# SOME METABOLIC EFFECTS OF GLUCOCORTICOIDS
# IN CATTLE AND RATS

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SOME METABOLIC EFFECTS OF GLUCOCORTICOIDS IN

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# CHAPTER I

## INTRODUCTION

In recent years the use of glucocorticoids as therapy for ketosis in dairy cattle has become widely employed. Their advantages lie in the ease of their administration and prolonged blood glucose-elevating properties when compared to many treatments currently employed. More recently synthetic hydrocortisone derivatives have become available for use in mammalian experiments. Many investigators have used laboratory animals to study changes in glucocorticoid and mineralocorticoid properties which accompany certain alterations in the cortisone and hydrocortisone molecule such as unsaturation, halogenation and methylation. Less is known of the physiological properties of these synthetic corticoids in ruminant animals. Because of the profound effects of synthetic steroids in laboratory animals, an experiment was designed to compare the effects of intramuscular (i.m.) administration of two dosages of four synthetic corticoids on blood glucose, leukocytes and plasma sodium and potassium in the lactating cow. The compounds chosen represented hydrocortisone which had been altered by unsaturation, fluorination, unsaturation and fluorination, or methylation.

Although protein hormones have been shown to be ineffective when administered orally to monogastric animals, steroid hormones may retain their activity even though subjected to digestive processes of the simple stomached animal. In the ruminant animal, however, any compound admin-

istered per os is subjected to attack by the microflora and microfauna of the rumen as well as the usual digestive enzymes. For this reason it seemed desirable to study the effects of these highly potent synthetic corticoids upon blood glucose, leukocytes and plasma sodium and potassium in the lactating cow, when administered orally. Nine-alpha fluoroprednisolone, which has been reported to possess the greatest glucocorticoid activity in laboratory animals, was chosen as the steroid for use in the oral study.

Following the study of oral and i.m. administration of glucocorticoids in ruminant animals, the need of more basic knowledge concerning the effects of these steroid hormones became apparent. In vitro studies have shown that the activity of a number of enzymes involved in the glycolytic and Krebs cycles are affected by glucocorticoid treatment. Among these enzymes are phosphorylase, various phosphatases, several transaminases and certain respiratory enzymes. Since liver is known to be one of the most metabolically active tissues in the animal body, its use in future experiments designed to study basic metabolic effects of glucocorticoid treatment seemed appropriate. A thorough search of the literature failed to uncover any experiments which had been conducted to study the effects of glucocorticoids on oxygen uptake by liver tissue. It is logical to assume that any treatment which would alter respiration in a given tissue might also change the rate at which various metabolites such as glycogen, glucose, lactic acid and inorganic phosphate were broken down and formed.

To study the possible effects of glucocorticoids upon tissue respiration, an experiment was planned in which the effect of i.m., oral and in vitro administration of 9a-fluoroprednisolone upon oxygen consump-

tion by rat liver homogenates was determined. This was followed by a series of more elaborate experiments, using prednisolone and hydrocortisone as treatments, in which the metabolism of glucose, glycogen, lactic acid, inorganic phosphate and protein was studied during incubation under aerobic and anaerobic conditions.

## CHAPTER II

## REVIEW OF LITERATURE

### Metabolic Effects of Adrenal Cortical Hormones

Carbohydrate Metabolism: The effect of glucocorticoids upon blood glucose has been extensively studied. Some of the early work leading to a more thorough knowledge of the effects of adrenocortical hormones was reported by Long et al. (67) who found that the administration of adrenocortical extract (ACE) or crystalline adrenal steroids to either fasted normal or adrenalectomized mice and rats was followed by a large increase in glycogen and slight hyperglycemia. Muscle glycogen was unaffected. Britton and Silvette (12) noted a reduction in blood glucose following adrenalectomy in cats and rats, whereas ACEs were shown to maintain blood glucose levels in adrenalectomized animals (12, 13, 79, 95). Ingle and associates (43, 44), using intact rats, were the first to demonstrate that prolonged glycosuria and hyperglycemia could be produced with cortisone. A cortisone-induced hyperglycemic response in cattle has been reported by Shaw and co-workers (85, 87, 88, 89). Others (77, 111) have found that in adrenalectomized rats the glycogen content of the liver can be restored with cortisone, and even overcompensated. Kerppola (47) reported the glycogen content of livers from rabbits treated with 10 to 20 mg. cortisone daily for 1 week to be increased 20 to 30 fold.

In some of the early work using synthetic corticoids, Liddle et al. (65) noted that, when hydrocortisone bears a chlorine or fluorine in the

9-alpha position, it not only becomes more potent than hydrocortisone in its ability to alter carbohydrate metabolism and in producing eosinopenia but also becomes more potent than desoxycorticosterone (DOC) in its sodium retaining activity. Using adrenalectomized dogs as the experimental animal, it was found that 9a-fluorohydrocortisone (9a FF) was 20 times more potent than hydrocortisone on eosinopenic response and 4.7 times more potent than DOC in retaining sodium. Using intact rats, Perlman and Tolksdorf (80) reported 1-dehydrocortisone (prednisone) and 1-dehydrohydrocortisone (prednisolone, $\Delta^1$F) to be 3 to 4 times more active than cortisone or hydrocortisone when compared on the basis of eosinopenic response, liver glycogen deposition and thymus involution following intramuscular (i.m.) injection of these compounds. West (115) found prednisone and prednisolone to be five times as potent as cortisone in their hyperglycemic effects in human subjects, whereas their sodium-retaining ability was not increased above that of the parent compound in adrenalectomized rats (80) or in human beings (15).

Stafford et al. (94), using adrenalectomized rats, studied the relative potencies of hydrocortisone, desoxycorticosterone (DOC), prednisolone, 9a-fluorohydrocortisone and 9a-fluoroprednisolone ($\Delta^1$ 9aFF) in causing deposition of liver glycogen and reduction of sodium excretion. In their ability to produce glycogen deposition and to retain sodium, the compounds had activity of the following magnitude:

| | Glycogen deposition | Sodium retention |
|---|---|---|
| DOC | -- | 1.0 |
| Hydrocortisone | 1.0 | very slight |
| Prednisolone | 2.9 | very slight |
| 9a-Fluorohydrocortisone | 12.6 | 5.0 |
| 9a-Fluoroprednisolone | 50.0 | 4.6 |

It is significant that 9a-fluoroprednisolone was found to be 4 times more potent than 9a-fluorohydrocortisone in the glycogen deposition test but

no more potent than this synthetic corticoid in the sodium retention test. Nine alpha-fluoropredisisolone has been reported to be 25 times as potent as hydrocortisone in the mouse liver assay (41). Shaw et al. (90) found 9a-fluorohydrocortisone to be 15 to 20 times as potent as hydrocortisone in normal and ketotic cows when evaluated on the basis of its effect on blood glucose, eosinophil numbers and milk production. The use of prednisolone in the treatment of ketosis in dairy cattle has been reported by Link et al. (66). Blood glucose values averaged 31 mg. per cent at the time of treatment but had risen to an average value of 62 mg. per cent 24 hours after i.m. injection of this steroid.

Lyster et al. (68), using adrenalectomized rats, compared the activity of 6-methyl prednisolone (6M$\Delta^1$F) and prednisolone and reported 6-methyl prednisolone to be 3 times as potent as prednisolone on glycogen deposition assay but the 2 compounds were similar in their failure to induce sodium retention. More recently, Neff and associates (74) studied the effects of i.m. administration of 9a-fluoroprednisolone in normal lactating dairy cattle. This steroid was found to be 10 times as potent as prednisolone in its ability to elevate blood glucose. It was further noted that two 100 mg. doses of 9a-fluorprednisolone 24 hours apart caused a definite decrease in serum sodium. However, the hypokalemia was temporary and serum potassium values returned to normal values shortly after cessation of treatment.

Lipid Metabolism

Brady et al. (10) studied the hormonal control of fatty acid synthesis using $C^{14}$ labeled acetate. It was found that the rate of incorporation of labeled acetate into long chain fatty acids by rat liver slices was

greater in adrenalectomized rats than in normal animals. Cortisone administration to normal rats resulted in a profound inhibition of fatty acid synthesis by liver slices. Similar findings have been reported by White and Engel (116) in which hydrocortisone injections into adrenalectomized rats resulted in a decrease in epididymal and liver fat. These findings disagree with earlier reports by Stoerch and Porter (96), who found that cortisone inhibited loss of fat from adipose tissues of adrenalectomized rats, and by Li et al. (64) who found that the carcass fat of ACTH injected hypophysectomized rats increased over that of non-injected hypophysectomized rats. Duhlin (30) reported that chronic injection of cortisone or hydrocortisone daily for 10 days caused a marked increase in liver fat in birds.

Protein Metabolism

An effect of adrenocorticoids on protein metabolism was first suggested by Long et al. (67) when it was noted that ACE caused an increase in nitrogen excretion concommitant with a large increase in liver glycogen. It was postulated that the increased protein catabolism could be the source of the newly formed carbohydrate. It was further suggested that one of the properties of cortical hormones is a stimulation of protein catabolism and that the increased carbohydrate level and nitrogen excretion following corticoid injection into animals is an expression of this effect. This property of promoting gluconeogenesis and increased nitrogen excretion has been verified many times (7, 14, 46, 76).

More recently, Welt et al. (114) studied the effect of adrenocortical hormones on gluconeogenesis by the use of $C^{14}$ labeled glucose. Normal and cortisone-treated anesthetized rats were infused with labeled glucose and

the rate of formation of glucose from sources not derived from the infused glucose was determined. Treatment with cortisone resulted in a 7 fold increase in the rate of gluconeogenesis over that of the normal rat in that a far smaller fraction of liver glycogen was formed from body glucose stores in the cortisone-treated than in normal rats.

Sodium and Potassium Metabolism

The pronounced effect of certain adrenocortical hormones (DOC and aldosterone) on body sodium and potassium values is well documented (26, 32, 67, 93, 105, 106). The effects of unsaturation and/or halogenation of the hydrocortisone molecule upon mineralocorticoid activity has been the subject of more recent research. An increase in sodium retaining properties of 9 alpha-halo derivatives of hydrocortisone when compared to unaltered hydrocortisone has been noted in dogs (99) and rats (9, 94). Swingle et al. (99) reported that 9a-fluorohydrocortisone was 4.5 to 9 times more potent than DOC in maintaining adrenaloctomized dogs. Stafford and associates (94) found that unsaturation of the hydrocortisone molecule did not enhance its mineralocorticoid activity but that this property was markedly increased by fluorination. These workers reported 9a-fluorhydrocortisone and 9a-fluoroprednisolone to be similar in their ability to cause sodium retention and to be 4 to 5 times as potent DOC.

Arons et al. (3) demonstrated that hydrocortisone had no effect on the exchange-ability of body sodium and potassium in a normal human female whereas DOC and 9a-fluorohydrocortisone both elevated plasma sodium values and decreased plasma potassium values. Bunin et al. (15) were unable to demonstrate any mineralocorticoid properties of prednisone and prednisolone when these compounds were administered to human subjects at the rate of

30 mg. per day for 12 days and 50 mg. daily for 24 days. Halogenated derivities of cortisone and hydrocortisone have been shown to possess marked mineralocorticoid activity in dogs when compared to the parent steroid, the increase in activity being of the magnitude of 50 to 200 fold (100).

Liddle et al. (65), using adrenalectomized dogs, found that when hydrocortisone bears a chlorine or fluorine in the 9 alpha position it becomes more potent than DOC in sodium retaining activity. Repeated injections of 9a-fluorohydrocortisone to ketotic cows have been reported to result in death, apparently from hypokalemia (91). It was further noted that 100 mg. of this synthetic corticoid depressed plasma potassium in cattle as much as 50 per cent in 24 hours.

## Enzyme Activity

The profound effect of adrenocortical hormones on protein metabolism has led to considerable work directed toward studying the changes in activity of those enzymes known to be involved in protein anabolism and catabolism.

Roberts (82), using adrenalectomized and unoperated rats, explained the mechanism of action of cortical hormones by the observation that these hormones labilized tissue protein which can be used for anabolism or catabolism depending upon the tissue requirements for protein at the moment. Brin and McKee (11) found an increase in transaminase activity in livers from rats treated twice daily for 4 days with 2 to 4 mg. of cortisone. A 2 to 5 fold increase in glutamic-pyruvic transaminase activity in livers of rats given cortisone or hydrocortisone subcutaneously for 4 consecutive days was reported by Rosen et al. (83, 84). The

glutamic-pyruvic transaminase activity of rat livers was found to be directly related to the protein content of the diet and could be markedly increased by fasting (84).

Lecroix and Leusen (58) found an increase in hepatic arginase activity in rats treated for 14 to 16 days, with a daily dose of 5 mg. of cortisone acetate. This treatment did not alter liver glutamic-oxalacetic transaminase activity but a decrease in hepatic succinic dehydrogenase and cytochrome oxidase activities were noted. Adrenalectomy significantly lowered dipeptidase activity of surviving rat diaphram whereas cortisone resulted in increased dipeptidase activity (98).

Knox and Auerback (51) noted a decrease in liver trypotophan peroxidase (TPO) activity in adrenalectomized rats. Cortisone administration caused an increase in liver TPO activity in both intact and adrenalectomized rats (51). This work was verified by Thomson and Mikuta (107) who studied the activity of hepatic TPO 6 hours after the injection of cortisone and hydrocortisone into adrenalectomized rats. Although both steroids caused an increase in activity, the effect of hydrocortisone was much more pronounced. Cortisone caused a many fold increase in TPO and picolinic carboxylase in livers from diabetic rats, however the increased activity of the former was evident 2 hours after cortisone administration whereas several days were required before any alteration in picolinic peroxidase activity was apparent (70). After adrenalectomy, the ability of rat kidney homogenates to oxidize proline was decreased whereas this ability was restored to normal by cortisone (47).

A significant increase in liver glucose-6-phosphatase activity in rats which had received 25 mg. cortisone i.m. daily for five days was reported by Weber et al. (112). It was proposed that this explained the

increase in blood glucose which follows cortisone administration, since this enzyme catalyzes the hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate. Other workers (59, 113) have also found that the liver homogenates from cortisone treated rats exhibited a marked increase in glucose-6-phosphatase activity, the increased enzymic activity being apparent in nuclear, mitochondrial and microsomal fractions of the homogenates (113). Other workers (4, 34) have shown an increase in glucose-6-phosphatase activity in liver slices from adrenalectomized rats treated with cortisone. The enzyme-stimulating response from cortisone administration was evident as early as two hours after treatment (4).

Froesch et al. (34) found that glucose-6-phosphatase activity was increased in a similar fashion in both liver and kidneys of intact rats in metabolic states associated with accelerated endogenous glucose production, such as in fasting. In adrenalectomized rats this response to fasting no longer occurred but could be restored by substitution therapy with cortisone. Kvam and Parks (52) noted that the marked increase in hepatic glucose-6-phosphatase and fructose-1, 6-diphosphatase activity observed after hydrocortisone administration in adrenalectomized rats is inhibited by treating the animals with the methionine antogonist, ethionine. This inhibition is, in turn, reversed by the administration of methionine. This is evidence that these increases represent enzyme formation involving the synthesis of new protein, since methionine had to be available before an increase in enzyme activity could be demonstrated.

Mokrasch et al. (72) found an increase in fructose-1,6-diphosphtase activity in liver slices taken from cortisone-treated rabbits. Diets low in glucose but rich in protein caused a similar increase in enzyme

activity. A decrease in liver phosphorylase activity of one-half to one-fifth of normal values following cortisone administration has also been reported (47).

Keppola and Pitkanen (49) studied the activity of several respiratory enzymes in the livers of normal and cortisone-treated rats of different ages and sex. The activity of cytochrome oxidase and DPN-cytochrome C reductase was decreased in old cortisone-treated rats whereas succinic dehydrogenase activity was unaltered in all groups. No cortisone-induced changes in the activities of the glycolytic enzymes were found.

Glycolysis and Respiration

Blecher and White (8) investigated the possible sites of action of adrenocortical hormones using lymphocyte suspensions from male rat thymuses. Oxidation of added alpha ketoglutarate was inversely related to the concentration of hydrocortisone or DOC. This suggests a possible direct action of adrenocortical hormones on oxidation in lymphoid cells. Overell et al. (78), using an experimental system designed for measuring the in vitro uptake of glucose by mouse skin, found that hydrocortisone depressed glucose uptake when added to the system in concentrations comparable to those which may occur in vivo.

In a second experiment, Blecher and White (8) noted that in rat lymphosarcoma homogenates the degree of anaerobic glucolysis represents a balance among a relatively low hexokinase activity, a high adenosine triphosphatase (ATPase) activity and a limited capacity for glycolytic regeneration of ATP. It was found that cortisone does not affect the hexokinase reaction directly but does affect it secondarily by a primary effect on the availability of ATP. It was suggested that a competition

exists between hexokinase and ATPase for the supply of ATP.

Grossfeld (40) used tissue cultures from mouse fibroblasts to study the effect of hydrocortisone upon respiration and aerobic glycolysis. In one experiment a single high dose of hydrocortisone (125 ug. per ml.) was added to cultured cells at the time of incubation in Warburg vessels and found to cause an inhibition of respiration coupled with a significant increase in aerobic glycolysis. It was suggested that the release of glycolysis may be due to the inhibition of respiration, in accordance with the Pasteur effect. In a second experiment, hydrocortisone, DOCA or cholesterol were added (1.50 ug. per ml.) to cultured cells at 96 and 24 hours before and at the time of incubation in Warburg flasks. In this instance hydrocortisone caused a significant increase in both respiration and aerobic glycolysis whereas DOCA or cholesterol did not affect respiratory or glycolytic rates.

Cortisone administered daily for 14 to 16 days to male rats at the rate of 5 mg. per 100 gm. body weight has been reported to cause only a slight decrease in endogenous respiration of cardiac muscle slices but the utilization of glucose and pyruvate as substrates was markedly impaired (57). Alpha ketoglutarate and succinate stimulated the oxygen consumption of the slices to the same extent in the cortisone group as in the control group (57). This indicates that at least one site for the impairment of glucose utilization during prolonged cortisone administration would probably be located at the level of the Krebs cycle between the pyruvate and alpha ketoglutarate steps. Clark and Pesch (22) reported that oxygen uptake by liver mitochondria from rats chronically treated with 2.5 mg. cortisone to be significantly less than in non-treated rats. It was also noted that cortisone inhibited mitochondrial oxidation of

alpha ketoglutarate, again indicating a partial block at some point in the Krebs cycle. Cortisone administered intraperitoneally (i.p.) to rats at the rate of 10 to 12.5 mg. per day for 10 to 16 days caused a significant decrease in respiration in heart (53, 54, 56) and diaphram slices (53, 54, 55, 56). Welt et al. (114) injected $C^{14}$ glucose into anesthetized cortisone-treated and untreated rats and found that the rate of oxidation of glucose to carbon dioxide was not affected by pretreatment of the rats with cortisone. Kerppola (48) and Kerppola and Pitkanen (49) have reported a decrease in oxygen uptake in liver mitochondria from rats previously treated daily for 7 days with 5.0 mg. cortisone. A simultaneous decrease in the P/O ratio was interpreted as evidence that cortisone may uncouple oxidative-phosphorylation.

Another criteria often used as an indicator of glycolytic and respiratory rates is inorganic phosphate level. Mills and Thomas (71) reported that inorganic phosphate concentrations in human plasma and whole blood were approximately the same and fell in a similar manner after intravenous (i.v.) injection of hydrocortisone, whereas DOCA injection produced no changes in phosphate concentrations.

## Miscellaneous Effects of Adrenocorticoids

The anti-inflammatory action of glucocorticoids and inflammatory action of mineralocorticoids are well documented (108). Duhlin (29) reported prednisolone, 9a-fluorohydrocortisone and 9a-fluoroprednisolone to be 3.1, 7.3 and 14.0 times as active respectively as hydrocortisone in their anti-inflammatory properties as determined by the cotton pellet implantation method. Lyster et al. (68), using adrenalectomized rats, compared the anti-inflammatory activity of 6-methyl prednisolone and

reported 6-methyl prednisolone to be twice as potent in this respect when measured by the granuloma pouch method.

The blood pressure elevating properties of hydrocortisone, 9a-fluorohydrocortisone and 9a-fluoroprednisolone have been investigated by Knowlton et al. (50). Nine-alpha fluorohydrocortisone and 9a-fluoroprednisolone were reported to be 5 and 10 times as potent respectively as hydrocortisone in this respect.

The effect of adrenocortical hormones on white blood cell numbers has been extensively studied. Zak (121) reported the administration of ACTH, cortisone, prednisone and prednisolone to rats, mice, rabbits and dogs regularly induced a fall in the absolute numbers of circulating eosinophils and lymphocytes and an increase in neutrophil numbers. Adenalectomy caused a reversal of these findings. A decrease in circulating eosinophils following ACTH administration has been reported in the cow (42) and dog (69).

## Theories of Increased Activity of Synthetic Corticoids

Several attempts have been made to explain the increase in potency which occurs when the hydrocortisone molecule is altered by fluorination and/or unsaturation. Nugent et al. (76) reported the plasma half time of hydrocortisone to be 113 minutes after I.V. administration to normal human subjects compared to 204 and 241 minutes for prednisolone. Collins et al. (25) found the plasma half life of prednisolone in the dog was 1.37 times that of hydrocortisone and suggested that the increased potency of prednisolone could be explained by decreased rate of metabolism of the compound by the liver. The rates of inactivation of hydrocortisone analogues by rat liver enzyme systems have been studied by Glenn et al.

(38) who found a fairly close correlation between increased biological activity (liver glycogen deposition) and the increased resistance of these analogues to enzymatic attack in the liver. This supports the hypothesis that increased activity of steroid analogues may result from a slower rate of their metabolism by the liver.

Pasteur and Crabtree Effects

The phenomenon which is now known as the "Pasteur effect" was first described when Pasteur noted that fermentative processes were inhibited under aerobic conditions. Recently, numerous attempts have been made to explain the Pasteur effect. Johnson (46) proposed the following mechanism for the Pasteur effect: In both aerobic and anaerobic phosphorylative processes, inorganic phosphate and a phosphate acceptor are essential reactants. In their absence neither glycolysis nor oxidation could proceed. The Pasteur effect could then be due to the cessation or reversal of glycolysis which takes place when concentrations of inorganic phosphate and phosphate acceptors become low because of the phosphorylative oxidations which occur in the presence of oxygen.

Gatt et al. (35) proposed that limiting amounts of ADP are shared by intra- and extramitochondrial systems and that it shuttles back and forth between them. Thus rapidly respiring mitochondria may deprive the glycolytic system of essential ADP, or vise versa. It was also proposed that pyruvate could act as the phosphate acceptor in which case inorganic phosphate would be taken up with an accumulation of phospho-enolpyruvate and other phosphate esters. Lardy and Wellman (60) in an attempt to determine the importance of phosphate acceptors in controlling rates of oxidation in enzyme systems found that proline, glutamate, citrate,

pyruvate, alpha keto glutarate, succinate, malate and beta hydroxybutyrate were more rapidly oxidized in the presence of phosphate acceptor systems such as adenylic acid, ADP, creatine plus its phosphorylating enzyme and glucose plus hexokinase. Further work by Lardy and Wellman (61) supported the concept that rates of oxidation in mitochondria are limited by the rates at which phosphate is transferred from the $Y \sim PO_3$ complex to acceptors. Agents which cause liberation of inorganic phosphate (such as 2,4-dinitrophenol) from the $Y \sim PO_3$ also accelerate mitochondrial respiration.

Terner (102) explained the Pasteur effect in terms of a competition between oxygen and pyruvate for the hydrogen of reduced DPN and related this to the relative capacity of certain tissues for glycolysis and oxidative processes. More recently Terner (103, 104) has demonstrated a Pasteur effect in cell-free suspensions of mammary gland in which glucose and yeast hexokinase were used as the phosphate acceptor system. The addition of p-nitrophenol caused an additional 50% increase in respiration.

Later work by Gatt et al. (35), using a reconstructed system consisting of glycolytic enzymes and respiring mitochondria, demonstrated that oxidation of glutamate by mitochondria is stimulated in the presence of a phosphate acceptor system such as glucose, hexokinase and catalytic amounts of ADP. At low ADP levels the addition of glucose and the complete glycolytic system resulted in a pronounced inhibition of mitochondrial respiration. There was no inhibition of respiration at high ADP concentration or when glycolysis was prevented by the omission of a single glycolytic enzyme. Dinitrophenol completely released the inhibition. Aisenberg and Potter (2), studying specific mechanisms of the

Pasteur reaction, concluded the underlying cause of this phenomenon was not competition for inorganic phosphate or phosphate acceptors or mitochondrial resynthesis of glucose but rather the oxidative system, in some manner, inhibited the phosphohexokinase and hexokinase reactions. The inhibition of glycolysis by respiring mitochondrial was shown to involve glucose disappearance as well as lactate accumulation.

