# THE EFFECTS OF EPINEPHRINE INJECTION AND COMPETITION BETWEEN MALES FOR 3, 5 AND 10 DAYS ON THE METABOLISM OF RABBIT TESTIS IN VITRO

By

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# CHAPTER I

## INTRODUCTION

Stressful conditions cause an alteration in the homeostatic mechanisms of an animal insuring its survival in an ever changing environment. These responses are mediated by the endocrine glands following stimulation by higher nervous systems. The change in homeostatic mechanisms is advantageous to the animal if only of a temporary nature. It proves deleterious if the animal cannot adapt to the stress.

Many workers have investigated the various effects of stress on the metabolism and histology of tissues in the body. They have determined the responses of various endocrine glands and the effects on their function and indirectly on target organs of their secretions.

A review of literature revealed that prolonged stress and simulated stress affected the male reproductive system in such a way as to decrease its efficiency. Changes noted were as follows: decreased sperm counts, decreased sperm viability, decrease in size of the seminiferous tubules, and a decrease in size of the testes and accessory organs. The review of literature further revealed that these changes could be brought about by: decreased gonadotrophic hormone output, increased adrenocorticosteroids, direct action of epinephrine,

et cetera. A further search of the literature revealed that metabolic changes that might be associated with this impairment of the male reproductive system had not been investigated.

Histological changes in rabbit testes due to subcutaneous injections of various levels of epinephrine for 45 days were noted by Vandemark and Boyd (143). Therefore, an experiment was designed to detect the effect of stress on metabolic activity of testis tissue preceding the effect of stress on histological changes in the testis. Along with histological changes, criteria of metabolic activity investigated were: oxygen uptake, lactic acid production and glucose uptake *in vitro*.

# CHAPTER II

## REVIEW OF LITERATURE

### General Effects of Stress

Whole organism: Cannon et al. (14) have shown that cold invokes added heat production of the body without shivering. Cori and Buchwald (24) working with dogs, rats, rabbits and man and Boothby and Sandiford (11) working with dogs found a similar effect in animals artifically stressed by injections of epinephrine (27). In addition there was an increase in pulse rate, respiratory depth, respiratory rate, blood pressure, oxygen utilization and carbon dioxide production (24, 25, 43, 102).

Adrenal weight and adrenal ascorbic acid: Many investigators have shown that natural stresses such as cold, exercise, late pregnancy, starvation, anoxia, shock, burns, infection, toxins, dietary deficiencies, crowding and hemorrhage cause an increase in adrenal size and deplete adrenal ascorbic acid (18, 19, 21, 33, 34, 59, 62, 63, 87, 120, 124, 126, 139, 150, 151). Simulating stress by injecting epinephrine will cause the same results as the above mentioned stresses (84, 90). Stress may also be simulated by injecting adrenocorticotropic hormone. This is characterized by adrenal enlargement and adrenal ascorbic acid depletion (4,

90, 99, 120, 122, 123, 125). The increase in size of the adrenal gland involves the cortex and not the medulla (21, 33). Cortex enlargement is primarily confined to the zona fasciculata and some contribution by the zona reticularis (19, 33). The depletion of ascorbic acid levels in the adrenal cortex quickly follows the onset of stress, but its return to previous levels is dependent upon the nature of the stress (151). At the beginning of stresses to which animals tend to adapt, adrenal ascorbic acid depletion is directly proportional to the stress intensity, but thereafter the animal adapts and depletion is reduced to a lesser degree (121, 122, 125, 144). The rat, an animal that can synthesize ascorbic acid, can recover normal ascorbic acid levels of the adrenal following stress in as short a time as nine hours (122, 123, 125, 151). During intense continuous stress, adrenal ascorbic acid of the adrenal cortex drops rapidly at first and then continues to fall until death (122).

Adrenal weight changes are not as rapid as adrenal ascorbic acid changes, nor do they return to normal as rapidly as the ascorbic acid levels. In the rat, Sayers and Sayers (122) found adrenal weight changes to begin three hours following the onset of prolonged injections of ACTH. In these animals adrenal ascorbic acid levels return to normal in 38 hours (125). Sayers and Sayers (122) found the increase in adrenal size to continue until termination of ACTH injection and then to begin a decline after several days. Other workers found that in rats exposed to cold, adrenal hy-

pertrophy did not begin until 20 hours or more and then did not show a decrease in size until a month or more (59, 62, 126, 150). D'Angelo et al. (33) found that starvation increased adrenal size proportional to the degree of body weight loss. Christian (19) working with male mice and Christian and Davis (21) working with wild Norway rats found that adrenal weights were directly related to population size. These findings are in agreement with the views of Selye (129) and Sayers (119) in which they outline adrenal changes taking place during adaptation to stress.

Adrenal medullae: The adrenal medullae are a part of the sympathetic branch of the autonomic nervous system. Stimulation of the autonomic nervous system by stress causes a release of endogenous epinephrine from the adrenal medullae (38, 47, 85, 92, 130). This hormone is then carried by the blood stream to the various target organs.

Adreno-pituitary system: Epinephrine released by autonomic nervous stimulation indirectly stimulates the release of ACTH from the anterior pituitary via the hypothalamus (89, 90, 91, 92, 98, 148). The fact that the hypothalamus is involved is supported by evidence showing that hypothalamic lesions block the pituitary response to stress (90, 91). Roberts and Keller (114) have shown that epinephrine stimulates *in vivo* respiration and aerobic glycolysis of posterior hypothalamic tissue and Keller and Roberts (69) have shown a similar response *in vitro*.

ACTH released from the pituitary causes the release of adrenocortical hormones from adrenal cortex, which is responsible for adrenal hypertrophy and depletion of adrenal ascorbic acid. A number of investigators have shown that the pituitary gland is essential to normal adrenal cortical response to stress (6, 15, 56, 68, 86, 90, 92, 109, 121, 124, 125, 128, 139, 148). Atrophy of the adrenal cortex occurs in the absence of the pituitary (16, 86, 139). However, this atrophy may be prevented and a normal response obtained by injections of ACTH (56, 86, 90).

The adrenal cortical hormones then exercise a feedback effect upon the pituitary and limit its release of ACTH (4, 47, 85, 92, 111, 120, 121, 138). Administration of these hormones reduces adrenal responses to stress (4, 111, 121, 138). Munson and Briggs (98) and Sayers and Sayers (120) suggest that feedback action is directly on the pituitary. Roberts and Keller (114) showed that cortisone injected intravenously reduced the oxygen consumption of the anterior pituitary, which suggests a possible indirect action via the hypothalamus. D'Angelo et al. (33) and Fortier (47) have shown that prolonged injection of adrenal cortical hormones will reduce pituitary ACTH to zero, which in turn causes adrenal atrophy. The adrenal steroids not only influence the release of ACTH from the pituitary but more markedly its synthesis. Fortier (48) has shown this to be true in an experiment with bilaterally-adrenalectomized rats in which he found pituitary ACTH to reach a peak of 575% of normal by the

thirty-second day following adrenalectomy.

The Effects of Stress on Reproduction in the Male

Stress invokes the production of epinephrine, which stimulates the release of ACTH from the pituitary. ACTH causes the adrenal cortices to release their hormones. Therefore, the effects of stress and the various hormones on male reproduction will be reviewed.

Selye (130) in a review reports that systemic stress produces testicular atrophy, degeneration of seminiferous epithelium with the formation of polynuclear giant spermatocytes and reduced spermatogenesis. Sand (117) reports that spermatogenesis is reduced in men sentenced to death. Meschoks (95) has found temporary damage to spermatogenesis caused by transportation of bulls over short distances. Harrison (58) found degenerative changes in the spermatogenic epithelium of rats stressed by daily subcutaneous injections of normal saline. Christian (18, 19, 20) working with mice found that overcrowding caused a decrease in the weights of the testes, preputial gland and seminal vesicles, decreased diameter of the seminiferous tubules and reduced rate of spermatogenesis. He also found an inverse relationship between population size and these effects of stress on the male reproductive system.

A number of workers experimenting with rats, rabbits, fowl and bulls have shown that epinephrine injections cause regression of the male accessory organs and testes with decreased spermatogenesis and reproductive capacity (41, 42,

66, 105, 106, 142, 143, 147). On the other hand, Lamar (81) did not find this to be the case. Vandemark and Boyd (143) found that subcutaneous injection in rabbits of 2 mg. of epinephrine daily for 45 days caused testicular atrophy, a reduction in the diameter of seminiferous tubules, reduction in the number of tubules containing spermatozoa, dissociation of the interstitial cells and the appearance of multinucleated giant cells.

The effects of the adrenal corticoids, which are also released during stress, have been investigated and the results have been found to be controversial. However, the general consensus is that they cause the same gross effects as epinephrine, but only if used in large doses (46, 49, 67, 130). Sawyer and Critchlow (118) observed no effect on the male reproductive system. Feeding of adrenal cortex to rats and chickens was found to increase testis size, as recorded by several investigators (41, 66, 93).

ACTH in rather large doses of 1-8 mg. injected daily in rats caused a slight reduction in testes weight with atrophy of the Leydig cells (5). These effects are probably due to the consequent release of the oxygenated steroids from the adrenal cortex (5).

During stress, the increased release of ACTH suppresses the release of gonadotrophin (20, 130). This hormone has been shown to be essential to normal development and function of testes and accessory organs (13, 97, 152). The cessation of sperm production following removal of the pitui-

tary offers further supporting evidence (104). Several investigators believe the effects of stress on the male reproductive system may be attributed in part to this suppression of gonadotrophin release (13, 20, 97, 106, 130, 152). Part of the testicular atrophy may be due to the action of the adrenal steroids on protein catabolism and metabolism (104).

Effects of Stress on Metabolism

The metabolic effects of the various hormones released following stress will be reviewed. Several changes in the organism are noted following epinephrine injection. These are as follows: decreased inorganic phosphate plasma concentration, elevated pyruvic and lactic acid content of plasma, hyperglycemia and glucosuria (8, 10, 11, 24, 26, 27, 28, 29, 30, 37, 57, 64, 88, 130, 131, 134, 145). Accompanying the hyperglycemia is a net loss of muscle glycogen (10, 12, 17, 25, 26, 37, 134, 145, 148). Following epinephrine injection an early decrease in liver glycogen was noted, followed by a later net gain in liver glycogen (10, 12, 17, 26, 27, 134, 148).

The increased amounts of lactate and pyruvate are an indication that glycolysis is accelerated by epinephrine (37, 114). Cori and Cori (25) presented the theory that lactate produced in the extra hepatic tissues was the source of the increased glycogen deposit noted earlier. Drury and Wick (40) have shown that increased circulating lactate and pyruvate are not converted to liver or muscle glycogen, but rather enter the Krebs cycle and are oxidized rapidly to

carbon dioxide. Many other investigators have found that respiration is accelerated by epinephrine injection both *in vitro* and *in vivo* (2, 25, 43, 57, 88, 114).

Hyperglycemia results from decreased uptake of glucose by the tissues and increased glycogenolysis in liver and other tissues (12, 25, 26, 27, 28, 37, 50, 64, 133, 134, 136, 145, 148, 149). These effects are largely due to the influence of epinephrine upon various enzymes. The enzyme protein known as the adenyl cyclase system readily converts ATP to cyclic 3' - 5' AMP and pyrophosphate (76, 100, 108). This system, which is present in liver and many extra hepatic tissues including muscle, is readily stimulated to higher activity by epinephrine (76, 100, 108). Cyclic 3' - 5' AMP readily stimulates the formation of phosphorylase a (60, 61, 79, 132). This is accomplished by a stimulation of phosphorylase b kinase, which converts the phosphorylase b to phosphorylase a, the active form of the enzyme (45, 77, 78, 79, 137). Other workers have noted this activation of phosphorylase in liver and in muscle (3, 107, 132, 136). Phosphorylase is the rate limiting step in the series of enzymes responsible for the conversion of glycogen to glucose (137). Phosphatase, the enzyme responsible for the final step of converting glycogen to glucose, is not found in muscle (140). This final step involves the conversion of glucose-6-phosphate to glucose. Therefore, muscle, instead of forming glucose and releasing it into the blood stream as does liver, forms lactate or pyruvate via glycolysis and these enter the blood stream

(7). Glucose-6-phosphate has been shown to accumulate in muscle under stimulation by epinephrine (75, 140). The depressing effect of epinephrine on glucose utilization and uptake by the tissues may be explained in part by an inhibitory action of accumulated glucose-6-phosphate on the hexokinase of muscle and other tissues (31, 32, 101, 135, 140). This enzyme system is involved in the utilization of blood glucose (140). Increased muscle glycogenolysis obviously is seen not only to be in part responsible for decreased glucose uptake by the tissues, but is also responsible for the increased glycolytic rate. The increased liver glycogenolysis partly explains the hyperglycemia and glucosuria.

