EFFECTS OF PROGESTERONE ON CARBOHYDRATE METABOLISM AT REST AND DURING EXERCISE IN THE RAT

by

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This thesis is dedicated to my parents.

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ABSTRACT

The possible role of sex steroids in intermediary metabolism has been the subject of numerous studies for the past twenty years (Beck, 1969; Sutter-Dub et al., 1981; Mendez, 1985). Attention concerning these effects of sex steroids on carbohydrate metabolism has centered around the role played by progesterone in pregnancy-induced diabetes, the effects of synthetic hormones found in contraceptive preparations on carbohydrate and lipid metabolism and finally the effects of the naturally occuring hormones of the menstrual cycle, progesterone and estrogen, on metabolism. However , the results of these studies are not in agreement and focused more on the effects of estrogens rather than those of progesterone on metabolism.

Therefore, a study was undertaken to determine the effects of progesterone on carbohydrate metabolism at rest and during exercise in the rat. The animals used in this study were female rats of the Wistar strain and weighed between 175 and 200 grams when the study began. In order to better isolate the effects of progesterone, the animals were ovariectomized and divided into three goups. Two groups of animals received a hormone replacement therapy consisting of surgical implantation of Silastic tubing filled with crys-

talline progesterone (P4). One group of rats received tubes measuring 1.5 cm whereas the other group received 4.0 cm long Silastic implants. It was thought that this difference in the length of the Silastic tubing would produce significantly different plasma levels of progesterone since it was shown that the circulating levels of homone produced by implantation of Silastic tubes vary according to the length and diameter of the Silastic tubing (Weick, 1977, 1984). The third groups of rats received no progesterone but were fitted with an empty implant measuring 4.0 cm. The treatment lasted for either 7, 14 or 21 days.

The exercise protocol consisted of the rats running on a rodent motorized treadmill five days a week for the duration of the treatment. Blood collection was done three days after ovariectomy to verify the success of the surgery (determined by low levels of progesterone), three days after implantation of the Silastic tubing and again at sacrifice in order to determine the progesterone levels achieved through this method.

On the last day of the treatment the animals were either sacrificed at rest or after a run to exhaustion on the treadmill. Muscle samples from the soleus, plantaris and white vastus lateralis muscles and tissue sample from the liver were taken at the time of sacrifice in order to determine the resting and depleted levels of glycogen of these tissues. Plasma levels of glucose were also measured. The

running times and weight gains of the animals were also recorded.

The results of the present study show that implantation of Silastic tubing filled with progesterone results in highly variable levels of the hormone among rats of the same groups and in the same rats over a period of time. It was also found that the levels of P4 achieved through implantation of Silastic tubing were consistently lower after 21 days than after 7 or 14 days. Finally, analyses of progesterone levels also show that P4 levels are higher in animals receiving a 1.5 cm long Silastic implant than in rats receiving a 4.0 cm implant.

It was found that long term progesterone treatment results in elevated plasma glucose levels at rest. These results are in agreement with those of Sutter-Dub et al. (1981, 1982). Furthermore, it was found that this P4 induced hyperglycemic state was more important in animals which received a 1.5 cm implant.

It was also found that P4 treatment results in lower glycogen levels in both soleus and liver at rest when compared to glycogen levels in ovariectomized non-treated rats. Glycogen levels of white vastus lateralis and plantaris muscle were also found to be lower in progesterone treated rats than in rats which were ovariectomized but did not receive the progesterone treatment. However, these differences were not found to be significant.

Finally results concerning running performances show that rats which received the progesterone treatment ran significantly longer than the untreated animals therefore suggesting that progesterone treatment may result in a sparing of the carbohydrate reserves during work and/or in a greater utilization of fats as a source of fuel during exercise, since Sutter-Dub et al. (1981, 1982) have consistently showed that progesterone resulted in an accelerated rate of lipolysis.

Lasty it was found that P4 treatment results in a greater weight gain in rats. These results confirm those of Mendes et al, (1985) and those of Ashby et al, (1978).

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INTRODUCTION

1.1 Introduction:

The female menstrual cycle and its characteristic hormonal fluctuations have concerned researchers for a century now. The possible relationship between ovarian hormones and physical exercise has been observed as early as 1890 by Van Ott when his findings demonstrated an influence of the menstrual cycle phase on energy balance in women.

Because of women's increasing participation in sports, there has been renewed interest in the link between female performance and menstrual function. Research stemming from this interest has, over recent years, addressed three main questions. The first area of interest concerns the effect of acute exercise and/or training on menstrual function and the ovarian-pituatary axis hormones. A survey has suggested that exercise and training affect menstrual function. Zaharieva (1965) surveyed a number of female athletes at the Tokyo Olympics and found that 27 out of 65 competitors experienced disturbances in their menstrual cycle, all of which were related to training.

A second important question resulting from the interest in performance and the menstrual cycle is the effect of the

latter on exercise. Jurkowski et al. (1978) in a study on the hormonal responses to exercise found that time to exhaustion at maximum power output was increased in the luteal phase i.e. when levels of progesterone are high. This discovery and other findings on the effects of natural and synthetic estrogens on carbohydrate and lipid metabolism at rest led researchers (Matute, 1973, Beck, 1973) to study the effects of the menstrual cycle on blood metabolites and substrate utilization. Lastly, in a study done by Gorski et al. (1976) in which the effects of another steroid, estradiol, on carbohydrate utilization during exercise in rats were studied, it was found through muscle samples and blood metabolite analyses that the steroid had a sparing effect on carbohydrate reserves during exercise in rats.

1.2 Rationale:

The discovery and subsequent widespread use of many preparations of oral contraceptives have prompted researchers to study the physiological effects of female sex steroids, both natural and synthetic. A great deal of research on the subject is still being done today. Furthermore, the increasing use of anabolic steroids as a training tool for endurance and strength training has generated a great deal of research on the effects of both natural and synthetic anabolic steroids at rest, but especially during acute or chronic exercise. However, little information is available on the

effects of either natural or synthetic female sex steroids on both carbohydrate and lipid metabolism during acute and/or chronic exercise. Therefore the rationale for this study was to fulfill the need to gain further information on the effects of progesterone administration on carbohydrate metabolism using a rat model. Furthermore the available information on the subject contains several discrepancies between studies concerning method of administration, levels of hormone administered and/or achieved and duration of treatment.

1.3 Statement of the problem:

The existence of major differences between studies concerning the method of administration, the widely varying levels of circulating hormone achieved with these different methods and the duration of treatment necessary in order to obtain results made it virtually impossible to draw any conclusions regarding the effects of progesterone administration on carbohydrate metabolism at rest and during exercise.

The problem, therefore, was to verify the effects of progesterone administration on carbohydrate metabolism at rest and during exercise using the implantation of Silastic capsules measuring either 1.5 cm or 4.0 cm in length for either 7, 14 or 21 days. The exercise regiment that the animals will be submitted to consists of a run on a motorized rodent treadmill 5 days/week at a speed of 35 m/min and an incline

of 15 %. The effects of progesterone on carbohydrate metabolism during rest and exercise will be verified by measuring blood glucose levels as well as muscle glycogen levels in soleus, plantaris and white vastus lateralis muscles and liver glycogen levels.

1.4 Hypothesis:

It is hypothesized that chronic administration of progesterone (7 to 21 days) results in changes in carbohydrate metabolism:

1) It will result in lower glycogen concentrations in both muscle and liver due to a progesterone induced insulin resistance in tissue

2) Higher resting glucose levels (creating a state of hyperglycemia).

3) Rats treated with progesterone will exhibit greater running times than the animals which did not receive the progesterone treatment.

4) Rats treated with progesterone will gain more weight over the course of the study than the non treated rats.

1.5 Scope of the study:

This study was an analysis of the effects of progesterone on carbohydrate metabolism at rest and during exercise. The subjects were rats to which were administered either empty implants or implants of varying lengths, filled with progesterone and for different durations of treatment.

1.6 Limitations of the study:

Tissue analyses were limited to the soleus, the plantaris and the white vastus lateralis muscles and the liver. A major limitation in studies in which Silastic implants are used as the method of hormone administration is that circulating levels of hormone produced by the implants are dependent upon the weight of the animal (especially the proportion of tissue that is fat tissue). In addition, the length and diameter of tubing filled with progesterone, will somewhat vary among animals of the same group. Another limitation of the study, is that even though surgical removal of the ovaries (ovariectomies) results in an almost complete decrease in circulating levels of sex steroids (progesterone and estrogens) there is still a minor contribution to the circulating pool by the adrenal production of steroids and conversion of androgens to estrogens. The exercise regiment was limited to an aerobic type of exercise of moderate intensity.

1.7 Definition of terms:

Progesterone:

A steroid hormone produced by the corpus luteum, the adrenal glands and the placenta. The circulating levels of this hormone are measured by way of radioimmunoassay (RIA) and the units are ng/ ml plasma.

Glucose:

A simple carbohydrate found in circulation in the blood which constitutes an important source of fuel for metabolism. Its circulating levels are measured by a enzyme (colorimetric) assay and its units are mg/ dL.

Glycogen:

A polymer of glucose which constitutes the storage form for carbohydrates in muscle and in liver. The glycogen is available for metabolism to glucose whenever there is need for reserve energy. Its concentration in tissue is measured through an assay technique and its units are mg/ g tissue.

Exhaustion:

Exhaustion is defined as the time during the run to exhaustion at which the rats were unable to keep pace with the treadmill and avoid the shock grid

Chapter II

REVIEW OF THE LITERATURE

2.1 Introduction:

The purpose of this chapter was to review current concepts in carbohydrate metabolism, general research on exercise in the rat and the effect of verified menstrual phases as determined by progesterone levels on substrate utilization during exercise.

2.2 Carbohydrate Metabolism:

Carbohydrates represent a major source of caloric intake and fuel in humans and for most animals. Carbohydrates can either circulate freely in the blood in the form of hexose phosphates or be stored in tissue as glycogen. Many factors affect carbohydrate metabolism and its regulation. Such factors include hormones, diet and exercise.