Wu and Racker (117) presented data in favor of the hypothesis that inorganic phosphate is the limiting factor in glycolysis and respiration. These workers concluded that glycolytically produced ATP is used more efficiently for glucose phosphorylation than ATP generated in mitochondria. Since DNP alone increases both glucose utilization and inorganic phosphate levels, and since the inorganic phosphate level aerobically is well below saturation of ascites tumor glyceraldehyde phosphate dehydrogenase, it was proposed that the glycolytic regeneration of ATP is limited by the availability of inorganic phosphate. Later studies by Gatt and Racker (36) and Wu and Racker (118) revealed that with limiting adenine nucleotide concentrations and excessive inorganic phosphate, addition of mitochondria to a glycolyzing system resulted in a pronounced inhibition of lactic acid production. However, glucose utilization was unimpaired and fructose 1,6 diphosphate accumulated. With limiting inorganic phosphate concentrations ATPase had to be added to maintain respiration and glycolysis.

In 1929 Crabtree (27) reported a 12% lowering of respiration in animal tissues incubated in glucose containing Ringers when compared to xylose containing Ringers. Shorr et al. (92), using excised renal tissue, failed to demonstrate a Crabtree effect when glucose was added to the incubating media. Elliot and Baker (31) were able to confirm the

Crabtree effect in tumor tissue when it was found that the addition of glucose caused a retardation of respiration which was accompanied by an increase in the R.Q. Rosenthal et al. (85) demonstrated that the inhibition of respiration by the addition of glucose occurs also in normal tissues such as rat renal papillae and bovine articular cartilage. These workers postulated that the addition of glucose causes a decrease in oxygen consumption in such tissues in which the hexose is not oxidized with the same velocity as the cellular substrates, the oxidation of which it replaces.

Cohen (24) reported a failure to detect a Crabtree effect in adult rabbit retina, whereas study of the postnatal development of the retina revealed that prior to the formation of the sensory elements of the visual cells glucose inhibits respiration as much as 40 per cent. This inhibition was abolished by dinitrophenol.

Gatt et al. (35) and Gatt and Racker (37), using a reconstructed system of glycolytic enzymes and liver mitochondria, found at low ADP levels the addition of glucose and the complete glycolytic system resulted in a pronounced inhibition of mitochondrial respiration. No respiratory inhibition resulted when ADP levels were high. With limiting inorganic phosphate concentration a Crabtree effect was observed provided that suitable amounts of ATPase were added to maintain respiration. These workers concluded that an active glycolytic system may deprive respiration of essential ADP. Wu and Racker (117, 120) reported the addition of glucose to washed ascites tumor cells resulted in a marked fall in intracellular inorganic phosphate as well as a decrease in respiratory rate. Determination of intracellular concentration of adenine nucleolides, hexose phosphates and inorganic phosphate pointed to inorganic

phosphate as the limiting factor in glycolysis and respiration.

Using Ascites cells, Chance and Hess (19) noted a 3 phase respiratory response to glucose addition. A 2 and 3 fold immediate stimulation of respiration which lasted for about 2 minutes was followed by an intense inhibition of respiration which, in turn, lasted about 3 minutes after which the inhibition diminished to a level characteristic of the Crabtree effect. It was suggested that ADP produced during phosphorylation of the added glucose was responsible for the activation of the mitochondrial electron transfer system and the reverse process occurred during the inhibited phase of oxygen metabolism. Spectroscopic studies showed that the response of the Ascites cell suspension to the addition of excess glucose was typical of the response of the mitochondrial respiratory chain to an increase in phosphate or phosphate acceptor.

# CHAPTER III

## MATERIALS AND METHODS

### EXPERIMENT I:

Series 1. Eight multiparous, nonpregnant, lactating Holstein-Fresian cows, grazing on Bermuda grass pasture and averaging 940 pounds body weight, were randomly assigned to one of the following treatments: 50 mg. prednisolone, 100 mg. prednisolone, 25 mg. 9a-fluorohydrosortisone, 100 mg. 9a-fluorohydrocortisone, 25 mg. 9a-fluoroprednisolone, 100 mg. 9a-fluoroprednisolone, 25 mg. 6-methyl prednisolone or 100 mg. of 6-methyl prednisolone. The compounds were in aqueous suspension and were the alcoholic form, except for methyl prednisolone, which was in the acetate form. All compounds were injected i.m. in the gluteal region. Blood samples were drawn from the jugular vein 72, 24 and 0 hours prior to the injection to obtain pre-treatment values and at 24, 48, 96 and 144 hours postinjection, to determine treatment effects. This procedure was replicated 6 times at 2 week intervals, using the same 8 animals for each replicate.

Blood glucose, plasma sodium and potassium, total leukocytes and differential leukocytes were determined for each blood sample. Blood glucose was determined by the method described by Nelson (75). Plasma sodium and potassium were determined by the Department of Biochemistry, Oklahoma State University, with a Perkin-Elmer flame photometer, using a 1000 p.p.m. lithium internal standard. Total leukocytes were deter-

mined by the usual dilution method whereas blood smears to be used for differential counts were prepared by staining with Wright's stain.

Series 2: Four multiparous, nonpregnant, lactating Holstein-Fresian cows grazing on Bermuda grass pasture were randomly assigned to receive either 100 or 200 mg. boluses of 9a-fluoroprednisolone by mouth. Blood samples were drawn by jugular vein puncture, 24 and 0 hours before treatment and 24, 48 and 72 hours after treatment. Four replicates of this procedure were conducted, using the same animals for each replicate. Blood samples were subjected to the same analyses as in Series 1.

EXPERIMENT II:

Mature female albino rats weighing approximately 350 gm. were randomly selected to receive 0.16 mg. 9a-fluoroprednisolone i.m., 0.32 mg. 9a-fluoroprednisolone orally or to remain untreated and serve as controls. Blood samples to be used for blood glucose determinations were taken by cardiac puncture at the time of treatment and 5 hours post-treatment. Following treatment all rats were returned to their original pens and allowed free access to food and water. Five hours after treatment each animal was killed by decapitation. An incision was quickly made into the abdominal cavity and the left half of the cystic lobe of the liver removed. The liver tissue was immediately placed in 9 ml. of cold (0 to 5 degrees C) isotonic potassium chloride and homogenized for approximately 30 seconds with a chilled Potter-Elvehjem homogenizer. One and one-half ml. of the homogenate was then pipetted into a standard Warburg flask which had previously been prepared by placing in it one and one-half ml. of phosphate buffer solution. The phosphate buffer was prepared by dissolving 1.49 gm. potassium chloride, 0.203 gm. magnesium chloride and 0.136 gm. potassium acid phosphate in water, adjusting

to pH 7.5 with potassium hydroxide and diluting to 150 ml. with distilled water. To study the effects of the addition of the corticoid directly to homogenates another series of flasks was prepared to which 0.04 mg. 9a-fluoroprednisolone had previoulsy been added to the phosphate buffer. Liver homogenates from untreated rats were introduced into these flasks. Two tenth ml. of ten per cent KOH was placed in the center well of all Warburg flasks to absorb any carbon dioxide released during incubation.

The Warburg flasks were then placed on manometers and oxygenated for 5 minutes with pure oxygen. Following oxygenation, the flasks were immediately placed in a constant temperature water bath at 37°C. and allowed to equilibrate for 8 minutes. Readings to determine oxygen uptake were taken at 10 minute intervals for 30 minutes. Dry weight of the liver tissue in each homogenate was determined by placing 1 ml. of the homogenate in a tared planchet and drying in an oven at approximately 100°C for one hour.

To study the levels of certain other metabolites during incubation a second series of experiments using mature female albino rats was conducted. The experimental animals were divided into two groups, one group receiving a single i.m. injection of 5.0 mg. prednisolone, whereas the other group served as the control and remained untreated. Blood samples were collected just prior to treatment and again 5 hours following treatment and were used to determine blood glucose, lactic acid and inorganic phosphate. Liver homogenates were prepared as before but samples of each homogenate were removed prior to and following incubation and used to determine liver glycogen, glucose, lactic acid, protein and inorganic phosphate. Oxygen uptake was determined as before. In one phase of the experiment, however, the flasks were flushed with nitrogen instead of

oxygen to determine the various metabolite levels when incubation was carried out under anaerobic conditions.

To ascertain if the administration of natural corticoids produced effects similar to synthetic corticoids, another experiment was conducted using intramuscularly injected hydrocortisone at the level of 5 mg. or 50 mg. as treatments. Oxygen consumption during 30 minutes of incubation was determined. Finally, to determine if liver homogenates from chronically treated rats reacted in a manner similar to those treated with a single injection, another group of rats was treated with 5.0 mg. prednisolone for 7 days prior to removing the liver sample. Oxygen uptake and liver inorganic phosphate levels were compared with those following single prednisolone injections. To ascertain if carbohydrate levels in liver homogenates may affect oxygen consumption, a series of Warburg flasks were prepared for incubation by adding glucose to the phosphate buffer prior to innoculation with corticoid-treated and nontreated rat liver homogenates. Glucose was added at the level of 1.5 mg. per ml. of homogenate. The ability of 2,4 dinitrophenol (DNP) to uncouple oxidative-phosphorylation was studied by adding this compound to the Warburg vessels. Final DNP concentration was $3 \times 10^{-4}$ M.

Blood glucose was determined by the method described by Nelson (75). Liver glucose values were determined by first preparing a protein-free filtrate using 0.3 N barium hydroxide and 10 per cent zinc sulfate. The protein-free filtrate was then subjected to identical procedures as used in blood glucose determination. Liver glycogen was isolated by boiling equal quantities of liver homogenate and 30 per cent potassium hydroxide for 15 to 20 minutes, followed by precipitation of glycogen with 95 per cent ethyl alcohol. The glycogen was hydrolyzed by boiling in 5 ml. 2 N

sulfuric acid for 3 to 4 hours. After neutralization with 1 N sodium hydroxide, the hydrolyzed glycogen was analyzed for glucose, again using the method described by Nelson (75). Protein was determined by the biuret test. Liver and blood inorganic phosphate was determined by the method of Fiske and SubbaRow (33). Lactic acid was ascertained by the method described by Barker and Summerson (6). To determine if treatment effects existed, all data were subjected to Student's "t" test.

## CHAPTER IV

## RESULTS AND DISCUSSION

EXPERIMENT I.

The average blood glucose values of all replicates prior to and following the i.m. injection of the various steroids to lactating dairy cattle are shown (Table I) and depicted graphically (Fig. 1 and 2). Fifty and 100 mg. of prednisolone produced significant increases ($P \doteq 0.05$) in blood glucose at 24 hours postinjection, when compared with those obtained at the time of injection. However, at 48 hours postinjection, these values had returned to essentially preinjection levels. At the 25-mg. dosage, 9a-fluorohydrocortisone did not produce a significant change in blood glucose, whereas, at the 100-mg. level, this compound produced very highly significant increases. This elevation was transitory, however, since 48-hour blood samples contained nearly normal quantities of glucose. The most potent compound with respect to its ability to elevate blood glucose was 9a-fluoroprednisolone. Both 25- and 100-mg. dosages of this material produced highly significant increases 24 and 48 hours postinjection and, at 96 hours, the higher dosage still maintained highly significant values. The methylated corticoid did not alter blood glucose values significantly at either dosage. The maximum blood glucose elevation following the administration of the various compounds is shown graphically (Fig. 3).

At both dosages, prednisolone and 9a-fluoroprednisolone produced

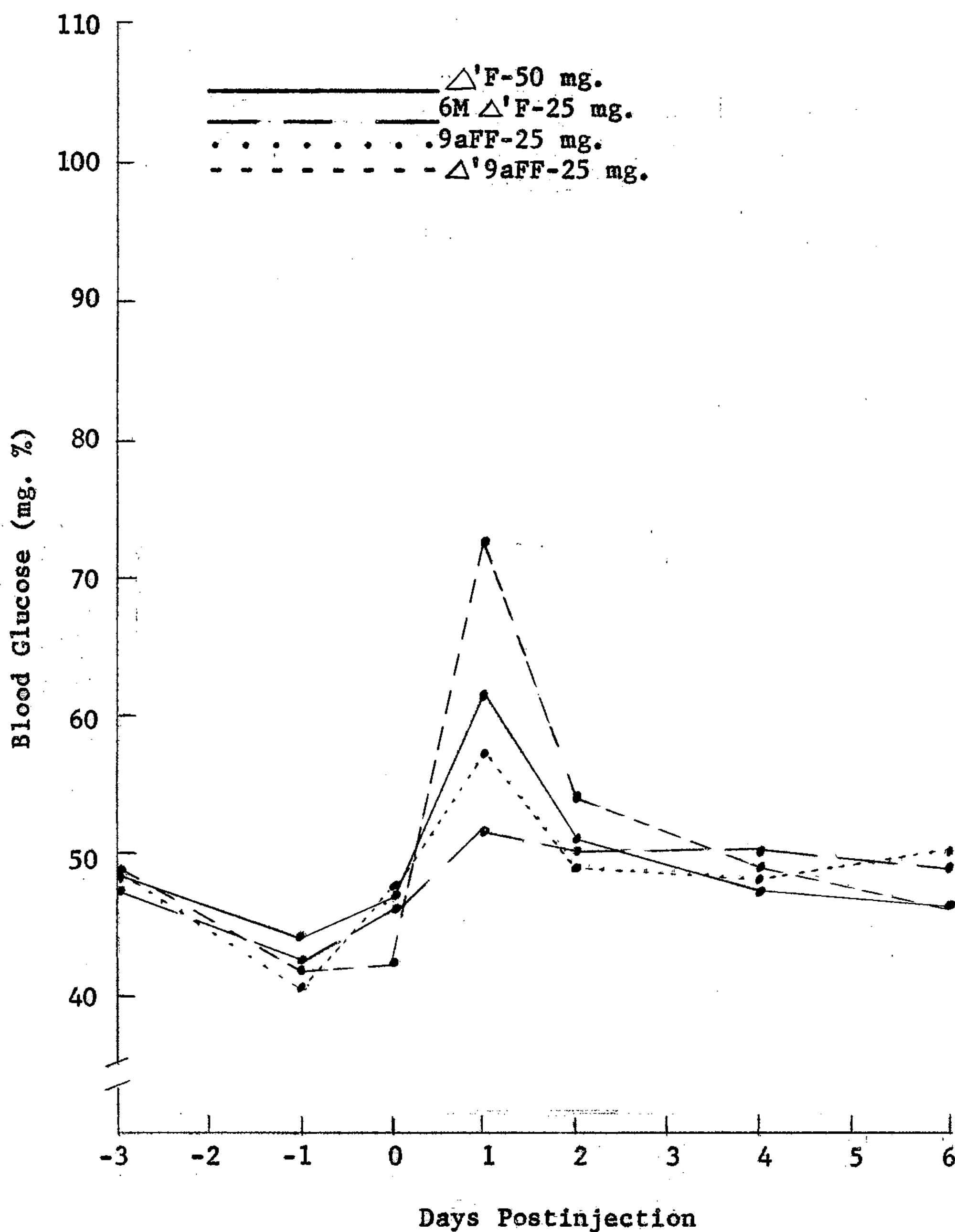



Figure 1. Average blood glucose (mg. %) prior to and following i.m. injection of 4 synthetic corticoids.

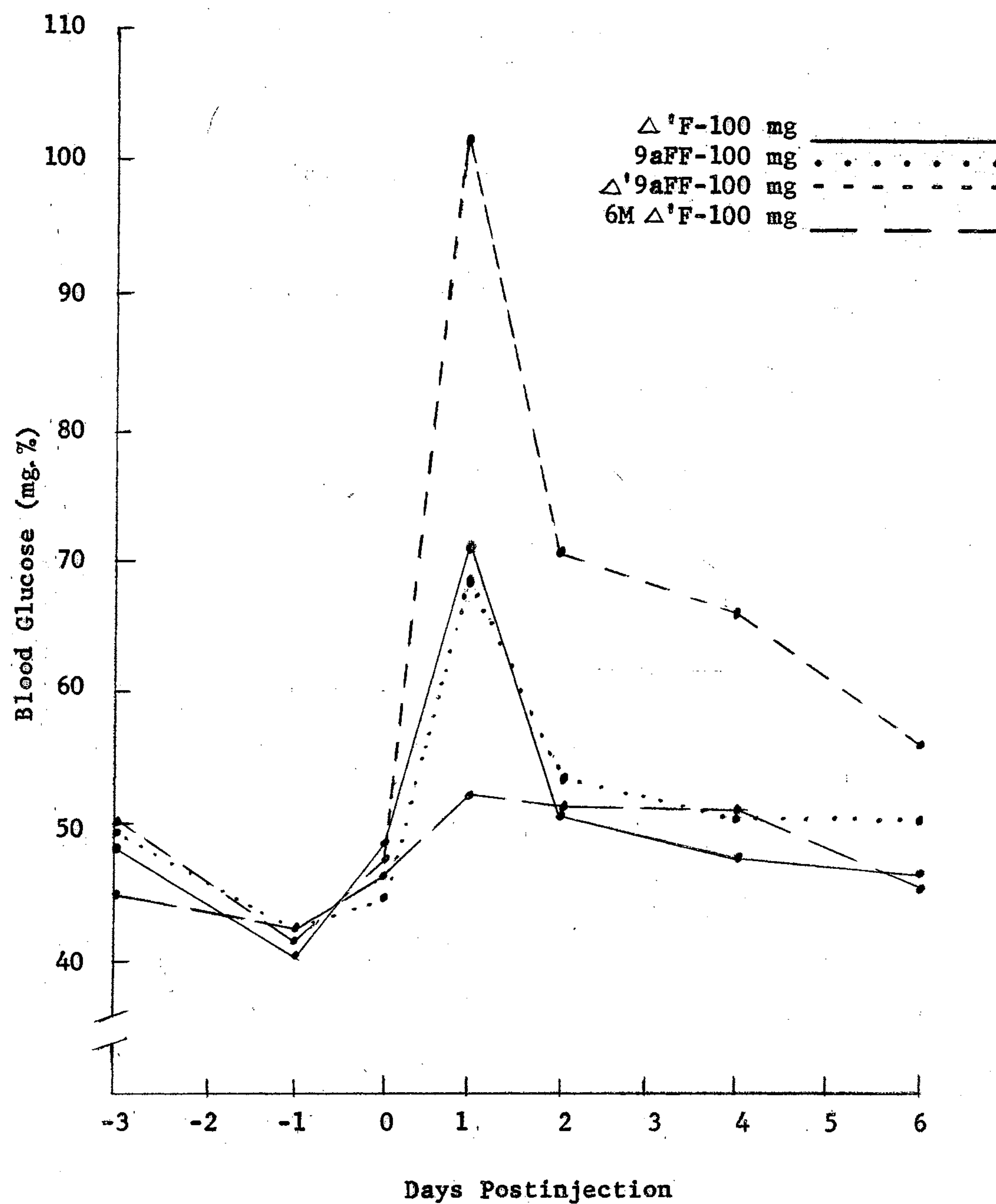



Figure 2. Average blood glucose (mg. %) prior to and following i.m. injection of four synthetic corticoids.

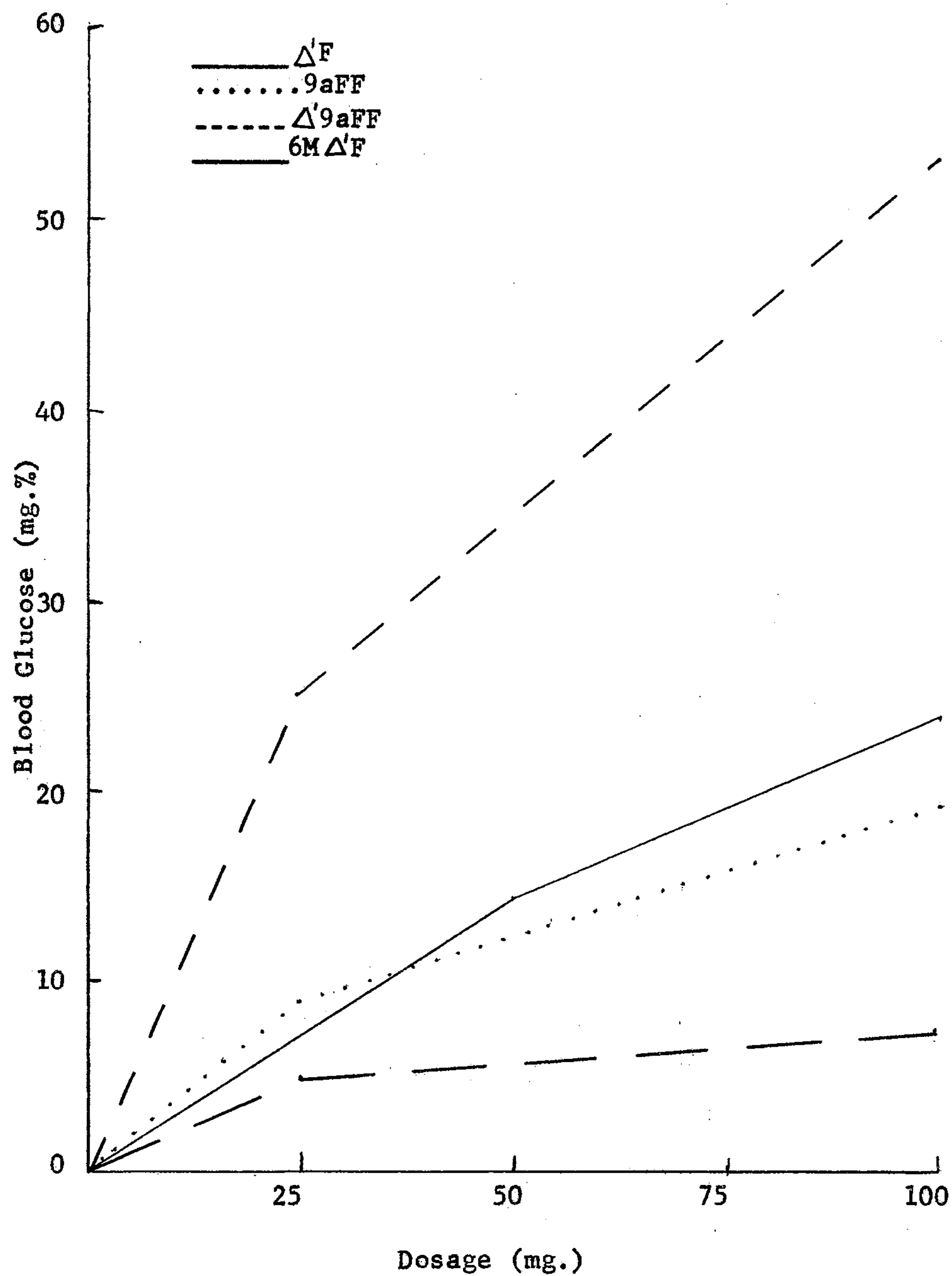



Figure 3. Maximum blood glucose elevation (mg. %) following i.m. injection of 4 synthetic corticoids.

TABLE I

AVERAGE BLOOD GLUCOSE (MG. %) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta^1$F-50 | 48.0 | 44.5 | 47.5 | 61.8* | 51.2 | 47.8 | 46.8 |
| $\Delta^1$F-100 | 48.0 | 40.3 | 48.3 | 71.3* | 50.8 | 47.3 | 46.5 |
| 9aFF-25 | 48.0 | 43.2 | 47.8 | 57.3 | 49.3 | 48.2 | 50.2 |
| 9aFF-100 | 49.8 | 43.2 | 44.8 | 68.8** | 53.5 | 50.3 | 50.0 |
| $\Delta^1$ 9aFF-25 | 48.6 | 43.4 | 43.8 | 73.7# | 54.1# | 49.1 | 46.8 |
| $\Delta^1$ 9aFF-100 | 50.0 | 42.5 | 47.0 | 103.2** | 70.3# | 66.8# | 56.0 |
| 6M $\Delta^1$ F-25 | 47.5 | 43.7 | 46.5 | 52.8 | 50.4 | 51.0 | 49.5 |
| 6M $\Delta^1$ F-100 | 45.2 | 42.7 | 46.0 | 52.5 | 51.1 | 50.3 | 46.1 |

*P = 0.05 #P = 0.01 **P = 0.001

significant increases in leukocyte numbers 24 hours postinjection (Table II). One hundred milligrams of 9a-fluoroprednisolone produced the most prolonged response, since 96-hour samples contained significantly more leukocytes when compared to preinjection blood samples. Although 9a-fluorohydrocortisone and 6-methyl prednisolone caused a slight increase in leukocyte numbers this rise was not statistically significant.