The rise in liver glycogen following a transient decline noted earlier is due primarily to the adrenal corticosteroids. As has been noted, this glycogen does not come from blood glucose, lactate and pyruvate (40, 146). A number of investigators have shown that the adrenal corticosteroids cause a negative nitrogen balance, loss of weight, reduced healing of wounds and retarded growth rate (5, 22, 39, 54, 67, 72, 82, 83, 103, 113, 115, 116, 139, 146). These factors indicate that gluconeogenesis may be occurring with protein acting as the source for the newly formed glucose. This glucose formation from protein may account for the gain in liver glycogen noted several hours after stress (55). Adrenalectomized animals do not show this glycogen deposition, when injected with epinephrine, unless they have been pretreated with adrenal corticosteroids (148, 149).

The increased glycogen deposition is thought to be brought about by the action of the adrenal glucocorticoids on the cellular concentration of certain enzyme proteins (55). The amounts of several transaminases have been shown to be increased by these hormones (44, 51, 55, 70, 115, 116, 127). The elevation of the level of the transaminases may be responsible for enhanced gluconeogenesis (55). Puromycin, an inhibitor of protein formation in general, and actinomycin, an inhibitor of DNA dependent RNA synthesis, inhibit the formation of liver glycogen (55, 65, 110). This strongly suggests that the action of the hormones is on RNA synthesis of enzyme protein (55, 110).

The adrenal steroids cause hyperglycemia when injected into animals (1, 67, 82, 103, 139, 146). They do not affect glucose uptake by the tissues (1, 53, 115, 130, 146). They have been shown to inhibit phosphorylase activity in liver and in muscle (71, 83, 148). The hyperglycemia cannot be explained on the basis of decreased peripheral uptake of glucose nor on the basis of glycogenolysis, but is explained by its gluconeogenic effect (83, 115, 130, 146). Also, adrenal steroids would be expected to reduce muscle glycogenolysis. Winternitz et al. (149) and Winternitz and Long (148) have shown that adrenalectomized animals had twice as much loss of muscle glycogen, when stressed, than did the controls.

Goetsch and McDonald (53) did not find that the glycolytic rate was affected by adrenal corticosteroids in liver

homogenates. Kerppola and Pitkanen (73) did not find any changes in the glycolytic enzymes following injections of cortisone.

Goetsch and McDonald (53) found that injection of various glucocorticoids five hours before sacrifice resulted in highly significant increases in oxygen uptake by rat liver homogenates. These two workers and others have shown that chronic administration of the glucocorticoids causes decreased uptake of oxygen in liver (23, 53, 72, 73, 114). This decreased oxygen uptake is partially explained by the work of Kerppola and Pitkanen (73), who found that cortisone injections decreased the activity of cytochrome oxidase in rat liver. Kerppola (72) also found that cortisone frequently inhibited oxidative phosphorylation and ATP production. It follows then that reduced ATP production may account for the inhibition of protein synthesis caused by the glucocorticoids (72).

In summary, stress has been shown to cause increased size of the adrenal cortices, depletion of adrenal ascorbic acid and decreased efficiency of the male reproductive system. Epinephrine, which is released in response to stress, causes decreased uptake of glucose, increased circulating lactate and pyruvate, and increased respiration. The adrenal glucocorticoids, which are also released following stress, are thought to cause hyperglycemia and increased deposition of liver glycogen by increasing gluconeogenesis. The glucocorticoids do not affect glycolytic rate, but upon

chronic administration, caused decreased uptake of oxygen in liver homogenates.

# CHAPTER III

## MATERIALS AND METHODS

Mature New Zealand White male rabbits were chosen as the experimental animals. Upon receiving the animals from the supplier, they were placed singly in different cages and allowed to remain in these cages approximately two weeks prior to beginning the experiments. In the two week rest period and throughout the experiments, the rabbits were offered free choice of water and a commercial grade of rabbit pellets.

Forty-two rabbits were obtained and used in these experiments. The rabbits were randomly placed into seven groups of six animals. Each animal was individually caged. One of the groups served as controls. Three of the groups of rabbits were injected approximately every 12 hours with one mg. of epinephrine suspended in peanut oil for periods of 3, 5 and 10 days. The remaining three groups of rabbits were paired at random and allowed to compete for dominance for periods of 3, 5 and 10 days. The control rabbits were given injections of peanut oil twice daily for 5 days to simulate stress of injection procedure.

At the end of the experimental periods, the animals were killed by dislocation of cervical vertebrae and then

weighed. The testes and a kidney were quickly removed and placed in ice cold Locke's minus glucose solution. The adrenals were removed and placed in 10 ml. of 2.5% metaphosphoric acid.

After the testes were weighed, slices of both testis and kidney tissues were prepared with the Stadie-Riggs hand microtome. These slices were then divided into portions of approximately 70-85 mg. wet weight. Oxygen uptake was determined by the direct method of Warburg (141), for a period of three hours at a temperature of 37° C. The tissues were run in duplicate with and without addition of exogenous glucose.

Cross-sectional slices from one testis were fixed in Bouin's solution for two days. They were then washed for one day in running tap water, placed in 10% formalin solution and later prepared for microscopic examination. Other portions of testis tissue were used to determine the percentage dry weight. Dry weights were found after weighted amounts of wet tissue had been dried overnight at 100° C.

After the oxygen uptakes had been determined and the tissues removed from the flasks, aliquots of the incubation media were saved and lactic acid was determined colorimetrically by the method of Barker and Summerson (9), and glucose uptake by the method of Keston (74).

The adrenals were weighed and their wet weights were recorded. They were then homogenized with a Potter-Elvehjem homogenizer in 10 ml. of a freshly prepared 2.5% solution of

metaphosphoric acid. The ascorbic acid content was then determined by the method of Mindlin (96).

# CHAPTER IV

## RESULTS AND DISCUSSION

This experiment was designed to investigate metabolic effects that precede stress induced degenerative changes in the male reproductive system. The methods employed in stressing the animals were epinephrine injection and crowding of rabbits as outlined in the previous chapter. Criteria measured determined the following: efficiency of the stressors, gross changes in the testis and metabolic effects of stress.

Basic to any changes in testis was the need for evidence indicating the efficacy of epinephrine injections and crowding as stressors. Of several methods employed in determining stress, adrenal ascorbic acid depletion has been found adequate by a number of investigators (18, 19, 21, 33, 34, 40, 59, 62, 87, 120, 124, 126, 139, 150, 151). The graph showing adrenal ascorbic acid levels in Figure 1 indicates that there was a depletion of adrenal ascorbic acid due to the treatments. Levels of ascorbic acid were lowest at day 3, below normal at day 5, and near normal at day 10. Statistical analysis reveals that: (1) there was no significant difference between the adrenal ascorbic acid levels of the controls and all others; (2) epinephrine injection caused a significantly greater adrenal ascorbic acid depletion than

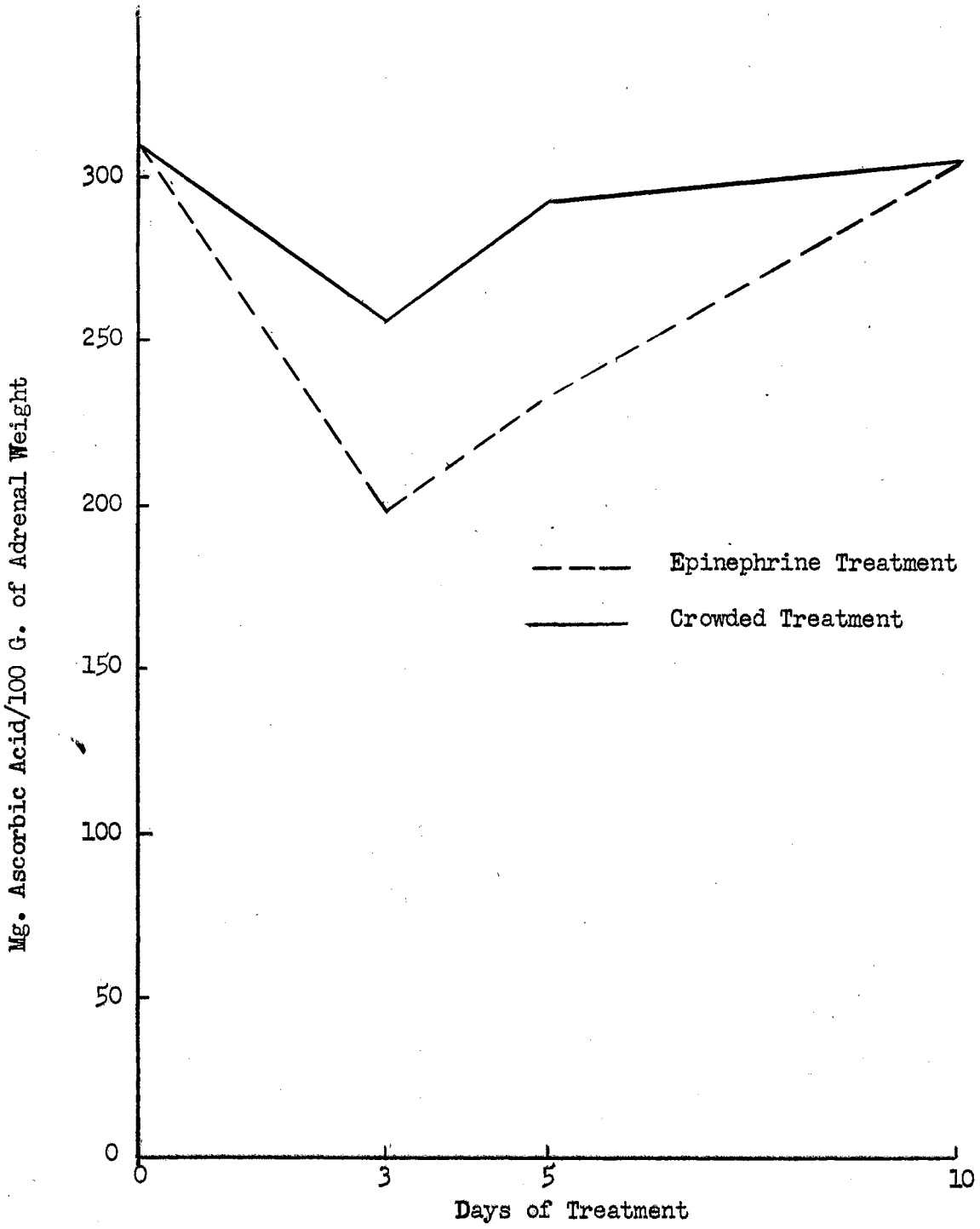


Figure 1. Adrenal ascorbic acid levels.

did crowding ($P<.05$); and (3) there was a significant difference between 3, 5 and 10 days. The controls would certainly have been significantly different from all treated animals had they been compared only to days 3 and 5. Day 10 was near normal and caused this comparison to fall slightly below significance. This is further emphasized in that there was a highly significant difference ($P<.01$) between the 3, 5 and 10 day periods of stress. The work of Selye (128, 129, 130) leads one to think that the return to near normal ascorbic acid levels of the adrenals by day 10 may be a response of adaptation to stress.

