140.

Appendix

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY BRAIN HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
41	83		52	80	104
95	191	262	52	186	258
102	200	287	108	192	216
64	124	138	73	130	186
74	157	239	93	165	232
82	130	223	50	85	126
56	115	170	68	122	177
49	95	139	52	118	173
66	139	196	62	126	146
46	94	130	53	100	146
48	108	153	51	103	150
36	85	133	50	98	139
53	94	113	45	94	138
67	128	179	37	75	113
42	78	121	62	125	174
38	93	139	62	113	156
35	83	125	25	71	113
79	141	193	42	90	131
45	92	129	57	114	169
43	90	131	47	88	123
63	114	166	45	82	123
87	152	212	41	82	123
110	201	256	67	126	178
67	136	182	70	124	168
73	148	211	79	135	183
			67	131	193
Mean					
62	122	176	58	113	155
S.D.					
21.2	36.8	50.4	17.4	32.2	34.7

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
85	144	182	70	103	128
109	161	209	198	297	366
160	245	331	83	124	182
76	124	167	55	93	
104	168	265	180	235	285
77	150	218	136	198	223
33	65	124	64	126	158
30	74	127	161	248	313
178	251	295	121	166	194
92	129	154	86	130	156
19	29	53	58	85	112
154	195	222	134	196	233
82	114	144	102	152	169
109	175	222	117	201	259
140	210	281	122	189	240
136	200	245	143	215	265
83	139	180	175	240	283
97	161	208	129	196	246
169	293	384	149	241	303
150	241	304	75	111	143
37	96	101	121	177	198
106	237	249	158	239	297
120	198	249			
Mean					
102	165	213	119	180	226
S.D.					
45.2	66.0	77.0	41.5	58.4	44.2

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY DIAPHRAGM HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
40	74	84	53	65	75
91	137	170	51	66	76
25	46	72	84	98	156
17	43	47	71	96	135
83	111	158	105	155	180
	35	113		18	30
08	19	48	16	20	57
18	76	121	27	53	66
	36	66	25	48	72
24	43	64	13	24	44
13	33	50		27	16
47	78	102	07	22	32
21	44	54	04	20	29
30	57	79	63	96	124
25	44	57	62	70	91
42	73	94	08	15	18
100	141	168	41	59	75
44	74	94	81	118	148
63	93	115	59	99	121
134	172	202	18	26	37
60	89	110	45	65	81
11	33		03	40	86
53	115	135			
	23	53			
Mean					
43	70	98	41	59	79
S.D.					
33.9	40.6	44.4	30.0	38.2	47.0

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
103	209	256	99	117	143
188	332	423	208	267	334
227	436	561	231	394	479
160	247	331	149	220	318
159	283	365	166	273	308
222	279	389	186	266	310
167	302	431	149	228	283
129	269	390	61	111	165
127	192	250	39	79	108
213	432	553	74	129	174
122	220	280	74	122	161
30	64	93	16	55	87
119	174	217	38	55	83
157	296	408	137	276	373
132	205	251	141	225	268
130	261	386	93	146	189
128	232	280	119	200	247
125	220	276	136	215	274
134	266	348	114	173	215
107	205	265	103	163	211
188	291	363	146	266	334
100	155	207	140	230	282
128	208	272	91	149	206
163	262	341	103	169	253
127	312	271	181	288	356
Mean					
143	249	328	119	193	246
S.D.					
42.8	78.6	104	53.0	82.2	96.3

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY KIDNEY HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG. CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
64	90	125	55	85	111
84	140	218	164	275	361
103	211	296	99	173	255
78	130	198	76	132	207
84	151	215	105	204	256
91	120	187	85	140	183
76	120	174	59	157	215
61	113	158	75	135	177
57	110	154	59	106	148
66	116	162	35	68	101
92	166	222	72	126	179
97	158	204	54	97	130
46	93	127	36	78	108
33	67	96	39	70	97
58	106	140	75	133	176
32	62	91	51	90	111
59	110	149	51	94	134
60	113	147	58	107	136
70	127	165	67	123	166
62	114	156	57	101	130
57	106	138	50	84	114
88	161	207	72	113	148
99	161	206	96	151	189
148	234	279	80	136	176
112	198	249	116	179	213
72	130	181	104	185	242
Mean					
70	131	178	74	128	171
S.D.					
25.2	40.8	51.3	28.7	47.0	61.0