TABLE II

AVERAGE LEUKOCYTE NUMBERS (THOUSANDS/$MM^3$) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta^1$F-50 | 9.9 | 8.4 | 8.8 | 11.8* | 9.7 | 8.2 | 7.2 |
| $\Delta^1$F-100 | 7.8 | 8.0 | 7.9 | 11.1* | 9.1 | 7.1 | 7.5 |
| 9aFF-25 | 7.4 | 8.2 | 9.5 | 10.5 | 8.2 | 6.9 | 6.7 |
| 9aFF-100 | 7.8 | 9.1 | 8.9 | 11.2 | 10.0 | 8.1 | 7.7 |
| $\Delta^1$9aFF-25 | 8.4 | 9.3 | 9.6 | 13.2* | 10.8 | 8.8 | 8.8 |
| $\Delta^1$9aFF-100 | 7.9 | 8.7 | 8.1 | 15.5* | 11.8* | 12.1* | 9.1 |
| 6M $\Delta^1$ FF-25 | 8.7 | 8.2 | 8.0 | 9.4 | 8.1 | 7.6 | 7.3 |
| 6M $\Delta^1$ FF-100 | 8.3 | 9.2 | 7.8 | 10.1 | 10.0 | 8.3 | 8.1 |

*P = 0.05

To determine whether the increase in leukocyte numbers was due to an increase in all types of white cells or to one or more specific cell types, differential counts were performed. These values, and total leukocyte numbers, were used to compute the average total number of each type of white cells per cubic millimeter of blood. It was evident (Table III) that circulating lymphocte numbers were not altered by any of the treatments. The average numbers of eosinophils prior to and following the various treatments are also shown (Table IV). Although all compounds with the exception of 6 methyl prednisolone produced what appeared to be various degrees of eosinopenia, at no time were these changes significant. All compounds caused increases in neutrophil numbers (Table V) at 24 hours postinjection, although the changes produced by the lower dosage of 9a-fluorohydrocortisone and 6 methyl prednisolone were not significant. Forty-eight hours after treatment, only those animals treated with the higher dosage of 6 methyl prednisolone and 9a-fluoroprednisolone still retained a significant increase in curculating neutrophils. The most prolonged effect on neutrophil numbers was caused by 100 mg. of 9a-fluoroprednisolone, since significant increases were still evident 96 hours after treatment.

TABLE III

AVERAGE LYMPHOCYTE NUMBERS (THOUSANDS/MM$^3$) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 3 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta^1$ F-50 | 5.4 | 4.1 | 5.2 | 4.7 | 5.0 | 4.8 | 4.2 |
| $\Delta^1$ F-100 | 4.7 | 4.6 | 4.5 | 4.7 | 4.1 | 4.2 | 4.6 |
| 9aFF-25 | 4.2 | 4.9 | 5.5 | 4.9 | 4.6 | 3.7 | 3.9 |
| 9aFF-100 | 4.1 | 4.6 | 5.1 | 5.3 | 5.8 | 4.6 | 4.9 |
| $\Delta^1$ 9aFF-25 | 4.0 | 4.7 | 5.0 | 5.6 | 5.3 | 4.6 | 4.7 |
| $\Delta^1$ 9aFF-100 | 4.5 | 4.6 | 4.7 | 4.2 | 4.5 | 4.7 | 4.8 |
| 6M $\Delta^1$ FF-25 | 4.5 | 4.5 | 4.6 | 4.6 | 4.9 | 4.1 | 4.3 |
| 6M $\Delta^1$ FF-100 | 5.0 | 5.1 | 4.3 | 4.8 | 4.6 | 4.2 | 4.7 |

TABLE IV

AVERAGE EOSINOPHIL NUMBERS (HUNDREDS/$MM^3$) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta'$ F-50 | 4.2 | 4.4 | 6.2 | 5.1 | 8.1 | 4.7 | 6.5 |
| $\Delta'$ F-100 | 5.3 | 7.1 | 8.2 | 1.5 | 6.8 | 4.5 | 5.0 |
| 9aFF-25 | 3.8 | 3.7 | 8.1 | 4.9 | 5.7 | 6.7 | 6.7 |
| 9aFF-100 | 4.7 | 8.1 | 5.2 | 3.7 | 7.1 | 5.4 | 7.5 |
| $\Delta'$ 9aFF-25 | 9.9 | 9.2 | 9.4 | 6.5 | 8.2 | 9.9 | 8.0 |
| $\Delta'$ 9aFF-100 | 5.9 | 7.6 | 8.6 | 1.4 | 2.2 | 4.1 | 5.9 |
| 6M $\Delta'$ FF-25 | 9.1 | 6.4 | 5.5 | 6.0 | 7.9 | 8.1 | 6.6 |
| 6M $\Delta'$ FF-100 | 6.5 | 8.3 | 5.8 | 7.8 | 9.3 | 8.7 | 9.2 |

TABLE V

AVERAGE NEUTROPHIL NUMBERS (THOUSANDS/$MM^3$) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta'$ F-50 | 3.9 | 3.6 | 2.8 | 6.2** | 3.6 | 2.8 | 2.3 |
| $\Delta'$ F-100 | 2.3 | 2.6 | 2.6 | 6.1** | 3.5 | 2.2 | 2.2 |
| 9aFF-25 | 2.7 | 2.9 | 3.0 | 4.8 | 2.8 | 2.4 | 2.1 |
| 9aFF-100 | 2.9 | 3.4 | 3.2 | 5.3* | 3.4 | 2.9 | 2.2 |
| $\Delta'$ 9aFF-25 | 3.2 | 3.2 | 3.5 | 6.6* | 4.5 | 3.0 | 3.0 |
| $\Delta'$ 9aFF-100 | 2.5 | 3.0 | 2.6 | 10.9** | 7.0** | 4.2* | 3.5 |
| 6M $\Delta'$ FF-25 | 2.9 | 2.9 | 2.7 | 4.0 | 2.2 | 2.4 | 2.2 |
| 6M $\Delta'$ FF-100 | 2.5 | 3.0 | 2.5 | 4.4** | 4.4* | 3.0 | 2.3 |

*P = 0.05 **P = 0.01

Average plasma sodium and potassium levels before and after treatment are indicated (Table VI). No changes in either sodium or potassium values were evident in postinjection blood samples when compared with preinjection samples. At no time did any of the animals show objective clinical signs, such as anorexia and muscular weakness, which

are usually associated with hypokalemia.

The increased potency of these hydrocortisone analogues over the parent compound may be due to their resistance to metabolic degradation. Collins et al. (25) found the half-life of prednisolone to be 1.37 times that of hydrocortisone and suggested that, since it was more slowly metabolized, this prolonged half-life may explain, in part, the enhanced potency of this steroid. Glenn et al. (38) reported that for analogues active as glucocorticoids, a fairly close correlation existed between increased biological activity and increased resistance to enzymatic attack in the liver.

TABLE VI

AVERAGE PLASMA SODIUM AND POTASSIUM LEVELS (MG %) PRIOR TO AND FOLLOWING THE ADMINISTRATION OF FOUR SYNTHETIC CORTICOSTEROIDS

| Ion | Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|---|
| Na | $\Delta^1$F-50 | 312 | 317 | 314 | 316 | 333 | 312 | 311 |
| Na | $\Delta^1$F-100 | 317 | 305 | 315 | 317 | 321 | 309 | 314 |
| Na | 9aFF-25 | 318 | 310 | 318 | 315 | 319 | 308 | 315 |
| Na | 9aFF-100 | 316 | 311 | 314 | 320 | 316 | 310 | 317 |
| Na | $\Delta^1$ 9aFF-25 | 318 | 311 | 320 | 321 | 316 | 318 | 316 |
| Na | $\Delta^1$9aFF-100 | 313 | 311 | 314 | 318 | 320 | 309 | 318 |
| Na | 6M $\Delta^1$ F-25 | 321 | 311 | 310 | 312 | 306 | 306 | 311 |
| Na | 6M $\Delta^1$ F-100 | 320 | 305 | 312 | 320 | 318 | 302 | 312 |
| K | $\Delta^1$F-50 | 17.7 | 14.5 | 17.6 | 18.2 | 18.3 | 16.7 | 17.7 |
| K | $\Delta^1$F-100 | 17.0 | 17.0 | 18.2 | 18.3 | 18.9 | 17.7 | 17.5 |
| K | 9aFF-25 | 17.3 | 17.4 | 17.8 | 17.6 | 17.3 | 17.7 | 16.3 |
| K | 9aFF-100 | 17.4 | 18.1 | 17.1 | 16.5 | 16.9 | 17.5 | 16.0 |
| K | $\Delta^1$ 9aFF-25 | 17.4 | 17.6 | 16.6 | 17.3 | 18.2 | 17.3 | 17.6 |
| K | $\Delta^1$9aFF-100 | 16.4 | 17.8 | 17.8 | 17.2 | 16.4 | 15.8 | 16.3 |
| K | 6M $\Delta^1$ F-25 | 17.7 | 16.9 | 17.4 | 17.5 | 18.4 | 18.4 | 17.7 |
| K | 6M $\Delta^1$ F-100 | 17.2 | 17.4 | 17.7 | 18.8 | 18.3 | 17.6 | 16.5 |

The blood glucose responses following the administration of the four synthetic compounds indicates that 9a-fluoroprednisolone is the most

potent and the most prolonged in this respect. Similar responses in blood glucose values were manifested by the administration of 100 mg. of prednisolone and 9a-fluorohydrocortisone, and by 25 mg. of 9a-fluoropre-dnisolone, indicating that prednisolone and 9a-fluorohydrocortisone are about equal in their hyperglycemia-producing properties, whereas 9a-fluoropresnisolone is approximately four times as potent as either of these.

Link et al. (66) found an average increase in blood glucose of 30 mg. per 100 ml. over a 24 hour period when 100 mg. of prednisolone was administered to ketotic dairy cattle. This increase is slightly greater than that found in the normal animal but is not too surprising in view of the fact that ketotic cows usually exhibit a marked hypoglycemia. The blood glucose-elevating properties of 9a-fluorohydrocortisone have been reported to be 15 to 20 times as great as hydrocortisone in the cow (91).

The eosinopenic response to the administration of adrenal hormones and to stressors is well known. The decrease in circulating eosinophils following ACTH administration has been suggested as a possible method for evaluating adrenal cortical function in the cow (42) and dog (69). In the cow, the mild eosinopenia but marked neutrophilia reported herein following the administration of hydrocortisone analogues indicated that the neutrophilic response may be a more reliable indicator of adrenal function than decreased eosinophil numbers, although the compounds used in this study are not natural corticosteroids.

Some confusion exists on the effects and role of mineralocorticoids in cattle. Carlstrom (16) reported an increase in plasma potassium values following the administration of adrenal cortical extract or

desoxycorticosterone to ketotic cows, whereas Shaw (91) found that the administration of 100 mg. of 9a-fluorohydrocortisone to keotic cows depressed plasma potassium levels in normal cattle as much as 50 per cent in 24 hours. The effects of adrenal cortical hormones on plasma potassium levels in normal cattle have been reported to be irregular (16). Neff and co-workers (74) reported a marked decrease in serum potassium values when 9a-fluoroprednisolone was administered i.m. to normal lactating dairy cows whereas Davidson et al. (28) found that serum potassium values tended to undulate following the oral administration of this steroid to normal cattle and followed no distinct pattern which could be attributed to the therapy. In the present study, the failure to detect any changes in plasma sodium or potassium levels following the administration of synthetic corticoids does little to clarify the role of mineralocorticoids in cattle, and further study is needed.

The effects of oral administration of 9a-fluoroprednisolone on blood glucose values are shown (Table VII and Fig.4 ). Blood samples contained significantly more glucose 24 hours after oral administration of 100 mg. and 200 mg. of this steroid when compared to pretreatment blood samples. Forty-eight hours after treatment a hyperglycemia was no longer evident, blood glucose values being almost identical with pretreatment values. The animals receiving 200 mg. of the corticoid responded with a somewhat higher average blood sugar level at 24 hours than those receiving 100 mg. of the compound. However, due to wider sample variation, no greater significance could be attached to the increased hyperglycemic response to the higher dosage.

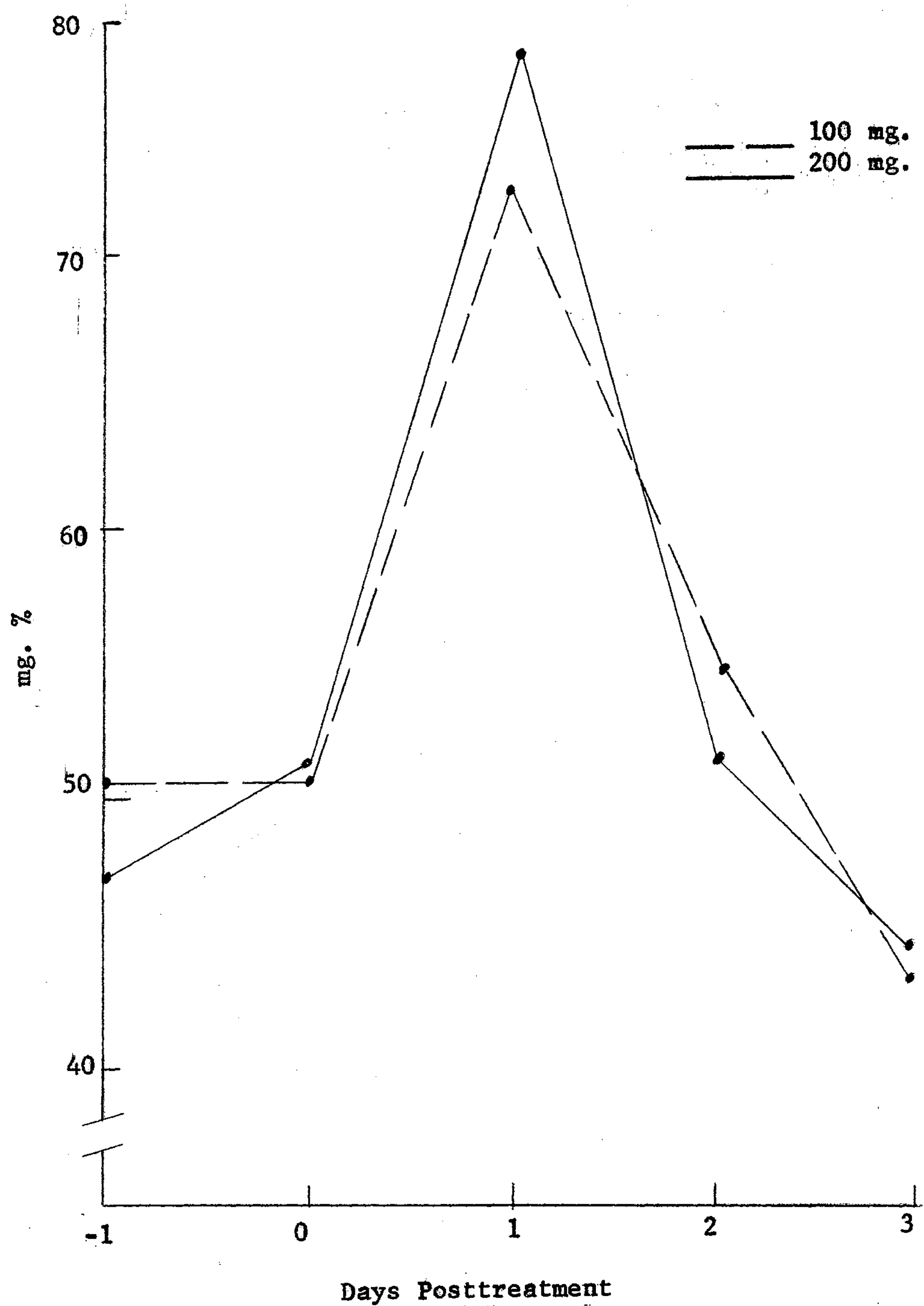



Figure 4. Average blood glucose (mg. %) prior to and following the oral administration of 9a-fluoroprednisolone.

TABLE VII

AVERAGE BLOOD GLUCOSE (MG. %) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 9a-FLUOROPREDNISOLONE

| Dosage | No. of Observations | -1 | 0 | Days Posttreatment 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 100 mg. | 8 | 50.7 | 50.7 | 72.6* | 54.6 | 43.5 |
| 200 mg. | 8 | 47.5 | 51.0 | 78.7* | 51.0 | 44.8 |

*P = 0.01

Total white blood cell numbers were not altered significantly by the oral administration of 100 mg. of 9a-fluoroprednisolone, whereas 200 mg. caused a highly significant (P = 0.005) increase in leukocytes 24 hours after treatment. Total lymphocyte numbers were not altered significantly by either oral dosage of 9a-fluoroprednisolone in that these cells remained essentially constant throughout the experiment (Table VIII). At 24 hours posttreatment the oral administration of the synthetic corticoid at both dosage levels caused a marked eosinopenia and neutrophilia (Table VIII). However, these changes were somewhat transitory in that 48 hours after treatment no changes in eosinophil or neutrophil numbers were apparent.

Plasma sodium and potassium values remained unchanged following the oral administration of 9a-fluoroprednisolone regardless of dosage (Table IX). At no time during the experiment did any of the animals show signs of hypokalemia. These findings agree with those reported earlier following the i.m. administration of this steroid and indicate that this compound possesses little or no mineralocorticoid activity when administered to the cow.

TABLE VIII

AVERAGE CIRCULATING LEUKOCYTE, LYMPHOCYTE, EOSINOPHIL AND NEUTROPHIL NUMBERS (THOUSANDS/$MM^3$) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 9a-FLUOROPREDNISOLONE

| Cell Type | Dosage | No. of Observations | Days Postinjection | | | | |
|---|---|---|---|---|---|---|---|
| | | | -1 | 0 | 1 | 2 | 3 |
| Leukocyte | 100 mg. | 8 | 8.0 | 8.6 | 10.2 | 8.2 | 7.5 |
| Leukocyte | 200 mg. | 8 | 7.4 | 7.5 | 12.8*** | 9.2 | 9.0 |
| Lymphocyte | 100 mg. | 8 | 4.3 | 4.6 | 4.3 | 4.3 | 4.2 |
| Lymphocyte | 200 mg. | 8 | 4.1 | 4.0 | 4.0 | 4.7 | 4.6 |
| Eosinophil | 100 mg. | 8 | 0.7 | 1.0 | 0.3* | 0.9 | 0.7 |
| Eosinophil | 200 mg. | 8 | 0.5 | 0.7 | 0.2** | 0.7 | 1.0 |
| Neutrophil | 100 mg. | 8 | 2.9 | 2.9 | 5.4** | 2.8 | 2.4 |
| Neutrophil | 200 mg. | 8 | 2.7 | 2.7 | 8.4# | 3.5 | 3.3 |

*P = 0.05 **P = 0.01 ***P = 0.005 #P = 0.001

TABLE IX

AVERAGE SODIUM AND POTASSIUM VALUES (MG. %) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 9aFLUOROPREDNISOLONE

| Ion | Dosage | No. of Observations | Days Postinjection | | | | |
|---|---|---|---|---|---|---|---|
| | | | -1 | 0 | 1 | 2 | 3 |
| Na | 100 mg. | 8 | 327 | 324 | 324 | 321 | 320 |
| Na | 200 mg. | 8 | 316 | 316 | 316 | 324 | 319 |
| K | 100 mg. | 8 | 19.7 | 18.5 | 19.5 | 18.8 | 19.1 |
| K | 200 mg. | 8 | 17.9 | 17.9 | 18.4 | 19.2 | 19.3 |

The hyperglycemic response following the oral administration of 9a-fluoroprednisolone was similar to that found following the i.m. injection of this corticoid. However, it appears that the oral effect was not as long lasting since significant differences in blood glucose values were found only at 24 hours posttreatment whereas significant changes were evident 96 hours after i.m. injection. The difference in length of response

may lie in the difference of dosage since most materials are absorbed more slowly from the gastro-intestinal tract than from intramuscular administration. In fact, it is surprising that any effects following oral administration of the steroid could be demonstrated since any substance administered by way of the mouth to the cow may be subjected to microbiological attack in the rumen. A thorough search of the literature has not revealed a previous report of a hyperglycemic response in ruminant animals to orally administered glucocorticoids. Subsequent to this finding a report (74) has appeared in which the hyperglycemic response to oral 9a-fluoroprednisolone was used to treat ketosis successfully in dairy cattle.

EXPERIMENT II

To study the effects of glucocorticoids upon oxygen consumption by liver homogenates, 104 rats were randomly assigned to the following groups: Group 1 received 0.16 mg. 9a-fluoroprednisolone i.m.; Group 2 received 0.32 mg. of the same steroid orally; Group 3 received no treatment but 0.08 mg. 9a-fluoroprednisolone was added to the liver homogenate at the time of incubation, and; Group 4 received no treatment and served as the control group. To ascertain that sufficient quantities of the steroid were being given to produce a physiological effect, blood glucose values were determined prior to treatment and again 5 hours after treatment at the time of sacrifice. Preliminary trials indicated sham injections of physiological saline did not affect blood glucose values. The results of the blood glucose determinations are shown (Table X). The significant increase ($P = 0.05$) in blood glucose values in the control animals may be due to the release of adrenal cortical hormones and/or epinephrine which would accompany handling and the stress of cardiac puncture. The fact that

TABLE X

AVERAGE BLOOD GLUCOSE VALUES (MG. %) BEFORE AND 5 HOURS AFTER TREATMENT WITH 9a-FLUOROPREDNISOLONE I.M. (0.16 MG.) OR ORALLY (0.32 MG.). CONTROL RATS WERE UNTREATED

| Hours after Treatment | No. of Rats | I.M. | Oral | Control |
|---|---|---|---|---|
| 0 | 26 | 66 | 65 | 66 |
| 5 | 26 | 85*** | 78** | 74* |

*P = 0.05 **P = 0.01 ***P = 0.001
P = 0.1 between oral and control at 5 hours
P = 0.05 between I.M. and control at 5 hours

higher and more significant increases (P = 0.01 and 0.001) in blood glucose values were obtained in the treated animals indicates that doses of the steroid sufficient to expect physiological responses had been given.

It is evident (Table XI and Fig. 5) that the in vivo administration of 9a-fluoroprednisolone caused marked changes in oxygen uptake by rat liver homogenates. The greatest stimulation of respiration was obtained

TABLE XI

AVERAGE OXYGEN UPTAKE BY RAT LIVER HOMOGENATES EXPRESSED AS MICROLITERS $O_2$ TAKEN UP PER MG. LIVER ON A DRY WEIGHT BASIS

| Treatment and Dosage | Route of Administration | No. of Rats | Incubation Time (Minutes) 10 | 20 | 30 | $Qo_2$ |
|---|---|---|---|---|---|---|
| 0.16 mg. $\Delta^1$9aFF | I.M. | 26 | 14.8*** | 27.0*** | 34.0*** | 88.0*** |
| 0.32 mg. $\Delta^1$9aFF | Orally | 26 | 12.7** | 22.5*** | 28.5*** | 76.2*** |
| 0.08 mg. $\Delta^1$9aFF | In vitro | 26 | 5.9* | 10.4* | 12.1* | 35.4* |
| None | — | 26 | 9.2 | 14.1 | 16.0 | 55.2 |

*P = 0.05 **P = 0.01 ***P = 0.001

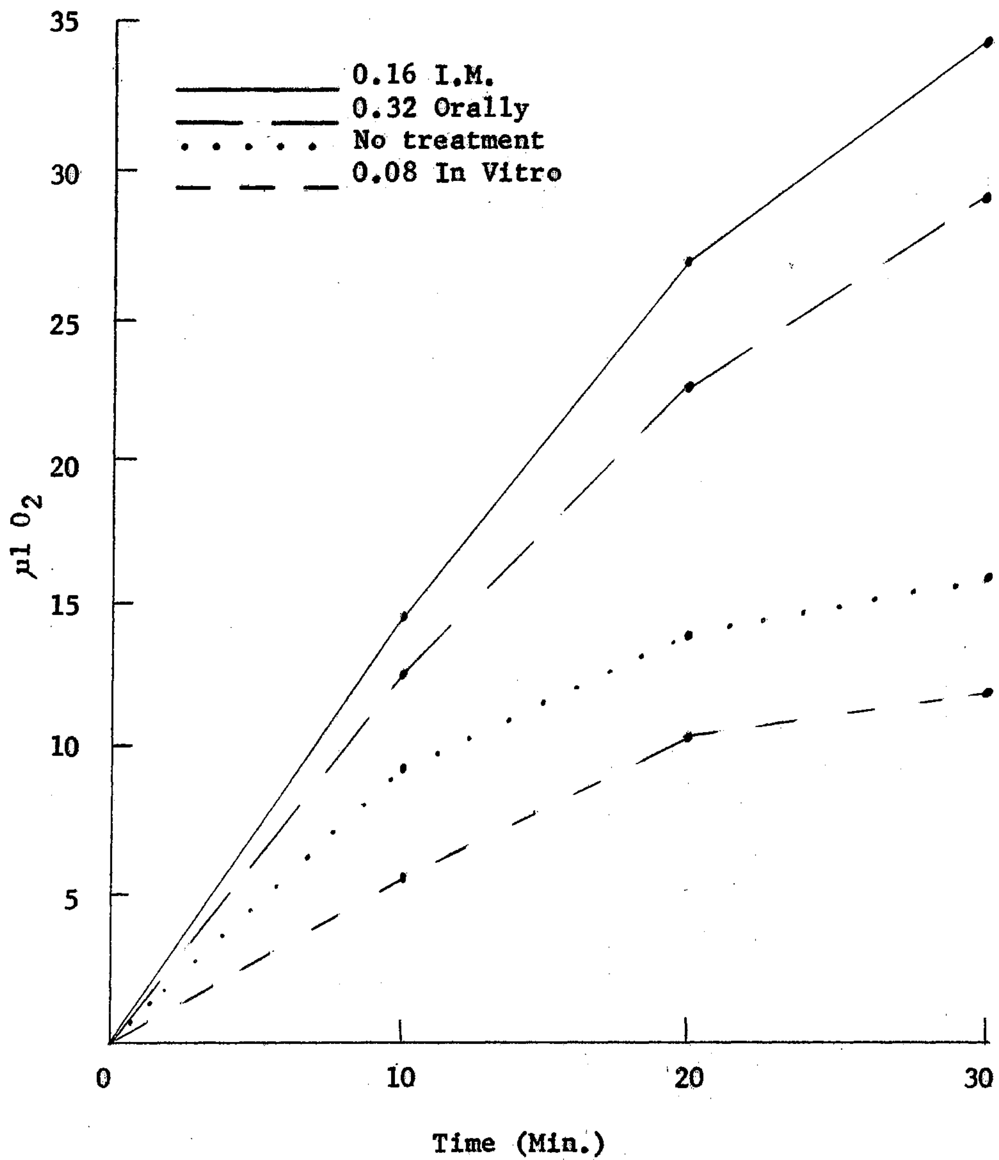



Figure 5. Average oxygen uptake (µl/mg. dry liver) by rat liver homogenates expressed as microliters $O_2$ taken up per mg. liver on a dry weight basis.

following the i.m. administration of the steroid although oral administration of the material also produced a very highly significant increase in the respiratory rate. It is also of interest to note that the in vitro addition of the synthetic corticoid at the level used caused a significant decrease in the rate of oxygen consumption. Similar results were reported by Grossfeld (40) in which hydrocortisone added to fibroblast tissue cultures at the time of incubation caused an inhibition of respiration whereas pretreatment of the cultured cells with hydrocortisone resulted in a significant increase in respiration.