Viewing the graph of adrenal weight changes in Figure 2, it can be seen that adrenal hypertrophy in general is the inverse of adrenal ascorbic acid levels. This response in general usually accompanies stress. These changes although not significant follow the trend expected.

Analysis of variance of testis weights showed that there was no significant difference among controls and treated animals. This is emphasized further by viewing testis weights in Figure 3, testis per kilogram of rabbit weight in Figure 4 and percentage dry weight of testis tissue in Figure 5. In addition microscopic examination of histological preparations of these testes showed no changes in seminiferous tubules. Therefore, it can be concluded that the treatments have not caused any gross changes in the testes of these animals.

Since it has been shown that the animals were being stressed and that no gross changes occurred in the testes,

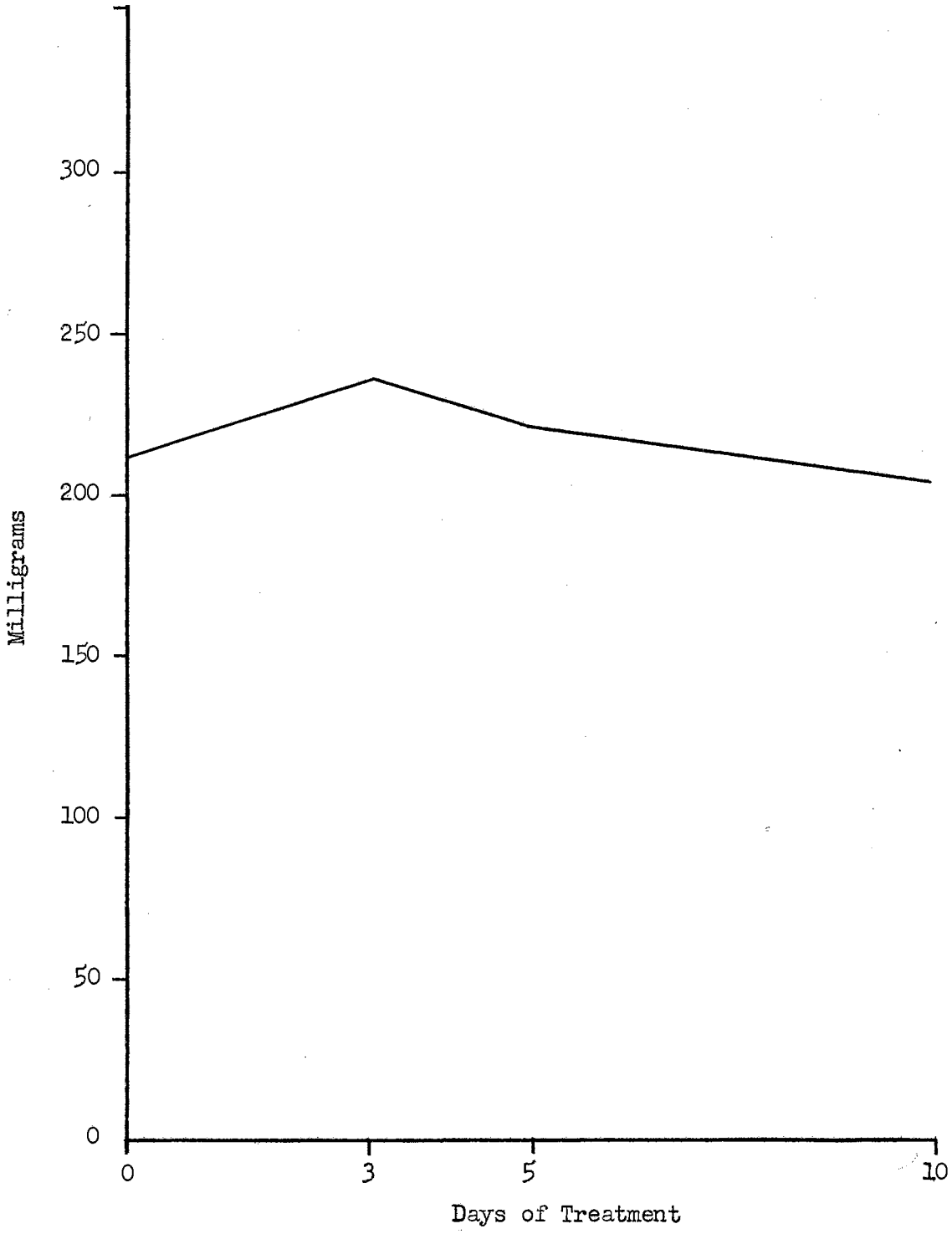


Figure 2. Adrenal gland weight of both treatments combined.

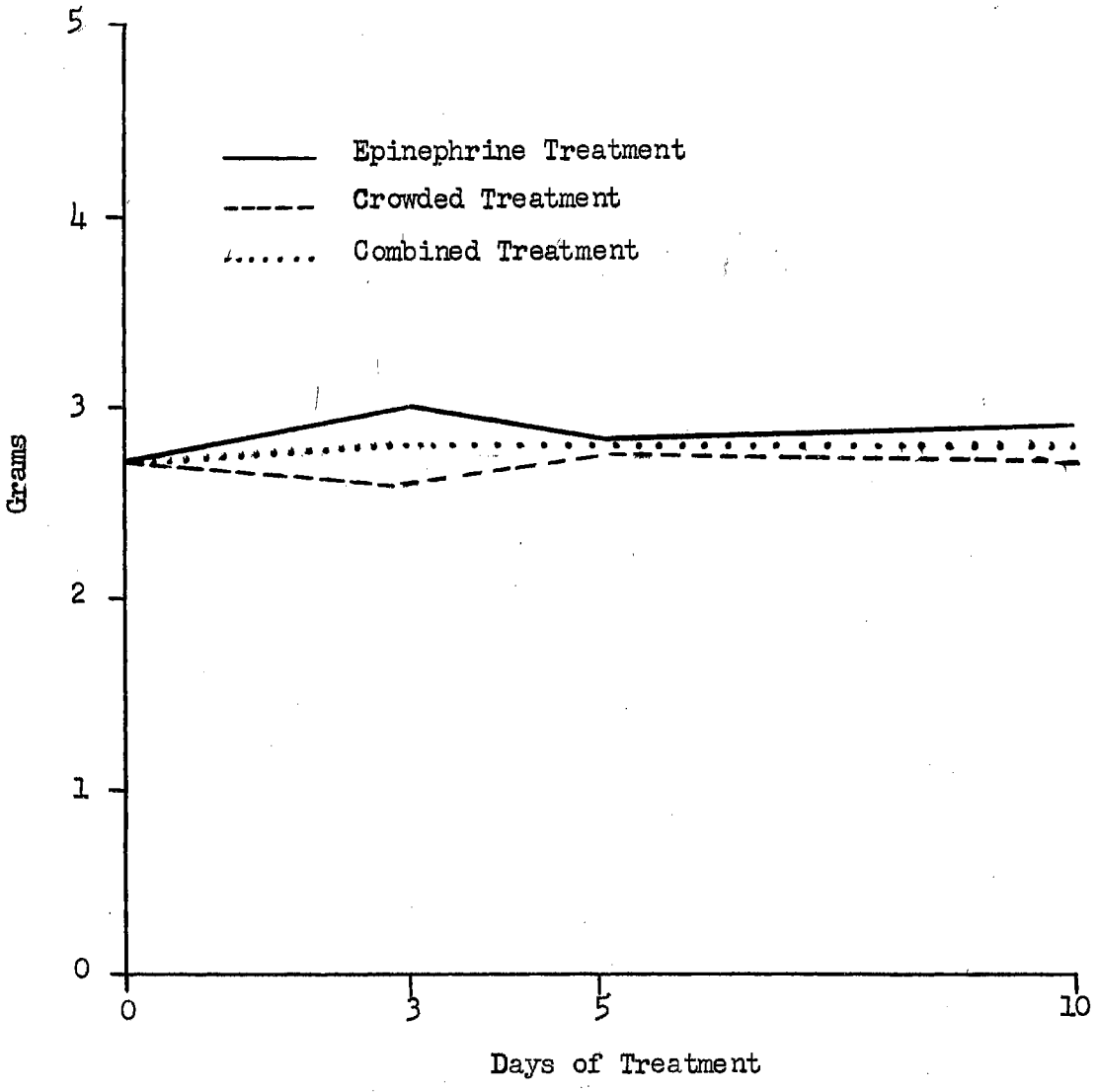


Figure 3. Testis weights of both treatments separate and combined.

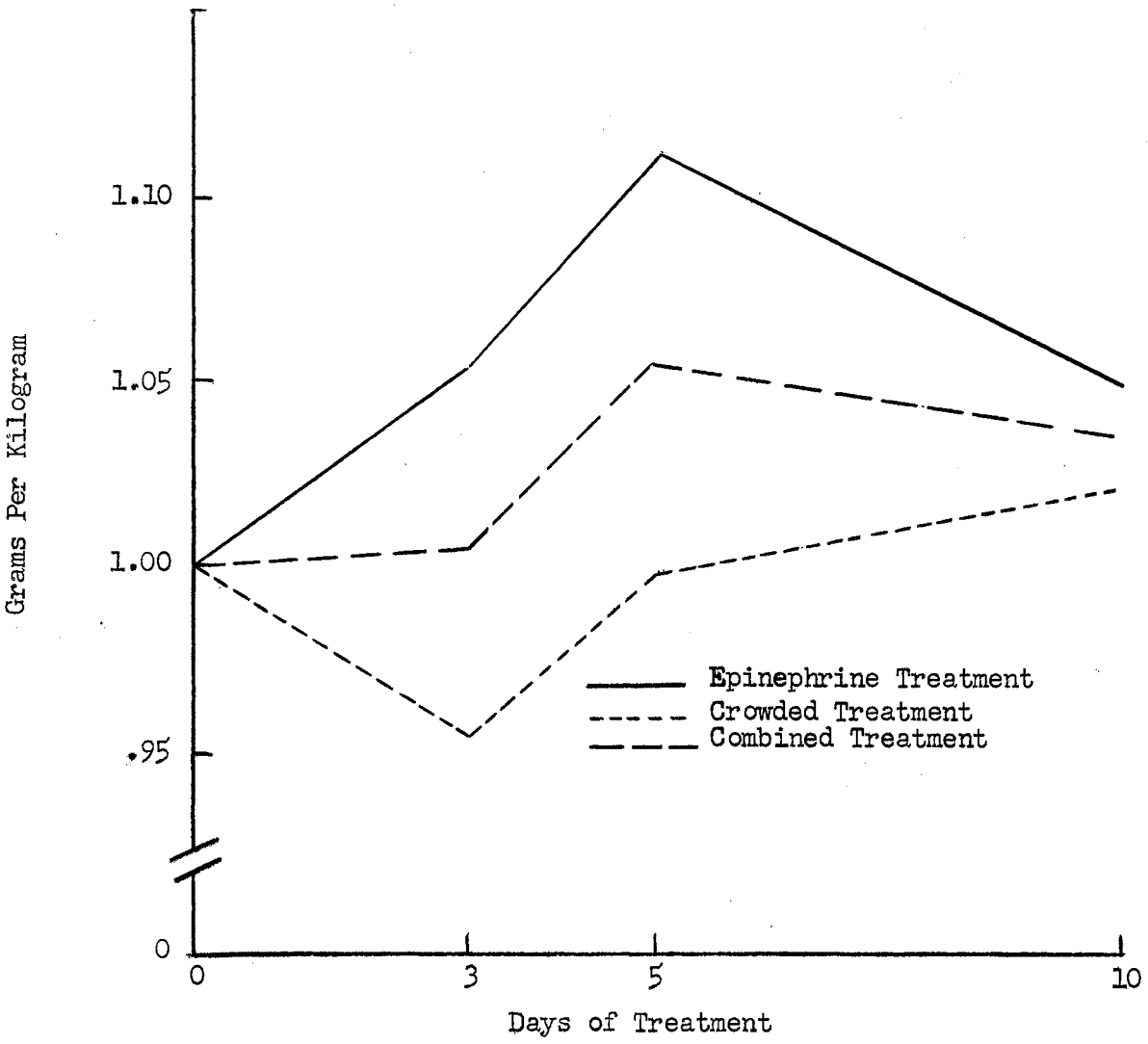


Figure 4. Grams of testis per kilogram of rabbit weight.