2.2.1 Endocrine control:

Endocrine control of carbohydrate metabolism is mainly regulated by hormones of the pancreatic islets, insulin and glucagon. Insulin stimulates glucose utilization and storage and inhibits its production. The result is a decrease in circulating levels of glucose. Insulin also affects carbo-

hydrate metabolism in tissue, mainly in liver, muscle and fat. The liver is the site for many carbohydrate transformations (pathways) which are mediated by insulin. Insulin increases glucose uptake by the liver and its storage in the form of glycogen by inducing the hepatic enzyme glucokinase and promoting the glycogen synthase enzyme complex. Insulin lowers blood glucose by inhibiting glycogenolysis and gluconeogenesis by decreasing glycogen phosphorylase activity and decreasing uptake of amino acids by the liver. Promotion of glycogen storage by insulin is accomplished by stimulation of glycogenesis.

In muscle, insulin regulation of carbohydrate metabolism is accomplished through carrier mediated transport located in the membrane. The process is called facilitated diffusion (Berne & Levy, 1983). The exact effect of insulin on the transport mechanism and/or the membrane receptors is unclear, but it is known that insulin promotes carbohydrate storage in muscle in the form of glycogen. The effects of insulin on adipose tissue is similar to that of muscle. Glucose in adipose tissue is metabolized into glycerophosphate used in the esterification of fatty acids and stored as triglycerides.

Glucagon, which is also secreted by the pancreatic islets, but from a different cell type, acts as an antagonist to insulin in the regulation of carbohydrate metabolism. Its secretion is stimulated by low levels of plasma

glucose. This effect appears to be modulated by insulin. Since glucagon is a hormone antagonist to insulin, its actions are directly opposite to those of insulin. Glucagon acts to mobilize carbohydrates rather than promote their storage. This effect of glucagon is mainly directed at the liver. Glucagon promotes hepatic glucose output by stimulating glycogenolysis and gluconeogenesis through various enzymes. Finally glucagon promotes B-oxydation of free fatty acids in the liver rather than their synthesis into triglycerides.

Endocrine control of carbohydrate metabolism is dominated by insulin actions but also includes effects of catecholamines and growth hormone (GH). The B-adrenergic effects of epinephrine and the A-adrenergic effects of norepinephrine on glucose metabolism are directed at the liver and at the muscle. In the liver, epinephrine increases glucose production by stimulating glycogenolysis through activation of the enzyme phosphorylase. Epinephrine also stimulates gluconeogenesis. In muscle, epinephrine stimulates glycogenolysis resulting in the production of lactate which can be reutilized by the liver as substrate (Santiago, 1980).

Growth hormone (GH) is secreted by the cells of the anterior pituatary and is responsible for somatic growth. GH also affects carbohydrate and fat metabolism in tissue. In liver, GH acts to stimulate gluconeogenesis. In muscle and in adipose tissue, GH action consists in decreasing glucose

uptake. In the presence of a prolonged GH excess, plasma glucose levels rise. Thus GH is a diabetogenic hormone.

Considerable attention has been focused in recent years on the hormones of the ovarian-pituatary axis and their role in metabolism. Over the past decade, research has been pointing towards a possible link between naturally occurring and synthetic ovarian steroids on both carbohydrate and lipid metabolism.

Research was first directed towards the study of the possible metabolic effects of contraceptive steroids. In a review of the effects of contraceptive steroids on carbohydrate and lipid metabolism, Beck (1973) reported that changes in glucose tolerance with contraceptive therapy are usually small and related to the age of the patient. He also reported that neither estrogens nor progesterone derivatives have any effect on glucose tolerance of normal young women when taken individually. However, Spellacy et al. (1970) found that when women receive a single large injection of medroxyprogesterone, deterioration in glucose tolerance occurs 6 months after the injection. On the other hand, Beck (1973) reported that Goldman et al (1969) found no deterioration in glucose tolerance when normal women were administered a combination of progesterone and testosterone derivatives for three months, whereas it was reported by Spellacy et al. (1970) that administration of a nortestosterone derivative in the birth control pill (in replacement

of a progesterone derivative) resulted in a greater incidence in deterioration in glucose tolerance.

When glucose tolerance tests were performed in subclinical diabetic women using contraceptive steroids, it was found that these women had a higher incidence of abnormal glucose tolerance tests (Szabo et al., 1970; Beck et al., 1969). It would appear though, that administration of large doses of insulin may neutralize the hyperglycemic effect of the various contraceptives. This ability of insulin has been confirmed by animal studies (Lewis et al. 1950). These animal studies have also demonstrated the ability of sex steroids to stimulate insulin production. Finally, it was found that estrogens potentiate the hyperglycemic effect of nortestosterone, but the mechanism by which this is accomplished remains obscure. It was postulated that estrogens may possibly inhibit the biliary excretion of nortestosterone derivatives or slow their catabolism (Beck, 1973). Finally it can be said that when contraceptive steroid administration results in alterations in carbohydrate tolerance, the magnitude of the change is small and is age related (Beck, 1973). It should also be noted that when contraceptive steroid treatment is discontinued, changes in glucose tolerance are reversed.

Contraceptive steroids have been shown to have an effect on lipid as well as on carbohydrate metabolism. Beck's review revealed that synthetic estrogen administration

resulted in elevated plasma triglycerides in postmenopausal women, whereas testosterone derivatives lowered them. Furthermore, other studies have demonstrated an increase in very low density lipoprotein (VLDL) as well as an increase in triglycerides (Gustafson et al., 1972). It was also reported that when progesterone derivatives were used as the sole contraceptive agent no changes in triglycerides were observed (Beck, 1973). Furthermore, it has been shown that administration of a combination of synthetic estrogens and progestins results in a twofold increase in serum triglycerides and that the hypertriglyceridemia was related to the estradiol dose. Also Beck's study revealed that the larger the concentration of synthetic testosterone in the contraceptive preparation , the lower the serum triglyceride concentration.

The mechanism by which contraceptive steroid administration results in elevated plasma triglycerides remains somewhat unclear. It was reported in Beck's review that contraceptive steroid use may impair the clearing rate of triglycerides. It was also found that triglyceride turnover and removal rate are accelerated in women using contraceptive steroids and that the increase in triglyceride concentrations are due to the rate of triglyceride synthesis exceeding the rate of triglyceride removal. Furthermore, the increase in triglyceride concentration is seen predominantly in the low density lipoprotein fraction (LDL). It

would appear that the effects of contraceptive steroids on both carbohydrate and lipid metabolism are not distinct but related. The same contraceptive agents (namely nortestosterone derivatives) that impair glucose tolerance are also agents which counteract the hypertriglyceridimic effects of synthetic estrogens.

Concerning cholesterol metabolism, Beck reports that while a combination of estrogen-progestin modestly increases levels of serum cholesterol, administration of estradiol alone either lowered or did not affect plasma cholesterol concentrations. However, large and constant doses of estrogen (50 ug/day) have been shown to increase fasting serum cholesterol levels.

In his review of contraceptives and carbohydrate metabolism, Kalkhoff (1975) found that the contraceptive steroids (particularly estrogens) are capable of diverging effects. First they may create a state of hyperinsulemia (thereby lowering blood glucose levels and improving glucose tolerance), or they may act as insulin antagonists, in a glucocorticoid like fashion, either alone or in concert with endogenous adrenal glucocorticoids. Estrogen and progesterone also affect other hormones with the combination resulting in changes in carbohydrate metabolism. Estrogens are known to increase plasma growth hormone levels and GH has been demonstrated to have contrainsulin effects in women taking contraceptives containing estrogens. Progestins, on

the other hand, have been found to significantly suppress plasma GH, and can also alter glucose homeostasis when administered in doses considerably above that found in contraceptive steroids. These can induce hyperinsulemia and decrease glucose tolerance.

Matute et al. (1973) studied sex steroid influence on hepatic gluconeogenesis and glycogen formation in female rats. The animals were divided into for 4 groups and received subcutaneous injections twice daily for 21 days. Control rats received sesame oil alone. Rats from the other three groups received either estradiol benzoate in sesame oil in total daily dosages of 2.5 ug for 14 days and 5 ug for the final 7 days, progesterone in oil in a total daily dose of 2.5 mg for 14 days and 5 mg during the final 7 days, or a combination of estradiol benzoate and progesterone for 21 days in daily dosages equivalent to those given to the animals receiving either hormone alone. Results showed that when estradiol and progesterone are administered in combination, in vivo incorporation of alanine and pyruvate into glucose is suppressed. It was shown that rats receiving progesterone alone or in combination with estradiol showed a significantly greater weight than control rats, although rats receiving estradiol alone gained slightly less weight than control animals, the difference was not significant. All hormone treatments resulted in greater liver weights than those of control rats with the greatest values being

seen in the rats either receiving progesterone alone or in combination with estradiol. Each hormone treatment resulted in an increased liver glycogen deposition, with the increase being significant in rats receiving either progesterone alone or in combination with estradiol. It was also found that hormone treatment did not result in changes in plasma glucose, but significantly higher levels of insulin were found in rats receiving sex steroids. Hyperinsulemia resulting from sex steroid administration was associated with pancreatic islet hypertrophy in the rat. In this experiment, progesterone administration by itself did not result in glucose intolerance even though the steroid had an inhibitory effect on liver gluconeogenesis.

Finally Matute et al. conclude: " These observations suggest that natural estrogens improve carbohydrate tolerance on the basis of three primary effects: 1) hyperinsulemia, 2) depressed hepatic glucose release, 3) increased peripheral utilization of substrate. Progesterone shares the first two actions with estrogens, but its ameliorative effects on carbohydrate tolerance from these standpoints may be counterbalanced by opposing contrainsulin actions in extrahepatic tissue."