The glucocorticoid-induced increase in oxygen uptake does not agree with the findings of Clark and Pesch (22) and Kerppola and Pitkanen (49) in which a decrease in liver mitochondrial respiration following cortisone injections in rats was noted. However, it should be pointed out that the results reported by these workers were based on chronic administration of the steroid for periods of 7 to 14 days whereas the work reported herein was based on a single injection of the corticoid 5 hours before sacrifice.

To determine if different responses in the uptake of oxygen do occur in livers from chronically treated rats and livers of rats treated with single injections, an experiment was conducted in which rats were injected i.m. daily for 7 days with 5.0 mg. prednisolone. Under these conditions, a decrease in oxygen consumption by the liver homogenates from chronically treated rats was found (Table XII and Fig. 6), whereas a marked stimulation in respiration occurred in "single-injection" rats. The decrease in oxygen uptake by liver homogenates from chronically treated rats agrees with those cited earlier (22, 49) and points out the fact that variations in corticoid-induced changes in oxygen consumption may depend upon the duration of treatment.

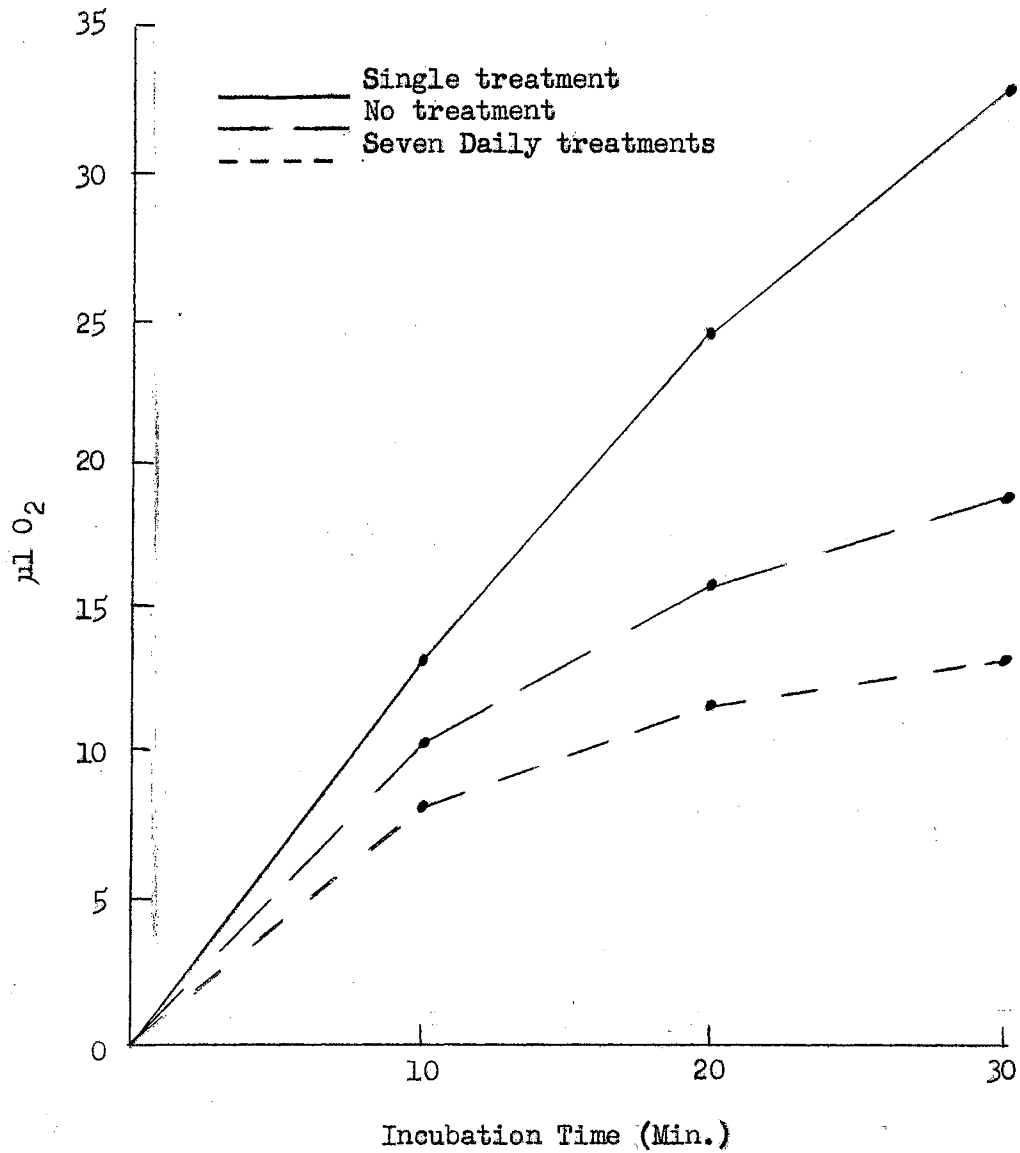



Figure 6. Average oxygen uptake (µl/mg. dry liver) by liver homogenates from rats treated daily for 7 days with 5.0 mg. prednisolone or a single injection of 5.0 mg. prednisolone 5 hours before sacrifice. Control rats untreated.

TABLE XII

AVERAGE OXYGEN UPTAKE (MICROLITERS/MG. DRY LIVER) BY LIVER HOMOGENATES FROM RATS TREATED DAILY FOR 7 DAYS WITH 5.0 MG. PREDNISOLONE OR A SINGLE INJECTION OF 5.0 MG. PREDNISOLONE 5 HOURS BEFORE SACRIFICE. CONTROL RATS WERE UNTREATED

| No. of Treatments | No. of Rats | Incubation Time (Minutes) 10 | 20 | 30 | $Qo_2$ |
|---|---|---|---|---|---|
| 7 | 12 | 8.35 | 11.89 | 13.81 | 51.10 |
| 1 | 10 | 12.87 | 24.80* | 33.35** | 77.00 |
| None | 10 | 10.27 | 15.77 | 18.43 | 61.60 |

*P = 0.01 **P = 0.001

The foregoing experiments were not designed to ascertain where in the metabloic cycles the synthetic corticoid was having the effect of causing increased respiration. Since no additional substrate was being added to the incubation flasks, the most obvious answer seemed to lie in the well known fact that glucocorticoids cause an increased storage of liver glycogen. This in turn could supply more endogenous substrate to the liver homogenate which conceivably could explain the enhanced respiratory rate. However, examination of the literature reveals that under certain conditions in vitro respiration of body tissues may be depressed by the addition of glucose (12, 19, 24, 27, 35, 37, 97, 117, 120). This phenomenon has been termed the "Crabtree effect." Other workers (21, 35, 36, 60, 61, 85, 102, 103, 104, 117, 120) have demonstrated that aerobic conditions (oxygen) may have an inhibitory effect upon the rate of glycolysis (Pasteur effect). Therefore, the question arose as to whether the presence of increased levels of liver glycogen due to glucocorticoid administration was inhibitory, stimulatory or had no effect on oxygen uptake by liver homogenates and whether aerobic conditions decreased the rate of glycolysis

in homogenized liver tissue. In order to compare the rate at which glycolysis occurs in treated and untreated rat liver homogenates, a series of experiments was conducted in which glycogen and glucose disappearance, lactic acid formation and inorganic phosphate levels during incubation were determined. The level of these metabolites in the blood before and after treatment was also studied.

TABLE XIII

AVERAGE BLOOD GLUCOSE, LACTIC ACID AND INORGANIC PHOSPHATE VALUES (MG. %) OF UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE SAMPLES TAKEN BEFORE AND 5 HOURS AFTER TREATMENT

| Analysis | Treatment | No. of Rats | Hours Posttreatment 0 | 5 |
|---|---|---|---|---|
| Glucose | 5.0 mg. $\Delta'$F | 20 | 78.6 | 114.1* |
| Glucose | None | 20 | 73.7 | 78.6 |
| Lactic Acid | 5.0 mg. $\Delta'$F | 33 | 8.32 | 7.44 |
| Lactic Acid | None | 31 | 8.51 | 7.46 |
| Inorg. $PO_4$ | 5.0 mg. $\Delta'$F | 20 | 5.18 | 5.61 |
| Inorg. $PO_4$ | None | 20 | 5.09 | 5.91 |

*P = 0.001

Average blood glucose, lactic acid and inorganic phosphate values before and 5 hours after treatment with 5.0 gm. prednisolone are shown (Table XIII). The steroid caused a very highly significant increase (P = 0.001) in blood glucose 5 hours posttreatment. This finding verifies many earlier reports of a hyperglycemic response to glucocorticoids and indicates sufficient quantities of the steroid had been used to bring about a response. The treatment did not alter blood lactic acid or blood inorganic phosphate levels. In fact, treated and control animals exhibited almost identical values for these metabolites at the termination of the experiment.

Liver homogenates from rats treated with 5.0 mg. prednisolone exhibited a very highly significant increase (P = 0.001) in oxygen uptake during 30 minutes incubation when compared to homogenates from untreated rats (Table XIV). This increase is of the same magnitude as that reported earlier following treatment with 9a-fluoroprednisolone and points to the probability that all naturally occurring and synthetic glucocorticoids have the ability to stimulate respiration. To investigate this proposal further, rats were injected i.m. with 5.0 mg. or 50.0 mg. hydrocortisone 5 hours before removal of a liver sample or left untreated to serve as control animals. The results of this experiment are shown in Table XV. Both levels of treatment caused a significant increase in respiration of the incubated homogenate when compared to homogenates from untreated animals and furnishes additional evidence for a respiratory stimulating property of glucocorticoids.

TABLE XIV

AVERAGE OXYGEN UPTAKE BY RAT LIVER HOMOGENATES EXPRESSED AS MICROLITERS $O_2$ TAKEN UP PER MG. LIVER ON A DRY WEIGHT BASIS. TREATED RATS INJECTED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE REMOVAL OF LIVER

| Treatment | No. of Rats | Incubation Time (Minutes) 10 | 20 | 30 | $Qo_2$ |
|---|---|---|---|---|---|
| 5.0 mg. F | 20 | 15.19* | 25.46** | 34.00** | 91.14* |
| None | 20 | 11.45 | 17.21 | 21.64 | 68.70 |

*P = 0.005 **P = 0.001

TABLE XV

AVERAGE OXYGEN UPTAKE (uL/MG.) BY RAT LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED I.M. WITH 5.0 OR 50.0 MG. HYDROCORTISONE

| Treatment | No. of Rats | Incubation Time (Minutes) 10 | 20 | 30 | $Qo_2$ |
|---|---|---|---|---|---|
| None | 16 | 9.15 | 15.22 | 18.92 | 54.90 |
| 5.0 mg. F | 10 | 10.38 | 18.25 | 25.99** | 62.28 |
| 50.0 mg. F | 10 | 12.75** | 19.96* | 26.07* | 76.50** |

*P = 0.1 **P = 0.05

The effects of i.m. treatment with prednisolone on liver carbohydrate values are shown in Tables XVI and XVII and further summarized in Table XVIII. The steroid caused an increase in liver glycogen but because of wide variation these values are not significant (Table XVI). A wide variation in liver glycogen values is not surprising in view of the well known fact that glycogen storage fluctuates markedly during a 24 hour period, depending primarily upon diet and absorptive state of the intestine.

TABLE XVI

AVERAGE LIVER GLYCOGEN VALUES (GM. %) IN UNTREATED RATS AND RATS TREATED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE REMOVAL OF LIVER SAMPLE. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | Oxygen | 20 | 14.34 | 8.77* | 5.57 |
| None | Oxygen | 20 | 11.14 | 5.73* | 5.41 |
| 5.0 mg. $\Delta^1$F | Nitrogen | 20 | 9.92 | 6.53* | 3.39 |
| None | Nitrogen | 20 | 8.36 | 4.91* | 3.45 |

*P = 0.001

TABLE XVII

AVERAGE LIVER GLUCOSE VALUES (GM. %) IN UNTREATED RATS AND RATS TREATED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE REMOVAL OF LIVER SAMPLE. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | Oxygen | 20 | 0.73 | 2.60* | 1.87 |
| None | Oxygen | 20 | 0.77 | 2.38* | 1.61 |
| 5.0 mg. $\Delta^1$F | Nitrogen | 20 | 0.43 | 1.60* | 1.17 |
| None | Nitrogen | 20 | 0.44 | 1.73* | 1.29 |

*P = 0.001

In order to determine total carbohydrate values, liver homogenates were also analyzed for glucose (Table XVII). Glucose values of liver samples taken at the time of killing the animals were almost identical in treated and control animals, indicating no treatment effect. The differences between liver glucose values of those rats used in the oxygen phase of the experiment and nitrogen phase of the study are difficult to explain. It is possible that two different populations of rats were used since approximately one month elapsed between these two studies. Experimental techniques were identical, however.

Liver glycogen values decreased markedly (P = 0.001) during the 30 minute incubation period (Table XVI), however, the rate at which glycogen was hydrolyzed in treated rat livers was no different from that of control rats. Although it appears that glycogen was hydrolyzed more rapidly when the homogenates were incubated under oxygen when compared to nitrogen, these differences are not significant. The fact that liver glucose values increased considerably during incubation (Table XVII) indicates that not all the glycogen which was hydrolyzed immediately

underwent glycolysis. The rate at which glucose appeared in the homogenate during incubation was not affected by treatment or the gas under which they were incubated.

In order to get an overall picture of carbohydrate metabolism, average liver glucose and glycogen values were added together to give total carbohydrate values (Table XVIII). No differences were apparent between the rate at which liver homogenates from control and treated rats metabolyzed carbohydrate material during incubation. A Pasteur effect was not demonstrated in that treated rat livers tended to use more carbohydrate when incubated under oxygen than when incubated under nitrogen (P = 0.1). This same trend was apparent in control livers but wider variation prevented these differences from being classed as significant. Since treated and control rat liver homogenates utilized similar quantties of glucose and glycogen, the explanation for the marked increase in respiration following corticoid administration does not appear to lie in this area of metabolism.

TABLE XVIII

SUMMARY OF TABLES XVI AND XVII SHOWING AVERAGE TOTAL LIVER CARBOHYDRATE VALUES (GM. %) IN UNTREATED RATS AND RATS TREATED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE REMOVAL OF LIVER SAMPLE. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$ F | Oxygen | 20 | 15.07 | 11.37* | 3.70# |
| None | Oxygen | 20 | 11.91 | 8.11* | 3.80 |
| 5.0 mg. $\Delta^1$ F | Nitrogen | 20 | 10.35 | 8.13* | 2.22# |
| None | Nitrogen | 20 | 8.80 | 6.64* | 2.16 |

*P = 0.001 #P = 0.1 between $O_2$ and $N_2$

Liver inorganic phosphate values in control and prednisolone-treated rat liver homogenates before and after incubation were determined to disclose if there were treatment effects on phosphorylation and/or phosphorolysis (Table XIX). When the homogenates were incubated under aerobic conditions, a very highly significant increase (P = 0.001) in inorganic phosphate occurred in control samples during incubation. This increase did not appear in homogenates from prednisolone-injected animals thus indicating a treatment effect.

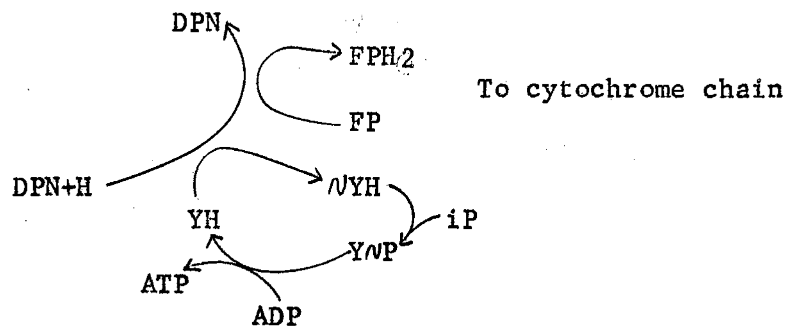
TABLE XIX

AVERAGE LIVER INORGANIC PHOSPHATE VALUES (MG. %) OF UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE. SAMPLES TAKEN FROM HOMOGENATES BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG VESSELS UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | Oxygen | 21 | 7.81 | 8.41 | 0.60** |
| None | Oxygen | 21 | 7.05 | 17.46*** | 10.41 |
| 5.0 mg. $\Delta^1$F | Nitrogen | 19 | 8.46 | 17.71* | 9.25 |
| None | Nitrogen | 20 | 9.10 | 19.34* | 10.24 |

*P = 0.05 **P = 0.005 ***P = 0.001

The explanation for this finding may well lie in the fact that, in oxidative processes which occur during the functioning of Krebs cycle, there is an obligatory phosphorylation of adenosine diphosphate (ADP). The process of oxidative-phosphorylation may be depicted in the following manner (115):

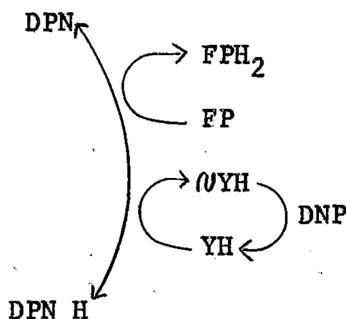



In this scheme DPN is reduced during the oxidation of the various intermediates of Krebs cycle. As reduced DPN is re-oxidized, so that it may once more accept hydrogen and electrons from further oxidative processes, it gives up its electrons to flavoprotein (FP) and this compound is reduced. However, comcommitant with these reactions there is a coupled phorylative process. This is shown above by some unknown compound, noted above as YH (possibly vitamin K or Coenzyme Q), being energized to form a high energy complex (∿YH). In the presence of inorganic phosphate (iP), ∿YH is phosphorylated to form a high energy phosphate bond (Y∿P). This high energy phosphate bond can, in turn, be transferred to ADP to form adenosine triphosphate (ATP).

The increase in inorganic phosphate in untreated rat liver homogenates could thus be explained on the basis of a failure of phosphorylative processes to keep pace with the rate at which ATP is being broken down by ATPase (109). On the other hand, in treated livers these processes must have proceeded at almost equal rates since pre-and postincubation inorganic phosphate levels were similar. This hypothesis appears even more attractive in view of the earlier reported increase in respiration in treated livers since an increase in respiration undoubt-

edly points to an increase in oxidative processes and, therefore, an increase in the rate of oxidative-phosphorylation. It should be noted, however, that another possible explanation for the differences in inorganic phosphate levels in control and treated rat liver homogenates following 30 minute incubation does exist. It is possible that corticoid treatment may not stimulate phosphorylative processes under aerobic conditions but rather that it may decrease the activity of ATPase. This would result in a tendency for phosphate to remain in the organic form at the expense of inorganic phosphate in liver homogenates from treated rats. The net result would be a decrease in inorganic phosphate levels following incubation.

Under steady state conditions any of the reactants in the oxidative-phosphorylation process could conceivably become rate limiting. Levels of ADP and inorganic phosphate are especially critical and have been cited as being involved in both the Pasteur and Crabtree effects (60, 104, 118). The reactions involved in oxidative-phosphorylation are known to be uncoupled by several chemical compounds (37, 61, 103, 104), one of which is 2,4 dinitrophenol (DNP). Using the previously shown scheme for oxidative-phosphorylation, it is thought that DNP may uncouple these reactions in the following manner.



In this scheme there is no longer an obligatory phosphorylation of ADP which must accompany oxidation and thus ADP and/or inorganic phosphate levels would no longer be rate limiting. The energy loss which accompanies treatment with DNP undoubtedly appears as heat since animals so treated develop a very high body temperature.

Kerppola (48) used changes in the phosphate/oxygen ratio (P/O) in liver mitochondria from glucocorticoid-treated rats as evidence for the hypothesis that corticoids may also uncouple oxidative-phosphorylation. If glucocorticoids actually do uncouple oxidative- phosphorylation undoubtedly this is not accomplished in the same manner as with 2,4 dinitrophenol. Tepperman and Tepperman (101) have suggested that several ways of uncoupling these processes may exist. A decrease in both oxidation and phosphorylation was cited by Keppola (48); however, the decrease in oxidation was of a greater magnitude which served to alter the normal P/O ratio and permit the conclusion of an uncoupling effect. These findings do not agree with the work reported herein since an increase in both oxidation and phosphate utilization in liver homogenates from rats treated with 9a-fluoroprednisolone or prednisolone has been found. In order to determine P/O ratio of a respiring system it is necessary to stop ATPase activity so that, when ADP is phosphorylated to form ATP, it is not immediately reconverted to ADP by this enzyme. In this experiment no attempt was made to block ATPase activity so an accurate measurement of P/O ratio is not available. It should be noted, however, that Keppola (48) treated his rats chronically for 7 days with 5.0 mg. cortisone acetate and used liver mitochondria for his studies. It has been pointed out earlier (Table XII) that duration of glucocorticoid treatment may have a marked effect upon oxygen consumption by liver

homogenates and presumably also by liver mitochondria.

To eliminate or confirm the possibility that the presence of increased amounts of carbohydrate present in corticoid-treated rat livers may in some manner stimulate oxygen consumption during incubation, an experiment was conducted in which glucose was added to Warburg flasks innoculated with homogenates from either prednisolone-treated or untreated rats. Sufficient glucose was added to the untreated rat liver homogenates so that the total carbohydrate present in these flasks was approximately equal to that which appeared endogenously in the treated vessels. Concurrently with this experiment, in order to study oxidation and phosphorylation, a third group of Warburg vessels were prepared which contained DNP. The results of this experiment appear in Tables XX and XXI.

TABLE XX

AVERAGE OXYGEN UPTAKE BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS TREATED I.M. WITH 5.0 MG. PREDNISOLONE

| Treatment | Added Substrate# | No. of Rats | Incubation Time (Minutes) 10 | 20 | 30 | $Qo_2$ |
|---|---|---|---|---|---|---|
| 5.0 mg. Δ'F | None | 11 | 13.17 | 24.71 | 32.91 | 79.02 |
| 5.0 mg. Δ'F | Glucose | 11 | 10.59* | 16.18** | 20.20** | 63.54* |
| 5.0 mg. Δ'F | DNP | 10 | 4.27*** | 6.51*** | 7.62*** | 25.62*** |
| None | None | 11 | 8.64 | 15.39 | 20.30 | 51.84 |
| None | Glucose | 11 | 8.00 | 12.09 | 14.63* | 48.00 |
| None | DNP | 10 | 2.70*** | 5.05*** | 5.65*** | 16.20*** |

# Glucose added at the rate of 1.5 mg. per ml. of homogenate
DNP (2,4 dinitrophenol) added to final concentration of $3 \times 10^{-4}$M
*P = 0.1 **P = 0.05 ***P = 0.001

TABLE XXI

AVERAGE LIVER INORGANIC PHOSPHATE VALUES (MG. %) OF UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE. SAMPLES TAKEN FROM HOMOGENATES BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG VESSELS UNDER OXYGEN

| Treatment | Added Substrate# | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | None | 11 | 7.36 | 5.41 | -1.95 |
| 5.0 mg. $\Delta^1$F | Glucose | 11 | 6.19 | 6.89 | 0.70 |
| 5.0 mg. $\Delta^1$F | DNP | 10 | 7.04 | 10.89* | 3.85* |
| None | None | 11 | 5.37 | 8.55 | 3.18 |
| None | Glucose | 11 | 6.08 | 10.61 | 4.53** |
| None | DNP | 10 | 5.64 | 14.61* | 8.97*** |

#Glucose added at the rate of 1.5 mg. per ml. of homogenate.
DNP added to final concentration of $3 \times 10^{-4}$M.
*P = 0.05 **P = 0.01 ***P = 0.001

Rather than causing an increase in respiration, the addition of glucose to liver homogenates from treated and untreated rats resulted in a decrease in respiration. This inhibition of respiration by glucose is undoubtedly an expression of the Crabtree effect. The phenomenon was most pronounced in homogenates from corticoid-treated rats since significant decreases in oxygen consumption at 20 and 30 minutes (P = 0.05) were evident in these homogenates when compared to flasks where no additional substrate had been added. This same trend was also evident in liver homogenates from control rats to which glucose had been added (P = 0.1 at 30 min.). These results provide evidence that the mere presence of additional substrate which is present in livers from glucocortiocoid-treated rats does not, in itself, stimulate respiration in homogenates prepared from these livers. In fact, the presence of additional endogenous carbohydrate material in treated livers may actually have a depressing effect

upon respiration.