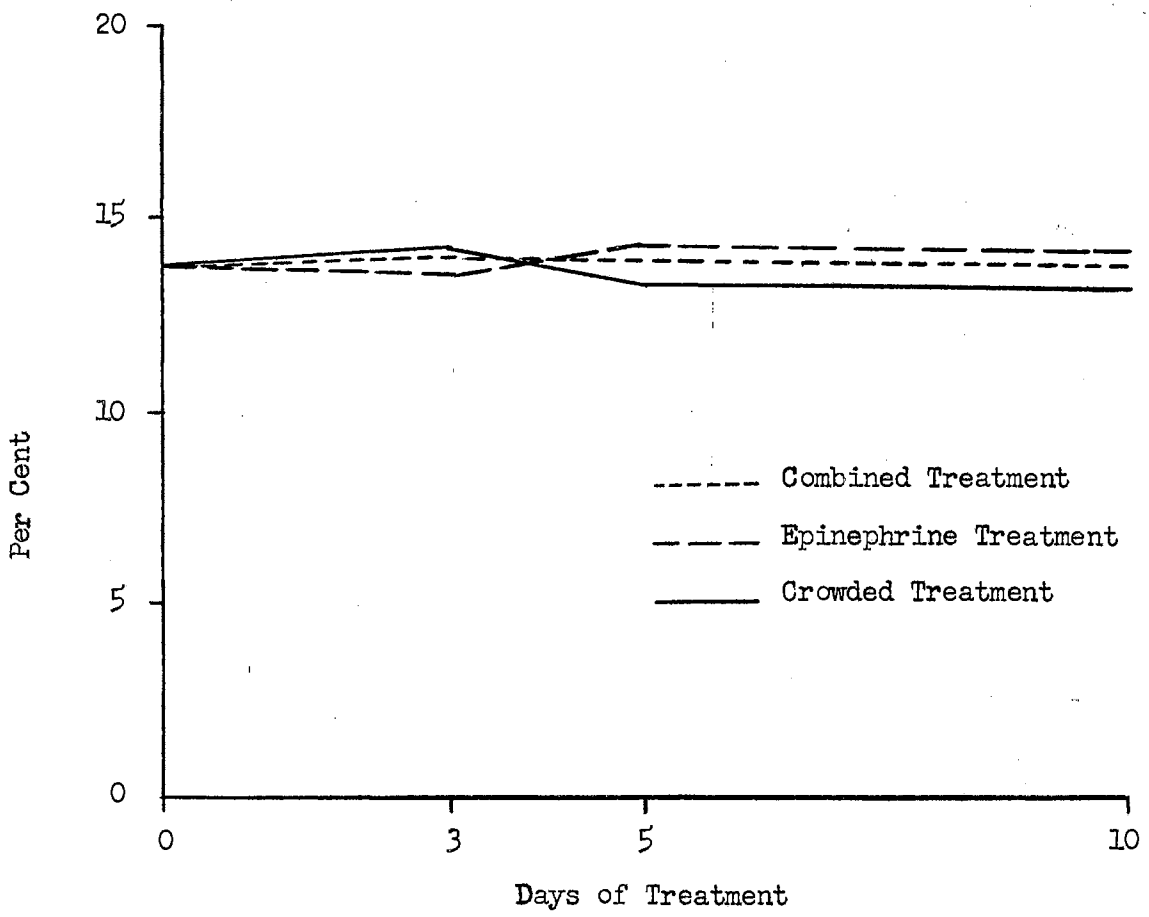


Figure 5. Percentage dry weight of testis.

it is possible to draw some conclusions from the results obtained in the metabolism study of testis and kidney cortex slices *in vitro*. Criteria investigated in this study were oxygen uptake, glucose uptake and lactic acid production. Metabolic activity of kidney cortex tissue was also investigated to serve as a comparison for testis tissue.

When a comparison of oxygen uptake of control tissue versus treated tissue was made, it was found that treated tissue was significantly higher ($P<.05$) than control tissue. However, no significant differences were found among the stressed animals. Kidney cortex slices exhibited significantly higher ($P<.01$) oxygen uptake than testis tissue. No significant differences in exogenous or endogenous substrates were noted in either tissue. The absence of an interaction between crowded tissue oxygen uptake and epinephrine treated oxygen uptake and the lack of significant difference between oxygen uptakes of the crowded tissues and the epinephrine treated tissues indicate that both types of treatment had the same general effects on the animals.

A study of Figure 6, $Q_{O_2}$ for testis tissue; Figure 7, $Q_{O_2}$ for kidney tissue; Figure 8, $Q_{O_2}$ for both tissues combined; and Figure 9, progressive oxygen uptake for both tissues, reveals a number of general trends. These are as follows: oxygen uptake of kidney cortex is higher than testis as shown by Figure 8; oxygen uptake by kidney cortex is about the same with and without glucose as a substrate as observed in Figure 9; oxygen uptake by testis tissue in glucose is

slightly higher than without glucose, also shown in Figure 9; and in both tissues oxygen uptake is elevated at day 3, highest at day 5 and near normal, as shown in Figure 8, by day 10. Therefore, these data show epinephrine injection and crowding cause an increase in oxygen uptake of testis and kidney cortex slices *in vitro*. This finding agrees with that of other investigators working with cardiac muscle, striated muscle, brain tissue, liver and adipose tissue (2, 25, 43, 57, 88, 114).

A rise in oxygen uptake in brain tissue, adipose tissue, liver, heart muscle and striated muscle has been shown to be brought about by epinephrine (2, 25, 43, 57, 88, 114). However, Goetsch and McDonald (53) observed an increase in oxygen uptake in rat liver homogenates following the injection of various glucocorticoids. Since stress causes the release of glucocorticoids as well as epinephrine, it would not be safe to specifically attribute the rise in oxygen uptake, noted in kidney cortex and testis slices following stress, to either of these hormones.

The decrease in oxygen uptake following the rise could be an adaptation to stress as discussed by Selye (129, 130). However, in rat liver homogenates, Goetsch and McDonald (53) found a decrease in oxygen uptake was brought about by injection of a glucocorticoid for seven days. Other workers got a similar response in liver homogenates by preinjection of glucocorticoids for 12 to 14 days (23, 72, 73, 114). Therefore, it is possible that the decrease in oxygen uptake

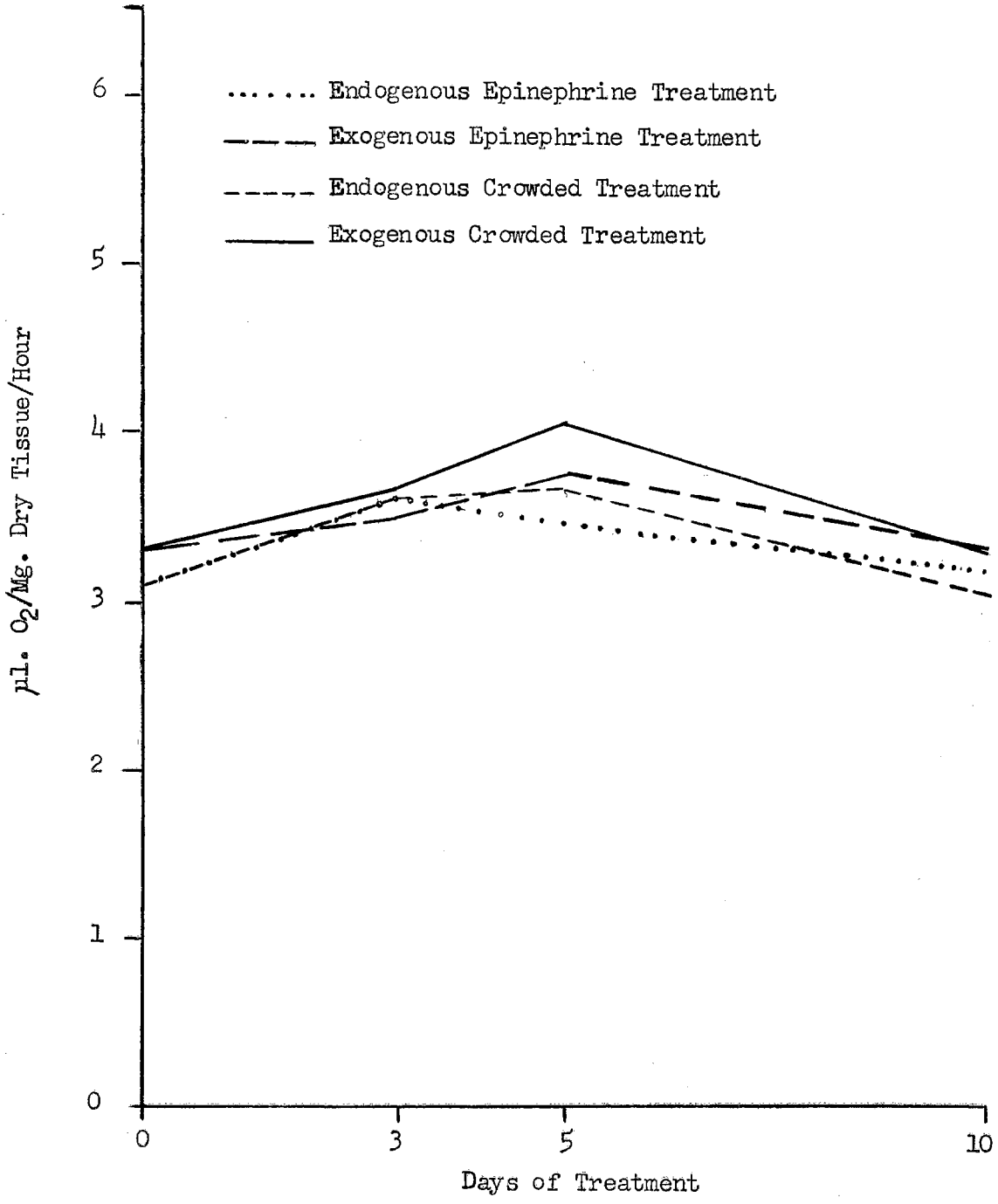


Figure 6. $Q_{O_2}$ for all testis tissues.