Mandour et al. (1977) studied the mechanism of estrogen and progesterone effects on carbohydrate and lipid metabolism in ovariectomized rats receiving either 17-B estradiol or progesterone daily. They found that 17-B estradiol admin-

istration resulted in elevated plasma triglycerides and reduced plasma glucose levels. Progesterone administration however did not produce any significant results. Furthermore, estrogen treatment resulted in decreased circulating levels of insulin with doses of 10 and 100 ug but had no significant effect on insulin in doses of 1 ug/kg/day. The two larger doses also produced a reduction in plasma glucagon levels whereas the smaller dose did not produce a significant effect. Progesterone administration in doses of 0.5 and 5 mg/kg/day resulted in a significant increase in circulating insulin levels. The plasma glucagon levels were proportionally increased.

Estrogen treatment was also reported to have increased the limiting enzymes of lipogenesis in liver while gluconeogenetic activity was inhibited. On the other hand, progesterone administration did not produce any changes in plasma glucose or triglyceride levels. It should be noted, however, that in order to obtain significant results Mandour et al. used considerably larger doses of the steroids and did not define the duration of the treatment.

In a study in which glucose tolerance and plasma insulin of the rat were investigated in relation to the estrous cycle, Bailey et al. (1972) reported that at estus, plasma insulin levels were highest and glucose tolerance greatest. At diestrus, plasma insulin levels were decreased and glucose tolerance was reduced. At both proestrus and metestrus, no changes were observed.

In order to verify the possible contribution of sex steroids in the manifestation of these effects, adminstration of both estradiol and progesterone was undertaken. Gonadectomized rats of both sexes were given daily injections of 17-B estradiol-3-benzoate in doses of 5 ug or progesterone in doses of 5 ug. The injections were given intramuscularly for 14 days. It was found that in the female rat, both estradiol and progesterone administration resulted in improved glucose tolerance and increased plasma insulin levels. In the male rats, progesterone tended to lower plasma insulin levels and impair glucose tolerance while estrogen did not produce any significant effect.

Sladek (1974) in a study on gluconeogenesis and liver glycogen in the rat in relation to the oestrus cycle found that gluconeogenesis was highest at diestrus as demonstrated by elevated blood glucose levels and lower serum insulin levels. Liver glycogen content was found to be highest during proestrus, lowest at estrus and slighly increased during metestrus and diestrus. Sladek's results concerning gluconeogenesis are in accordance with those of Bailey et al.

The review on the effects of sex steroids on carbohydrate metabolism has provided evidence suggesting that sex steroid's influence on carbohydrate metabolism is most probably mediated by insulin and glucocorticoids such as cortisol. Studies in human subjects suggest a possible role of sex steroids in the control of insulin receptors. This control

appears to exist at the level of receptor concentration rather than receptor affinity.

2.2.2 Effect of diet:

The second major factor affecting carbohydrate metabolism is diet. Studies have shown that diet content and frequency of feeding play an important role in glycogen metabolism. Carbohydrates are a major source of fuel both at rest and during work. Therefore, they form the larger part of the diet consumed in humans. Also, until recently, it was believed that carbohydrate storage in the form of glycogen in the liver and muscle remained unaffected by nutritional conditions. However, recent studies have demonstrated an effect of frequency of feeding on carbohydrate metabolism. Cohn et al. (1969) studied the relationship between frequency of feeding and the quantity of food and its effect on carbohydrate metabolism. It was found that when animals have free access to food rather than being meal fed at specific times, the average amount of food consumed each day is greater in the rats fed ad libitum than in the meal fed animals. The effect of a greater food intake as well as differences in feeding frequency was that in animals fed ad libitum there was greater removal of glucose and a greater deposition of tissue glycogen. Furthermore, the underfed meal fed rats exhibited a greater sensitivity to the hypoglycemic effect of insulin and removed glucose at a greater rate than the animals fed ad libitum. However, when the meal

fed rats were force fed to increase their caloric intake to that of the rats fed ad libitum, the results obtained were opposite: the force fed animals were less sensitive to insulin and showed a delay in the clearance of glucose. In a study where the effects of glucose ingestion on food intake were studied, it was found that when glucose is administered orally or intravenously, there results an inhibition of food intake for some time , even after complete absorption of the stomach load. Food intake suppression could therefore not be attributed to stomach distention and a feeling of satiety. It was concluded from the study that when animals are fed a 100% carbohydrate meal, the metabolic effects are to increase the intensity and duration (subsequent meals are smaller and usually delayed) of satiety and result in a suppression of appetite (Booth, 1976).

Other studies have demonstrated similar alterations in metabolic pathways due to feeding pattern. Leveille (1967) studied the effects of meal feeding in rats versus ad libitum feeding on tissue glycogen and liver weight. Contrary to other findings (Cohn 1969), Leveille found that the meal fed animals consumed about 30% more food than the animals fed ad libitum. Furthermore, it was found that the meal fed rats had higher fasting liver glycogen levels than the rats fed ad libitum. These results are in accordance with earlier results reported by Tepperman & Tepperman (1958). Leveille also reported a greater rate of glycogen deposition in

the diaphragm muscle and in adipose tissue than in tissues of the animals having free access to food. In contrast to the findings in diaphragm and adipose tissue, the rate of glycogen accumulation in liver was found to be similar in both groups. Liver weight increase after feeding was also found to be similar in both groups with glycogen showing the greatest percentage increase. Finally, intraperitoneal administration of glucose resulted in a 128% increase in glycogen content of adipose tissue in meal fed rats whereas glycogen content of adipose tissue of rats fed ad libitum was not altered. These results tend to demonstrate a definite metabolic adaptation to feeding frequency with adipose tissue showing the greatest adaptability to meal feeding with respect to glycogen accumulation and with meal fed rats showing the greatest ability to dispose of glucose and a more rapid lipogenic capacity.

Conlee et al. (1976) studied the effects of food consumption on skeletal muscle glycogen in different types of muscle. It was found that when rats are fasted for a 24 hour period, a marked decline in glycogen concentration of all muscle samples followed. However there were marked differences between glycogen concentrations in different muscle types in relation to fasting. The difference in glycogen content between the fed and the fasted states was twice as great in the soleus and red vastus as compared with the gastronemius muscle, with the fed animals exhibiting the highest glycogen levels.

2.2.3 Effects of diurinal variations:

Another factor which has been found to affect carbohydrate metabolism consists in a circadian cycle or a diurinal variation. Sollberger (1964) found that glycogen content in liver of rats was not stable over a 24 hour period, but fluctuated widely and that these variations were related to feeding. It was also believed at the time that liver was the only tissue in which these wide fluctuations could be seen and that skeletal muscle glycogen content was stable (Androvny, 1969, Russel, 1956). However, more recent studies (Conlee, 1976 and Conlee, 1979) have demonstrated that skeletal muscle glycogen as well as liver glycogen fluctuates over a 24 hour period and is affected by food consumption.

Conlee (1976) found in a study where rats were kept on a light schedule from 7:00-19:00 (dark period 19:00-7:00) that food consumption was greater during the dark period. It was also found that liver glycogen concentration fluctuated greatly over a 24 hour period, from a peak value at 12:00 h (light period) to a nadir at 20:00 h (dark period). Skeletal muscle glycogen content exhibited a similar diurinal variation. It was determined that in the red vastus lateralis, glycogen values ranged from a peak of 8.3 mg/g at 8:00 h to a nadir of 5.7 mg/g at 20:00 h. The most rapid decline in glycogen values was seen between 8:00 h and 12:00 h whereas values between 12:00 h and 24:00 h were found to be relatively stable. In soleus muscle the diurinal fluctuations in

glycogen content proved to be similar to those of red vastus lateralis. Glycogen values rose from a nadir of 3.2 mg/g at 20:00 h to a peak of 6.6 mg/g at 8:00 h, with the sharpest decline observed between 8:00 h and 12:00 h. Finally, the white portion of the vastus lateralis muscle did not exhibit sharp fluctuations in glycogen concentration, showing a peak value of 7.3 mg/g at 8:00 h and declining gradually to a low level of 5.7 mg/g at 24:00 h. Clark (1979) also looked at the effects of diurinal variations on glycogen content of liver and skeletal muscle in rats. The study established that fed animals had considerably more glycogen stored in skeletal muscle in the morning than in the afternoon when the light cycle was established from 7:00 h to 19:00 h. Different muscle types exhibited different variations in glycogen levels. The decline in glycogen level due to diurinal variations was found to be 16% in the fast twitch white, 35% in fast twitch red and 55% in slow twitch red muscle. Liver glycogen content also exhibited a diurinal fluctuation and showed a decrease of 46% from morning to evening.

2.2.4 Effect of exercise:

Finally, exercise has been found to significantly affect carbohydrate metabolism. Carbohydrate metabolism provides the major source of fuel for the skeletal muscles during work. The stimulatory effect of exercise on glucose uptake by skeletal muscle was first noted by Chevreau & Kauffman in 1886. Since then, studies have shown that during physical

exercise, both liver and skeletal muscle glycogen content decreases significantly, in both human and animal subjects (Bergstrom,1967, Blacwacka, 1978, Clark, 1979). It was demonstrated that the steepest fall in glycogen content occurs in the initial period of exercise (Bergstrom, 1967, Blacwacka, 1978, Hermansen, 1967). Liver and skeletal muscle carbohydrate metabolism during work is dependent upon two components of exercise: intensity and duration. It has been shown that during heavy exercise in man blood glucose may supply 30-40% of the total energy required by the exercising muscles to sustain work (Felig, 1975). During prolonged moderate exercise, liver and skeletal muscle glycogen stores provide between 40 and 50% of the energy required for work (Bergstrom, 1967). It has been demonstrated in rats that the rate at which muscle glycogen depletion occurs depends on the relative intensity of exercise (Armstrong et al., 1974, Gollnick, 1974). Therefore when glycogen stores are depleted during prolonged severe exercise in rats (60-70 % VO2 max.), the intensity of exercise had to be reduced in order for work to be continued (Bagby et al., 1975). Furthermore, Baldwin et al. (1975), found that during severe prolonged exercise in the rat, the rate of glycogen depletion in skeletal muscle differs among the muscles involved in the activity and among different muscle fiber types.