The addition of glucose to both treated and control flasks caused a slight decrease in the rate of phosphorylation as evidenced by the fact that these flasks contained greater amounts of inorganic phosphate at the end of the incubation period than did the flasks to which additional substrate had not been added. It may be that under these conditions glycolysis deprives the respiring mitochondria of their phosphate acceptor, ADP, thus limiting the rate at which oxidative-phosphorylation can occur (35).

The addition of DNP to the flasks caused a very highly significant decrease (P = 0.001) in oxygen uptake in both treated and control homogenates (Table XX), the decrease being evident during all periods of incubation. DNP also caused a decrease (P = 0.05) in phosphorylation as evidenced by the fact that DNP flasks contained more inorganic phosphate at the end of the 30 minute incubation period when compared to flasks which did not contain DNP (Table XXI). P/O ratios were not determined but, since respiration appeared to be more depressed than phosphorylation, an "uncoupling" may have occurred.

TABLE XXII

AVERAGE LIVER LACTIC ACID VALUES (MG. %) OF UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE. SAMPLES TAKEN FROM HOMOGENATE BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG VESSELS UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | Oxygen | 18 | 5.1 | 9.6* | 4.5 |
| None | Oxygen | 18 | 4.9 | 11.3** | 6.4 |
| 5.0 mg. $\Delta^1$F | Nitrogen | 18 | 6.9 | 18.0** | 11.1 |
| None | Nitrogen | 17 | 5.2 | 9.0# | 3.8 |

*P = 0.01 **P = 0.001 #P = 0.01 between treated and control

Liver lactic acid values under aerobic and anaerobic conditions are summarized in Table XXII. Lactic acid accumulated in the homogenates during incubation under aerobic conditions but the rate at which this metabolite was formed was not affected by pretreatment of the rat with prednisolone. Under anaerobic conditions lactic acid was formed more rapidly in homogenates from treated rat livers than in untreated homogenates. The origin of this increased amount of lactic acid is baffling. Under anaerobic conditions, one would not expect the Krebs cycle to be functioning. That the lactic acid could be of glucose or glycogen origin is eliminated by the fact that carbohydrate disappearance was similar in treated and control homogenates (Table XVIII). One remaining alternative is that this lactic acid could arise from the deamination of certain amino acids by way of pyruvic acid. This hypothesis seems entirely possible since the ability of glucocorticoids to stimulate gluconeogenesis is well established (114). The fact that cortical hormones increase the activity of certain transaminases has been cited earlier (11, 58, 83, 84, 98).

Further experimentation in which homogenate protein levels before and after incubation were determined make the foregoing hypothesis untenable, however (Table XXIII). Treatment of the experimental animals with prednisolone did not alter the rate of protein synthesis or degradation during incubation of the homogenates. A somewhat surprising finding was that anaerobic conditions favored protein synthesis whereas incubation under oxygen tended to favor protein breakdown slightly. This difference was most pronounced in untreated rat liver homogenates ($P = 0.1$) but the trend was also apparent in corticoid-treated rat liver homogenates.

TABLE XXIII

AVERAGE PROTEIN (MG./GM. DRY LIVER) IN LIVER HOMOGENATES FROM UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN OR NITROGEN

| Treatment | Gas Phase | No. of Rats | Preincubation | Postincubation | Difference |
|---|---|---|---|---|---|
| 5.0 mg. $\Delta^1$F | Oxygen | 16 | 354 | 353 | -1 |
| None | Oxygen | 15 | 362 | 358* | -4 |
| 5.0 mg. $\Delta^1$F | Nitrogen | 16 | 354 | 363 | +9 |
| None | Nitrogen | 15 | 360 | 367* | +7 |

*P = 0.1 for difference between aerobic and anaerobic conditions

The work reported herein undoubtedly leaves many questions unanswered. Many avenues for future experimentation remain open. More work is needed to clarify the role of adrenal steroids in sodium and potassium in the ruminant animal. Additional enzyme studies will be necessary to understand and explain the in vitro respiration-stimulating activity of glucocorticoids. The effects of cortical hormones upon liver lactic acid and protein levels need further study. Since ruminant animals differ markedly from monogastric animals in their metabolism, a study similar to the current work but using bovine liver homogenates should be conducted.

## CHAPTER V

## SUMMARY AND CONCLUSIONS

It has previously been shown that unsaturation, fluorination or methylation of hydrocortisone will markedly increase the glucocorticoid and mineralocorticoid activity of this compound in laboratory animals; however, little is known concerning their activity in large domestic animals. To study the effects of intramuscular injection of synthetic corticoids in dairy cattle, a randomized complete block design was used in which each of eight lactating Holstein-Fresian cows was injected with one of the following: 50 or 100 mg. of prednisolone, 25 or 100 mg. of 9a-fluorohydrocortisone, 25 or 100 mg. 9a-fluoroprednisolone or 25 or 100 mg. of 6 methyl prednisolone. This procedure was replicated six times with two week intervals between replications. Blood samples were taken at 72, 24, and 0 hours prior to injection and 24, 48, 96, and 144 hours following treatment and analyzed for glucose, total leucocytes, differential leucocytes, sodium and potassium.

Nine alpha-fluoroprednisolone exhibited the most pronounced glucocorticoid activity in that both 25 and 100 mg. dosages produced highly significant increases in blood glucose levels at 24 and 48 hours post-injection when compared to pre-injection levels. Significant increases in blood glucose values were detected at 24 hours but not at 48 hours following the injection of 100 mg. of 9a-fluorohydrocortisone and 50 and 100 mg. of prednisolone. Six-methyl prednisolone did not alter

blood glucose levels significantly at either dosage. All compounds produced varying degrees of leucocytosis, neutrophilia and eosinopenia, whereas lymphocyte numbers remained unchanged when compared to pre-injection values. Although numerous reports indicate that these compounds possess mineralocorticoid activity in laboratory animals, no changes in plasma sodium or potassium values were detected following any of the treatments.

The most potent synthetic corticoid, 9a-fluoroprednisolone, was used to determine the effects of oral administration in cattle and to compare intramuscular injection with oral administration as to dosage and duration of action. Four lactating dairy cows were randomly assigned to receive 100 or 200 mg. of this steroid per os. This procedure was replicated four times with two week intervals between replication. Results following oral administration were very similar to those following intramuscular injection in that significant increases in blood glucose levels were detected 24 hours post-treatment; 48 hour post-treatment blood glucose values were normal, however. Intramuscular injection of 100 mg. of 9a-fluoroprednisolone caused a 100 % increase in blood glucose 24 hours post-treatment, whereas the same dosage given orally produced a 75% increase at this time. A marked neutrophilia and eosinopenia were again observed following the oral treatment with 9a-fluoroprednisolone. Plasma sodium and potassium values remained unchanged. At no time in either experiment did any of the animals show objective clinical signs, such as anorexia and muscular weakness, which usually are associated with hypokalemia. These results indicate that in the cow, prednisolone, 9a-fluorohydrocortisone and especially 9a-fluoroprednisolone possess marked glucocorticoid activity but little

mineralocorticoid activity and that the glucocorticoid activity of 9a-fluoroprednisolone following oral administration is comparable to a similar intramuscular dose.

In an attempt to clarify further the actions and mechanisms of action of cortical hormones, a more basic experiment, using rat liver homogenates, was designed in which the effects of corticoid administration on blood and liver inorganic phosphate, lactic acid, glucose, glycogen, oxygen consumption and protein levels were studied under aerobic and anaerobic conditions.

A single intramuscular injection of prednisolone caused an increase in blood glucose. No corticoid-induced changes in blood lactic acid or inorganic phosphate were detected. Chronic i.m. administration of 5.0 mg. prednisolone daily for 7 days caused a marked inhibition of respiration by rat liver homogenates, whereas a single injection of hydrocortisone, prednisolone or 9a-fluoroprednisolone 5 hours before sacrifice resulted in a very highly significant increase in oxygen uptake by rat liver homogenates. Efforts to find the place in the glycolytic cycle or Krebs cycle which was responsible for the stimulation of respiration revealed that endogenous carbohydrate utilization was similar in liver homogenates from control and treated animals, thus eliminating the possibility that increased liver glycogen levels in treated animals may, in some manner, stimulate respiration. A Crabtree effect, in which the addition of glucose to liver homogenates caused a marked inhibition of respiration, has been demonstrated.

Incubation of liver homogenates under aerobic conditions disclosed that inorganic phosphate levels were decreased in homogenates from corticoid-treated rats when compared to untreated liver homogenates.

This may be due to a corticoid-induced stimulation of phosphorylation or a corticoid-induced depression in liver ATPase activity. The former hypothesis is favored since, in biological systems, oxidation and phosphorylation are normally coupled and since previous work had shown oxidative processes to be increased under the influence of glucocorticoid treatment. The fact that similar quantities of inorganic phosphate appeared in treated and untreated rat liver homogenates incubated under nitrogen offers further evidence of phosphorylation-stimulating property of glucocorticoids. Under anaerobic conditions oxidative-phosphorylation by Kreb's cycle would be completely inhibited whereas ATP synthesis by glycolysis would be expected to continue. Under these conditions a corticoid-induced depression of ATPase activity would manifest itself by increased levels of inorganic phosphate in untreated rat liver homogenates.

Corticoid treatment did not alter the rate of glycolysis as evidenced by the fact that carbohydrate utilization was similar in liver homogenates from treated and non-treated rats and by the fact that inorganic phosphate levels were similar in treated and control liver homogenates incubated under nitrogen.

Under anaerobic conditions, liver homogenates from treated rats accumulated lactic acid more rapidly than untreated rat liver homogenates. Glucocorticoid treatment did not appear to affect protein metabolism under the conditions of the experiment since no difference between protein levels in treated and untreated rat liver homogenates were detected following incubation. Aerobic conditions favored protein breakdown, whereas anaerobic conditions favored protein synthesis.

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# A P P E N D I X

EXPERIMENT I

BLOOD GLUCOSE VALUES (MG. %) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF PREDNISOLONE AND 9a-FLUOROHYDROCORTISONE

| Treatment and Dosage | | Days Postinjection | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | -3 | -1 | 0 | 1 | 2 | 4 | 6 |
| $\Delta^1$F - 50 mg. | | | | | | | | |
| | | 52 | 44 | 56 | 58 | 56 | 43 | 60 |
| | | 56 | 54 | 48 | 60 | 46 | 44 | 50 |
| | | 47 | 40 | 42 | 50 | 47 | 50 | 49 |
| | | 46 | 38 | 48 | 75 | 49 | 56 | 42 |
| | | 49 | 49 | 49 | 75 | 56 | 45 | 40 |
| | | 38 | 42 | 42 | 53 | 53 | 49 | 40 |
| | Mean | 48.0 | 44.5 | 47.5 | 61.8 | 51.2 | 47.8 | 46.8 |
| | SD | 6.1 | 6.0 | 5.2 | 4.7 | 4.5 | 4.7 | 7.8 |
| $\Delta^1$F - 100 mg. | | | | | | | | |
| | | 58 | 40 | 58 | 56 | 67 | 47 | 57 |
| | | 56 | 41 | 48 | 108 | 42 | 41 | 48 |
| | | 46 | 40 | 55 | 78 | 40 | 50 | 49 |
| | | 42 | 40 | 45 | 56 | 50 | 45 | 40 |
| | | 46 | 41 | 42 | 68 | 50 | 52 | 45 |
| | | 40 | 40 | 42 | 62 | 56 | 49 | 40 |
| | Mean | 48.0 | 40.3 | 48.3 | 71.3 | 50.8 | 47.3 | 46.5 |
| | SD | 7.4 | 0.1 | 5.1 | 13.8 | 9.8 | 2.7 | 6.4 |
| 9a-FF- 25 mg. | | | | | | | | |
| | | 50 | 41 | 56 | 58 | 58 | 43 | 62 |
| | | 48 | 45 | 43 | 80 | 46 | 42 | 56 |
| | | 61 | 44 | 42 | 47 | 47 | 50 | 53 |
| | | 44 | 45 | 52 | 53 | 53 | 59 | 50 |
| | | 40 | 42 | 49 | 50 | 50 | 45 | 40 |
| | | 45 | 42 | 45 | 56 | 42 | 50 | 40 |
| | Mean | 48.0 | 43.2 | 47.8 | 57.3 | 49.3 | 48.2 | 50.2 |
| | SD | 7.2 | 1.7 | 5.5 | 11.8 | 5.6 | 6.3 | 8.8 |
| 9a-FF- 100 mg. | | | | | | | | |
| | | 56 | 50 | 50 | 56 | 60 | 53 | 60 |
| | | 56 | 41 | 40 | 82 | 68 | 50 | 62 |
| | | 56 | 44 | 42 | 63 | 50 | 54 | 49 |
| | | 46 | 42 | 48 | 68 | 42 | 50 | 49 |
| | | 40 | 42 | 49 | 75 | 56 | 50 | 42 |
| | | 45 | 40 | 40 | 69 | 45 | 45 | 38 |
| | Mean | 49.8 | 43.2 | 44.8 | 68.8 | 53.2 | 50.3 | 50.0 |
| | SD | 7.0 | 3.7 | 4.5 | 9.1 | 9.7 | 3.1 | 9.5 |

EXPERIMENT I

BLOOD GLUCOSE VAUES (MG. %) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF 9a-FLUOROPREDNISOLONE AND 6-METHYL PREDNISOLONE

| Treatment and Dosage | | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|---|
| $\Delta^1$9aFF - 25 | | | | | | | | |
| | | 56 | 42 | 52 | 50 | 64 | 53 | 53 |
| | | 56 | 40 | 41 | 67 | 53 | 41 | 56 |
| | | 50 | 46 | 46 | 68 | 56 | 56 | 50 |
| | | 43 | 40 | 42 | 87 | 50 | 53 | 42 |
| | | 42 | 42 | 40 | 75 | 53 | 50 | 42 |
| | | 45 | 50 | 42 | 95 | 50 | 42 | 38 |
| | Mean | 48.6 | 43.4 | 43.8 | 73.7 | 54.1 | 49.1 | 46.8 |
| | SD | 6.4 | 4.0 | 4.5 | 15.9 | 5.2 | 6.3 | 7.2 |
| $\Delta^1$9aFF - 100 | | | | | | | | |
| | | 52 | 46 | 58 | 88 | 76 | 81 | 82 |
| | | 60 | 41 | 42 | 94 | 78 | 56 | 50 |
| | | 56 | 46 | 40 | 106 | 84 | 87 | 62 |
| | | 48 | 40 | 48 | 87 | 50 | 53 | 53 |
| | | 40 | 42 | 49 | 112 | 59 | 62 | 49 |
| | | 45 | 40 | 45 | 132 | 75 | 62 | 40 |
| | Mean | 50.0 | 42.5 | 47.0 | 103.2 | 70.3 | 66.8 | 56.0 |
| | SD | 7.3 | 2.8 | 6.4 | 17.3 | 13.0 | 13.9 | 14.6 |
| 6M$\Delta^1$F - 25 | | | | | | | | |
| | | 52 | 42 | 56 | 67 | 81 | 53 | 67 |
| | | 56 | 47 | 46 | 47 | 44 | 43 | 46 |
| | | 47 | 49 | 46 | 47 | 50 | 56 | 42 |
| | | 40 | 42 | 42 | 61 | 50 | 56 | 49 |
| | | 50 | 42 | 50 | 53 | 50 | 45 | 53 |
| | | 40 | 40 | 50 | 42 | 49 | 53 | 40 |
| | Mean | 47.5 | 43.7 | 46.5 | 52.8 | 54.0 | 51.0 | 49.5 |
| | SD | 6.5 | 3.5 | 4.7 | 9.5 | 13.4 | 5.6 | 9.7 |
| 6M$\Delta^1$F - 100 mg. | | | | | | | | |
| | | 40 | 36 | 46 | 44 | 70 | 57 | 62 |
| | | 56 | 45 | 42 | 53 | 50 | 42 | 46 |
| | | 46 | 40 | 48 | 59 | 50 | 50 | 40 |
| | | 40 | 45 | 49 | 62 | 50 | 53 | 40 |
| | | 42 | 49 | 49 | 50 | 49 | 50 | 40 |
| | | 47 | 42 | 42 | 47 | 40 | 50 | 49 |
| | Mean | 45.2 | 42.7 | 46.0 | 52.5 | 51.5 | 50.3 | 46.1 |
| | SD | 6.1 | 4.5 | 3.2 | 7.0 | 9.9 | 4.9 | 8.6 |

EXPERIMENT I

LEUKOCYTES (THOUSANDS/$MM^3$) IN CATTLE PRIOR TO AND FOLLOWING THE I.M. INJECTION OF PREDNISOLONE AND 9a-FLUOROHYDROCORTISONE

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta'$F - 50 mg. | | | | | | | |
| | 16.5 | 13.0 | 10.0 | 15.2 | 9.3 | 9.0 | 11.0 |
| | 10.0 | 7.4 | 6.7 | 10.5 | 7.6 | 6.7 | 5.6 |
| | 7.9 | 7.3 | 6.2 | 8.9 | 12.2 | 7.6 | 4.4 |
| | 10.0 | 9.2 | 8.5 | 14.9 | 13.0 | 8.8 | 7.7 |
| | 8.0 | 8.5 | 11.0 | 11.3 | 10.5 | 10.6 | 7.6 |
| | 7.3 | 4.7 | 10.4 | 9.7 | 5.5 | 6.6 | 7.1 |
| Mean | 9.9 | 8.4 | 8.8 | 11.8 | 9.7 | 8.2 | 7.2 |
| SD | 3.4 | 2.8 | 2.0 | 2.7 | 2.8 | 1.5 | 2.3 |
| $\Delta'$F - 100 mg. | | | | | | | |
| | 9.5 | 8.0 | 8.9 | 9.5 | 8.4 | 7.5 | 7.5 |
| | 9.2 | 8.3 | 10.0 | 14.0 | 13.4 | 8.6 | 9.5 |
| | 7.0 | 7.2 | 5.0 | 7.6 | 9.5 | 6.7 | 7.1 |
| | 5.1 | 7.0 | 9.0 | 9.1 | 6.8 | 5.1 | 5.5 |
| | 7.4 | 6.8 | 7.8 | 12.4 | 6.5 | 5.7 | 4.6 |
| | 8.3 | 11.0 | 6.9 | 14.1 | 9.8 | 8.6 | 10.7 |
| Mean | 7.8 | 8.0 | 7.9 | 11.1 | 9.1 | 7.1 | 7.5 |
| SD | 1.6 | 1.6 | 1.8 | 2.7 | 2.5 | 1.5 | 2.3 |
| 9a-FF - 25 mg. | | | | | | | |
| | 9.5 | 12.6 | 11.3 | 12.6 | 10.5 | 9.0 | 8.3 |
| | 9.9 | 10.2 | 10.9 | 15.4 | 11.5 | 8.7 | 10.1 |
| | 7.7 | 7.2 | 6.9 | 9.6 | 7.8 | 8.1 | 6.2 |
| | 7.6 | 7.3 | 9.0 | 10.2 | 7.9 | 5.1 | 4.5 |
| | 5.5 | 7.0 | 11.1 | 7.0 | 4.8 | 6.0 | 5.6 |
| | 4.2 | 5.1 | 8.0 | 8.1 | 6.7 | 4.6 | 5.6 |
| Mean | 7.4 | 8.2 | 9.5 | 10.5 | 8.2 | 6.9 | 6.7 |
| SD | 2.6 | 1.1 | 1.9 | 3.1 | 2.5 | 1.9 | 2.9 |
| 9a-FF - 100 mg. | 9.9 | 13.8 | 12.2 | 13.8 | 9.8 | 10.2 | 12.0 |
| | 9.3 | 7.9 | 6.5 | 12.2 | 11.6 | 8.1 | 8.1 |
| | 6.4 | 7.6 | 7.0 | 8.2 | 8.8 | 6.4 | 5.0 |
| | 6.4 | 8.8 | 9.1 | 10.9 | 12.8 | 6.2 | 7.0 |
| | 5.3 | 6.8 | 7.9 | 9.0 | 7.6 | 6.7 | 2.7 |
| | 9.5 | 9.8 | 10.6 | 13.0 | 9.3 | 11.1 | 11.2 |
| Mean | 7.8 | 9.1 | 8.9 | 11.2 | 10.0 | 8.1 | 7.7 |
| SD | 2.0 | 2.5 | 2.2 | 3.1 | 1.9 | 2.1 | 3.5 |

EXPERIMENT I

LEUKOCYTES (THOUSANDS/$MM^3$) IN CATTLE PRIOR TO AND FOLLOWING THE I.M. INJECTION OF 9a-FLUOROPREDNISOLONE AND 6-METHYLPREDNISOLONE

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| Δ'9aFF - 25 mg. | | | | | | | |
| | 9.8 | 8.4 | 8.4 | 13.8 | 7.6 | 8.8 | 7.0 |
| | 9.2 | 12.8 | 13.5 | 17.8 | 10.8 | 10.9 | 10.5 |
| | 10.4 | 9.5 | 11.1 | 11.0 | 9.8 | 11.3 | 12.9 |
| | 5.4 | 6.5 | 7.4 | 10.5 | 7.3 | 4.9 | 4.8 |
| | 8.4 | 10.5 | 9.8 | 17.1 | 13.1 | 9.0 | 10.2 |
| | 7.1 | 8.1 | 7.6 | 10.8 | 8.9 | 7.7 | 7.0 |
| Mean | 8.4 | 9.3 | 9.6 | 13.2 | 10.8 | 8.8 | 8.8 |
| SD | 1.8 | 2.2 | 2.3 | 2.8 | 4.0 | 2.3 | 3.0 |
| Δ'9aFF - 100 mg. | | | | | | | |
| | 9.3 | 7.4 | 7.9 | 14.3 | 9.0 | 10.5 | 7.9 |
| | 7.8 | 7.4 | 7.1 | 27.2 | 16.4 | 14.3 | 9.0 |
| | 9.4 | 11.0 | 9.8 | 8.1 | 13.9 | 12.8 | 11.0 |
| | 5.4 | 8.9 | 8.9 | 15.2 | 9.4 | 6.8 | 4.9 |
| | 8.1 | 10.3 | 9.7 | 14.9 | 11.8 | 18.1 | 10.6 |
| | 7.4 | 7.1 | 5.2 | 13.0 | 10.5 | 10.7 | 11.1 |
| Mean | 7.9 | 8.7 | 8.1 | 15.5 | 11.8 | 12.1 | 9.1 |
| SD | 1.5 | 1.7 | 1.7 | 6.3 | 2.8 | 3.7 | 2.4 |
| Δ'6MF - 25 mg. | | | | | | | |
| | 9.4 | 7.0 | 7.2 | 10.9 | 6.3 | 8.8 | 7.1 |
| | 8.7 | 7.9 | 6.6 | 9.9 | 7.6 | 8.9 | 7.1 |
| | 10.7 | 11.2 | 11.4 | 10.2 | 10.8 | 8.6 | 7.2 |
| | 10.6 | 11.0 | 10.5 | 13.6 | 11.2 | 9.3 | 9.4 |
| | 9.2 | 5.3 | 6.3 | 7.2 | 7.5 | 4.8 | 6.0 |
| | 3.7 | 6.7 | 5.8 | 4.3 | 4.9 | 5.1 | 6.9 |
| Mean | 8.7 | 8.2 | 8.0 | 9.4 | 8.1 | 7.6 | 7.3 |
| SD | 2.6 | 2.4 | 2.4 | 3.2 | 2.5 | 2.0 | 1.1 |
| Δ'6MF - 100 mg. | 8.1 | 7.8 | 7.3 | 7.8 | 8.3 | 7.6 | 6.4 |
| | 9.7 | 10.7 | 9.4 | 12.8 | 13.1 | 11.2 | 10.0 |
| | 11.3 | 12.7 | 10.0 | 11.6 | 9.9 | 9.5 | 8.9 |
| | 10.2 | 9.7 | 9.0 | 10.0 | 11.2 | 8.1 | 8.0 |
| | 6.8 | 6.7 | 5.6 | 8.6 | 7.8 | 6.4 | 6.4 |
| | 4.0 | 7.3 | 5.7 | 9.7 | 9.7 | 6.7 | 8.6 |
| Mean | 8.3 | 9.2 | 7.8 | 10.1 | 10.0 | 8.3 | 8.1 |
| SD | 3.0 | 2.4 | 1.9 | 1.8 | 1.9 | 2.0 | 1.4 |