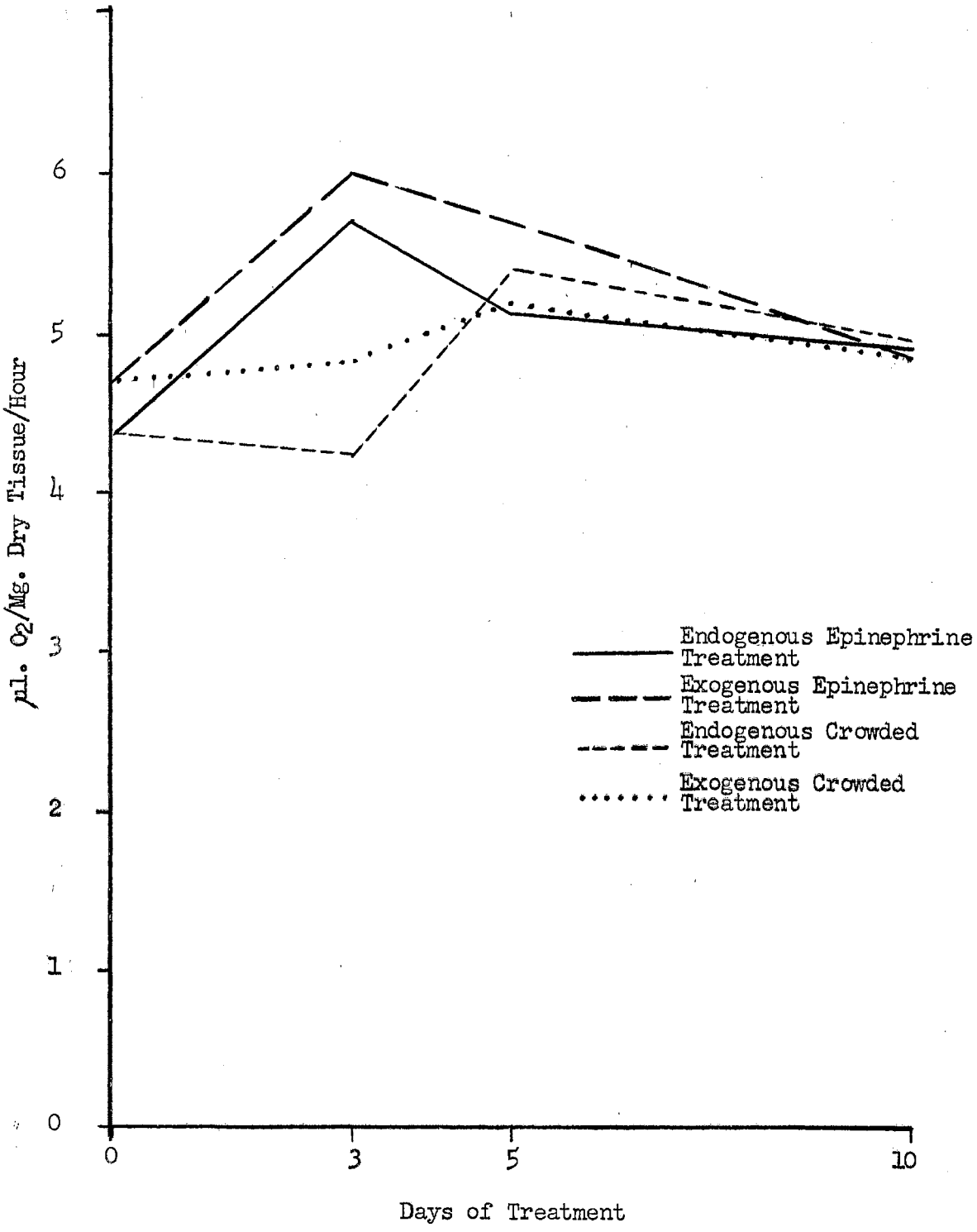


Figure 7. $Q_{O_2}$ for all kidney tissues.

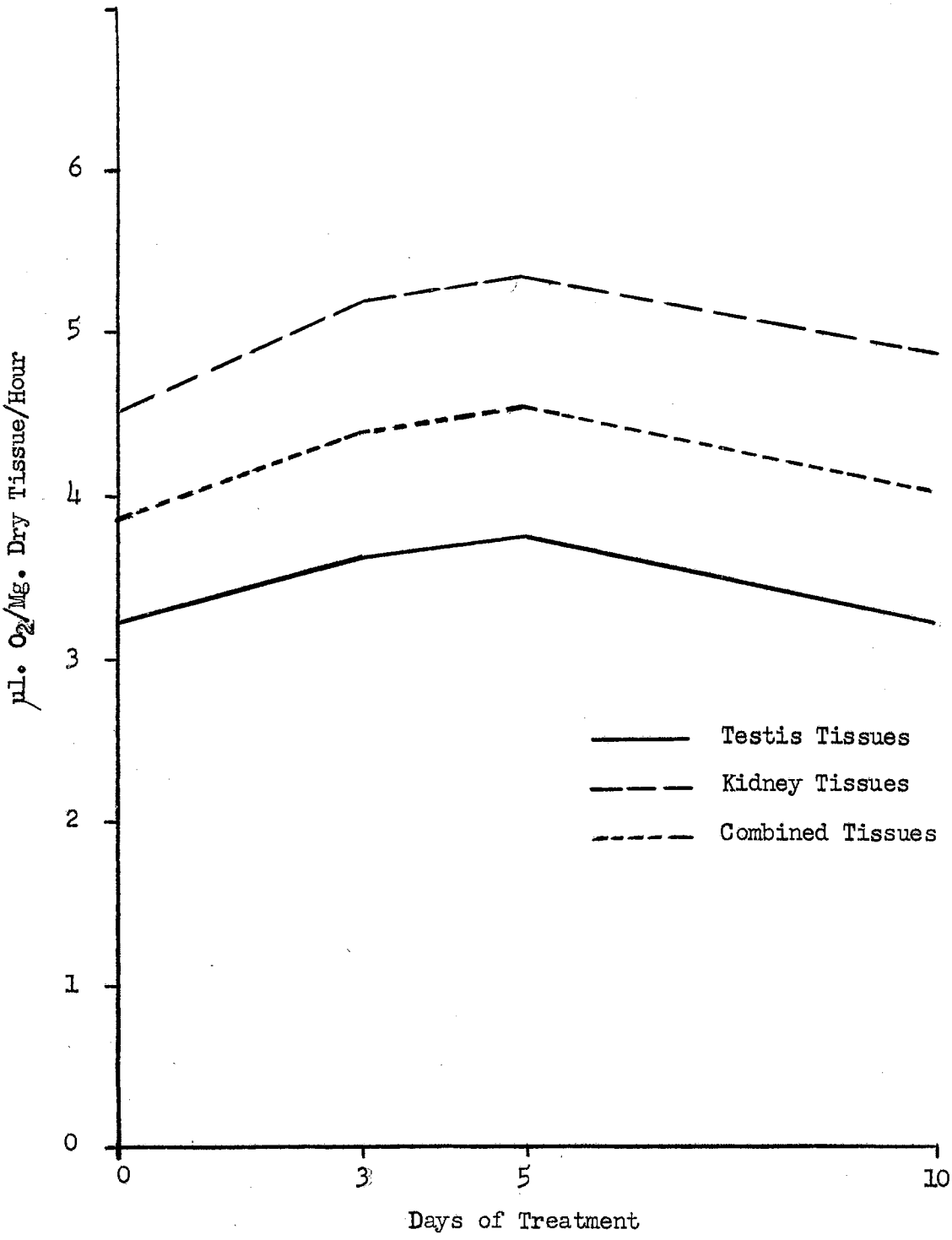


Figure 8. $Q_{O_2}$ for testis tissues, kidney tissues, and both tissues combined.

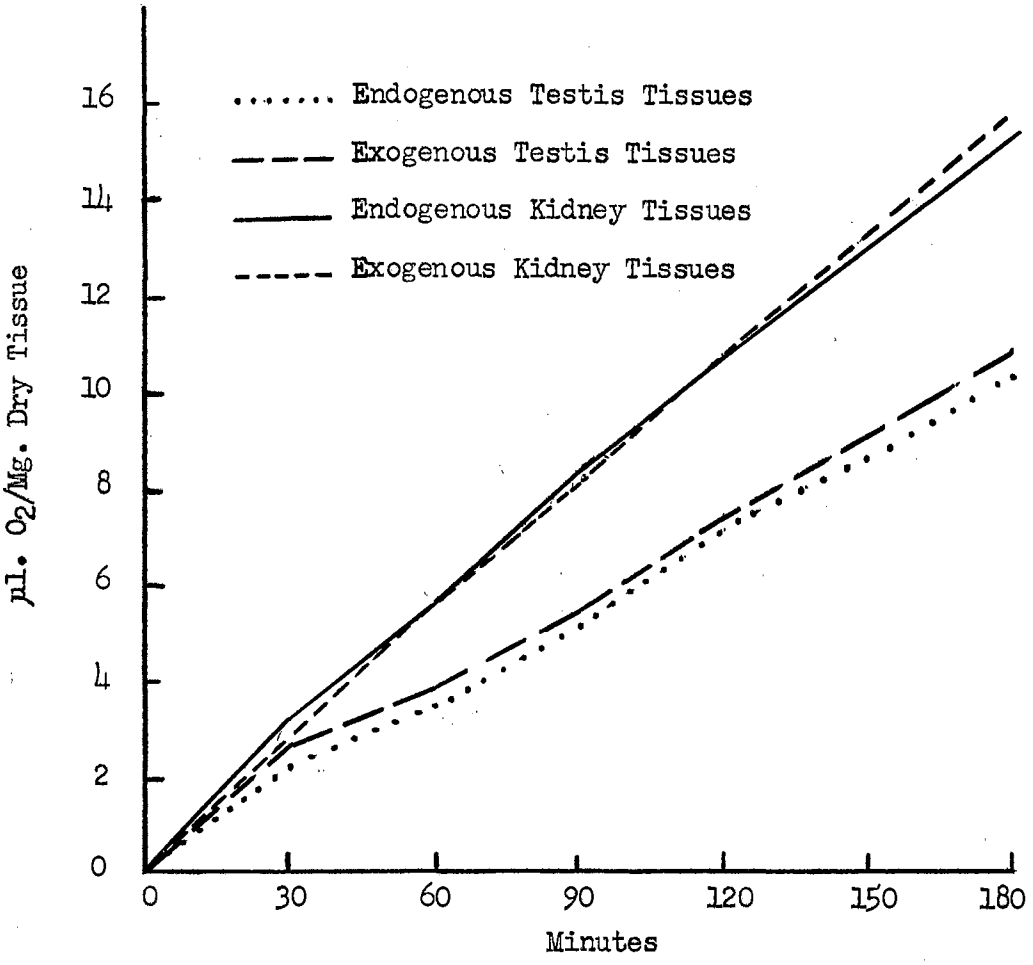


Figure 9. Progressive oxygen uptake for all tissues.

by day 10 in testis and kidney cortex slices is in response to the release of endogenous adrenal glucocorticoids.

In order to get some idea of substrate utilization by testis and kidney cortex slices *in vitro*, the slices were run in the Warburg with and without glucose as a substrate. Glucose uptake by slices *in vitro*, as shown by Figure 10, decreased at day 3, increased at day 5 and, in general, was lower at day 10. In all cases, glucose uptake in stressed animals was lower than in the controls.

The decreased glucose uptake following stress is in agreement with the findings of other workers studying muscle tissue (26, 27, 28, 37, 50, 145). It was found that decreased glucose uptake in muscle was caused by the activation of phosphorylase by epinephrine which then led to excess production of glucose-6-phosphate from glycogen (3, 60, 61, 75, 78, 79, 107, 132, 136, 140). Glucose-6-phosphate inhibits hexokinase, an enzyme system involved in the utilization of glucose. The effect of this is to shift the source of energy in muscle from a primary dependence on blood glucose to endogenous glycogen. The decreased glucose uptake found in testis tissue and kidney cortex tissue suggests that this effect may also be occurring in testis and kidney cortex as well as in muscle tissue.