Studies on the development of physical exhaustion during prolonged strenuous exercise have indicated that depletion

of liver and skeletal muscle glycogen stores plays an important role in the development of fatigue and the cessation of work (Ahlborg et al.,1967, Bergstom et al., 1967, Hermansen et al., 1967). Other investigations have revealed that hypoglycemia secondary to hepatic glycogen depletion develops before complete exhaustion of skeletal muscle glycogen stores during prolonged exercise in rats and may be responsible for the development of fatigue. (Hermansen et al., 1967, Bergstom, 1967). If exercise plays an important role in the depletion of carbohydrate stores, this role is greatly influenced by a factor that has already been discussed: diurinal fluctuations. In a study on diurinal variations and endurance, Clark et al (1979) reported that in experimental rats, endurance exercise is influenced by diurinal variations in liver and skeletal muscle glycogen content. It was found that there was a 39% difference in exercise time between animals exercised at night (at the end of the light cycle) and the animals exercised in the morning (at the beginning of the light cycle), with the rats killed in the evening showing the lowest glycogen values. These differences in glycogen content after exercise between the two groups can only be attributed to diurinal variations. Furthermore, the study revealed that exercise caused an almost complete depletion (96-97%) of liver glycogen in animals exercised at both time periods. It was also found that while exercise caused a significant decrease in skeletal muscle glycogen

stores, there was still considerable glycogen remaining in the muscle, with the animals exercised in the morning showing the greatest residue. These effects of diurinal variation in muscle glycogen stores on exercise were particularly evident for fast twitch white muscle. Since skeletal muscle glycogen content was not completely depleted at the end of exercise and thus severe hepatic glycogen depletion rendered the animals hypoglycemic, the authors postulated that fatigue results from hypoglycemia secondary to liver glycogen depletion.

The liver and muscle glycogen stores depleted as a result of severe prolonged exercise are later replenished in the hours following the cessation of work. In a study in rats (Terjung,1974) where carbohydrate repletion after exhaustive exercise was studied, it was found that blood glucose levels returned to resting levels two hours after exercise. It was also found that the rate of skeletal muscle glycogen repletion varies with the type of muscle fiber studied: the fast twitch red muscle showed the most rapid repletion of glycogen, with the depleted levels returning to pre exercise control values between 1 to 2 hours after exercise. Glycogen repletion was slowest in white muscle fibers with values equal only to 74% of the preexercise control levels 4 hours after exercise. The rate of glycogen repletion in slow twitch red muscle was determined to be intermediate between that of fast twitch red and fast twitch white. The rate of

liver glycogen repletion was very slow, reaching only 47% of the initial control levels four hours post exercise, and is probably due to the greater capability of the liver to store glycogen as compared to skeletal muscle (Terjung, 1974).

Considerable evidence has been gathered suggesting that muscle and liver glycogen repletion is dependent upon substrates from exogenous sources. Fell et al., (1980) compared glycogen repletion in fasted and fed rats. The author found that when animals were fasted for 24 hours after exercise, muscle glycogen values returned to between 66 and 100% of the resting control values whereas liver glycogen was still essentially completely depleted in both the fasted and the fed animals, 24 hours after exercise. In the fed animals, muscle glycogen values reached 169% and 353% of the pre exercise control levels in the white vastus and the plantaris muscle respectively. The authors concluded by postulating that muscle glycogen resynthesis was heavily favoured over liver glycogen repletion when exogenous sources of carbohydrates were not available and that carbohydrate starvation did not prevent rapid glycogen repletion. However, in Terjung's study, the rats that were fasted for 24 hours prior to exercise were administered 0.5 g of glucose by stomach tube immediately after exercise and again one hour later and were allowed to eat ad libitum. Furthermore none of the resting control rats were fasted. In a similar study, Gaesser et al. (1980) studied the glycogen repletion rate in

muscle and liver after continuous (CE) and intermittent exercise (IE). The rats in the experiment were fasted for twelve hours before the exercise and were not administered exogenous substrates nor were allowed food post exercise. It was found that an almost complete glycogen depletion (94-98%) occurred in skeletal muscle and liver. Cardiac muscle glycogen depletion was also demonstrated. The exercise to exhaustion combined with the resulting depletion in tissue glycogen rendered the animals severely hypoglycemic, with a decrease of 63% in blood glucose. The study also revealed that intermittent exercise appears to be more stressful than continuous exercise. Time to exhaustion was significantly longer for CE than for IE. Since liver and muscle glycogen levels at exhaustion were similar for both types of exercise, it would appear that glycogen utilization took place at a faster rate during IE. The high intensity sprints characteristic of IE call upon a substantial recruitment of fast twitch glycogenolitic muscle fibers.

The study also revealed that a substantial cardiac muscle glycogen depletion took place (49-61%). Cardiac muscle also demonstrated the ability to replenish its stores of glycogen within 4 hours after exercise while relying solely on endogenous substrate sources. However in skeletal muscle, glycogen stores were restored to only half of their pre exercise control levels at 4 hours post exercise when exogenous sources of substrate were not available. It would then

appear that while skeletal muscle requires exogenous sources of substrate in order to replenish its stores of glycogen, cardiac muscle has the ability to do so by relying exclusively on endogenous sources of substrate.

2.2.5 The menstrual cycle, progesterone and exercise:

The menstrual cycle in humans and its characteristic hormonal fluctuations have concerned researchers for over a century now. The hormones of the ovarian-pituatary axis are regulated by a system of feedback control. These hormones are luteinizing hormone (LH) follicle stimulating hormone (FSH); estradiol (E2) and progesterone (P). These hormones are responsible for the regulation of the events taking place during the menstrual cycle.

Briefly, one of the follicles produced by the ovary starts to grow until the fourteenth day of the menstrual cycle when the follicle is ruptured and the ovum expelled in the fallopian tube. This event is referred to as ovulation. From the time of ovulation through the occurrence of menses, concentrations of P and E2 are markedly increased. If the ovum is not fertilized, the corpus luteum degenerates and levels of P decrease. Menstruation follows and the layers of the endometrium are shed. Concentrations in LH are quite stable throughout the first portion of the menstrual cycle but surge on one day where the increase is in the order of 300-400%. FSH concentrations are increased at about the same time. It remains unclear why a surge in LH concentration is

observed on the day prior to ovulation, but it is thought to play a role in the occurrence of ovulation. The portion of the cycle prior to ovulation is dominated by the development of the follicle and so is called the follicular phase whereas the portion of the cycle that is dominated by the activity of the corpus luteum is called the luteal phase (Bonen et al., 1978).

LH and FSH are glycoprotein hormones that are secreted by the pituitary gland and whose function consists of regulating gonadal growth, maturation, reproductive cycles and steroid secretion in both sexes. The regulation of LH and FSH secretion is highly complex, since it is done in a pulsatile, cyclic, periodic and diurinal fashion.Their secretion by the pituitary gland is stimulated by a hypothalamic releasing factor-luteinizing hormone (LHRH) which creates a greater secretion in LH than in FSH. LH and FSH secretion is also regulated by a feedback system from the gonads. The major estrogen, estradiol, inhibits the release of LH. Negative feedback control of FSH release is accomplished by a specific substance produced by the gonads and is called inhibin. Progesterone also participates in the regulation of gonadotropin release. Progesterone administration results in a rise in plasma LH levels 24 to 48 hours later.

The sex steroids found in both sexes originate from a common pathway. Testosterone, estrogens and progesterone are all synthesized from a common precursor, cholesterol.

Cholesterol can either be synthesized from acetyl-COA or taken up from the circulating pool.

Estrogens and progesterone have extraovarian actions. During puberty estradiol stimulates growth of the internal organs and breasts but also accelerates linear growth. Estrogens are also responsible for the greater adipose mass found in women. Futhermore, estrogens inhibit bone resorbtion. Estradiol also stimulates sodium reabsorbtion from the renal tubules and increases the synthesis of a number of proteins by the liver. These include thyroid, cortisol and sex steroid binding globulins, angiotensinogen and finally very low density lipoprotein (VLDL). Actions of progesterone are not very well understood, but it is known that progesterone increases body temperature by 0.5 degree C at the time of ovulation.. Actions of progesterone are mainly centered around the central nervous system and consist of an increase in appetite and an increase in sensitivity of the respiratory center to stimulation by CO2. Progesterone is also a aldosterone antagonist.

The mechanism of action of the sex steroids consists involves the molecule freely entering the cell and binding to cytoplasmic receptors. Following a conformational change, the receptor-hormone complex enters the nucleus where it induces transcriptional changes resulting in an increase or a decrease in protein synthesis. Estrogens and progesterone also have actions on the receptors themselves.

Estrogens, on one hand, increase the number of progesterone receptors thus priming the tissues for progesterone actions. On the other hand, progesterone decreases the number of estrogen receptors, possibly explaining the anti-estrogen effects of progesterone.

The metabolism of ovarian steroids can be described as such: estrogens circulate largely bound to albumin, since their affinity for sex steroid binding globulins is much lower than that of testosterone. Their metabolic clearance rates (MCR) are very high. Most of the circulating estradiol is derived from ovarian secretion while the remaining fraction is metabolized from testosterone originating in the liver and adipose tissue. Estradiol can also be further metabolized into estrone, which if still metabolized turns to estriol. Estrone is the major estrogen compound found in post menopausal women.

Progesterone also circulates loosely bound to albumin even though it can bind to cortisol binding globulin but it is prevented from doing so because of the much higher levels of cortisol. During the luteal phase of the menstrual cycle when progesterone levels are at their highest, progesterone secretion originates almost exclusively from the ovaries. During the follicular phase, 50% of the circulating progesterone is produced by the ovaries while the other half originates from the adrenal cortex.