EXPERIMENT I

NEUTROPHILS (THOUSANDS/MM$^3$) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF PREDNISOLONE AND 9a-FLUOROHYDROCORTISONE

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta^1$F - 50 mg. | | | | | | | |
| | 7.9 | 5.6 | 1.3 | 8.2 | 2.6 | 2.5 | 3.5 |
| | 3.6 | 3.0 | 2.2 | 4.9 | 2.5 | 3.4 | 1.9 |
| | 2.3 | 2.5 | 2.1 | 3.4 | 4.7 | 2.7 | 1.1 |
| | 3.5 | 4.7 | 3.7 | 9.4 | 5.1 | 1.9 | 2.2 |
| | 3.6 | 4.2 | 4.0 | 6.7 | 4.6 | 4.7 | 2.7 |
| | 2.4 | 1.7 | 3.3 | 4.7 | 2.4 | 1.8 | 2.2 |
| Mean | 3.9 | 3.6 | 2.8 | 6.2 | 3.6 | 2.8 | 2.3 |
| SD | 2.1 | 1.4 | 1.1 | 2.3 | 1.3 | 1.1 | 0.8 |
| $\Delta^1$F - 100 mg. | | | | | | | |
| | 1.1 | 1.4 | 1.4 | 5.1 | 1.9 | 1.5 | 1.6 |
| | 3.2 | 2.3 | 3.0 | 6.6 | 5.4 | 2.0 | 2.8 |
| | 2.2 | 2.6 | 1.7 | 3.9 | 3.6 | 2.7 | 2.6 |
| | 1.6 | 2.5 | 3.9 | 5.1 | 2.4 | 1.9 | 1.0 |
| | 2.4 | 2.3 | 3.1 | 7.3 | 3.1 | 1.8 | 1.7 |
| | 3.2 | 4.4 | 2.5 | 8.6 | 4.9 | 3.2 | 3.9 |
| Mean | 2.3 | 2.6 | 4.9 | 6.1 | 3.6 | 2.2 | 2.3 |
| SD | 0.8 | 1.0 | 1.0 | 1.7 | 1.4 | 1.4 | 1.0 |
| 9aFF -25 mg. | | | | | | | |
| | 3.2 | 4.7 | 2.9 | 8.2 | 3.8 | 3.1 | 2.7 |
| | 3.3 | 3.1 | 3.4 | 6.2 | 2.9 | 3.0 | 3.0 |
| | 4.6 | 2.9 | 2.6 | 5.0 | 3.7 | 3.7 | 3.3 |
| | 1.1 | 1.5 | 2.1 | 2.8 | 1.9 | 1.4 | 0.6 |
| | 2.7 | 2.6 | 3.6 | 4.4 | 2.8 | 1.7 | 1.4 |
| | 1.5 | 2.4 | 3.3 | 2.4 | 1.6 | 1.8 | 1.6 |
| Mean | 2.7 | 2.9 | 3.0 | 4.8 | 2.8 | 2.5 | 2.1 |
| SD | 1.3 | 1.0 | 0.2 | 2.2 | 0.9 | 0.9 | 1.1 |
| 9aFF - 100 mg. | | | | | | | |
| | 3.3 | 6.2 | 4.3 | 9.4 | 3.0 | 3.3 | 3.5 |
| | 3.8 | 3.0 | 1.9 | 3.9 | 4.3 | 3.6 | 2.5 |
| | 2.1 | 2.4 | 2.2 | 2.6 | 3.1 | 1.9 | 1.8 |
| | 2.3 | 2.5 | 3.8 | 5.3 | 3.7 | 2.4 | 1.8 |
| | 2.0 | 2.8 | 3.2 | 5.1 | 2.4 | 2.9 | 0.7 |
| | 4.0 | 3.6 | 3.7 | 5.8 | 3.8 | 3.6 | 3.1 |
| Mean | 2.9 | 3.4 | 3.2 | 5.4 | 3.4 | 3.0 | 2.2 |
| SD | 0.9 | 1.4 | 0.9 | 1.8 | 0.7 | 1.5 | 1.1 |

EXPERIMENT I

NEUTROPHILS (THOUSANDS/$MM^3$) IN CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF 9a-FLUOROPREDNISOLONE AND 6-METHYLPREDNISOLONE

| Treatment and Dosage | -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| | | | Days Postinjection | | | | |
| $\Delta^1$9aFF - 25 mg. | | | | | | | |
| | 3.9 | 3.9 | 3.4 | 9.8 | 2.0 | 3.9 | 2.8 |
| | 4.2 | 5.9 | 5.5 | 5.8 | 9.8 | 3.4 | 3.3 |
| | 3.3 | 1.8 | 3.1 | 4.0 | 4.0 | 2.9 | 4.6 |
| | 1.7 | 2.0 | 3.0 | 4.1 | 3.0 | 1.0 | 2.0 |
| | 3.3 | 2.6 | 3.3 | 9.7 | 4.1 | 3.9 | 3.5 |
| | 2.7 | 3.0 | 3.0 | 6.0 | 4.3 | 2.8 | 2.1 |
| Mean | 3.2 | 3.2 | 3.6 | 6.6 | 4.5 | 3.0 | 3.1 |
| SD | 0.9 | 1.6 | 1.0 | 2.6 | 3.4 | 1.1 | 1.0 |
| $\Delta^1$9aFF - 100 mg. | | | | | | | |
| | 2.6 | 3.1 | 2.8 | 11.1 | 5.2 | 6.5 | 3.7 |
| | 2.2 | 2.2 | 2.1 | 20.4 | 11.1 | 9.1 | 2.7 |
| | 1.9 | 3.7 | 3.1 | 5.3 | 6.9 | 6.7 | 5.8 |
| | 2.0 | 3.6 | 2.8 | 11.4 | 6.0 | 3.3 | 2.0 |
| | 3.8 | 3.1 | 3.3 | 9.5 | 7.8 | 10.8 | 4.0 |
| | 2.6 | 2.6 | 1.6 | 7.7 | 5.0 | 4.3 | 3.0 |
| Mean | 2.5 | 3.1 | 2.6 | 10.9 | 7.0 | 6.8 | 3.5 |
| SD | 0.7 | 0.6 | 0.2 | 5.1 | 2.3 | 2.8 | 1.3 |
| 6M$\Delta^1$F - 25 mg. | | | | | | | |
| | 2.2 | 2.3 | 2.2 | 8.1 | 1.9 | 3.5 | 1.8 |
| | 2.4 | 2.4 | 2.0 | 3.3 | 2.5 | 2.8 | 3.0 |
| | 3.8 | 5.1 | 3.8 | 3.8 | 3.9 | 2.9 | 2.5 |
| | 2.6 | 4.0 | 3.8 | 5.8 | 2.1 | 1.8 | 2.0 |
| | 4.9 | 1.3 | 2.2 | 1.9 | 1.3 | 1.5 | 1.8 |
| | 1.4 | 2.5 | 2.0 | 1.2 | 1.5 | 1.8 | 2.1 |
| Mean | 2.9 | 2.9 | 2.7 | 4.0 | 2.2 | 2.4 | 2.2 |
| SD | 1.2 | 1.3 | 0.9 | 2.6 | 0.9 | 0.8 | 0.5 |
| 6M$\Delta^1$F - 100 mg. | | | | | | | |
| | 1.9 | 2.3 | 1.7 | 4.8 | 3.2 | 3.1 | 2.5 |
| | 3.5 | 3.7 | 2.3 | 4.9 | 3.9 | 4.5 | 2.7 |
| | 3.8 | 4.1 | 2.9 | 5.7 | 6.0 | 2.9 | 2.4 |
| | 2.2 | 2.3 | 3.6 | 3.3 | 7.2 | 2.7 | 2.0 |
| | 2.6 | 3.2 | 2.7 | 4.2 | 3.0 | 3.0 | 1.9 |
| | 1.0 | 2.2 | 1.7 | 3.4 | 3.0 | 2.0 | 2.3 |
| Mean | 2.5 | 3.0 | 2.5 | 4.4 | 4.4 | 3.0 | 2.3 |
| SD | 1.0 | 0.8 | 0.7 | 0.9 | 1.8 | 1.8 | 0.3 |

EXPERIMENT

LYMPHOCYTES (THOUSANDS/MM$^3$) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF PREDNISOLONE AND 9a-FLUOROHYDROCOTRISONE

| Treatment and Dosage | -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| | | | Days Postinjection | | | | |
| $\Delta^1$F - 50 mg. | | | | | | | |
| | 8.1 | 7.2 | 8.3 | 6.4 | 5.9 | 6.1 | 6.8 |
| | 3.5 | 4.1 | 3.8 | 5.0 | 4.0 | 2.6 | 2.9 |
| | 4.4 | 3.8 | 3.5 | 3.4 | 4.9 | 4.4 | 3.0 |
| | 6.1 | 3.8 | 4.3 | 4.3 | 7.2 | 6.4 | 4.0 |
| | 3.8 | 3.5 | 6.1 | 4.3 | 5.0 | 5.1 | 4.3 |
| | 4.7 | 2.5 | 5.5 | 4.9 | 2.9 | 4.0 | 4.0 |
| Mean | 5.4 | 4.2 | 5.3 | 4.7 | 5.0 | 4.8 | 4.2 |
| SD | 1.5 | 1.6 | 1.8 | 1.0 | 1.6 | 1.3 | 1.4 |
| $\Delta^1$F - 100 mg. | | | | | | | |
| | 8.2 | 6.2 | 7.1 | 4.2 | 6.4 | 5.6 | 5.9 |
| | 3.8 | 4.1 | 4.7 | 7.4 | 6.0 | 5.6 | 5.5 |
| | 4.1 | 3.4 | 2.7 | 3.2 | 4.8 | 3.4 | 3.6 |
| | 2.9 | 4.2 | 4.5 | 3.9 | 3.9 | 3.0 | 4.0 |
| | 4.8 | 4.1 | 4.0 | 4.5 | 3.2 | 3.4 | 2.6 |
| | 4.2 | 5.4 | 3.7 | 5.4 | 4.0 | 4.6 | 6.3 |
| Mean | 4.7 | 4.6 | 4.5 | 4.8 | 4.7 | 4.2 | 4.6 |
| SD | 1.8 | 1.0 | 1.5 | 1.6 | 1.2 | 1.2 | 1.5 |
| 9a-FF - 25 mg. | 6.0 | 6.6 | 7.1 | 3.8 | 5.7 | 3.5 | 3.7 |
| | 5.5 | 6.2 | 6.4 | 7.9 | 7.0 | 4.4 | 6.0 |
| | 2.9 | 4.0 | 3.7 | 4.3 | 3.8 | 3.8 | 2.7 |
| | 2.5 | 3.2 | 4.0 | 4.1 | 4.0 | 2.9 | 4.1 |
| | 4.7 | 5.1 | 5.0 | 5.7 | 4.8 | 3.2 | 2.5 |
| | 3.5 | 4.1 | 6.9 | 3.5 | 2.5 | 3.2 | 3.3 |
| Mean | 4.2 | 4.7 | 5.6 | 4.9 | 4.6 | 3.7 | 3.9 |
| SD | 1.4 | 1.2 | 1.5 | 1.7 | 1.6 | 2.2 | 1.3 |
| 9aFF - 100 mg. | | | | | | | |
| | 6.0 | 6.9 | 7.3 | 4.0 | 5.9 | 6.3 | 7.4 |
| | 3.8 | 2.8 | 4.0 | 7.8 | 5.6 | 3.5 | 4.3 |
| | 3.5 | 4.0 | 4.6 | 4.7 | 5.7 | 4.2 | 2.5 |
| | 3.7 | 5.7 | 5.1 | 5.1 | 9.0 | 3.4 | 4.9 |
| | 2.6 | 3.0 | 3.8 | 3.6 | 3.9 | 3.5 | 3.5 |
| | 5.2 | 5.4 | 5.9 | 6.5 | 4.8 | 6.6 | 6.4 |
| Mean | 4.1 | 4.6 | 5.1 | 5.3 | 5.8 | 3.6 | 4.9 |
| SD | 1.2 | 1.6 | 1.3 | 1.6 | 1.7 | 1.5 | 1.8 |

EXPERIMENT

LYMPHOCYTES (THOUSANDS/$MM^3$) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. INJECTION OF 9a-FLUOROPREDNISOLONE AND 6-METHYLPREDNISOLONE

| Treatment and Dosage | Days Postinjection -3 | -1 | 0 | 1 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| $\Delta$9aFF - 25 mg. | | | | | | | |
| | 4.9 | 4.0 | 4.5 | 3.3 | 5.2 | 4.2 | 4.1 |
| | 3.5 | 5.5 | 6.3 | 8.4 | 5.9 | 3.5 | 4.5 |
| | 5.1 | 5.9 | 6.6 | 6.2 | 5.4 | 7.6 | 7.1 |
| | 2.5 | 3.2 | 3.4 | 5.0 | 3.5 | 3.4 | 2.4 |
| | 4.0 | 5.3 | 5.3 | 6.8 | 6.7 | 4.7 | 6.0 |
| | 3.8 | 4.2 | 3.7 | 4.1 | 5.0 | 4.0 | 4.2 |
| Mean | 4.0 | 4.7 | 5.0 | 5.6 | 5.3 | 4.6 | 4.7 |
| SD | 0.9 | 1.0 | 1.3 | 1.8 | 1.0 | 1.6 | 1.6 |
| $\Delta^1$9aFF - 100 mg. | | | | | | | |
| | 6.2 | 3.8 | 4.6 | 2.9 | 3.5 | 4.0 | 3.7 |
| | 4.4 | 4.6 | 5.0 | 6.3 | 4.9 | 3.8 | 4.6 |
| | 5.9 | 5.0 | 4.8 | 2.8 | 6.3 | 5.4 | 5.1 |
| | 2.8 | 4.4 | 4.5 | 3.5 | 3.4 | 3.3 | 2.6 |
| | 4.0 | 6.6 | 6.1 | 5.2 | 4.3 | 6.9 | 5.7 |
| | 3.8 | 3.8 | 3.1 | 4.6 | 4.7 | 5.2 | 7.1 |
| Mean | 4.5 | 4.7 | 4.7 | 4.2 | 4.5 | 418 | 4.8 |
| SD | 1.3 | 1.0 | 1.0 | 1.4 | 1.1 | 1.3 | 1.6 |
| 6M$\Delta^1$F - 25 mg. | | | | | | | |
| | 6.7 | 4.4 | 4.8 | 2.5 | 4.0 | 4.3 | 4.5 |
| | 5.2 | 5.1 | 4.2 | 5.9 | 4.3 | 4.3 | 3.1 |
| | 5.4 | 4.8 | 6.7 | 5.7 | 5.5 | 4.2 | 4.5 |
| | 5.1 | 5.5 | 5.9 | 6.8 | 6.9 | 6.6 | 6.4 |
| | 3.0 | 3.7 | 2.9 | 3.9 | 6.1 | 2.3 | 3.6 |
| | 1.9 | 3.4 | 3.3 | 2.6 | 2.9 | 2.9 | 3.8 |
| Mean | 4.6 | 4.5 | 4.6 | 4.6 | 4.9 | 4.1 | 4.3 |
| SD | 1.7 | 0.8 | 1.4 | 1.8 | 1.4 | 1.5 | 1.1 |
| 6M$\Delta^1$F - 100 mg. | | | | | | | |
| | 6.0 | 5.2 | 3.7 | 2.8 | 4.1 | 4.3 | 3.2 |
| | 5.5 | 6.0 | 5.6 | 6.4 | 6.6 | 4.1 | 5.3 |
| | 6.7 | 6.8 | 6.7 | 5.2 | 3.3 | 5.7 | 5.8 |
| | 5.7 | 5.6 | 4.1 | 4.8 | 4.0 | 4.1 | 4.4 |
| | 3.5 | 3.0 | 2.6 | 3.7 | 4.4 | 2.9 | 3.6 |
| | 2.8 | 4.4 | 3.2 | 5.7 | 5.3 | 3.8 | 5.7 |
| Mean | 5.0 | 5.2 | 4.3 | 4.8 | 4.6 | 4.2 | 4.7 |
| SD | 1.5 | 1.3 | 1.5 | 1.3 | 1.2 | 0.8 | 1.1 |

EXPERIMENT I

EOSINOPHILS (THOUSANDS/$MM^3$) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. ADMINISTRATION OF PREDNISOLONE AND 9a-FLUOROHYDROCORTISONE

| Treatment and Dosage | Days Postinjection | | | | | | |
|---|---|---|---|---|---|---|---|
| | -3 | -1 | 0 | 1 | 2 | 4 | 6 |
| $\Delta^1$F - 50 mg. | | | | | | | |
| | 0 | 0 | 0.20 | 0.15 | 0.93 | 0.90 | 0.44 |
| | 0.40 | 0.22 | 0.68 | 0.32 | 0.99 | 0.61 | 0.56 |
| | 1.03 | 0.95 | 0.50 | 1.52 | 1.96 | 0.15 | 0.27 |
| | 0.30 | 0.37 | 0.34 | 0.75 | 0.78 | 0.44 | 1.31 |
| | 0.56 | 0.68 | 0.66 | 0.11 | 0.84 | 0.85 | 0.46 |
| | 0.22 | 0.43 | 1.35 | 0.19 | 0.22 | 0.66 | 0.86 |
| Mean | 0.42 | 0.44 | 0.62 | 0.51 | 0.81 | 0.47 | 0.65 |
| SD | 0.32 | 0.28 | 0.28 | 0.54 | 0.35 | 0.27 | 0.38 |
| $\Delta^1$F - 100 mg. | | | | | | | |
| | 0 | 0 | 0.36 | 0.95 | 0.84 | 0.46 | 0.75 |
| | 1.56 | 1.67 | 2.00 | 0.14 | 0.88 | 0.95 | 0.95 |
| | 0.49 | 1.09 | 0.51 | 0.76 | 0.96 | 0.27 | 0.86 |
| | 0.46 | 0.21 | 0.63 | 0.91 | 0.41 | 0.15 | 0.44 |
| | 0. | 0.41 | 0.63 | 0.37 | 0.26 | 0.29 | 0.37 |
| | 0.66 | 0.88 | 0.83 | 0.14 | 0.49 | 0.61 | 0.32 |
| Mean | 0.53 | 0.71 | 0.83 | 0.55 | 0.68 | 0.45 | 0.50 |
| SD | 0.56 | 0.62 | 0.60 | 0.37 | 0.59 | 0.29 | 0.27 |
| 9aFF - 25 mg. | | | | | | | |
| | 0.95 | 0.88 | 0.90 | 0.25 | 0.53 | 1.17 | 0.92 |
| | 0.90 | 0.62 | 0.88 | 0.62 | 0.92 | 1.13 | 0.80 |
| | 0.77 | 0.72 | 0.35 | 0.96 | 0.24 | 0.41 | 0.12 |
| | 0.55 | 0.26 | 1.60 | 1.05 | 0.81 | 0.28 | 0.90 |
| | 0.15 | 0.73 | 0.27 | 0.46 | 0.31 | 0.15 | 0.58 |
| | 0.44 | 0.35 | 0.88 | 0.91 | 0.62 | 0.91 | 0.68 |
| Mean | 0.63 | 0.59 | 0.81 | 0.49 | 0.57 | 0.67 | 0.67 |
| SD | 0.30 | 0.23 | 0.47 | 0.34 | 0.27 | 0.45 | 0.30 |
| 9aFF - 100 mg. | | | | | | | |
| | 0.20 | 0 | 0.61 | 0.14 | 0.69 | 0.61 | 0.84 |
| | 0.74 | 1.99 | 0.46 | 0.37 | 1.74 | 1.05 | 1.13 |
| | 0.71 | 0.99 | 0.14 | 0.66 | 0 | 0.19 | 0.35 |
| | 0.32 | 0.27 | 0.18 | 0.33 | 0.13 | 0.25 | 0.28 |
| | 0.64 | 0.96 | 0.87 | 0.90 | 1.14 | 0.27 | 0.44 |
| | 0.19 | 0.69 | 0.85 | 0.65 | 0.56 | 0.89 | 1.46 |
| Mean | 0.47 | 0.81 | 0.52 | 0.51 | 0.71 | 0.54 | 0.75 |
| SD | 0.26 | 0.69 | 0.32 | 0.28 | 0.65 | 0.36 | 0.47 |

EXPERIMENT I

EOSINOPHILS (THOUSANDS/$MM^3$) OF CATTLE PRIOR TO AND FOLLOWING THE I.M. INJECTION OF 9a-FLUOROPREDNISOLONE AND 6-METHYLPREDNISOLONE

| Treatment and Dosage | Days Postinjection | | | | | | |
|---|---|---|---|---|---|---|---|
| | -3 | -1 | 0 | 1 | 2 | 4 | 6 |
| Δ'9aFF - 25 mg. | | | | | | | |
| | 0.29 | 0.84 | 0.50 | 0.28 | 0.15 | 0.53 | 0 |
| | 1.01 | 0.90 | 1.22 | 1.42 | 1.60 | 3.60 | 2.42 |
| | 1.87 | 1.62 | 1.22 | 0.55 | 0.29 | 0.79 | 0.90 |
| | 1.14 | 0.46 | 0.96 | 1.16 | 0.80 | 0.34 | 0.24 |
| | 1.18 | 1.68 | 0.89 | 0.17 | 1.97 | 0.36 | 0.61 |
| | 0.57 | 0.82 | 0.84 | 0.32 | 0.36 | 0.62 | 0.63 |
| Mean | 1.01 | 0.93 | 0.94 | 0.65 | 0.82 | 1.04 | 0.80 |
| SD | 0.54 | 0.47 | 0.27 | 0.51 | 0.75 | 1.19 | 0.85 |
| Δ'9aFF - 100 mg. | | | | | | | |
| | 0.19 | 0.30 | 0.55 | 0.14 | 0 | 0 | 0.87 |
| | 0.47 | 0.45 | 0.50 | 0 | 0.33 | 0.54 | 1.17 |
| | 1.32 | 1.98 | 1.76 | 0 | 0.24 | 0.70 | 0 |
| | 0.32 | 0.62 | 1.51 | 0.15 | 0 | 0.68 | 0.98 |
| | 0.24 | 0.52 | 0.29 | 0.15 | 0.23 | 0.18 | 0.64 |
| | 0.96 | 0.72 | 0.52 | 0.39 | 0.53 | 0.96 | 0.78 |
| Mean | 0.59 | 0.76 | 0.86 | 0.14 | 0.22 | 0.41 | 0.59 |
| SD | 0.45 | 0.61 | 0.62 | 0.14 | 0.20 | 0.35 | 0.40 |
| 6MΔ'F - 25 mg. | | | | | | | |
| | 0.94 | 0 | 0.14 | 0 | 0.32 | 0.53 | 0.57 |
| | 0.44 | 0.32 | 0.20 | 0.50 | 0.76 | 1.42 | 0.79 |
| | 1.28 | 1.13 | 0.68 | 0.82 | 1.30 | 1.03 | 0.22 |
| | 2.33 | 1.42 | 0.74 | 0.54 | 1.79 | 0.56 | 0.94 |
| | 1.01 | 0.27 | 1.12 | 1.30 | 0.75 | 0.96 | 0.54 |
| | 0.30 | 0.68 | 0.41 | 0.43 | 0.50 | 0.36 | 0.90 |
| Mean | 0.91 | 0.64 | 0.55 | 0.60 | 0.79 | 0.81 | 0.66 |
| SD | 0.72 | 0.55 | 0.37 | 0.43 | 0.54 | 0.40 | 0.27 |
| 6MΔ'F - 100 mg. | | | | | | | |
| | 0 | 0 | 0.15 | 0.78 | 0.33 | 0.15 | 0.51 |
| | 0.39 | 0.75 | 1.22 | 1.41 | 2.10 | 2.13 | 1.60 |
| | 0.68 | 1.68 | 0.20 | 0.58 | 0.20 | 0.67 | 0.53 |
| | 2.14 | 1.46 | 1.26 | 1.60 | 1.34 | 1.13 | 1.52 |
| | 0.54 | 0.54 | 0.22 | 0.51 | 0.47 | 0.32 | 0.84 |
| | 0.16 | 0.59 | 0.45 | 0.48 | 1.16 | 0.80 | 0.52 |
| Mean | 0.65 | 0.83 | 0.58 | 0.78 | 0.93 | 0.87 | 0.92 |
| SD | 0.77 | 0.63 | 0.51 | 0.48 | 0.58 | 0.71 | 0.50 |