EXPERIMENT I

OXYGEN UPTAKE (MICROLITERS/100 MG PROTEIN) BY TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. HOMOGENATES INCUBATED IN WARBURG FLASK UNDER AIR

Treated			Control		
Incubation Time (Minutes)			Incubation Time (Minutes)		
10	20	30	10	20	30
13	28	49	22	32	46
32	48	93	97	132	166
33	92	148	39	68	97
19	41	73	24	57	102
43	71	101	38	80	96
26	56	63	34	64	85
30	57	91	44	68	84
19	41	62	24	48	62
25	56	76	29	54	81
24	49	73	19	41	56
17	56	57	18	39	48
23	36	47	22	41	56
33	60	77	10	33	49
34	62	79	32	58	76
34	62	79	28	45	57
29	56	73	17	34	55
26	49	63	21	43	56
27	40	67	26	46	62
22	41	58	33	56	72
21	41	58	16	24	30
39	63	81	32	47	64
50	81	105	37	65	83
21	37		26	35	51
35	69	91	44	49	52
15	36	54	23	39	58
Mean					
27	53	71	30	51	69
S.D.					
8.4	14.8	26.2	16.5	21.2	26.8

EXPERIMENT I

PROTEIN (MG/GM DRY TISSUE WT.) IN BRAIN AND HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Brain				Heart			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
382	403	389	389	325	244	358	303
399	479	346	348	507	340	452	452
409	450	448	390	435	508	520	486
476	409	373	435	444	384	457	472
397	410	369	384	499	526	514	587
533	416	506	422	570	484	513	513
432	328	556	620	191	191	289	289
356	248	565	451	318	237	306	286
498		563	498	236	177	301	252
376	412	673	602	220	191	374	251
400		606	471	250	190	319	265
340	409	514	440	396	497	436	348
427	467	340	438	297	254	580	506
477	558	383	452	367	367	326	365
585	494	502	545	394	373	325	344
735	677	578	591	540	469	478	521
398	407	556	505	514	514	657	597
458	407	600	659	585	552	523	523
421	377	492	540	628	463	507	635
428	485			397	356	549	641
420	450			516	364	454	454
				320	273	539	497
				345	345	355	296
				440	440	351	310
						400	460
Mean							
445	436	492	483	405	364	435	426
S.D.							
88.2	87.5	101	89	123	138	101	125

EXPERIMENT I

PROTEIN (MG/GM DRY TISSUE WT.) IN DIAPHRAGM AND LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Diaphragm				Liver			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
446	254	305	407	446	485	399	317
305	376	453	502	545	665	448	561
418	386	441	481	530	626	450	468
528	497	561	601	847	484	531	531
461	577	290	290	515	461	574	798
173	173	210	143	356	283	508	524
400	285	364	229	437	347	389	389
285	285	404	353	338	377	337	297
438	571	387	387	341	389	426	380
327	410	402	402	280	280	493	493
456	492	432	516	395	346	425	319
672	575	711	711	390	407	427	304
491	532	750	683	448	415	512	512
722	826	546	656	621	507	478	585
641	479	645	645	686	731	426	456
403	307	708	497	683	611	607	650
509	361	457	307	763	794	833	799
275	200	493	393	703	742	714	910
396	396	284	235	807	745	834	787
290	350	352	352	493	493	822	722
		300	260	700	844	684	627
				623	574	816	729
				487	487	616	556
				530	490	510	553
						490	560
Mean							
431	417	452	430	540	524	550	553
S.D.							
140	156	152	163	159	163	151	171

EXPERIMENT I

PROTEIN (MG/GM DRY TISSUE WT.) IN KIDNEY AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Kidney				Testis			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
464	510	456	407	417	472	425	398
412	457	470	484	449	479	502	502
476	524	468	406	483	483	498	468
489	423	604	529	485	566	438	383
455	599	534	509	468	482	510	518
505	452	369	369	445	480	455	380
326	311	406	291	368	368	412	425
306	261	481	428	397	342	490	453
446	317	627	543	436	363	653	603
428	428	620	581	657	567	669	585
466		491	491	550	504	669	585
428	428	350	385	502	502	459	478
470	352	346	371	429	429	500	464
350	484	580	549	438	423	595	595
266	266	640	532	574	718	600	600
612	543	692	692	693	655	765	851
575	532	760	684	583	583	901	704
730	669	671	586	703	717	708	661
662	592	666	616	719	719	688	619
367	343	435	298	518	509	555	508
552	483	448	539	492	492	575	642
368	346	520	555	545	520	570	570
448	448			678	678		
430	430			510	550		
Mean							
460	443	529	498	522	525	570	546
S.D.							
51	108	119	124	105	110	122	116