With women's increasing participation in sports and exercise, considerable attention has been given to the relationship between the menstrual cycle and exercise. Research on the subject started in the form of surveys. One investigation revealed that 30% of the women surveyed at the Tokyo Olympics experienced menstrual cycle disturbances due to training. Erdelyi (1975) in a study of endurance athletes found them to be more inclined to develop menstrual disorders such as oligomenorrhea and amenorrhea. Shangold (1979) in a case study involving a long distance female runner, studied the relationship between long distance running plasma progesterone and luteal phase length. The study revealed that luteal phase length varies inversely with the average weekly mileage run, i.e. the longer the distances run, the shorter the luteal phase. It was also found that progesterone levels were decreased during the training cycles.

Bonen & Belcastro (1978) in a study on the effects of exercise on the menstrual cycle in teenage swimmers found that the athletes experienced a much shorter luteal phase length as compared with control subjects. Furthermore, LH concentrations were found to be higher and FSH concentrations lower in the follicular phase than that of the control subjects. The authors found no differences in the levels of progesterone and estradiol between the two groups during the follicular phase. However, significantly lower levels of both progesterone and estradiol were found during the luteal

phase of the swimmers as compared with the control subjects. However, it should be noted that the control subjects used in Bonen's study were women of adult age and it has been demonstrated that 17.8% of teenage subjects experience short luteal phase length without training. Therefore it is possible that the shorter luteal phase length experienced by the teenage swimmers may not be due to training but more so to their age. Finally, Bonen's study revealed that when training is stopped, menstrual disturbances disappear.

In a second study, Bonen et al. (1981) studied the influence of training on menstrual cycle hormones in 4 teenage swimmers. It was found that the menstrual cycles of the athletes were shorter than those of the age matched controls or of the adult controls. The shorter menstrual cycles of the athletes were attributed to an abbreviated luteal phase. LH levels were shown to be higher in the follicular phase of the swimmers as compared with the control groups, whereas the FSH values were lower during both phases of the menstrual cycle of the swimmers as compared to both control groups. Furthermore, the characteristic FSH peak was not observed in any of the swimmers. Concentrations of estradiol were found to be significantly lower during the luteal phase in the swimmers as compared to the controls. Furthermore, progesterone values were significantly depressed during the luteal phase of the menstrual cycle in the swimmers. The authors concluded that the cycles of the swimmers must have been

anovulatory and that the corpus luteum failed to work properly. Finally, it should be noted that the number of experimental subjects used in the study was small.

Other investigations studied the acute effects of exercise on the menstrual cycle hormones. Bonen et al. (1979) studied the effects of 30 minutes of exercise at 75% of VO2 max. on the response of FSH, LH, estradiol (E2), and progesterone (P) in 10 women. It was found that exercise of such intensity was a powerful stimulus for increasing the plasma levels of progesterone and estradiol. P concentrations were raised by 37.6% above the resting levels and estradiol concentrations increased by 13.5% above resting levels. The exercise bout did not exert any effect on the concentration of either FSH or LH. Furthermore, hormone measurements were taken when the subjects exercised at different times in the menstrual cycle. The results showed that increases (of the same order of magnitude) in progesterone occurred during both menses and the luteal phase. However, the exercise bout did not result in an increase in estradiol levels during menses. At the end of the experiment, the subjects were trained for a 8-11 week period and were subsequently retested. It was found that concentrations of P and E2 were no longer increased as a result of the exercise period. However, it should be noted that as a result of training, the workload imposed no longer represented 75% of VO2 max. but only 68%. Therefore it cannot be determined

whether the differences in P4 and E2 levels before and after training are due to a training effect or to a decrease in intensity of work. Furthermore, even though all of the subjects exhibited normal menstrual cycles prior to training, the cycles became irregular during the training period. It was therefore impossible for the investigators to determine in which phase of the menstrual cycle the subjects were in when retesting took place.

Jurkowski et al. (1978) examined the effects of exercise on the ovarian and gonadotropic hormones as well as the effect of exercise intensity and menstrual cycle phase on hormonal, cardiovascular and respiratory responses. The testing protocol consisted of the subjects exercising on a bicycle ergometer for 20 minutes at 30-35% of maximum power output, 20 minutes at 60-65% and finally to exhaustion at 80-95% of max. power output. The exercise intensities were considered to be light, heavy and exhaustive. It was found that progressive exercise was associated with low levels of progesterone and estradiol in the follicular phase and with high levels of the steroids during the luteal phase and did not result in any differences in heart rate or ventilation between the two phases. This result is in contradiction with the results found at rest where it was found that during the luteal phase, where progesterone levels are highest, arterial partial pressure of carbon dioxide (PaCO2) is lowered due to an alteration in sensitivity of the respiratory control center.

In the follicular phase, progesterone levels were low and changes were detected with exercise, whereas changes in E2 concentrations were significant only at exhaustion. During the luteal phase, progesterone levels were increased significantly at all exercise intensities. Increases in E2 levels were observed for all intensities of work, but the results were significant only during heavy and exhaustive exercise. FSH concentrations did not change with exercise during the luteal phase but changed significantly at the end of heavy exercise or at exhaustion. LH levels did not change with exercise in the follicular phase or the luteal phase. Finally, the study revealed that time to exhaustion was increased in all subjects during the luteal phase (at 90% of maximum power output). The physiological reason for this is not apparent. However, as previously mentioned, it would appear that ovarian steroids, particularly estrogens, affect carbohydrate metabolism in such a way as to increase peripheral glucose uptake by insulin sensitive tissue such as muscle, most likely by increasing the number of insulin receptors. Furthermore, some studies have shown that high levels of circulating ovarian steroids, particularly progesterone, stimulate hepatic glycogen deposition which can be used as fuel during exercise.

A question can be raised concerning the increase in ovarian steroid levels with exercise observed in several of the studies reviewed thus far. The increases in circulating lev-

els of ovarian steroids with exercise can either result from an increase in secretion by the ovaries and/or adrenal cortex, or from a decrease in metabolic clearance. Most of the studies in which an increase in the circulating levels of the hormones was found did not attempt to investigate the origin or cause of these increases, mostly because of the limitations in the techniques used. Furthermore, it has been demonstrated that exercise produces a 30% decrease in hepatic blood flow (Rowell, 1974). Therefore, many have postulated that the increases in plasma levels of ovarian steroids observed during exercise must result from a decrease in metabolic clearance since the steroid hormones are degraded in the liver.

Keiser et al. (1980) studied the effect of physical exercise on the metabolic clearance rate of estradiol (E2). Six subjects were exercised on a bicycle ergometer for a period of 10 minutes at 70% of VO2 max.. Their results showed an average of 36% decrease in the metabolic clearance rate (MCR) of E2. The variation between the subjects for MCR was considerable, the range falling between 18-67 %. The authors postulated that their results explain the findings of Bonen et al. and Jurkowski et al. However, Keiser et al. found in their study that trained subjects experienced a sharper decrease in MCR than did untrained subjects. From this, it would appear safe to speculate that trained subjects should exhibit higher circulating levels of the sex steroids than

untrained subjects during exercise. However, the literature is unanimous in demonstrating the lower levels of P and E2 during exercise after training (Bonen et al., 1978, Shangold, 1979).

Since changes in hepatic and extrahepatic clearance cannot solely account for the changes observed in MCR of the sex steroids during exercise, Keiser et al. (1982) reviewed the relationship between exercise, MCR and biological activity of the sex seroids. Blood production rate of steroids (the rate at which the substance appears in the blood) is derived mostly from the ovaries with a small fraction being secreted by the adrenal cortex. Metabolic clearance is accomplished through hepatic and extrahepatic metabolism. Biological activity on the other hand is determined by the ovarian steroids association to plasma proteins. The ovarian hormone dynamics have been reviewed in a previous section. It was revealed that the sex steroids circulate loosely bound to albumin. The hormone fractions that are responsible for biological activity of the hormone are the free fraction and the fraction which is associated to low affinity binding protein such as albumin. Hormone-protein binding dynamics are affected by physiological factors such as temperature. An increase in blood temperature will result in a higher percentage of unbound hormone. Exercise of moderate intensity has been shown to result in an increase in blood temperature and therefore result in a higher percentage of

unbound and biologically active steroids. Since the hormone measurement techniques used in most studies only measure the free fraction, it appears possible that the increases in ovarian steroids observed during exercise may be partly due to a higher free fraction of the hormone brought about by an increase in blood temperature.

In a study on the metabolic and thermoregulatory responses to exercise during the menstrual cycle, Stephenson et al. (1982) found, contrary to results reported elsewhere (Jurkowski et al., 1978, 1982), that time to exhaustion during exercise was not affected by menstrual cycle phase. Furthermore, all of the cardiovascular and respiratory parameters measured in Stephenson's study remained unchanged during the menstrual cycle. Variables such as oxygen pulse, oxygen consumption (VO2), carbon dioxide production (VCO2), tidal volume (Vt), respiratory rate (Fr) and respiratory exchange ratio (R) were unaltered by menstrual cycle phase both at rest and durig exercise. However, thermoregulatory data revealed a significant increase in core temperature at rest and at all intensities of work during the follicular phase.