EXPERIMENT I

BLOOD GLUCOSE VALUES (MG. %) IN CATTLE PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. Of 9a-FLUOROPREDNISOLONE

| | | Days Postinjection | | | | |
|---|---|---|---|---|---|---|
| Dosage | | -1 | 0 | 1 | 2 | 3 |
| 100 mg. | | 52 | 48 | 85 | 60 | 44 |
| | | 50 | 48 | 86 | 46 | 46 |
| | | 50 | 42 | 70 | 40 | 44 |
| | | 54 | 48 | 72 | 53 | 42 |
| | | 46 | 60 | 70 | 67 | 44 |
| | | 60 | 60 | 70 | 70 | 40 |
| | | 48 | 52 | 66 | 50 | 44 |
| | | 46 | 46 | 62 | 51 | 44 |
| | Mean | 50.7 | 50.7 | 72.6 | 54.6 | 43.5 |
| | SD | 4.6 | 6.5 | 8.5 | 10.3 | 1.8 |
| 200 mg. | | 56 | 40 | 100 | 60 | 62 |
| | | 55 | 56 | 85 | 50 | 42 |
| | | 46 | 50 | 70 | 40 | 44 |
| | | 52 | 48 | 70 | 40 | 46 |
| | | 42 | 60 | 95 | 64 | 44 |
| | | 40 | 62 | 85 | 60 | 40 |
| | | 43 | 42 | 69 | 45 | 40 |
| | | 48 | 50 | 56 | 48 | 42 |
| | Mean | 47.5 | 51.0 | 78.7 | 51.0 | 44.8 |
| | SD | 6.0 | 7.9 | 14.9 | 9.4 | 7.4 |

EXPERIMENT I

LEUCOCYTES (THOUSANDS/$MM^3$) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. 9a-FLUOROPREDNISOLONE

| Dosage | | Days Postinjection -1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 100 mg. | | | | | | |
| | | 8.6 | 7.6 | 10.6 | 8.2 | 7.3 |
| | | 11.3 | 12.1 | 12.4 | 9.5 | 9.8 |
| | | 5.7 | 7.2 | 9.4 | 6.1 | 6.9 |
| | | 9.3 | 9.9 | 10.5 | 9.9 | 8.1 |
| | | 7.2 | 7.5 | 6.8 | 7.6 | 5.2 |
| | | 6.3 | 7.6 | 10.7 | 6.2 | 6.1 |
| | | 7.1 | 7.2 | 8.5 | 9.5 | 8.5 |
| | | 8.2 | 9.8 | 12.5 | 8.2 | 7.7 |
| | Mean | 8.0 | 8.6 | 10.2 | 8.2 | 7.5 |
| | SD | 1.8 | 1.8 | 1.9 | 1.4 | 1.4 |
| 200 mg. | | | | | | |
| | | 9.8 | 9.3 | 11.4 | 9.4 | 10.3 |
| | | 7.2 | 7.3 | 16.1 | 10.0 | 8.2 |
| | | 7.0 | 5.4 | 11.0 | 6.9 | 8.0 |
| | | 6.5 | 6.1 | 11.6 | 9.4 | 6.1 |
| | | 6.8 | 7.3 | 14.4 | 6.4 | 7.7 |
| | | 6.1 | 6.4 | 14.3 | 10.3 | 10.2 |
| | | 6.4 | 7.3 | 10.9 | 10.4 | 7.7 |
| | | 9.1 | 10.8 | 12.5 | 10.5 | 13.4 |
| | Mean | 7.4 | 7.5 | 12.8* | 9.2 | 9.0 |
| | SD | 1.3 | 1.8 | 1.9 | 1.6 | 1.9 |

*P = 0.005

EXPERIMENT

NEUTROPHILS (THOUSANDS/MM$^3$) IN CATTLE PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. 9a-FLUOROPREDNISOLONE

| Dosage | | Days Postinjection -1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 100 mg. | | | | | | |
| | | 0.31 | 0.29 | 0.62 | 0.33 | 0.28 |
| | | 0.50 | 0.25 | 0.69 | 0.35 | 0.17 |
| | | 0.22 | 0.25 | 0.56 | 0.22 | 0.28 |
| | | 0.35 | 0.37 | 0.48 | 0.37 | 0.30 |
| | | 0.17 | 0.20 | 0.25 | 0.16 | 0.16 |
| | | 0.25 | 0.22 | 0.49 | 0.22 | 0.26 |
| | | 0.20 | 0.24 | 0.39 | 0.26 | 0.24 |
| | | 0.33 | 0.45 | 0.85 | 0.32 | 0.26 |
| | Mean | 0.29 | 0.29 | 0.54 | 0.28 | 0.24 |
| | SD | 0.10 | 0.08 | 0.19 | 0.07 | 0.05 |
| 200 mg. | | | | | | |
| | | 0.33 | 0.13 | 0.77 | 0.25 | 0.34 |
| | | 0.29 | 0.25 | 0.87 | 0.26 | 0.19 |
| | | 0.18 | 0.15 | 0.64 | 0.31 | 0.28 |
| | | 0.23 | 0.20 | 0.82 | 0.39 | 0.26 |
| | | 0.29 | 0.30 | 1.04 | 0.22 | 0.27 |
| | | 0.21 | 0.29 | 1.08 | 0.43 | 0.51 |
| | | 0.23 | 0.31 | 0.64 | 0.40 | 0.30 |
| | | 0.41 | 0.51 | 0.89 | 0.55 | 0.46 |
| | Mean | 0.27 | 0.27 | 0.84 | 0.35 | 0.33 |
| | SD | 0.08 | 0.12 | 0.16 | 0.10 | 0.11 |

EXPERIMENT I

LYMPHOCYTES (THOUSANDS/MM$^3$) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. OF 9a-FLUOROPREDNISOLONE

| Dosage | | Days Postinjection -1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 100 mg. | | | | | | |
| | | 4.0 | 2.5 | 3.8 | 4.2 | 4.0 |
| | | 5.3 | 8.7 | 5.2 | 3.5 | 6.5 |
| | | 2.9 | 3.7 | 3.4 | 3.1 | 3.8 |
| | | 4.8 | 5.0 | 4.9 | 5.7 | 4.1 |
| | | 5.0 | 4.8 | 3.9 | 5.5 | 3.1 |
| | | 3.5 | 4.2 | 4.9 | 3.0 | 2.6 |
| | | 4.9 | 4.3 | 4.3 | 5.9 | 5.4 |
| | | 3.9 | 3.8 | 4.0 | 3.4 | 4.0 |
| | Mean | 4.3 | 4.6 | 4.3 | 4.3 | 4.2 |
| | SD | 0.8 | 1.8 | 0.6 | 1.2 | 1.2 |
| 200 mg. | | | | | | |
| | | 6.3 | 7.0 | 3.4 | 5.6 | 5.9 |
| | | 3.7 | 3.7 | 6.4 | 5.5 | 4.2 |
| | | 4.8 | 2.8 | 4.5 | 3.5 | 4.5 |
| | | 3.6 | 3.7 | 3.4 | 4.3 | 3.1 |
| | | 3.2 | 3.6 | 3.5 | 3.5 | 4.3 |
| | | 3.4 | 3.1 | 2.9 | 6.1 | 4.5 |
| | | 3.5 | 3.6 | 4.1 | 5.2 | 3.8 |
| | | 4.1 | 4.5 | 3.4 | 4.2 | 6.7 |
| | Mean | 4.1 | 4.0 | 4.0 | 4.7 | 4.6 |
| | SD | 1.0 | 1.3 | 1.1 | 1.0 | 1.1 |

EXPERIMENT I

EOSINOPHILS (THOUSANDS/$MM^3$) IN CATTLE PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. OF 9a-FLUOROPREDNISOLONE

| Dosage | | Days Postinjection | | | | |
|---|---|---|---|---|---|---|
| | | -1 | 0 | 1 | 2 | 3 |
| 100 mg. | | | | | | |
| | | 1.54 | 2.19 | 0.64 | 0.65 | 0.44 |
| | | 0.90 | 0.84 | 0.25 | 2.09 | 1.47 |
| | | 0.62 | 0.94 | 0.94 | 0.73 | 0.21 |
| | | 0.74 | 0.98 | 0.52 | 0.39 | 0.97 |
| | | 0.21 | 0.22 | 0.20 | 0.38 | 0.10 |
| | | 0.25 | 0.83 | 0.43 | 0.79 | 0.92 |
| | | 0.21 | 0.43 | 0.26 | 0.56 | 0.60 |
| | | 0.82 | 1.26 | 0 | 1.39 | 0.77 |
| | Mean | 0.66 | 0.96 | 0.30 | 0.87 | 0.68 |
| | SD | 0.44 | 0.60 | 0.26 | 0.59 | 0.44 |
| 200 mg. | | | | | | |
| | | 0.20 | 0.93 | 0.23 | 1.22 | 0.72 |
| | | 0.58 | 1.10 | 0.64 | 1.90 | 2.13 |
| | | 0.28 | 0.86 | 0 | 0.34 | 0.64 |
| | | 0.39 | 0.49 | 0 | 0.94 | 0.30 |
| | | 0.54 | 0.58 | 0 | 0.38 | 0.46 |
| | | 0.49 | 0.32 | 0.29 | 0.62 | 0.61 |
| | | 0.64 | 0.44 | 0 | 0.94 | 0.77 |
| | | 0.73 | 0.86 | 0.13 | 0.52 | 2.01 |
| | Mean | 0.48 | 0.70 | 0.16 | 0.86 | 0.96 |
| | SD | 0.17 | 0.26 | 0.16 | 0.51 | 0.70 |

EXPERIMENT I

PLASMA SODIUM (MG. %) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. 9a-FLUOROPREDNISOLONE

| | | Days Postinjection | | | | |
|---|---|---|---|---|---|---|
| Dosage | | -1 | 0 | 1 | 2 | 3 |
| 100 mg. | | | | | | |
| | | 368 | 308 | 308 | 302 | 337 |
| | | 305 | 316 | 300 | 32. | 316 |
| | | | 335 | 354 | 332 | 325 |
| | | 338 | 348 | 339 | 334 | 315 |
| | | 315 | 305 | 321 | 298 | 298 |
| | | 338 | 329 | 332 | 324 | 314 |
| | | 319 | 316 | 324 | 340 | 336 |
| | | 307 | 316 | 317 | 320 | 316 |
| | Mean | 327 | 322 | 324 | 321 | 320 |
| | SD | 22 | 14 | 17 | 14 | 13 |
| 200 mg. | | | | | | |
| | | 306 | 302 | 292 | 300 | 310 |
| | | 341 | 312 | 316 | 329 | 342 |
| | | 312 | 339 | 338 | 320 | 306 |
| | | 318 | 344 | 325 | 338 | 338 |
| | | 339 | 315 | 311 | 323 | 315 |
| | | 336 | 305 | 323 | 341 | 302 |
| | | 316 | 305 | 328 | 343 | 340 |
| | | 307 | 306 | 302 | 300 | 310 |
| | Mean | 322 | 316 | 317 | 324 | 320 |
| | SD | 15 | 16 | 15 | 17 | 17 |

EXPERIMENT I

PLASMA POTASSIUM (MG. %) PRIOR TO AND FOLLOWING THE ORAL ADMINISTRATION OF 100 AND 200 MG. 9a-FLUOROPREDNISOLONE

| Dosage | | Days Postinjection -1 | 0 | 1 | 2 | 3 |
|---|---|---|---|---|---|---|
| 100 mg. | | | | | | |
| | | 22.7 | 19.1 | 17.2 | 18.1 | 19.8 |
| | | 17.9 | 23.9 | 22.6 | 18.6 | 21.4 |
| | | | 19.1 | 20.4 | 21.0 | 19.7 |
| | | 19.3 | 1818 | 22.9 | 21.2 | 17.5 |
| | | 18.9 | 18.3 | 20.2 | 18.1 | 17.1 |
| | | 18.3 | 17.8 | 20.6 | 19.0 | 19.8 |
| | | 21.9 | 15.3 | 17.0 | 16.7 | 18.0 |
| | | 18.1 | 15.8 | 15.6 | 16.8 | 19.4 |
| | Mean | 19.6 | 18.5 | 19.6 | 18.8 | 19.1 |
| | SD | 1.9 | 2.6 | 2.7 | 1.3 | 1.4 |
| 200 mg. | | | | | | |
| | | 20.1 | 22.5 | 22.2 | 23.3 | 20.1 |
| | | 21.9 | 18.6 | 19.9 | 21.5 | 19.4 |
| | | 19.1 | 22.8 | 20.0 | 21.1 | 20.8 |
| | | 15.8 | 20.7 | 16.9 | 18.6 | 19.0 |
| | | 16.7 | 15.6 | 18.0 | 17.0 | 17.5 |
| | | 16.3 | 12.3 | 15.5 | 15.3 | 18.4 |
| | | 20.8 | 15.8 | 17.4 | 16.8 | 20.0 |
| | | 15.7 | 15.1 | 17.1 | 19.9 | 19.4 |
| | Mean | 18.3 | 17.9 | 18.4 | 19.2 | 19.3 |
| | SD | 2.5 | 3.8 | 2.1 | 2.7 | 1.0 |

EXPERIMENT II

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS TREATED WITH 0.16 MG. 9a-FLUOROPREDNISOLONE 5 HOURS BEFORE SACRIFICE. WARBURG FLASKS FLUSHED WITH OXYGEN

| I.M. Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 12.3 | 22.4 | 31.9 | 5.4 | 8.3 | 10.5 |
| 5.0 | 8.0 | 9.8 | 0.9 | 4.3 | 13.0 |
| 6.7 | 15.1 | 15.5 | 11.3 | 22.7 | 27.7 |
| 5.8 | 16.4 | 21.3 | 9.9 | 10.5 | 8.4 |
| 14.0 | 30.8 | 41.9 | 9.9 | 12.4 | 15.3 |
| 19.9 | 44.0 | 44.8 | 10.0 | 15.0 | 20.6 |
| 19.3 | 31.6 | 33.6 | 3.6 | 6.2 | 7.1 |
| 15.9 | 29.5 | 36.4 | 9.0 | 10.9 | 14.2 |
| 17.2 | 30.5 | 42.9 | 5.7 | 7.8 | 9.6 |
| 14.1 | 30.6 | 35.9 | 5.0 | 9.6 | 10.8 |
| 20.4 | 32.4 | 37.0 | 13.5 | 16.9 | 17.2 |
| 14.0 | 25.2 | 31.4 | 13.8 | 16.6 | 16.9 |
| 18.3 | 34.0 | 43.5 | 15.3 | 24.1 | 26.5 |
| 16.1 | 30.6 | 38.9 | 11.2 | 16.0 | 16.3 |
| 5.9 | 19.8 | 34.8 | 10.7 | 16.1 | 17.5 |
| 14.1 | 27.8 | 37.1 | 8.7 | 16.3 | 18.8 |
| 13.1 | 24.8 | 32.4 | 12.7 | 22.6 | 29.8 |
| 11.1 | 22.3 | 30.0 | 9.7 | 14.1 | 15.9 |
| 17.2 | 22.9 | 27.6 | 1.2 | 2.0 | 4.2 |
| 15.2 | 30.6 | 36.9 | 9.8 | 17.8 | 28.6 |
| 14.8 | 31.0 | 39.3 | 9.4 | 15.4 | 16.4 |
| 16.3 | 30.4 | 40.6 | 10.0 | 14.3 | 17.0 |
| 23.6 | 41.1 | 56.1 | 8.7 | 11.8 | 15.9 |
| 13.3 | 16.5 | 16.5 | 13.2 | 21.5 | 22.5 |
| 14.6 | 28.2 | 40.5 | 10.0 | 19.7 | 21.7 |
| 15.7 | 25.3 | 28.0 | 9.4 | 13.9 | 15.5 |
| Mean | | | | | |
| 14.4 | 27.0 | 34.0 | 9.2 | 14.1 | 16.9 |
| SD 1.4 | 2.5 | 3.1 | 1.1 | 1.5 | 2.1 |

EXPERIMENT II

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM RATS TREATED ORALLY WITH 0.32 MG. 9a-FLUOROPREDNISOLONE OR FROM UNTREATED RATS TO WHICH 0.08 MG. 9a-FLUOROPREDNISOLONE WAS ADDED TO THE INCUBATION FLASKS. FLASKS FLUSHED WITH OXYGEN.

| Oral | | | In Vitro | | |
|---|---|---|---|---|---|
| Incubation Time (Minutes) | | | Incubation Time (Minutes) | | |
| 10 | 20 | 30 | 10 | 20 | 30 |
| 9.5 | 17.3 | 24.2 | 0.6 | 4.2 | 8.3 |
| 17.8 | 31.7 | 39.3 | 7.5 | 8.4 | 7.7 |
| 0.9 | 9.1 | 12.6 | 14.3 | 25.0 | 28.3 |
| 6.9 | 14.5 | 15.1 | 10.3 | 24.8 | 36.2 |
| 1.9 | 13.7 | 15.8 | 4.1 | 9.4 | 11.7 |
| 19.1 | 34.8 | 42.8 | 6.8 | 9.0 | 11.2 |
| 10.4 | 16.3 | 15.0 | 4.3 | 6.9 | 6.6 |
| 7.1 | 10.9 | 15.4 | 4.8 | 5.9 | 6.3 |
| 12.4 | 21.1 | 26.5 | 6.2 | 9.2 | 9.4 |
| 24.0 | 35.9 | 42.0 | 7.7 | 11.7 | 12.5 |
| 15.7 | 25.4 | 27.4 | 0.9 | 3.8 | 6.3 |
| 10.4 | 12.9 | 14.3 | 5.4 | 10.9 | 14.6 |
| 17.3 | 30.9 | 43.6 | 1.0 | 5.6 | 6.8 |
| 17.2 | 27.7 | 40.4 | 1.4 | 3.4 | 4.7 |
| 4.2 | 7.2 | 7.9 | 2.2 | 3.2 | 4.4 |
| 13.0 | 27.6 | 30.0 | 2.7 | 5.1 | 4.8 |
| 15.9 | 27.9 | 37.0 | 4.4 | 8.9 | 9.5 |
| 18.8 | 31.1 | 42.7 | 12.6 | 17.3 | 20.3 |
| 10.2 | 21.5 | 30.7 | 10.5 | 14.7 | 18.3 |
| 11.5 | 20.6 | 25.5 | 8.2 | 14.3 | 18.1 |
| 14.7 | 19.5 | 21.3 | 7.0 | 10.9 | 12.7 |
| 18.2 | 19.1 | 20.4 | 4.5 | 5.1 | 5.1 |
| 14.1 | 30.7 | 39.0 | 8.3 | 16.2 | 16.2 |
| 12.6 | 23.6 | 33.5 | 7.9 | 16.7 | 16.7 |
| 13.6 | 26.3 | 34.3 | 5.1 | 11.1 | 10.4 |
| 12.7 | 28.8 | 44.9 | 5.1 | 7.8 | 8.0 |
| Mean | | | | | |
| 12.7 | 22.5 | 28.5 | 5.9 | 10.4 | 12.1 |
| SD | | | | | |
| 1.7 | 2.5 | 4.5 | 1.1 | 1.9 | 2.6 |

EXPERIMENT II

BLOOD GLUCOSE VALUES (MG. %) BEFORE AND 5 HOURS AFTER ORAL ADMINISTRATION OF 0.32 MG. OR I.M. INJECTION OF 0.16 MG. 9a-FLUOROPREDNISOLONE. CONTROL RATS UNTREATED

| I.M. | | Oral | | Control | |
|---|---|---|---|---|---|
| Before | After | Before | After | Before | After |
| 80 | 92 | 80 | 75 | 62 | 76 |
| 74 | 88 | 80 | 78 | 62 | 76 |
| 80 | 100 | 74 | 92 | 80 | 60 |
| 82 | 78 | 80 | 112 | 74 | 90 |
| 70 | 84 | 74 | 74 | 74 | 80 |
| 65 | 91 | 75 | 65 | 70 | 75 |
| 70 | 86 | 68 | 65 | 62 | 70 |
| 73 | 165 | 59 | 104 | 81 | 89 |
| 76 | 78 | 75 | 73 | 59 | 65 |
| 70 | 84 | 50 | 60 | 65 | 76 |
| 65 | 52 | 59 | 50 | 50 | 93 |
| 62 | 62 | 86 | 75 | 50 | 78 |
| 64 | 70 | 50 | 81 | 90 | 50 |
| 54 | 93 | 52 | 75 | 50 | 73 |
| 54 | 87 | 49 | 81 | 49 | 81 |
| 52 | 91 | 50 | 91 | 70 | 86 |
| 50 | 83 | 50 | 80 | 50 | 68 |
| 63 | 95 | 70 | 91 | 60 | 70 |
| 50 | 98 | 52 | 57 | 60 | 80 |
| 59 | 84 | 68 | 62 | 75 | 80 |
| 63 | 72 | 57 | 67 | 68 | 62 |
| 62 | 80 | 72 | 121 | 70 | 89 |
| 72 | 68 | 72 | 91 | 68 | 67 |
| 72 | 97 | 59 | 75 | 59 | 60 |
| 75 | 81 | 70 | 75 | 75 | 59 |
| 57 | 60 | 65 | 65 | 68 | 64 |
| Mean | | | | | |
| 66 | 85 | 65 | 78 | 66 | 74 |
| SD | | | | | |
| 9.4 | 20.3 | 11.5 | 18.6 | 10.7 | 11.0 |

EXPERIMENT II

BLOOD GLUCOSE AND LACTIC ACID VALUES (MG. %) FROM CONTROL RATS AND RATS INJECTED I.M. WITH 5.0 MG. PREDNISOLONE. BLOOD DRAWN BY CARDIAC PUNCTURE BEFORE AND 5 HOURS AFTER TREATMENT

| | Glucose | | | | Lactic Acid | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 92 | 92 | 90 | 82 | 7.7 | 7.4 | 10.7 | 15.6 |
| | 86 | 108 | 88 | 86 | 11.2 | 10.4 | 15.5 | 8.3 |
| | 94 | 134 | 92 | 60 | 11.4 | 6.1 | 11.5 | 4.8 |
| | 76 | 128 | 95 | 86 | 13.7 | 5.1 | 7.9 | 7.5 |
| | 96 | 106 | 96 | 92 | 11.6 | 6.9 | 9.6 | 6.4 |
| | 76 | 94 | 91 | 80 | 9.6 | 10.2 | 11.1 | 10.8 |
| | 84 | 112 | 84 | 70 | 8.6 | 6.1 | 12.1 | 12.5 |
| | 86 | 92 | 74 | 88 | 7.6 | 8.3 | 8.1 | 8.3 |
| | 78 | 116 | 72 | 70 | 9.6 | 10.4 | 7.6 | 8.8 |
| | 70 | 108 | 74 | 84 | 8.6 | 11.1 | 6.4 | 5.6 |
| | 68 | 156 | 96 | 73 | 7.9 | 8.7 | 5.9 | 6.2 |
| | 72 | 124 | 74 | 90 | 9.8 | 10.6 | 6.2 | 9.0 |
| | 50 | 98 | 64 | 82 | 11.6 | 6.6 | 11.8 | 12.4 |
| | 78 | 118 | 56 | 80 | 5.9 | 4.5 | 11.7 | 8.1 |
| | 82 | 104 | 64 | 87 | 6.9 | 4.8 | 8.4 | 6.8 |
| | 74 | 124 | 56 | 64 | 8.2 | 6.2 | 8.1 | 8.1 |
| | 62 | 136 | 68 | 69 | 7.2 | 6.3 | 10.8 | 8.8 |
| | 74 | 100 | 62 | 84 | | | | |
| | 82 | 134 | 82 | 76 | | | | |
| | 74 | 108 | 91 | 64 | | | | |
| Mean | 78.6 | 114.1 | 73.7 | 78.6 | 9.2 | 7.6 | 9.6 | 8.7 |
| SD | 11.4 | 16.8 | 12.4 | 9.3 | 2.1 | 2.2 | 2.6 | 2.8 |