EXPERIMENT I

GLYCOGEN (MG/100 G PROTEIN) IN BRAIN AND HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Brain				Heart			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
37	57	84	13		104	360	39
166		36	49	780	360	163	
234	194	303	284	396	247	180	120
173	69	197	109	52	52	315	118
325	367	267	89	404	173	447	83
466	311	109	219	521	51	339	121
145		106	127	702		107	5
160	80	145	109	278	34	45	36
236	325	228	93	513	128	256	85
818	391	780	314	610	106	428	107
985	532	528	251	427	77	484	42
307	185	122	70	345	49	729	91
566	300	464	355	370		473	37
625	530	559	345	114	575	245	117
197	164	397	233	596	66	845	211
Mean							
362	269	288	177	436	156	361	86
S.D.							
272	155	212	114	208	150	114	52

EXPERIMENT I

GLYCOGEN (MG/100 G PROTEIN) IN DIAPHRAGM AND LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Diaphragm				Liver			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
133	66	209	71	1400	120	23	
360	257	221	181	5746	70	180	135
488	292	346	173	2811	648	176	131
41	41	232	93	552	237	105	104
435	256	312		793	104	54	26
262	82	176	70	1018	87	58	90
193		145	35	1696	99	65	21
238	29	128	42	2015	51	130	65
278	46	285	95	906	69	47	42
304	60	112	32	1708	153	42	10
216	54	382	54	1893	118	100	50
619	61	306	74	3883	446	66	21
205	24	648	404	3866	317	123	82
498	43	507	253	1118	112	90	135
	222			3279	693		
				2531	545		
Mean							
305	109	286	121	2200	241	89	70
S.D.							
159	985	149	107	1420	224	48	46

EXPERIMENT I

GLYCOGEN (MG/100 G PROTEIN) IN KIDNEY AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Kidney				Testis			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
59	46	41	41	44	44	146	36
211		108	96	150	150	112	90
197	164	131	98	140	140	125	125
69	69	64	80	47	78	60	49
122	122	41	62	54		60	60
211	105	77	76	34	34	26	25
48		69	52	85	85	45	48
78	39	107	45	50	25	59	71
109	104	64	42	37	37	21	21
84	42	50	25	70	84	51	38
79	66	110	54	56	56	52	26
214		133	26	87	56	49	60
30	46	105	70	125	133	100	120
104	140	136	67				
112	67						
Mean							
114	84	88	59	76	79	69	59
S.D.							
63	40	33	36	37	41	37	33

EXPERIMENT I

GLUCOSE (MG/100 G PROTEIN) IN BRAIN AND HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Brain				Heart			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
58	58	65	22	245	245	404	269
41	13	46	46	198	279	335	447
24	24	111	57	322	402	144	248
16	16	58	16	145	440	192	193
14		64	31	280	370	114	95
46		18	56	174	135		81
53		39	17	175	232	222	177
62	31	43		78	19	205	307
42	21	65	29	197	229	75	105
7	7	36	27	240	191	223	191
46	23	27	10	46	72	104	46
53	21	51	38	151	152	120	6
58	39	63	21	115	90	114	63
8		4	4	170	138	80	63
10	21	31	31	102	52	172	203
86	35	26	24	51	89	100	100
74	23	57	78	209	176	209	265
36	80	51	5	143	172	189	236
91	3	79	23	203	238	62	149
42	20	143	135	277	221	72	108
		42	22	23	47		
		76	23	74	47		
Mean							
41	28	54	34	164	183	165	167
S.D.							
25	19.8	30	29	81	118	91	106