Jurkowski et al. (1981) studied the effects of menstrual cycle phase on blood lactate, O2 delivery and performance during exercise. The authors found no differences in maximum power output, heart rate (HR), stroke volume (SV), cardiac output (Q), oxygen consumption (VO2) and carbon dioxide pro-

duction (VCO2), between the follicular and the luteal phase for all exercise intensities. There were no differences observed in partial pressure of carbon dioxide (PCO2), pH and bicarbonate (HCO3-) between the follicular phase and the luteal phase at rest. At exhaustion, a decrease in pH was observed in both the follicular and the luteal phase but the decrease was significant only in the follicular phase. A decrease in plasma bicarbonate was also observed with exercise in both phases this decrease was significantly greater in the follicular phase than in the luteal phase. Lactate levels were found to be slightly but significantly elevated both at rest and during exercise in the follicular phase as compared with the luteal phase. The magnitude of the rise in lactate levels was found to parallel the fall in plasma bicarbonate observed in both phases. Consistent with their previous findings (Jurkowski et al., 1978), the authors found that time to exhaustion was doubled in the luteal phase at maximum power output. It was postulated that the lower plasma lactate levels observed during the luteal phase are a result of a decrease in efflux of lactate from the muscle. This has only been demonstrated though in the presence of much greater changes in bicarbonate than the changes observed in the luteal phase at rest. It is unlikely then that decrease in lactate efflux from the muscle is responsible for the lower lactate levels observed both at rest and during exercise during the luteal phase. The role of pro-

gesterone and estradiol in carbohydrate metabolism may provide a mechanism by which lower lactate levels in the luteal phase can be explained. It has been demonstrated that plasma catecholamine levels increase in a greater proportion when exercise is performed in the follicular phase as compared with the luteal phase (Sutton, 1978). It is known that the catecholamines, particularly epinephrine, increase the rate of glycolysis during exercise resulting in higher plasma levels of lactate. It is therefore possible that the differences in plasma catecholamines observed between the follicular and the luteal phase may mediate the effects of sex steroids on carbohydrate metabolism and blood metabolites during exercise.

Dalsky (1982) studied the effects of progesterone levels on substrate utilization during exercise in untrained, moderately trained and highly trained female subjects. When progesterone measurements were taken at rest to determine the phase of the menstrual cycle in each subject, the expected elevated progesterone levels in the luteal phase were not obtained. Consenquently, the author divided the data into four subphases: follicular low-P4, luteal low-P4, luteal medium-P4 and luteal high-P4 instead of by menstrual cycle phase. The results obtained show that serum glucose levels were unaffected by subphase or group (untrained, moderately trained and highly trained). Blood lactate analysis revealed an effect of subphase and group, with the trained

subjects and the subjects in the high-P4 subphase showing the lowest lactate levels, but the results did not reach statistical significance. Contrary to results obtained at rest by other investigators (Reinke et al., 1972)), FFA levels were found to be higher in the luteal medium-P4 subphase than in the luteal high-P4 subphase. Respiratory data collection revealed that no differences between groups or subphases occured in minute ventilation, oxygen consumption and respiratory exchange ratio. The similarities in R values indicate that there were no differences in the percent of calories derived from fat between subphases. The author concludes that there are no apparent effects of progesterone levels on substrate utilization and blood metabolites during exercise.

A study on the effects of menstrual cycle on the metabolic responses to exercise (Bonen et al.,1983) verified the relationship between nutritional state and menstrual cycle phase and its effect on certain metabolic parameters. The exercise protocol consisted of a 60 minute walk on a treadmill (30 minutes at 40% VO2 max. and 30 minutes at 80% VO2 max.). The subjects were divided into three groups: fasted, glucose loaded and control. Results concerning the endocrine response revealed that there were no differences between the nutritional groups for FSH, LH and cortisol in the two phases of the menstrual cycle. Metabolic responses such as glucose, lactate and glycerol were similar for all nutritional

groups in the two phases. However, in the glucose loaded group, FFA response was found to be lower in the luteal phase. The authors also observed an increase in insulin and GH response in the fasted subjects during the luteal phase. Furthermore, plasma progesterone levels rose significantly in the control and glucose loaded groups during the luteal phase whereas no alterations in P 4 concentrations were found in the fasted group in either phase. It is not known whether the fasting was responsible for the lack of progesterone response. Finally the authors advise that the nutritional status of the subjects should be taken into account when studying metabolic responses to exercise during the menstrual cycle, since FFA response in the luteal phase was different between the nutritional groups.

Gorski et al. (1976), in a study of the effect of estradiol on CHO metabolism during exercise in the rat, found that in the estradiol treated females, liver glycogen was lower than in oil injected females. However, at the end of one hour of swimming, it was found that liver glycogen levels in the estradiol treated rats were higher than in the oil injected rats. Muscle glycogen levels in the biceps femoris muscle were found to be significantly lower in the oil injected rats than in the hormone treated rats after one hour of exercise whereas in the masseter muscle, the resting glycogen concentrations were higher in the estradiol treated rats than in the oil injected rats. Similar results were

found after one hour of exercise. In the myocardium, it was observed that estradiol induces a supercompensatory glycogen increase after 5 hours of swimming. Blood glucose levels were shown to be significantly decreased in both groups during exercise, the decrease being of the order of 51.8% in the oil treated rats and only 25.5% in the estradiol treated rats. Therefore, it would appear that estradiol treatment results in a protective effect against hypoglycemia during prolonged exercise. Estradiol has been shown to increase glucocorticoid and GH secretion, which in turn decreases glucose uptake and increases free fatty acid mobilization.

A series of experiments were undertaken by Sutter-Dub et al. (1981) on the effect of pregnancy and/or progesterone administration on insulin, CHO and lipid metabolism in both adipose tissue and skeletal muscle.

In a study on the effects of progesterone on insulin action during pregnancy, it was found that circulating levels of progesterone and insulin vary in a parallel fashion. It was established that levels of insulin increase during pregnancy in the rat from day 11 to 19 and then decrease from day 19 til parturition to levels usually found in non pregnant rats. Changes in insulin levels during pregnancy are accompanied by morphological changes in pancreatic B cells such as an increase amount of light granules, swollen mitochondria and distented rough endoplasmic reticulum (RER) - cisternea. Sutter-Dub et al. (1977) observed that treat-

ment with estradiol and/or progesterone resulted in morphological B cells changes similar to those seen during pregnancy in the rat. The hyperactivity of the B cells resulted in an increased insulin secretion. It would appear that the increase in insulin secretion observed following progesterone treatment is necessary to counterbalance the inhibitory effect of progesterone on insulin action i.e. to maintain euglycemia.

Accompanied by the insulin resistance that results from pregnancy and progesterone treatment is the inhibition of glucose uptake and the oxidation to CO2 in fat cells and in muscle and also glucose incorporation into glycogen of the muscle. Time-course studies of progesterone action on differentially labelled 14C-glucose utilization by adipose tissue suggest that steroid action on carbohydrate metabolism may be located at the pentose phosphate pathway since it was shown that progesterone specifically decreases 1-14C glucose (Sutter-Dub et al. 1981). In adipose tissue, glucose can be metabolized by two major pathways: the pentose phosphate cycle and the Embden-Meyerhof pathway connected with the Krebs cycle. The pentose phosphate pathway is the cycle in which C1 is decarboxylated whereas C2 and C6 are decarboxylated when glucose is metabolized by the glycolytic pathway and citric acid cycle. The order of appearance of labelled carbon atoms as CO2 will determine whether the pentose phosphate shunt or the Krebs cycle will be taken. As stated pre-

viously, progesterone inhibited the metabolism of 1-14C glucose but had no effect on either 2-14C or 6-14C suggesting an inhibitory effect of the steroid on the pentose phosphate pathway but no action on the Krebs cycle. Furthermore, the C6/C1 ratio which can be taken as an index of pentose phosphate cycle activity was increased by progesterone. This increase in the C6/C1 ratio translates into a decrease of the participation of the pentose phosphate pathway in the metabolism of glucose. Winegrad & Renold (1958) have shown that insulin increases the glucose metabolism in the adipose tissue by stimulating the pentose phosphate pathway. In this regard, progesterone has an antagonistic effect on insulin action. The antiinsulin action of progesterone results in a general decrease in glucose metabolism.

The step of the glucose metabolism at which this general decrease occurs in the presence of progesterone has been studied by Sutter-Dub et al. (1982) using glucose analogues, 2-deoxyglucose and 3-0-methylglucose. It was determined that progesterone impairs the normal rate of 2-deoxyglucose utilization by the fat cell, but has no effect on the entry of 3-0-methylglucose into the cell. The studies revealed that progesterone decreases glucose metabolism within a few minutes of administration. Traditionally, it was thought that the physiological actions of steroid hormones took several hours to develop since they were dependent on synthesis of new protein. It was also estab-

lished that progesterone acts at the early steps of glucose metabolism. Sutter-Dub's study revealed that progesterone acts as an inhibitor of hexokinase activity. The inhibition was of the non-competitive type (i.e. it is not overcome by an excess of substate). This hexokinase enzyme system is composed of two isoenzymes: hexokinase-I and hexokinase-II. The study determined that the change in hexokinase activity due to progesterone was due to a decrease in hexokinase-II activity with hexokinase-I activity being unaffected. Other studies have also shown decreased activity in hexokinase-II in insulin resistant states (Katzen, 1967; Bernstein & Kipnis, 1973). Decreases in hexokinase-II activity have been shown to be correlated with decreased glucose oxidation and lipogenesis. It therefore appears that hexokinase-II activity may be responsible for the mediation in the insulin antagonism on glucose metabolism. It would appear though that the inhibitory effect of progesterone on hexokinase activity is not direct but rather exists through an indirect feedback mechanism, resulting from an increase in the concentration of the reaction product glucose-6-phosphate. Finally the study also revealed that progesterone's inhibition of hexokinase activity does not act through its cofactor Mg^{+2} since inhibition of glucose oxidation by progesterone is the same whatever the Mg^{+2} concentration.