Several earlier workers were of the opinion that testis tissue contained very little glycogen (35, 36, 112). However, Means (94) found that rat testis contained near one per cent glycogen by wet weight measurements. The fact that

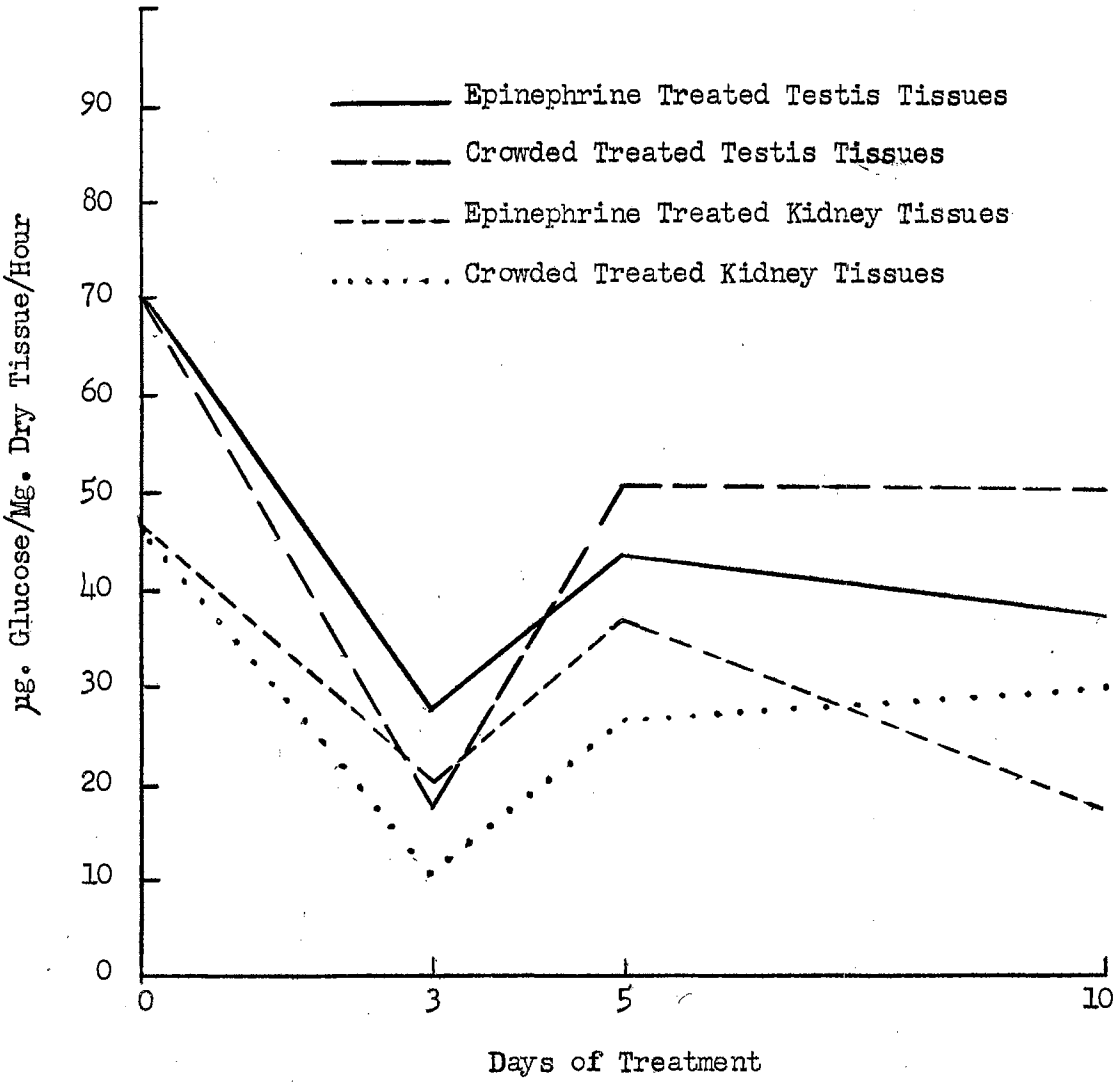


Figure 10. Glucose uptake by all treated tissues.

exogenous testis oxygen uptake was only slightly higher than endogenous testis oxygen uptake suggests that endogenous substrate was adequate for the needs of the testis tissue.

A general increase in glycolysis as detected by a rise in lactic acid production in liver, muscle, etc., following stress, was noted by several authors (8, 10, 24, 28, 37, 57, 88, 131, 134, 145). This rise in glycolysis was not observed in either the kidney or testis tissue following stress. A study of graphs of lactic acid production in testis slices, kidney slices and both tissues combined, as seen in Figures 11, 12 and 13, respectively, reveals several interesting observations. These are: that testis tissue has a higher glycolytic rate than kidney cortex, as shown in Figure 13; that lactic acid production is higher in exogenous substrate than in endogenous substrates in both tissues, as shown in Figures 11, 12 and 13; and, that there is no noticeable rise in lactic acid production caused by stress in either tissue, as shown in Figure 13.

If the decreased glucose uptake represents an increased concentration of glucose-6-phosphate, as it does in muscle, one would expect this also to be shown by an accumulation of lactic acid. In the case of testis and kidney cortex slices from stressed animals, this was not shown to be true. An increased utilization of oxygen was also obtained in these same tissues. These factors suggest that an increased utilization of other oxidative pathways is being employed or the glycolytic production of pyruvate from the glucose-6-

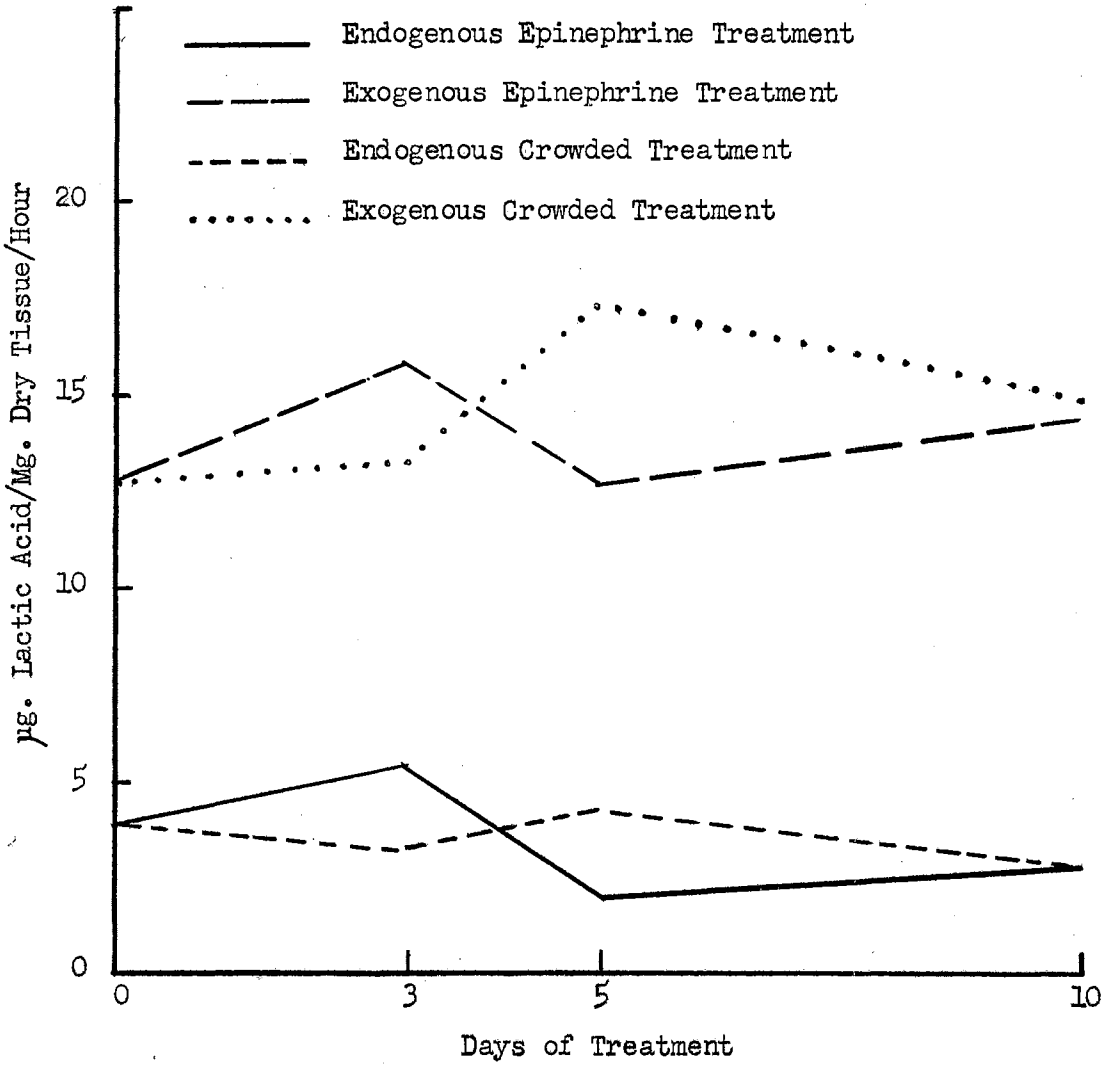


Figure 11. Lactic acid production by treated testis tissue.

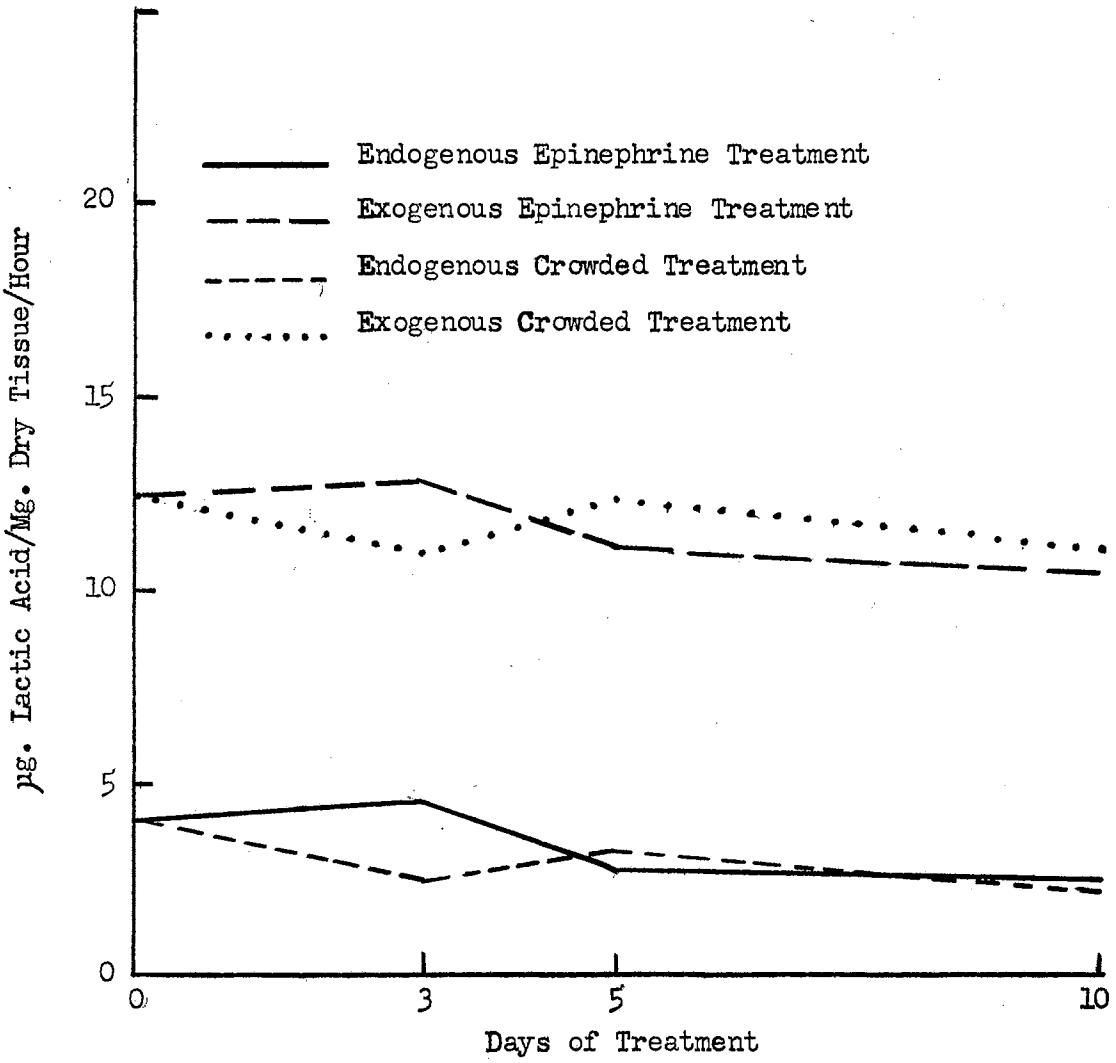


Figure 12. Lactic acid production by treated kidney cortex tissue.