EXPERIMENT I

GLUCOSE (MG/100 G PROTEIN) IN DIAPHRAGM AND LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Diaphragm				Liver			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
244	306	279	419	1254	3553	458	792
258	452	184	237	558	1257	559	838
170	307	288	288	993	1614	578	1052
290	290	336	336	864	2941	370	854
185	280	221		1518	4554	609	148
460	460	114	180	1547	4762	261	622
172	259	142	227	981	4257	291	421
114	127	24	73	564	1954	509	403
47	95	210	163	784	1176	766	508
88	215	63	79	672	2364	510	997
70	91	111	189	748	1940	218	454
108	145	63	74	601	1557	294	802
110	110	39	56	681	1846	324	934
58	65	109	131	428	5542	185	367
133	163	70	79	536	2111	311	765
144	198	143	143	529	2206	291	801
100	100	234	234	737	3070	204	715
310	344	70	104	717	1988	219	897
36	72	32	64	810	2466	167	657
56	56			478	3349	115	672
				498	1991		
Mean							
157	193	143	170	784	2690	361	745
S.D.							
108	130	94	103	318	1228	184	264

EXPERIMENT I

GLUCOSE (MG/100 G PROTEIN) IN KIDNEY AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Kidney				Testis			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
569	1424	354	780	52	78	134	134
507		457	1016	66	19	111	83
625	885	545	1304		44	47	47
517	682	282	736	63	15	60	6
891	1069	865	749	9	7	195	140
182	380	317	680		31	16	
273	460	383	587	9	9	18	45
194		254	526	23	47	15	19
413	565	568	568	15	7	40	3
445	317	178		44	7	14	14
	510	324		25	14	12	6
378	336	220	373	4	21	15	15
183	381	317	280	2		15	3
222	444	284	127	1	16	11	
149	483	120	376	59		20	20
126	355	271	514	10		4	12
218	408	241	508				
250	662	264	716				
250	590	373	644				
508	780	172	488				
143	402	120	301				
129	258						
Mean							
341	569	329	593	30	24	45	40
S.D.							
310	280	171	270	22	21	53	45.6

EXPERIMENT I

LACTIC ACID (MG/100 G PROTEIN) IN BRAIN AND HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Brain				Heart			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
61	37	65	58	9	86		82
47	25	47	44	68	59	71	59
42	72	32	35	49	37	133	104
21	15	24	8	80	94	77	58
38	63	54		98	96	89	80
43	31	26	19	9	30	115	115
43		33	23	92	92	71	78
32	32	41	35	7	81	43	36
37	37	32	25	120	95	80	96
31	4	48	28	69	90	36	50
102	73	26	40	64	68	67	81
44	31	33	35	59	82	75	81
44	34	50	47	55	85	76	92
44	44	53	50	49	58	81	84
35	22	34	29	48	58	48	75
37	29	4	2	91	91	67	86
56	25	43	33	81	42	77	109
41	33	48	41	43	68	33	45
48	43	8	2	16	25	42	28
21	30	19	11	53	47	44	59
50	35	17	27				
Mean							
43	37	36	30	69	69	69	74
S.D.							
16	12	14	14	24	22	26	24

EXPERIMENT I

LACTIC ACID (MG/100 G PROTEIN) IN DIAPHRAGM AND LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Diaphragm				Liver			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
80	83	118	48	1	1		9
151	222	72	72	4	7	6	6
49	26	28	38	5	5	28	46
274	82	116	116	6		1	4
197	197	89	92	3	27	21	19
66		70	56	3	29	27	20
92	100	80	105	3	4	20	12
260	116	86	55	.9	6	7	2
95	95	148	96	14	20	25	26
62	89	53	67	8	15	8	
5	49	105	13	17	13	16	28
73	78	116	112	8	11	10	9
131	131	65	67	6	7	1	10
60	60	64	38	5			13
66	61	86	86	4	50	11	11
116	133	118	126	5	7	1	1
101	93	55	55	5	12	6	10
54	6	41	16			14	5
38	31	60	60				
45	28						
Mean							
100	88	82	69	6.24	14	11	12
S.D.							
69	52	31	32	4.0	12.8	9	11