It was shown that progesterone action on glucose metabolism is rapid, soliciting a physiological response within 15

minutes. The early steps of such rapid hormonal action are usually mediated by cAMP and Ca++. Therefore, studies were undertaken to determine whether progesterone affects cAMP levels and Ca++. When levels of cAMP are varied in the presence of progesterone, there results no differences in the progesterone induced changes in carbohydrate metabolism. However, when levels of Ca++ are varied in the presence of progesterone, variations in the same way of glucose oxidation and lipogenesis follow. However, it was also found that in the complete absence of Ca++, the inhibitory effect of progesterone remains but is decreased in the presence of strong Ca++ fluxes blocking agents. Therefore, it would appear that progesterone effects on carbohydrate metabolism are related to transmembrane Ca++ fluxes but not to intracellular cAMP levels (Sutter-Dub et al., 1981). However, more recent studies by the same group (Sutter-Dub et al., 1984) tend to demonstrate an effect of progesterone on the cell membrane. When levels of cAMP were varied through manipulations of either adenyl cyclase activators , such as cholera toxin or inhibitors of phosphodiesterase such as caffeine and theophyllin, it was found that cholera toxin increases glucose-1-C14 oxidation and decreases progesterone's inhibitory action on this substrate's oxidation. There exists two types of phosphodiesterase inhibitors: the ones mentioned above, caffeine and theophyllin, which act also to increase lipolysis and others such as dipyridamol, papaverin

and imipramin which do not affect lipolysis. However, they all act to decrease progesterone action. Furthermore, it was shown that all substances that act to increase intracellular cAMP levels tend to decrease the steroid's action.

The disruptions observed in the glucose metabolism during pregnancy and/or progesterone treatment such as a decrease in glucose utilization, an increase in glucose oxidation through the pentose phosphate pathway and a decrease in the glucose dependent lipogenesis may be due to a defect in insulin binding and action in both muscle and adipose tissue (Sutter-Dub et al. 1984). The insulin resistance observed during pregnancy and progesterone treatment may be a result of disruptions at the level of the receptors or at the post-receptor level. It was shown that this insulin resistance during pregnancy was accompanied by a progressive increase in insulin binding to its receptors, suggesting a disruption of insulin metabolism at the post-receptor level (Sutter-Dub et al. 1984). It was also demonstrated that progesterone treatment decreases adipocyte sensitivity to insulin and carbohydrate metabolism in the absence of insulin. Furthermore, progesterone only slightly decreases the insulin receptor number, further suggesting that the change in carbohydrate metabolism during pregnancy originate at the post-receptor level.

The biochemical mechanisms by which the peripheral tissues become insulin resistant during pregnancy and/or pro-

gesterone treatment remains to be elucidated. Theoretically, insulin resistance may originate from three different sources: abnormal B-cell secretion, elevated levels of hormones which are antagonistic to insulin in their action and finally, target tissue defect. Also, insulin resistance as a syndrome can be divided into three subclasses, as described by Kahn (1978): insulin resistance can represent firstly a state or states of decreased responsiveness, in which maximal response to insulin is decreased but the dose response relationship between no hormonal effect and maximal effect is normal; secondly, states of decreased sensitivity, in which maximal response is unchanged, but greater than normal levels of hormone are required to elicit a quantitatively normal response and thirdly, states of decreased sensitivity and responsiveness.

In a study on insulin resistance during pregnancy, Sutter-Dub et al. (1984) have shown that insulin binding increases with the duration of pregnancy and that high and low affinity receptors were increased by pregnancy. Furthermore, insulin action on peripheral tissue was shown to be decreased by the fact that 1-14-C glucose oxidation was decreased by 28% and that CO2 production was also decreased at all insulin concentrations tested. However, the relationship between insulin binding and insulin action did not appear to be altered during pregnancy as compared to the non pregnant state, since the maximal insulin effect on glucose

oxidation was achieved by occupancy of approximately the same proportion of insulin receptors in both states. The study clearly demonstrates a shift to the right of the insulin dose-response curve for oxidation of 1-14C glucose indicating an impairment of sensitivity to insulin. Furthermore it was shown that the maximal response of the tissue over the basal level is depressed which represents a decreased responsiveness of the tissue to insulin and is generally considered as being a post-receptor defect. It can therefore be concluded that during pregnancy the insulin resistant state is characterized by an increase in receptor number, that the affinity constants of both high and low affinity receptors remain unchanged and that the peripheral tissue's maximal responsiveness to insulin is decreased.

Finally, in a study on lipid synthesis from glucose during pregnancy and progesterone treatment Sutter-Dub et al. (1983) found that in adipocytes of ovariectomized rats the ovariectomies resulted in a slight but not significant decrease in the glucose induced synthesis of triacylglycerols and fatty acids. It was also found that progesterone treatment in the absence or presence of insulin resulted in a decrease in fatty acid and triacylglycerol synthesis.

In summary, it can be said that many factors affect carbohydrate metabolism. These factors can be divided into two categories: hormonal and non-hormonal factors. Hormonal factors include the predominant effects of the hormones

secreted by the pancreatic islets, insulin and glucacon. Insulin promotes glucose uptake by peripheral tissues such as muscle and adipose tissue and its storage in these tissues as glycogen. On the other hand, glucagon acts as a catabolic hormone, promoting glucose production by the liver through the gluconeogenic pathway. The catecholamines secreted by the adrenal medulla and growth hormone are also hormones which affect carbohydrate metabolism. These hormones tend to rise the circulating levels of glucose.

Possible effects of the steroid hormones secreted by the gonads have been the subject of recent studies. It has been found that the female hormones, progesterone and estradiol affect, carbohydrate metabolism. The studies reviewed in this chapter tend to demonstrate a deterioration in the tolerance to a glucose load. In vitro studies on the effects of progesterone on glucose metabolism in isolated adipocytes have shown that progesterone treatment creates a state of insulin resistance in muscle and in adipose tissue resulting in elevated circulating levels of glucose, in decreased glycogen storage in muscle tissue and a decrease in the glucose oxidation via the pentose phosphate pathway in adipose tissue.

Non-hormonal factors which significantly affect carbohydrate metabolism include nutritional factors (food consumption, frequency of feeding), diurinal variations and exercise. Briefly, studies on the effects of frequency of

feeding on glycogen accumulation in muscle and in liver of rodents, tend to demonstrate that rats fed ad libitum exhibit higher levels of glycogen in both muscle and liver. Studies done on the effects of diurinal variations on glycogen levels in muscle and in liver of rats show that glycogen levels in these tissues is not held constant throughout the day but is submitted to daily variations. It was found that glycogen levels in these tissue are highest at the beginning of the dark period and are lowest at the beginning of the light period. Finally, studies on the effects of exercise on glycogen levels in skeletal muscle and in liver clearly demonstrate that strenuous exercise results in a severe depletion in both muscle and liver glycogen levels as well as a severe decrease in blood glucose levels.

Chapter III

METHODOLOGY

3.1 Introduction:

This chapter describes the methodology used in this study. The methodology is divided into four sections. These are: description of subjects, experimental procedure, analysis of blood and tissue and the statistical procedures used in the analysis of the results.

3.2 Description of subjects:

The subjects used in this study consisted of 72 female rats of the Wistar strain. The animals were approximately 6 weeks old and weighed between 175 and 200 grams when the study began. This study received the approval of the Animal Care Committee before it was undertaken.

3.3 Animal acclimatization procedure:

Upon arrival, the animals were housed in individual cages. Their diet consisted of standard laboratory rat chow and water ad libitum. The animals were maintained on a reverse light/dark cycle so that the time 6:00 pm to 6:00 am was light and the period 6:00 am to 6:00 pm was dark. The animals were allowed to adapt to their new environment for

five days. After the initial acclimatization period, ovariectomies were performed on all animals under anesthesia using Halothane. The success of the surgery was assessed by measuring circulating levels of progesterone. If plasma levels of progesterone were determined to be in the low physiological range (7.0-7.5 ng/ml) the surgery was taken as being successful. After at least four days of recovery from surgery, the rats were fitted with subcutaneous implants of Silastic tubing filled with progesterone (4 pregnene-3,20,dione acquired from Sigma chemicals). The tubes were of .062 in. inside diameter and .095 in. outside diameter and measured either 1.5 cm or 4.0 cm in length. A detailed description of the surgical procedures used when performing the ovariectomies and the implants is given in section 3.9.4.

Approximately one week after implantation of the Silastic tubing, the rats were introduced to a program designed to condition them to run on a calibrated motorized rodent treadmill (Quinton model 42-15). The rats ran five days a week for the duration of the treatment. The rats ran during the dark period of the cycle. The speed of the treadmill was set at 35 m/min and the incline remained constant at 15%. At the end of the treatment, the experimental protocol was begun.

3.4 Selection and description of experimental groups:

At the beginning of the treatment, the animals were assigned to one of nine groups. All of the animals received an implant of Silastic tubing. One group received an empty implant measuring 4.0 cm, a second group received an implant filled with progesterone and measuring 1.5 cm and a third group received a 4.0 cm long implant also filled with progesterone.

These three groups were further subdivided into three groups each consisting in three different durations of treatment i.e. the rats received the treatment for either 7, 14 or 21 days. Finally, on the day of the sacrifice the rats were either sacrificed at rest or after a run to exhaustion.

Blood samples were collected at 3 different times during the course of the study. The first blood sample was done while the animal was anesthetized for the implantation of the Silastic tubes. This sample was taken to verify the success of the ovariectomy. The second sample was taken three days after implantation of the capsules in order to verify the P4 levels achieved with the implants. The final blood sample was taken on experimental day when the animals were sacrificed. This blood sample was used to determine plasma progesterone and plasma glucose levels.

The first and second blood samples were taken from the heart in a volume of about 2 ml with a heparinized syringe and transferred to a heparinized tube. For the second and

third samples, the animals were anesthetized using Innovar (0.2 mg/kg) and Atropine (0.02 mg/kg). The Atropine which is an anticholinergic drug was used to maintain a high blood pressure and heart rate. The effect of Atropine was reversed after the blood collection with Naloxone (0.1 ml).