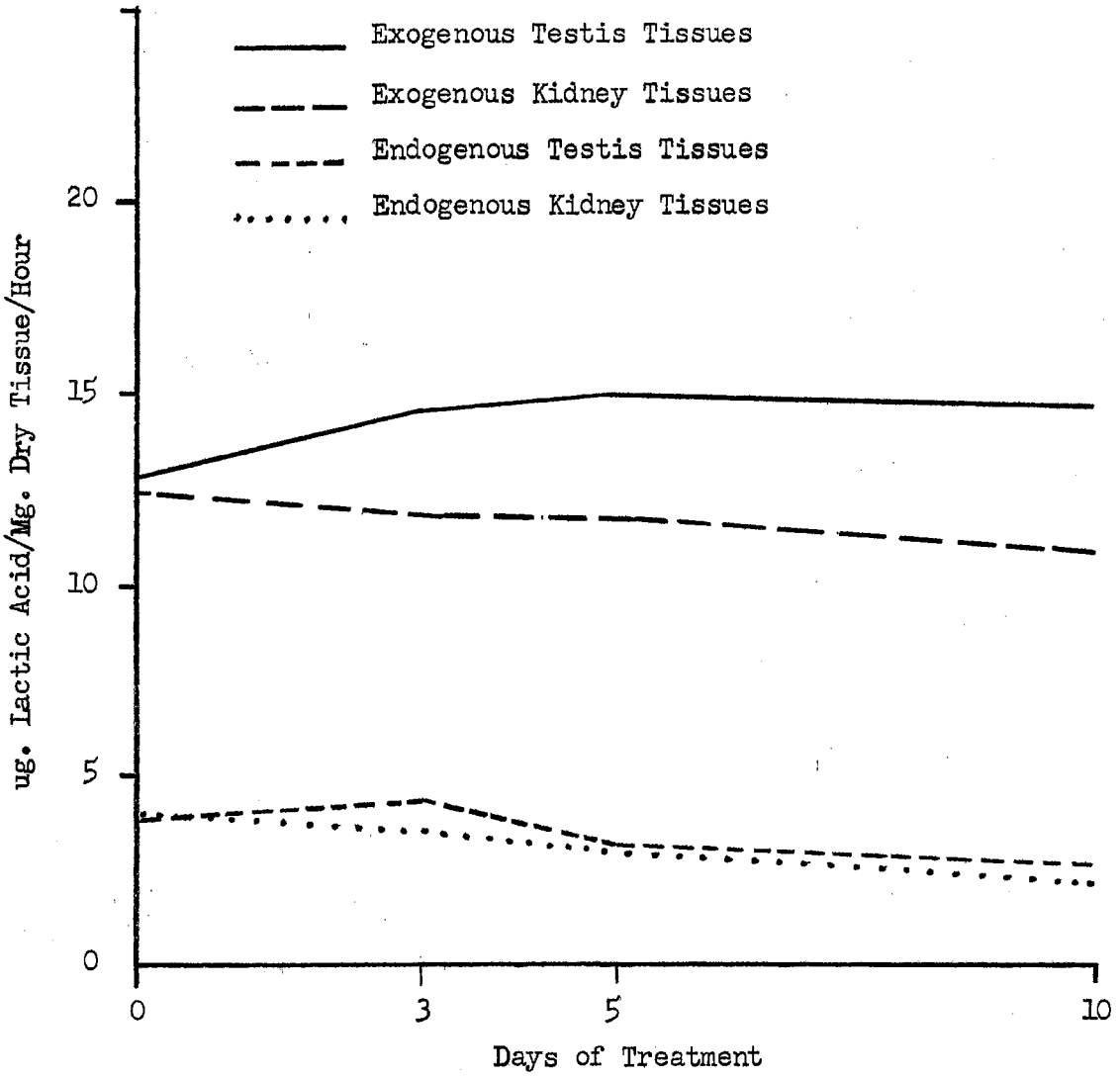


Figure 13. Lactic acid production; combined treatment effects on both types of tissues.

phosphate is being diverted more directly into the Krebs citric acid cycle and less into lactic acid.

This experiment was of a preliminary nature and as such has raised a number of questions that need further investigation. Some of these questions, with statements of possible means by which they may be answered, are as follows: (1) How is glycogenolysis affected by stress in the testis and kidney cortex? This could be measured by determination of glycogen levels, cyclic 3' - 5' adenosine monophosphate concentration, phosphorylase activity and glucose-6-phosphate concentration. The answer to this question could explain the reason for the decreased glucose uptake. (2) What hormones are actually responsible for the increased oxygen uptake? The effects of the adrenalcorticoids could be studied either by injection of ACTH or by injection of exogenous glucocorticoids. The effects of epinephrine could be studied by injection of epinephrine into either adrenalectomized, or hypophysectomized animals or animals with properly placed hypothalamic lesions. (3) Why is glycolysis not stimulated in testis and kidney cortex slices by stress? This question could be answered, in part, by measuring pyruvate concentrations and lactic dehydrogenase activity. (4) What routes are being utilized for oxidation? Answers for this could be obtained by measuring various intermediates and enzymes of the Krebs cycle and suggested alternate pathways. (5) Do the glucocorticoids affect oxidative phosphorylation in testis as in liver homogenates? This could be determined by a measure of en-

zymes and intermediates of the electron transport system. (6) What happens to metabolism after a stress longer than 10 days? An extended period of stress with adequate analytical procedures would answer this question.

# CHAPTER IV

## SUMMARY AND CONCLUSIONS

Since it has been shown that stress causes degenerative changes in testis tissue, an experiment was designed to study the metabolic changes that one would expect to precede gross degenerative changes in testis tissue. The methods employed in stressing are as in Chaper III. The metabolic criteria measured were oxygen uptake, glucose uptake and lactic acid production. Assays were also carried out to assure that the animals had been stressed and to determine what gross changes might have occurred in the testes of the stressed animals.

The efficacy of stressors was determined by measuring adrenal ascorbic acid depletion. It was found that adrenal ascorbic acid levels at days 3 and 5 were significantly lower ($P<.01$) than at day 10 or at the control level. This shows that the animals at days 3 and 5 were reacting to the stressors. The return to normal at day 10 is probably a response of adaptation. Adrenal hypertrophy, another measure of stress, showed the inverse response of adrenal ascorbic acid depletion, that is, adrenal hypertrophy at days 3 and 5.

No significant gross changes were detected in the testes. Testis weights, testis to rabbit weight ratios, per-

centage dry weights of testis tissue and morphology of testes were not significantly different in the treated animals from the control animals.

Oxygen uptake by both tissues in the treated animals was significantly higher ($P<.05$) than in both tissues of the controls. Oxygen uptake of kidney cortex was significantly higher ($P<.01$) than testis tissue. The lack of interaction between epinephrine injection and crowding, and the lack of significant differences between the two treatments indicates that both treatments had the same general effects on oxygen uptake. Therefore, in general, oxygen uptake was increased at day 3, further increased by day 5 and declined to levels near normal by day 10.

Glucose uptake of experimental tissues was significantly lower ($P<.05$) than in control tissues. There was a significant difference between days 3, 5 and 10. Glucose uptake by kidney cortex was significantly lower ($P<.01$) than in testis tissue. In general, glucose uptake was decreased considerably at day 3, increased from day 3 to day 5 and slightly decreased from day 5 to day 10.

Epinephrine causes decreased glucose uptake in muscle by its indirect action of increasing muscle phosphorylase activity, which causes increased glycogenolysis. In the process, glucose-6-phosphate concentration is elevated and this increased concentration of glucose-6-phosphate inhibits hexokinase. It is possible that the same action is responsible for decreased glucose uptake following stress noted in the

testis and kidney cortex slices.

Glycolysis as measured by lactic acid production was not noticeably altered by the treatments in testis or kidney cortex slices. This is different from the findings for other tissues in which lactic acid production is accelerated by epinephrine. There are a number of possible explanations: (1) lactic dehydrogenase activity has been decreased; (2) pyruvate is being directed immediately into the Krebs cycle; or, (3) more of the apparent accumulating glucose-6-phosphate is being directed into other oxidative pathways.

The response of testis metabolism to stress was similar to that of kidney cortex, in that both tissues showed the following: increased oxygen uptake, decreased glucose uptake, and no change in lactic acid production.

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# A P P E N D I X

ANALYSIS OF VARIANCE FOR ADRENAL ASCORBIC ACID

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Total | 41 | | | |
| Treatments | 6 | 68,198.0 | 11,366.3 | |
| Control vs. Others | 1 | 11,093.0 | 11,093.0 | 3.67 |
| With Others | 5 | 57,105.0 | 11,421.0 | |
| Type | 1 | 14,003.0 | 14,003.0 | 4.63* |
| Day | 2 | 36,330.0 | 18,165.0 | 6.01** |
| Type x Days | 2 | 6,772.0 | 3,386.0 | 1.12 |
| Error | 35 | 105,763.0 | 3,021.8 | |

ANALYSIS OF VARIANCE OF TESTIS WEIGHTS

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Total | 41 | | | |
| Treatments | 6 | .60320 | .10053 | |
| Control vs. Others | (1) | .02950 | .02950 | <1 |
| Within Others | 5 | .57370 | .11474 | |
| Type | (1) | .39480 | .39480 | <1 |
| Days | (2) | .00110 | .00055 | <1 |
| Type x Days | (2) | .17780 | .08890 | <1 |
| Error | 35 | 32.25140 | .92147 | |

ANALYSIS OF VARIANCE FOR $Q_{O_2}$ OF ALL ANIMALS

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Total | 335 | | | |
| Experiments | 6 | 30.4705 | 5.0784 | 2.53 |
| Control vs. Others | 1 | 8.3855 | 8.3855 | 4.18* |
| Within Others | 5 | 22.0850 | | |
| Type | 1 | 2.4090 | 2.4090 | 1.2 |
| Days | 2 | 13.0836 | 6.5418 | 3.26 |
| Days x Type | 2 | 6.5924 | 3.2962 | 1.64 |
| Error | 35 | 70.2868 | 2.0082 | |

ANALYSIS OF VARIANCE FOR $Q_{O_2}$ OF DIFFERENT TISSUES

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Tissues | 1 | 209.6660 | 209.6660 | 86.5** |
| Tissues x Experiments | 6 | 13.8097 | | |
| (Control vs. Other) x Tissue | 1 | 1.0451 | 1.0451 | <1 |
| (Within Others) x Tissue | 5 | 12.7646 | | |
| Type x Tissue | 1 | 5.5610 | 5.5610 | 2.30 |
| Days x Tissue | 2 | .0724 | .0362 | <1 |
| Type x Days x Tissue | 2 | 7.1312 | 3.5656 | 1.47 |
| Error | 35 | 84.8037 | 2.4230 | |

ANALYSIS OF VARIANCE FOR $Q_{O_2}$ OF DIFFERENT SUBSTRATES

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Substrates | 1 | 3.2411 | 3.2411 | 2.64 |
| Substrates x Experiments | 6 | 1.7483 | | |
| (Control vs. Other) x Substrates | 1 | .1269 | .1269 | <1 |
| (Within Others) x Substrates | 5 | 1.6214 | | |
| Type x Substrates | 1 | .0168 | .0168 | <1 |
| Days x Substrates | 2 | .5136 | .2568 | <1 |
| Type x Days x Substrates | 2 | 1.0910 | .5455 | <1 |
| Error | 35 | 43.0236 | 1.2292 | |

ANALYSIS OF VARIANCE FOR $Q_{O_2}$ OF SUBSTRATES x TISSUES

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Substrates x Tissues | 1 | .0060 | .0060 | <1 |
| Substrates x Tissues x Experiments | 6 | 3.2903 | | |
| x(Control vs. Other) (Substrates x Tissue) | 1 | .0105 | .0105 | <1 |
| x(Within Others) (Substrates x Tissues) | 5 | 3.2798 | | |
| Type x Substrates x Tissues | 1 | .4202 | .4202 | <1 |
| Days x Substrates x Tissues | 2 | 2.1290 | 1.0645 | <1.21 |
| Type x Days x Substrates x Tissues | 2 | .7306 | .3653 | <1 |
| Error | 35 | 30.5362 | .8725 | <1 |
| Duplicates (Pooled) | 168 | 147.8965 | .8803 | |