EXPERIMENT I

LACTIC ACID (MG/100 G PROTEIN) IN KIDNEY AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASK UNDER AIR

Kidney				Testis			
Treated		Control		Treated		Control	
Before	After	Before	After	Before	After	Before	After
14	14	8	8	19	17	18	14
1	15	36	17	13	14	14	7
57	43	36	35	96	70	3	38
50	47	6	7	4	4	2	10
9	19	43	18	20	23	16	24
101	23		23	72	16	18	18
7		19	16	13	27	9	6
7	7	13	8	14	9	11	5
28		24	17	6	10		16
13	13	31		16	16	22	19
22	14	34	11		13	16	12
12	37	62	40	18	19	14	14
20	16	24	22	18	19	22	27
22	23	30	17	21	10	17	15
12	11	20	13	3	11	9	6
2	5	5	6	7	129	11	10
16	110	24	9	16	15	10	12
17	15	2	12	21	21	5	2
29	37	7		40	2		2
	7	6	2				
2	2						
Mean							
18	24	21	14	19	22	12	12
S.D.							
23	25	16	10.5	24	43	8	9

EXPERIMENT I

DIFFERENCES IN PREINCUBATION AND POSTINCUBATION INORGANIC PHOSPHATE LEVELS (MICROMOLES/G PROTEIN) IN BRAIN AND HEART HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION UNDER AIR IN WARBURG FLASK

Brain		Heart	
Treated	Control	Treated	Control
90	-100	130	-240
140	-70	100	-160
00	120	120	-300
30	-30	-110	00
110	130	370	100
90	70	540	00
30	100	170	240
60	160	-10	-10
140	60	00	250
-120	220	160	240
120	-40	-80	350
220	60	-120	40
160	-30	180	520
320	40	190	-30
-130	60	00	60
110	-60	-130	160
00	-10	-140	240
00	170	210	340
10	100	60	-20
120	120	00	-170
26			-40
50			-90
Mean			
71.64	53.5	78.10	68.7
S.D.			
73	89	170	198

EXPERIMENT I

DIFFERENCES IN PREINCUBATION AND POSTINCUBATION INORGANIC PHOSPHATE LEVELS (MICROMOLES/G PROTEIN) IN DIAPHRAGM AND LIVER HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION UNDER AIR IN WARBURG FLASK

Diaphragm		Liver	
Treated	Control	Treated	Control
50	-40	-120	00
210	00	00	-90
00	00	-360	00
-400	00	-70	-70
-330	330	-90	330
-190	00	-80	00
-250	-230	-60	-180
00	260	210	-120
-250	70	100	-170
80	110	200	70
290	150	190	130
30	-290	80	-190
-140	-100	-100	100
40	-30	60	90
150	40	00	-80
-30	-310	30	100
70	00	10	00
500		70	350
-230		130	227
		310	90
		-320	-90
		-20	00
		100	-20
			60
			200
Mean			
-19.00	-2.4	11.74	31.2
S.D.			
219	109	157	151

EXPERIMENT I

DIFFERENCES IN PREINCUBATION AND POSTINCUBATION INORGANIC PHOSPHATE LEVELS (MICROMOLES/G PROTEIN) IN KIDNEY AND TESTIS HOMOGENATES FROM FASTED ADRENALECTOMIZED RATS TREATED IM WITH 5.0 MG CORTICOSTERONE AND FROM FASTED ADRENALECTOMIZED CONTROL RATS INJECTED IM WITH CARRIER VEHICLE 5 HOURS BEFORE SACRIFICE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION UNDER AIR IN WARBURG FLASK

Kidney		Testis	
Treated	Control	Treated	Control
00	00	50	-220
220	-90	-40	00
50	00	-40	-40
-80	-60	60	00
-90	80	150	140
180	40	00	120
-50	240	140	00
40	-270	80	160
100	-270	00	00
220	210	-270	40
-140	-130	-10	70
280	150	10	120
10	40	-20	100
280	-240	00	70
10	180	00	60
80	00	100	-50
40	-80	100	26
-30	230	-40	110
-60	50	230	160
120	210		
30	250		
Mean			
48	25.71	26.3	45.58
S.D.			
48.2	166	103	91.4