EXPERIMENT II

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE SACRIFICE

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 15.9 | 26.4 | 32.4 | 5.9 | 10.3 | 14.7 |
| 15.2 | 28.6 | 42.3 | 5.3 | 9.3 | 13.9 |
| 23.9 | 28.0 | 32.7 | 16.6 | 22.2 | 25.6 |
| 19.0 | 28.5 | 36.8 | 15.1 | 22.1 | 27.5 |
| 14.6 | 26.0 | 35.6 | 18.1 | 27.9 | 35.2 |
| 11.7 | 19.7 | 25.0 | 17.1 | 23.9 | 29.8 |
| 16.8 | 32.3 | 39.3 | 10.1 | 12.1 | 11.6 |
| 19.3 | 24.0 | 32.4 | 8.5 | 10.9 | 10.1 |
| 12.2 | 21.7 | 27.4 | 12.8 | 18.0 | 23.5 |
| 14.8 | 27.2 | 37.0 | 11.0 | 15.5 | 22.5 |
| 12.9 | 24.4 | 33.0 | 13.7 | 26.7 | 38.7 |
| 17.9 | 32.7 | 45.0 | 7.8 | 8.8 | 9.5 |
| 14.5 | 25.2 | 34.2 | 11.5 | 18.6 | 25.3 |
| 13.7 | 24.5 | 35.9 | 10.8 | 16.5 | 18.7 |
| 18.6 | 30.0 | 41.9 | 8.7 | 9.4 | 9.4 |
| 16.7 | 31.0 | 49.2 | 8.2 | 9.5 | 9.5 |
| 12.0 | 22.4 | 26.8 | 12.3 | 20.5 | 24.8 |
| 7.1 | 15.5 | 15.8 | 13.6 | 22.5 | 30.8 |
| 13.6 | 24.6 | 31.7 | 9.3 | 16.1 | 20.8 |
| 13.5 | 16.4 | 21.8 | 12.3 | 23.7 | 31.2 |
| Mean | | | | | |
| 15.2 | 25.5 | 34.0 | 11.5 | 17.2 | 21.6 |
| SD | | | | | |
| 3.5 | 4.7 | 8.3 | 3.6 | 6.3 | 9.1 |

EXPERIMENT II

BLOOD LACTIC ACID AND INORGANIC PHOSPHATE VALUES (MG. %) FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE. BLOOD SAMPLES DRAWN AT THE TIME OF TREATMENT AND 5 HOURS POSTTREATMENT

| | Lactic Acid | | | | Inorganic Phosphate | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 7.1 | 7.6 | 8.9 | 8.7 | 3.82 | 3.20 | 5.69 | 4.69 |
| | 10.4 | 14.7 | 6.0 | 9.4 | 5.03 | 5.33 | 4.55 | 4.21 |
| | 5.6 | 8.7 | 5.9 | 1.2 | 3.89 | 4.57 | 5.28 | 4.07 |
| | 10.3 | 12.0 | 5.0 | 7.3 | 4.57 | 6.36 | 5.39 | 4.57 |
| | 6.1 | 4.6 | 7.5 | 7.0 | 3.09 | 5.55 | 3.86 | 6.35 |
| | 8.3 | 6.3 | 7.1 | 6.2 | 4.59 | 5.07 | 3.84 | 5.26 |
| | 3.6 | 7.8 | 8.9 | 7.2 | 4.07 | 5.85 | 5.10 | 5.21 |
| | 6.9 | 5.6 | 7.3 | 7.0 | 6.13 | 5.49 | 4.94 | 7.09 |
| | 8.5 | 4.7 | 6.0 | 5.7 | 4.85 | 5.39 | 6.19 | 4.16 |
| | 10.3 | 4.4 | 7.0 | 5.4 | 5.07 | 5.10 | 4.14 | 5.99 |
| | 8.7 | 7.3 | 5.2 | 9.1 | 5.99 | 5.12 | 4.02 | 4.98 |
| | 7.8 | 9.6 | 6.7 | 2.9 | 4.64 | 5.78 | 4.98 | 6.54 |
| | 8.8 | 8.1 | 3.9 | 2.7 | 6.24 | 6.01 | 6.33 | 7.09 |
| | 6.3 | 3.9 | 8.3 | 3.3 | 6.63 | 5.21 | 6.06 | 8.53 |
| | 8.6 | 4.3 | | | 4.87 | 6.13 | 5.49 | 7.43 |
| | 8.0 | 7.4 | | | 4.80 | 5.42 | 5.90 | 6.84 |
| | | | | | 7.68 | 7.09 | 5.90 | 7.54 |
| | | | | | 6.45 | 7.11 | | |
| | | | | | 6.04 | 6.22 | | |
| Mean | 7.8 | 7.3 | 6.7 | 5.9 | 5.18 | 5.65 | 5.16 | 5.91 |
| SD | 1.8 | 2.9 | 1.6 | 3.5 | 1.14 | 0.90 | 0.80 | 1.24 |

EXPERIMENT II

LIVER GLUCOSE AND GLYCOGEN VALUES (GM. %) BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN. TREATED RATS INJECTED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE SACRIFICE. CONTROL RATS UNTREATED

| | Glucose | | | | Glycogen | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 0.25 | 2.10 | 0.34 | 2.41 | 12.98 | 9.89 | 9.60 | 6.17 |
| | 0.05 | 1.99 | 0.03 | 1.96 | 9.95 | 1.33 | 13.79 | 13.79 |
| | 0.81 | 2.64 | 0.71 | 3.36 | 16.67 | 19.29 | 11.53 | 6.83 |
| | 0.81 | 3.86 | 0.49 | 3.13 | 11.11 | 7.05 | 8.03 | 6.30 |
| | 0.76 | 3.15 | 0.63 | 2.65 | 14.05 | 7.94 | 9.92 | 3.60 |
| | 0.88 | 2.73 | 0.77 | 2.62 | 16.24 | 11.18 | 6.61 | 9.75 |
| | 0.70 | 2.18 | 2.33 | 1.84 | 6.00 | 7.16 | 10.74 | 9.17 |
| | 0.79 | 2.21 | 1.57 | 2.36 | 11.84 | 7.89 | 5.59 | 3.28 |
| | 0.52 | 2.06 | 0.53 | 1.97 | 13.83 | 10.75 | 5.89 | 3.68 |
| | 0.53 | 2.04 | 0.42 | 1.43 | 29.17 | 6.25 | 9.70 | 4.33 |
| | 0.47 | 1.76 | 0.45 | 3.59 | 14.85 | 4.24 | 16.62 | 4.50 |
| | 0.47 | 1.35 | 0.27 | 0.53 | 5.40 | 0.19 | 23.68 | 1.19 |
| | 0.91 | 2.52 | 0.29 | 2.59 | 12.92 | 14.32 | 2.23 | 0.39 |
| | 0.76 | 2.75 | 0.77 | 2.85 | 9.32 | 13.89 | 14.93 | 11.87 |
| | 1.36 | 4.07 | 1.53 | 2.40 | 4.87 | 2.62 | 7.50 | 1.92 |
| | 1.57 | 4.14 | 1.39 | 3.26 | 29.83 | 5.77 | 22.07 | 10.90 |
| | 1.67 | 4.04 | 0.81 | 0.56 | 16.92 | 11.19 | 14.96 | 4.50 |
| | 0.71 | 0.67 | 0.38 | 2.75 | 9.34 | 8.88 | 0.50 | 0.50 |
| | 0.38 | 2.33 | 1.38 | 2.35 | 22.24 | 15.28 | 11.59 | 7.30 |
| | 0.24 | 3.84 | 0.36 | 3.16 | 19.22 | 10.27 | 16.36 | 4.59 |
| Mean | 0.73 | 2.60 | 0.77 | 2.38 | 14.34 | 8.77 | 11.14 | 5.73 |
| SD | 0.42 | 0.95 | 0.57 | 0.83 | 6.80 | 4.70 | 6.00 | 3.90 |

EXPERIMENT

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. HYDROCORTISONE. WARBURG VESSEL FLUSHED WITH OXYGEN

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 9.9 | 18.8 | 26.0 | 4.0 | 10.0 | 15.6 |
| 7.2 | 11.1 | 16.5 | 12.7 | 19.1 | 20.7 |
| 12.6 | 17.5 | 22.7 | 6.3 | 8.0 | 8.9 |
| 14.3 | 21.0 | 24.4 | 17.0 | 27.9 | 33.0 |
| 15.2 | 26.7 | 37.4 | 6.0 | 6.6 | 6.9 |
| 15.0 | 23.1 | 31.5 | 9.7 | 13.0 | 14.2 |
| 14.2 | 21.0 | 25.7 | 8.3 | 12.0 | 14.3 |
| 13.5 | 21.6 | 27.4 | 6.5 | 10.3 | 12.9 |
| 13.7 | 18.1 | 22.8 | 8.4 | 10.5 | 12.6 |
| 11.9 | 20.7 | 26.2 | 7.5 | 11.0 | 13.0 |
| Mean | | | | | |
| 12.8 | 20.0 | 26.1 | 8.6 | 13.8 | 15.2 |
| SD | | | | | |
| 2.5 | 4.1 | 5.5 | 2.4 | 2.9 | 4.2 |

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS TREATED FOR 7 DAYS WITH 5.0 MG. PREDNISOLONE. WARBURG FLASKS FLUSHED WITH OXYGEN

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 4.8 | 4.6 | 4.4 | 10.8 | 17.9 | 22.6 |
| 14.4 | 23.7 | 31.5 | 7.4 | 7.2 | 7.0 |
| 4.6 | 4.4 | 4.3 | 4.3 | 7.3 | 7.9 |
| 7.9 | 11.7 | 12.1 | 4.9 | 6.3 | 6.0 |
| 13.7 | 17.9 | 18.7 | 11.9 | 20.0 | 24.3 |
| 14.5 | 25.2 | 36.7 | 12.0 | 20.0 | 24.5 |
| 4.3 | 5.3 | 4.3 | 13.1 | 17.0 | 22.4 |
| 8.4 | 14.1 | 16.3 | 11.1 | 18.1 | 23.5 |
| 3.3 | 4.1 | 5.2 | 12.3 | 20.1 | 24.6 |
| 9.8 | 11.4 | 10.6 | 13.2 | 20.3 | 24.9 |
| 8.2 | 13.5 | 15.5 | 11.0 | 18.3 | 22.4 |
| 6.3 | 7.1 | 6.3 | 13.0 | 19.0 | 25.0 |
| Mean | | | | | |
| 8.4 | 11.9 | 13.8 | 10.4 | 16.0 | 19.6 |
| SD | | | | | |
| 4.0 | 4.6 | 8.6 | 2.3 | 4.1 | 4.4 |

EXPERIMENT II

LIVER LACTIC ACID VALUES (MG. %) IN HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED I.M. WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE SACRIFICE. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASKS UNDER NITROGEN OR OXYGEN

| | Nitrogen | | | | Oxygen | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 1.0 | 4.5 | 2.8 | 30.7 | 8.3 | 6.3 | 6.5 | 6.0 |
| | 4.9 | 15.4 | 4.4 | 3.9 | 3.6 | 5.7 | 6.1 | 3.6 |
| | 5.9 | 15.0 | 6.5 | 10.9 | 2.2 | 6.5 | 9.1 | 18.8 |
| | 5.0 | 34.3 | 5.5 | 34.0 | 5.0 | 3.3 | 5.3 | 21.8 |
| | 7.5 | 16.6 | 6.0 | 7.1 | 4.4 | 12.8 | 2.9 | 4.3 |
| | 7.4 | 11.0 | 7.9 | 7.4 | 4.2 | 9.6 | 7.1 | 16.9 |
| | 5.8 | 24.5 | 9.8 | 9.6 | 5.4 | 11.4 | 7.5 | 4.9 |
| | 3.6 | 32.7 | 1.5 | 1.5 | 6.1 | 5.6 | 6.5 | 13.9 |
| | 6.0 | 26.0 | 5.2 | 6.9 | 3.6 | 4.6 | 5.7 | 18.4 |
| | 0.4 | 6.4 | 6.1 | 1.8 | 9.4 | 16.5 | 4.0 | 5.2 |
| | 6.0 | 2.7 | 2.9 | 4.3 | 12.3 | 5.3 | 6.0 | 14.6 |
| | 11.2 | 4.6 | 4.9 | 5.1 | 2.6 | 12.6 | 2.3 | 14.9 |
| | 13.2 | 31.6 | 2.9 | 6.8 | 3.8 | 18.7 | 2.1 | 15.1 |
| | 2.6 | 15.2 | 5.4 | 0.9 | 3.5 | 8.3 | 1.9 | 5.3 |
| | 19.7 | 23.2 | 3.5 | 14.7 | 4.3 | 5.7 | 7.3 | 8.9 |
| | 4.7 | 19.3 | 8.1 | 1.1 | 3.8 | 15.0 | 3.6 | 4.7 |
| | 12.1 | 26.7 | | | 3.6 | 13.1 | 1.1 | 17.4 |
| | | | | | 6.3 | 11.6 | 2.6 | 8.1 |
| Mean | 6.9 | 18.2 | 5.2 | 9.2 | 5.1 | 9.6 | 4.9 | 11.3 |
| SD | 4.7 | 10.0 | 2.2 | 8.6 | 2.6 | 4.5 | 2.3 | 6.1 |

EXPERIMENT II

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE. WARBURG VESSELS FLUSHED WITH OXYGEN AND CONTAINING 4.5 MG. GLUCOSE

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 13.9 | 22.1 | 27.4 | 10.6 | 10.9 | 10.9 |
| 6.8 | 13.2 | 16.6 | 4.6 | 6.7 | 8.5 |
| 9.7 | 6.9 | 8.4 | 9.7 | 12.1 | 13.1 |
| 15.6 | 28.5 | 37.0 | 8.6 | 15.7 | 18.8 |
| 10.0 | 18.4 | 23.9 | 8.9 | 17.1 | 22.9 |
| 5.8 | 2.5 | 4.4 | 3.8 | 4.0 | 4.4 |
| 8.8 | 18.4 | 26.5 | 8.9 | 16.1 | 19.9 |
| 11.0 | 20.8 | 28.3 | 10.8 | 18.5 | 24.6 |
| 15.7 | 22.2 | 21.5 | 3.8 | 2.8 | 4.7 |
| 7.7 | 11.5 | 15.0 | 7.1 | 11.9 | 13.4 |
| 11.4 | 13.4 | 13.2 | 11.1 | 17.4 | 19.8 |
| Mean | | | | | |
| 10.6 | 16.2 | 20.2 | 8.0 | 12.1 | 14.6 |
| SD | | | | | |
| 4.0 | 5.5 | 10.1 | 2.9 | 4.0 | 7.4 |

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 50.0 MG. HYDROCORTISONE. WARBURG VESSELS FLUSHED WITH OXYGEN

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 8.6 | 19.7 | 31.2 | 13.7 | 26.2 | 36.3 |
| 16.4 | 31.5 | 46.2 | 15.2 | 24.0 | 27.6 |
| 12.3 | 22.7 | 34.5 | 10.1 | 16.8 | 20.1 |
| 6.7 | 13.4 | 20.0 | 11.9 | 18.6 | 23.5 |
| 8.4 | 8.7 | 11.7 | 7.2 | 17.0 | 24.6 |
| 8.0 | 12.4 | 17.0 | 8.8 | 18.1 | 24.3 |
| 10.9 | 18.6 | 25.8 | 1.1 | 1.5 | 1.7 |
| 12.1 | 20.3 | 29.3 | 4.0 | 10.0 | 15.6 |
| 12.4 | 21.0 | 27.6 | 12.7 | 19.1 | 20.7 |
| 15.4 | 27.5 | 35.3 | 6.3 | 8.0 | 8.9 |
| Mean | | | | | |
| 11.1 | 19.6 | 27.9 | 9.1 | 15.9 | 20.3 |
| SD | | | | | |
| 3.6 | 7.7 | 11.6 | 4.5 | 7.5 | 9.6 |

EXPERIMENT II

LIVER INORGANIC PHOSPHATE VALUES (MG. %) IN UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE TAKING LIVER SAMPLE. DETERMINATIONS MADE BEFORE AND AFTER INCUBATION IN WARBURG FLASKS FLUSHED WITH OXYGEN OR NITROGEN

| | Oxygen | | | | Nitrogen | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 6.9 | 5.5 | 8.6 | 20.8 | 8.34 | 8.32 | 7.98 | 11.07 |
| | 9.5 | 6.8 | 10.4 | 37.3 | 7.54 | 16.59 | 7.18 | 21.05 |
| | 10.4 | 17.9 | 8.8 | 35.8 | 9.07 | 7.55 | 7.50 | 11.02 |
| | 6.6 | 23.7 | 9.5 | 37.4 | 8.12 | 21.16 | 8.87 | 20.66 |
| | 6.0 | 7.0 | 7.9 | 27.3 | 5.74 | 19.23 | 9.50 | 17.74 |
| | 13.0 | 1.0 | 7.1 | 18.2 | 7.38 | 14.77 | 9.12 | 15.70 |
| | 6.7 | 15.0 | 8.9 | 30.3 | 6.08 | 16.25 | 6.56 | 19.56 |
| | 7.3 | 5.6 | 9.9 | 25.2 | 11.34 | 19.56 | 8.94 | 21.32 |
| | 7.7 | 16.3 | 7.3 | 18.9 | 7.27 | 21.05 | 9.01 | 19.39 |
| | 8.8 | 18.3 | 10.6 | 21.5 | 9.67 | 20.22 | 9.94 | 18.40 |
| | | | | | 12.18 | 22.15 | 12.02 | 26.83 |
| | | | | | 11.06 | 22.04 | 9.94 | 18.79 |
| | | | | | 6.45 | 16.03 | 11.08 | 21.65 |
| | | | | | 9.37 | 21.65 | 9.53 | 21.54 |
| | | | | | 8.18 | 19.17 | 8.21 | 20.88 |
| | | | | | 7.28 | 17.63 | 9.78 | 21.60 |
| | | | | | | | 10.12 | 21.60 |
| Mean | 8.3 | 11.7 | 8.9 | 27.3 | 8.44 | 17.71 | 9.10 | 19.34 |
| SD | 2.1 | 7.4 | 1.1 | 8.0 | 1.7 | 4.4 | 1.3 | 3.9 |

EXPERIMENT II

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE. WARBURG VESSELS FLUSHED WITH OXYGEN

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 14.3 | 24.0 | 30.3 | 12.5 | 16.4 | 22.8 |
| 9.7 | 12.9 | 13.0 | 2.0 | 3.2 | 5.2 |
| 19.6 | 38.4 | 56.0 | 10.5 | 16.7 | 19.7 |
| 15.1 | 28.1 | 36.4 | 7.6 | 10.5 | 11.4 |
| 14.1 | 25.7 | 34.3 | 7.5 | 17.6 | 24.9 |
| 12.8 | 26.0 | 35.4 | 9.6 | 18.3 | 22.7 |
| 8.3 | 15.1 | 18.3 | 4.3 | 9.1 | 12.0 |
| 10.4 | 20.3 | 29.4 | 10.4 | 20.1 | 28.4 |
| 13.2 | 33.3 | 47.7 | 8.9 | 18.2 | 26.7 |
| 15.2 | 27.1 | 36.3 | 10.8 | 19.8 | 25.0 |
| 12.4 | 20.8 | 25.1 | 10.8 | 19.4 | 24.6 |
| Mean | | | | | |
| 13.2 | 24.7 | 32.9 | 9.5 | 15.4 | 20.3 |
| SD | | | | | |
| 3.2 | 7.4 | 12.1 | 3.3 | 2.2 | 7.9 |

OXYGEN UPTAKE (uL/MG. LIVER) BY LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE. WARBURG VESSELS FLUSHED WITH OXYGEN AND CONTAINING 3 X $10^{-4}$ M DNP

| Treated Incubation Time (Minutes) | | | Control Incubation Time (Minutes) | | |
|---|---|---|---|---|---|
| 10 | 20 | 30 | 10 | 20 | 30 |
| 9.0 | 13.5 | 14.6 | 6.1 | 8.9 | 7.4 |
| 6.1 | 7.2 | 7.7 | 3.1 | 4.5 | 6.8 |
| 1.3 | 1.6 | 1.9 | 1.8 | 2.9 | 3.7 |
| 3.2 | 5.1 | 6.4 | 1.9 | 2.6 | 2.8 |
| 3.7 | 7.2 | 8.7 | 5.3 | 6.7 | 7.1 |
| 3.0 | 4.8 | 5.8 | 1.2 | 3.4 | 4.0 |
| 2.4 | 3.4 | 3.8 | 2.5 | 2.5 | 2.9 |
| 5.4 | 9.2 | 12.2 | 4.9 | 8.8 | 10.6 |
| Mean | | | | | |
| 4.3 | 6.5 | 7.6 | 2.7 | 5.0 | 5.7 |
| SD | | | | | |
| 2.5 | 3.7 | 4.2 | 1.7 | 2.7 | 2.7 |

EXPERIMENT II

LIVER INORGANIC PHOSPHATE VALUES (MG. %) IN UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE TAKING LIVER SAMPLE. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASKS FLUSHED WITH OXYGEN AND CONTAINING 4.5 MG. GLUCOSE OR 3 X $10^{-4}$ M DNP

| | Glucose | | | | DNP | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 5.2 | 18.5 | 5.6 | 20.9 | 7.0 | 13.3 | 2.4 | 18.9 |
| | 0.7 | 14.0 | 10.9 | 13.2 | 2.1 | 12.8 | 8.6 | 14.9 |
| | 9.5 | 10.2 | 4.6 | 11.0 | 5.3 | 9.9 | 6.9 | 18.3 |
| | 1.7 | 1.1 | 8.7 | 12.4 | 8.2 | 13.8 | 8.5 | 14.8 |
| | 8.2 | 0.1 | 2.3 | 10.1 | 14.9 | 12.1 | 4.9 | 6.6 |
| | 7.3 | 3.0 | 5.8 | 7.3 | 3.6 | 6.2 | 7.7 | 21.8 |
| | 5.9 | 5.9 | 7.1 | 11.4 | 6.5 | 12.7 | 3.3 | 9.8 |
| | 7.2 | 4.4 | 1.3 | 4.4 | 8.8 | 6.2 | 2.8 | 11.7 |
| | 7.5 | 3.4 | 7.3 | 7.0 | | | | |
| | 8.8 | 0.4 | 6.5 | 12.4 | | | | |
| | | | 6.8 | 6.8 | | | | |
| Mean | 6.2 | 7.1 | 6.1 | 10.6 | 7.0 | 10.9 | 5.6 | 14.6 |
| SD | 3.3 | 6.4 | 2.7 | 4.4 | 3.8 | 3.1 | 2.6 | 5.1 |

LIVER INORGANIC PHOSPHATE VALUES (MG. %) IN UNTREATED RATS AND RATS TREATED WITH 5.0 MG. PREDNISOLONE 5 HOURS BEFORE KILLING. DETERMINATIONS MADE BEFORE AND AFTER 30 MINUTES INCUBATION IN WARBURG FLASKS FLUSHED WITH OXYGEN

| | Treated | | Control | |
|---|---|---|---|---|
| | Before | After | Before | After |
| | 14.1 | 13.0 | 1.6 | 15.3 |
| | 7.6 | 11.5 | 4.1 | 14.3 |
| | 6.0 | 2.2 | 8.4 | 9.4 |
| | 4.9 | 0.9 | 2.2 | 13.0 |
| | 6.8 | 3.9 | 7.4 | 5.7 |
| | 6.1 | 5.7 | 1.2 | 4.6 |
| | 7.3 | 0.8 | 4.6 | 6.7 |
| | 7.2 | 1.4 | 7.4 | 4.9 |
| | 3.9 | 2.6 | 10.4 | 7.6 |
| | 6.2 | 0.4 | 6.5 | 5.6 |
| Mean | 7.0 | 4.2 | 5.4 | 8.7 |
| SD | 2.9 | 3.5 | 3.0 | 3.9 |

## EXPERIMENT II

PROTEIN (MG./GM. DRY LIVER) IN LIVER HOMOGENATES FROM UNTREATED RATS AND RATS INJECTED WITH 5.0 MG. PREDNISOLONE. SAMPLES TAKEN BEFORE AND AFTER 30 MINUTES INCUBATION UNDER OXYGEN OR NITROGEN

| | Nitrogen | | | | Oxygen | | | |
|---|---|---|---|---|---|---|---|---|
| | Treated | | Control | | Treated | | Control | |
| | Before | After | Before | After | Before | After | Before | After |
| | 379 | 379 | 425 | 425 | 414 | 364 | 415 | 395 |
| | 357 | 370 | 428 | 430 | 414 | 352 | 448 | 400 |
| | 408 | 410 | 418 | 418 | 389 | 365 | 427 | 387 |
| | 390 | 388 | 301 | 339 | 401 | 371 | 318 | 351 |
| | 326 | 354 | 310 | 340 | 313 | 330 | 312 | 293 |
| | 337 | 354 | 307 | 307 | 311 | 359 | 294 | 307 |
| | 357 | 357 | 308 | 309 | 324 | 337 | 317 | 325 |
| | 349 | 349 | 353 | 355 | 332 | 368 | 353 | 355 |
| | 392 | 382 | 389 | 400 | 392 | 384 | 389 | 400 |
| | 356 | 356 | 327 | 341 | 356 | 364 | 327 | 341 |
| | 362 | 370 | 376 | 395 | 362 | 370 | 376 | 395 |
| | 350 | 360 | 421 | 417 | 350 | 358 | 421 | 421 |
| | 345 | 353 | 372 | 372 | 345 | 345 | 372 | 379 |
| | 363 | 396 | 324 | 326 | 363 | 334 | 324 | 308 |
| | 295 | 315 | 341 | 337 | 395 | 320 | 341 | 313 |
| | 297 | 318 | | | 397 | 333 | | |
| Mean | 354 | 363 | 360 | 367 | 354 | 353 | 362 | 358 |
| SD | 10.0 | 8.2 | 14.4 | 13.7 | 12.6 | 5.7 | 15.4 | 13.4 |

VITA

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