ANALYSIS OF VARIANCE OF MICROGRAMS GLUCOSE UPTAKE/MG. DRY TISSUE/HOUR ON ALL ANIMALS

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Total | 167 | 140,151.50 | 839.23 | |
| Experiments | 6 | 30,358.10 | 5,059.68 | 2.23 |
| Control vs. Others | 1 | 16,695.42 | 16,695.42 | 7.35* |
| Within Others | 5 | 13,662.68 | | |
| Days | 2 | 10,549.83 | 5,274.92 | 2.32* |
| Types | 1 | 7.49 | 7.49 | <1 |
| Days x Types | 2 | 3,105.36 | 1,552.68 | <1 |
| Error | 35 | 79,463.10 | 2,270.37 | |

ANALYSIS OF VARIANCE OF MICROGRAMS GLUCOSE UPTAKE/MG. DRY TISSUE/HOUR ON DIFFERENT TISSUES

| Source of Variation | df | SS | MS | F |
|---|---|---|---|---|
| Tissues | 1 | 10,418.20 | 10,418.20 | 57.55** |
| Tissues x Experiments | 6 | 2,517.40 | | |
| (Controls vs. Others) x Tissues | 1 | 560.67 | 560.67 | 3.10 |
| (Within Others) x Tissues | 5 | 1,956.73 | | |
| Days x Tissues | 2 | 1,069.44 | 534.72 | 2.95 |
| Types x Tissues | 1 | 263.30 | 263.30 | 1.45 |
| Days x Types x Tissues | 2 | 623.99 | 311.99 | 1.72 |
| Error | 35 | 6,335.90 | 181.03 | |
| Duplicates (Pooled) | 84 | 11,058.80 | 131.65 | |

MEAN ADRENAL WEIGHTS

| Controls | Epinephrine | | | Crowded | | |
|---|---|---|---|---|---|---|
| | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 210.5 | 263.6 | 180.9 | 204.2 | 218.6 | 261.5 | 203.4 |
| | Combined Effects | | | | | |
| | 3 Day | 5 Day | 10 Day | | | |
| 210.5 | 241.1 | 221.2 | 203.8 | | | |

MEAN GRAMS OF TESTIS TISSUE/KILOGRAM OF RABBIT WEIGHT

| Controls | Epinephrine | | | Crowded | | |
|---|---|---|---|---|---|---|
| | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| .950 | 1.004 | 1.061 | 1.000 | .906 | .948 | .972 |
| | Combined Effects | | | | | |
| | 3 Day | 5 Day | 10 Day | | | |
| .950 | .955 | 1.005 | .986 | | | |

MEAN GRAMS OF TESTIS TISSUE

| Controls | Epinephrine | | | Crowded | | |
|---|---|---|---|---|---|---|
| | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 2.71 | 2.97 | 2.81 | 2.89 | 2.59 | 2.76 | 2.70 |

ASCORBIC ACID LEVELS IN MG./100 GRAMS OF TISSUE

| Controls | Epinephrine | | | Crowded | | |
|---|---|---|---|---|---|---|
| | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 329 | 177 | 221 | 299 | 285 | 290 | 270 |
| 342 | 181 | 182 | 257 | 233 | 260 | 311 |
| 200 | 150 | 228 | 387 | 305 | 286 | 263 |
| 223 | 230 | 174 | 313 | 250 | 432 | 272 |
| 369 | 211 | 277 | 257 | 192 | 214 | 300 |
| 402 | 236 | 314 | 310 | 267 | 273 | 311 |
| Means 310 | 197 | 232 | 303 | 255 | 292 | 304 |

MEAN TESTIS $Q_{O_2}$

| Controls | | Epinephrine End.* | | | Epinephrine Exo.** | | | Crowd End. | | | Crowd Exo. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endogenous | Exogenous | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 3.10 | 3.32 | 3.63 | 3.44 | 3.16 | 3.49 | 3.75 | 3.34 | 3.62 | 3.68 | 3.03 | 3.68 | 4.07 | 3.30 |
| | | Combined Effects | | | | | | | | | | | |
| | | Endogenous | | | Exogenous | | | | | | | | |
| | | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | | | | | | |
| 3.10 | 3.32 | 3.63 | 3.56 | 3.09 | 3.58 | 3.91 | 3.32 | | | | | | |

* Endogenous
** Exogenous

MEAN KIDNEY CORTEX $Q_{O_2}$

| Controls | | Epinephrine End. | | | Epinephrine Exo. | | | Crowd End. | | | Crowd Exo. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endogenous | Exogenous | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 4.37 | 4.69 | 5.70 | 5.13 | 4.88 | 5.99 | 5.71 | 4.82 | 4.25 | 5.39 | 4.95 | 4.84 | 5.20 | 4.84 |
| | | Combined Effects | | | | | | | | | | | |
| | | Endogenous | | | Exogenous | | | | | | | | |
| | | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | | | | | | |
| 4.37 | 4.69 | 4.97 | 5.26 | 4.92 | 5.42 | 5.46 | 4.83 | | | | | | |

MEAN OXYGEN UPTAKE IN MICROLITERS/MG. OF DRY TISSUE:
COMBINED DATA ON TREATED TESTIS

| | Endogenous | | | | | | Exogenous | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Minutes | 30 | 60 | 90 | 120 | 150 | 180 | 30 | 60 | 90 | 120 | 150 | 180 |
| 3 Day Crowd | 2.87 | 4.13 | 5.28 | 7.25 | 8.82 | 10.59 | 3.02 | 4.39 | 5.70 | 7.51 | 9.63 | 11.22 |
| 5 Day Crowd | 2.12 | 3.98 | 5.96 | 7.50 | 9.48 | 11.09 | 2.38 | 4.17 | 6.14 | 7.65 | 9.95 | 12.51 |
| 10 Day Crowd | 1.83 | 2.46 | 4.76 | 6.04 | 7.27 | 9.09 | 2.25 | 2.80 | 5.43 | 6.70 | 8.60 | 10.11 |
| 3 Day Epine. | 2.50 | 3.16 | 5.52 | 7.09 | 8.96 | 10.64 | 2.24 | 3.03 | 5.12 | 6.81 | 9.01 | 10.47 |
| 5 Day Epine. | 2.32 | 4.15 | 5.13 | 7.65 | 8.73 | 10.48 | 2.40 | 4.48 | 5.17 | 7.86 | 9.23 | 10.95 |
| 10 Day Epine. | 1.97 | 3.24 | 4.49 | 6.46 | 8.35 | 9.47 | 2.65 | 3.14 | 4.48 | 6.39 | 8.45 | 9.87 |
| Epine. Mean | 2.26 | 3.52 | 5.05 | 7.07 | 8.68 | 10.20 | 2.43 | 3.55 | 4.92 | 7.02 | 8.90 | 10.43 |
| Crowd Mean | 2.27 | 3.52 | 5.33 | 6.93 | 8.53 | 10.26 | 2.55 | 3.79 | 5.76 | 7.29 | 9.39 | 11.28 |
| Overall Mean | 2.26 | 3.52 | 5.19 | 7.00 | 8.59 | 10.23 | 2.49 | 3.67 | 5.34 | 7.15 | 9.14 | 10.85 |
| Control | 1.55 | 2.58 | 3.83 | 6.13 | 7.11 | 9.29 | 2.10 | 3.12 | 4.48 | 6.17 | 7.35 | 9.97 |

MEAN OXYGEN UPTAKE IN MICROLITERS/MG. OF DRY TISSUE:
COMBINED DATA ON TREATED KIDNEY CORTEX

| | Endogenous | | | | | | Exogenous | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Minutes | 30 | 60 | 90 | 120 | 150 | 180 | 30 | 60 | 90 | 120 | 150 | 180 |
| 3 Day Crowd | 2.77 | 4.61 | 6.23 | 8.67 | 10.82 | 12.80 | 2.84 | 5.03 | 7.07 | 9.88 | 12.08 | 14.51 |
| 5 Day Crowd | 3.19 | 6.48 | 9.35 | 11.48 | 14.42 | 16.85 | 2.97 | 5.99 | 8.78 | 10.78 | 13.62 | 16.03 |
| 10 Day Crowd | 3.46 | 5.18 | 8.64 | 10.61 | 12.95 | 14.93 | 3.01 | 4.87 | 8.08 | 10.21 | 12.57 | 14.87 |
| 3 Day Epine. | 3.37 | 5.31 | 9.02 | 11.54 | 13.95 | 17.09 | 2.80 | 5.68 | 9.24 | 12.00 | 14.65 | 18.00 |
| 5 Day Epine. | 3.65 | 6.75 | 8.35 | 11.48 | 13.32 | 14.85 | 3.36 | 6.36 | 8.21 | 11.43 | 13.62 | 15.58 |
| 10 Day Epine. | 3.24 | 5.47 | 7.89 | 10.56 | 12.73 | 14.55 | 2.72 | 5.34 | 7.88 | 10.13 | 12.57 | 14.72 |
| Epine. Mean | 3.42 | 5.84 | 8.42 | 11.19 | 13.33 | 15.50 | 2.96 | 5.79 | 8.44 | 11.19 | 13.61 | 16.10 |
| Crowd Mean | 3.14 | 5.42 | 8.07 | 10.25 | 12.73 | 14.86 | 2.94 | 5.30 | 7.98 | 10.29 | 12.76 | 15.14 |
| Overall Mean | 3.28 | 5.63 | 8.25 | 10.72 | 13.03 | 15.18 | 2.95 | 5.55 | 8.21 | 10.74 | 13.19 | 15.62 |
| Control | 2.31 | 4.74 | 6.82 | 9.42 | 10.49 | 13.10 | 2.71 | 5.05 | 6.78 | 8.91 | 11.17 | 14.08 |

MEAN GLUCOSE UPTAKE BY TISSUES IN
MICROGRAMS/MG. DRY WEIGHT/HOUR

| Controls | | Epinephrine | | | | | | Crowded | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Testis | | | Kidney | | | Testis | | | Kidney | | |
| Testis | Kidney | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 71.43 | 46.72 | 27.63 | 43.73 | 37.06 | 19.93 | 36.50 | 17.32 | 17.20 | 50.57 | 50.11 | 11.00 | 26.23 | 29.83 |

MEAN LACTIC ACID PRODUCTION BY TESTIS IN MICROGRAMS/MG. DRY TISSUE/HOUR

| Controls | | Epinephrine End.* | | | Epinephrine Exo.** | | | Crowd End. | | | Crowd Exo. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endogenous | Exogenous | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 3.81 | 12.80 | 5.28 | 2.02 | 2.53 | 15.74 | 12.65 | 14.22 | 3.36 | 4.27 | 2.58 | 12.32 | 17.27 | 14.91 |
| | | Combined Effects | | | | | | | | | | | |
| | | Endogenous | | | Exogenous | | | | | | | | |
| | | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | | | | | | |
| | | 4.32 | 3.15 | 2.56 | 14.53 | 14.96 | 14.57 | | | | | | |

* Endogenous
** Exogenous

MEAN LACTIC ACID PRODUCTION BY KIDNEY CORTEX
IN MICROGRAMS/MG. DRY TISSUE/HOUR

| Controls | | Epinephrine End. | | | Epinephrine Exo. | | | Crowd End. | | | Crowd Exo. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endogenous | Exogenous | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day |
| 2.99 | 12.38 | 4.54 | 2.65 | 2.47 | 12.70 | 11.03 | 10.37 | 2.42 | 3.33 | 2.10 | 10.96 | 12.34 | 11.15 |
| | | Combined Effects | | | | | | | | | | | |
| | | Endogenous | | | Exogenous | | | | | | | | |
| | | 3 Day | 5 Day | 10 Day | 3 Day | 5 Day | 10 Day | | | | | | |
| | | 3.48 | 2.99 | 2.29 | 11.83 | 11.69 | 10.76 | | | | | | |

# VITA

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Master of Science

Thesis: THE EFFECTS OF EPINEPHRINE INJECTION AND COMPETITION BETWEEN MALES FOR 3, 5 AND 10 DAYS ON THE METABOLISM OF RABBIT TESTIS IN VITRO

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