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN BRAIN MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Succinate Used as Substrate					
Treated			Non-Treated		
O_2* Uptake	ATP** Formed	P:O	O_2* Uptake	ATP** Formed	P:O
63.5	67.5	1.06	77.0	77.0	1.00
114.0	125.5	1.10	74.5	113.0	1.52
102.5	108.5	1.06	84.5	117.5	1.39
66.0	80.5	1.22	75.5	82.0	1.08
49.6	66.6	1.34	94.0	107.0	1.14
61.3	75.3	1.23	63.5	54.0	0.85
38.0	45.3	1.19	47.3	69.3	1.46
38.6	44.6	1.16	52.6	62.9	1.20
38.0	43.7	1.15	37.6	49.3	1.31
33.3	40.0	1.20	37.3	30.6	0.82
Mean					
60.5	69.8	1.17	64.4	76.3	1.18
S.D.					
27.9	28.9	0.08	19.9	29.0	0.25

* Expressed as microatoms taken up per G. mitochondrial protein per minute.

** Micromoles of inorganic phosphate disappearing per G. mitochondrial protein per minute.

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN BRAIN MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
27.5	37.8	1.37	28.0	37.0	1.32
29.5	24.5	0.83	33.1	23.0	0.67
26.6	34.4	1.29	25.5	21.1	0.82
17.3	22.9	1.32	25.3	24.0	1.00
25.3	45.3	1.79	22.0	43.3	1.97
18.3	36.6	2.00	22.0	30.6	1.39
24.6	26.6	1.08	23.3	32.2	1.38
22.0	32.0	1.45	22.6	23.3	1.02
18.6	30.0	1.61	20.0	32.0	1.60
18.0	50.6	2.81			
18.6	29.3	1.57			
20.6	25.3	1.23			
Mean					
22.2	32.9	1.53	24.6	29.6	1.24
S.D.					
4.3	8.5	0.51	4.0	7.5	0.14

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN BRAIN MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate+Pyruvate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
41.3	86.6	2.10	35.3	62.8	1.8
33.6	80.0	2.38	40.6	114.0	2.8
42.0	78.0	1.86	45.3	91.3	2.01
39.3	123.3	3.14	40.0	110.0	2.75
33.3	74.6	2.24	20.7	56.0	1.16
42.6	88.6	2.08	39.3	89.3	2.27
Mean					
38.7		2.3	36.9	87.2	2.21
S.D.					
4.3		0.5	8.5	23.8	0.49

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN HEART MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Succinate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
140.0	98.0	0.70	130.5	85.0	0.65
123.5	80.0	0.65	108.0	106.5	0.99
124.0	29.0	0.24	142.0	105.5	0.74
156.6	59.3	0.39	139.0	63.0	0.45
76.6	38.6	0.50	148.0	73.3	0.5
140.0	96.0	0.69	65.2	26.2	0.4
144.6	50.0	0.35	105.3	40.0	0.38
			90.6	40.0	0.44
Mean					
129.3	64.3	0.5	116.1	67.4	0.57
S.D.					
26.0	27.8	0.2	28.9	30.6	0.2

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN HEART MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
25.7	62.5	2.4	39.0	72.0	1.85
22.5	80.0	2.13	46.0	95.0	2.07
35.3	22.6	0.64	51.5	118.0	2.29
23.3	73.3	3.14	20.5	49.5	2.41
17.3	68.6	3.96	20.6	29.8	1.45
28.6	52.0	1.83	23.3	46.6	2.00
21.3	24.0	1.12	26.0	33.3	1.28
28.6	65.3	2.28	36.0	86.0	2.40
30.0	83.3	2.78	43.3	66.6	1.54
			28.0	43.3	1.55
			24.0	24.0	1.00
			40.0	33.3	0.83
Mean					
25.8	59.1	2.25	33.2	58.1	1.72
S.D.					
5.4	22.3	1.0	10.7	29.6	0.53

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN HEART MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate+Pyruvate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
66.6	145.3	2.18	50.0	137.3	2.8
43.3	100.0	2.30	88.0	224.6	2.55
72.3	132.6	1.83	56.6	128.0	2.26
71.3	195.3	2.74	50.6	76.0	1.5
44.0	48.0	1.09	20.6	33.3	1.61
62.0	139.0	2.25			
Mean					
59.9	126.7	2.06	53.2	119.8	2.14
S.D.					
13.1	49.3	0.55	24.0	13.7	0.51

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN LIVER MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Succinate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
51.0	55.0	1.08	57.0	68.0	1.19
96.0	106.5	1.11	49.0	64.5	1.32
94.0	101.0	1.07	62.0	61.5	0.99
97.0	72.5	0.75	71.5	70.5	0.99
75.3	66.0	0.88	62.5	58.0	0.93
106.6	120.0	1.13	102.5	69.5	0.68
55.9	59.3	1.06	76.0	60.6	0.80
53.3	45.3	0.85	98.0	116.6	1.19
53.3	48.6	0.95	52.0	50.6	0.97
			50.6	31.3	0.62
			72.6	55.3	0.76
Mean					
75.8	74.9	0.98	68.5	64.2	0.95
S.D.					
22.8	27.4	0.13	18.2	20.6	0.23

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN LIVER MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
16.8	25.5	1.52	16.5	24.5	1.48
13.0	26.1	2.01	19.5	58.0	2.23
19.0	29.5	1.55	19.0	27.0	1.42
18.6	36.6	1.96	8.0	8.1	1.01
28.0	50.4	1.80	18.6	21.7	1.16
22.0	38.0	1.73	22.3	37.5	1.68
22.6	36.0	1.59	29.3	37.4	1.28
14.2	27.3	1.92	22.2	33.3	1.50
19.3	20.0	1.03	17.3	31.3	1.80
17.0	40.2	2.36	18.6	26.6	1.43
16.6	36.6	2.20	20.0	15.6	0.78
22.0	46.0	2.09	19.3	20.0	1.03
21.3	55.3	2.59	21.3	21.3	1.00
16.6	39.3	2.36	17.3	14.2	0.82
Mean					
19.1	36.2	1.94	19.2	26.9	1.33
S.D.					
3.9	9.9	0.41	4.6	12.0	0.4

EXPERIMENT II

OXYGEN CONSUMPTION, ATP FORMED, AND P/O RATIOS IN LIVER MITOCHONDRIA ISOLATED FROM ADRENALECTOMIZED CORTICOSTERONE-TREATED RATS AND ADRENALECTOMIZED NON-TREATED RATS

Malate+Pyruvate Used as Substrate					
Treated			Non-Treated		
O_2 Uptake	ATP Formed	P:O	O_2 Uptake	ATP Formed	P:O
30.0	61.0	2.02	20.0	47.6	2.40
24.6	60.0	2.43	25.3	41.3	1.63
21.3	36.6	1.72	25.3	33.3	1.32
28.0	66.1	2.36	22.0	22.2	1.00
22.6	39.3	1.73	20.6	24.0	1.16
24.6	49.3	2.00	20.6	25.3	1.23
Mean					
25.2	52.1	2.04	22.3	32.3	1.47
S.D.					
3.3	12.2	0.3	2.42	13.1	0.49

EXPERIMENT III

SUBCELLULAR DISTRIBUTION OF (3_H) CORTICOSTERONE IN RAT BRAIN 30 MIN. AFTER AN IV INJECTION OF 20 MICROCURIES OF (3_H) CORTICOSTERONE. DISTRIBUTION REPRESENTS DISINTEGRATIONS/MIN. FOR THE VARIOUS FRACTIONS ISOLATED FROM 1 GRAM WET WEIGHT OF TISSUE

BRAIN								
Homogenate	Nuclei		Mitochondria		Microsomes		Supernatant	
DPM	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*
3086	485	16.0	144	5.0	115	4.0	2196	72
2653	394	15.0	211	8.0	131	5.1	1803	68
6444	781	12.1	386	6.1	366	5.0	4431	69
Average		14.4		6.4		4.6		69

SUBCELLULAR DISTRIBUTION OF (3_H) CORTICOSTERONE IN RAT THYMUS 30 MIN. AFTER AN IV INJECTION OF 20 MICROCURIES OF (3_H) CORTICOSTERONE. DISTRIBUTION REPRESENTS DISINTEGRATIONS/MIN. FOR THE VARIOUS FRACTIONS ISOLATED FROM 1 GRAM WET WEIGHT OF TISSUE

THYMUS								
Homogenate	Nuclei		Mitochondria		Microsomes		Supernatant	
DPM	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*
691	67	9.6	28	4.0	35	5.0	628	91.0
3564	335	9.3	419	11.7	71	2.0	3315	93.0
1307	245	19.0	58	4.4	61	4.6	778	59.5
1615	288	17.8	119	7.3	71	4.3	1007	62.3
Average		13.9		6.8		4.0		76.5

* Values in percent of total contained in homogenate.

EXPERIMENT III

SUBCELLULAR DISTRIBUTION OF (3_H) CORTICOSTERONE IN RAT HEART 30 MIN. AFTER AN IV INJECTION OF 20 MICROCURIES OF (3_H) CORTICOSTERONE. DISTRIBUTION REPRESENTS DISINTEGRATIONS/MIN. FOR THE VARIOUS FRACTIONS ISOLATED FROM 1 GRAM WET WEIGHT OF TISSUE

HEART								
Homogenate	Nuclei		Mitochondria		Microsomes		Supernatant	
DPM	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*
2215	335	15.1	71	3.2	104	4.7	1787	80.7
6194	1050	16.9	158	2.5	390	6.2	5141	83.0
1500	227	15.1	69	4.6	155	10.3	1291	86.4
3807	523	13.8	81	2.1	127	3.3	2014	52.5
Average		15.2		3.1		6.12		75.65

SUBCELLULAR DISTRIBUTION OF (3_H) CORTICOSTERONE IN RAT LIVER 30 MIN. AFTER AN IV INJECTION OF 20 MICROCURIES OF (3_H) CORTICOSTERONE. DISTRIBUTION REPRESENTS DISINTEGRATIONS/MIN. FOR THE VARIOUS FRACTIONS ISOLATED FROM 1 GRAM WET WEIGHT OF TISSUE

LIVER								
Homogenate	Nuclei		Mitochondria		Microsomes		Supernatant	
DPM	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*	DPM	PERCENT*
15,265	4514	29.5	796	5.2	2502	16.3	6869	45.0
12,250	4799	39.1	819	6.6	1642	13.4	8211	67.0
5,191	634	12.2	219	4.2	414	7.9	1915	37.4
6,229	896	14.3	627	10.1	1212	19.9	2051	42.9
Average		23.8		6.53		14.4		48.0

* Values in percent of total contained in homogenate.

EXPERIMENT III

DISINTEGRATIONS PER MINUTE PER GRAM WET WEIGHT OF TISSUE THIRTY MINUTES AFTER AN IV INJECTION OF 20 MICROCURIES OF (3_H) CORTICOSTERONE

Rat Number	1	2	3	4	Aver-age	Tissue/ Blood
Blood dpm/g	7236	6042	3997	8498	6443	
Brain dpm/g	3086		2653	5370	3705	0.57
Thymus dpm/g	2303	3564	2178	2692	2684	0.42
Heart dpm/g	2215	7743	1500	4759	4056	0.59
Liver dpm/g	15265	12250	5191	6229	9734	1.51

VITA

Gerald Doyle Bottoms

Candidate for the Degree of

Doctor of Philosophy

Thesis: SOME METABOLIC EFFECTS OF CORTICOSTERONE IN DIFFERENT TISSUES OF THE RAT AND THE SUBCELLULAR DISTRIBUTION OF (^{3}H) CORTICOSTERONE IN THESE TISSUES

Major Field: Physiology

Biographical:

Personal Data: Born April 10, 1930, at Holdenville, Oklahoma, the son of Edgar and Lorene Bottoms.

Education: Attended elementary and secondary school at Spaulding, Oklahoma; graduated from Spaulding High School in 1948; received the Bachelor of Science degree from East Central State College, Ada, Oklahoma, in May, 1955, with a major in biology; completed the requirements for the degree of Master of Science at Oklahoma State University in May, 1959.

Professional Experience: Entered the United States Army in 1950 and was discharged as Sgt. First Class in 1952; taught high school science at Bowlegs, Oklahoma, during the academic year 1955-1956; taught high school science at Holdenville, Oklahoma, from 1956-1961; appointed science department chairman at Santiago High School, Garden Grove, California, in 1961; appointed science department chairman at Holdenville, Oklahoma, in 1962; accepted a position as a member of the Academic Year Institute for High School Science Consultants at Oklahoma State University in 1963; received NIH Predoctoral Fellowship in the Department of Physiology and Pharmacology at Oklahoma State University in 1964.