Table 1

Number of animals in each cell

Length of tube	Duration of treatment					
	7 days		14 days		21 days	
	rest	post	rest	post	rest	post
empty	4	4	4	4	4	4
1.5 cm	4	4	4	4	4	4
4.0 cm	4	4	4	4	4	4

3.5 Sacrifice day:

The sacrifice days always began at 8:00 a.m. and were set to take place at the end of each treatment period with progesterone (i.e. 7, 14 or 21 days). Animals from the resting groups were sacrificed at the begining of each experimental day. On experimental day, the animals from the exercise groups were submitted to a run to exhaustion on the rodent treadmill. The speed of the treadmill was set at 35 m/min and the grade was kept constant at 15%. Exhaustion was defined as the time at which the animals were unable to

keep the pace and to avoid the shock grid at the end of the treadmill.

3.6 Sacrifice methodology:

All animals received a 0.4 ml injection of sodium pentobarbital (65 mg/ml) at the time of sacrifice. Once the anesthetic had taken effect, blood and muscle sampling was begun.

3.7 Blood and muscle sampling:

Once the anesthetic had taken effect, the abdominal cavity of the animal was opened and the heart was exposed. Blood was collected directly from the heart. The blood samples were kept on ice until they could be transferred to a refrigerated centrifuge. Centrifugation was done in order to separate plasma from red blood cells. Plasma samples were kept frozen at -50 C until they could be assayed for glucose and progesterone levels. The assay techniques used for progesterone and glucose determination are given in section 3.8.1 and 3.8.2.

After the blood collection, the muscles of the hindlimb were rapidly exposed. The white vastus lateralis (WVL) the plantaris (P) and the soleus (S) muscles were dissected out. Muscles samples weighing between 35-75 mg were taken and frozen on dry ice immediately. The muscles samples were kept frozen in a freezer at -50 C until they could be assayed for glycogen levels. The methodology for the glycogen assay of Lo & Russel (1970) is given in appendix C.

3.8 Surgical procedures:

3.8.1 Ovariectomy:

The animals were anesthesized using Halothane (Fluothane) gas. The animal was laid on its ventral surface. Two small dorsal skin incisions were made approximately half way between the middle of the back and the base of the tail and one half to two thirds of the way down the side of the body. The skin incisions were made on a clean shaven back using a scalpel. The muscle incisions were made directly underneath the skin incisions using a pair of Allison forceps, making a blunt hole in the muscle. The ovaries were surrounded by an appreciable amount of fat and were found underneath the muscle incisions. The ovaries were pulled out through the muscle incisions by grasping the periovarian fat and not by the ovary itself since touching it with the surgical instruments may have caused small pieces of ovary to become detached and may reimplant themselves and carry on normal function. Two ties using 3.0 Vicryl absorbable suture material were tied around the uterine horn, distally to the ovary. A third tie was put in proximally to the ovary. The uterine horn and the surrounding fat and blood vessels were severed with a scalpel between the proximal tie and the first distal tie. The muscle incisions were closed by putting in one stitch. The skin incisions were closed by putting in one or two stitches using 3.0 silk suture material.

3.8.2 Silastic implants:

All implants were sterilized using gamma radiation before being filled with hormone. The filling of the Silastic tubes with progesterone was done in a sterile field. The tubes were sealed with silicone type A medical adhesive.

Subcutaneous implantation of the Silastic tubes was accomplished through a small dorsal skin incision. The tubes were placed between the skin and the muscle sheath. The small skin incision was closed using a staple. The staple was removed a few days later.

3.9 Statistical analysis:

Statistical analysis of the data consisted of two and three way analyses of variance followed by post-hoc analysis using Tukey's technique. The analyses of variances were performed on computer using SPSS (Statistical Package for the Social Sciences) software. The graphs of the results were done using SAS GRAPH software.

Chapter IV

RESULTS AND DISCUSSION

4.1 Introduction:

The purpose of this study was to determine the effects of progesterone administration on carbohydrate metabolism at rest and during exercise in the rat.

This chapter is divided in two sections: 1) the results and 2) the discussion.

4.2 Results:

This section is further divided into three subsections: a) results concerning the progesterone levels, b) results concerning plasma glucose levels and c) results concerning muscle and liver glycogen levels as well as results concerning the run times to exhaustion.

4.2.1 Progesterone implants:

In order to determine and isolate the effects of progesterone (P4) on glucose and glycogen levels in the rat, ovariectomies alone or in concert with implantation of Silastic tubing filled with progesterone were undertaken. Subsequent analysis of P4 levels achieved by this method was performed using radioimmunoassay.

As previously stated in the methodology, the rats either received a 4.0 cm long empty implant or implants of 1.5 cm or 4.0 cm filled with progesterone. The P4 levels were measured at three different times during the course of the study: first, blood sampling was done three days after ovariectomy to verify the success of the surgery, the second blood sample was taken three days after implantation of the Silastic tubing and finally the last blood collection was done when the animals were sacrificed. The results are presented in tables 2 to 6.

Table 2

Means and standard deviations for progesterone levels at ovariectomy, in ng/ ml plasma

Length of tube	7 days	14 days	21 days	MEAN
empty	10.41 + 6.20	10.42 +18.05	------	6.94
1.5 cm	12.46 +13.74	12.73 + 6.90	12.56 +7.30	12.54
4.0 cm	08.42 + 2.61	9.42 + 6.96	12.29 +4.47	12.71
MEAN	13.10	10.86	8.28	

n=4 for each cell

The results of the analysis of variance for levels of progesterone at ovariectomy showed that there were no dif-

ferences between means of groups i.e. the F ratio was not significant at a confidence level of 95% for both the main effects and the interaction effects.

Table 3 represents results obtained when levels of P4 are verified after implantation of the Silastic tubing. Statistical analysis of the results shows that there is a significant difference between the progesterone levels of the rats which received an empty implant and the rats which received implants of 1.5 cm filled with progesterone. There is also a significant difference between the rats which received an empty implant and the rats which received 4.0 cm long implant when P4 levels were measured. However, no significant difference was found between the levels of progesterone of the animals which received 1.5 cm implants and those which received 4.0 cm implants. Furthermore, no difference was found between levels of P4 achieved with the implantation of Silastic tubing when rats are grouped according to the duration of the progesterone treatment, i.e. 7, 14, 21 days. Finally, it can be seen from the results appearing in tables 3 and 4 that contrary to expectations, levels of progesterone achieved with implantation of 1.5 cm of Silastic tubing are consistently higher than the levels of progesterone achieved when rats are fitted with a 4.0 cm implant.

Table 3

Means and standard deviations for progesterone levels at implants, in ng/ ml plasma

Length of tube	7 days	14 days	21 days	MEAN
empty	12.83 + 2.44	11.61 +4.90	13.23 +13.52	12.56
1.5 cm	42.51 +13.90	32.76 +25.63	18.41 + 5.36	31.23
4.0 cm	32.69 +28.44	18.86 +11.60	35.02 +16.45	28.86
MEAN	29.34	21.08	22.22	

n=4 for each cell

The results of the analysis of variance for levels of progesterone at implants showed that there are significant differences between means for the effects of length of tubing. It was found that the F ratio for the variable time was not significant whereas the F ratio for the variable length was significant for a 95% confidence interval. The interaction effect between the factors time and length was not found to be significant.

Table 4

Results of Tukey's test following the ANOVA on P4 levels at implant

Length of tube		empty	1.5 cm	4.0 cm
	MEAN	12.56	31.23	28.86
empty	12.56	----	18.67*	16.30*
1.5 cm	31.23	----	-----	2.37
4.0 cm	28.86	----	----	----

n=4 for each cell
(*) significant at 0.05 level

The results presented in table 6 shows that there is a significant difference between levels of progesterone in rats receiving empty implants and rats receiving 1.5 cm of Silastic tubing as well as a significant difference between those receiving no progesterone and the rats fitted with 4.0 cm implants at the time of sacrifice. Results further show that there is a significant difference for levels of progesterone between rats implanted with 1.5 cm of Silastic tubing and rats implanted with 4.0 cm. Furthermore, it was shown that the duration of the treatment, i.e. 7, 14, 21 days did not result in significantly different levels of progesterone over time among groups which received the same length of tubing.

Table 5

Means and standard deviations for progesterone levels at sacrifice, in ng/ ml plasma

Length of tube	7 days	14 days	21 days	MEAN
empty	8.10 + 2.64	9.90 + 7.02	8.91 + 2.44	8.97
1.5 cm	24.78 +17.21	32.39 +20.00	25.33 +17.92	27.50
4.0 cm	17.92 +11.06	23.40 +16.97	23.21 +12.10	21.51
MEAN	16.94	21.90	19.15	

n=4 for each cell

The results of the ANOVA for levels of progesterone at sacrifice demonstrated that there were no significant differences between means for the variable time i.e. the F ratio was not significant at the 0.05 level, but was found to be significant for the variable length. Furthermore, the F ratio was not found to be significant when the interaction effect between length and time were studied.

Table 6

Results of Tukey's technique following ANOVA on progesterone levels at sacrifice

Length of tube		empty	1.5 cm	4.0 cm
	MEAN	8.97	27.50	21.51
empty	8.97	----	18.53*	12.54*
1.5 cm	27.50	----	-----	5.99*
4.0 cm	21.51	----	----	-----

n=4 for each cell
(*) significant at 0.05 level

4.2.2 Plasma glucose:

Results concerning the plasma glucose levels are presented in tables 7 and 8.

8

Table 7

Means and standard deviations for glucose levels, in mg/ dL plasma

Length of tube		7 days	14 days	21 days	MEAN
empty	(1)	115.41 +12.77	112.81+ 4.07	137.99+22.60	122.07
	(2)	91.82 +28.54	61.75+30.18	89.44+36.25	81.00
1.5 cm	(1)	139.50 +11.11	175.83+25.26	228.62+80.55	181.32
	(2)	42.52 +12.58	79.90+26.26	53.12+15.94	58.51
4.0 cm	(1)	152.76 +15.09	169.24+26.81	115.68+22.25	145.89
	(2)	74.14 +11.56	47.81+10.57	78.46+36.20	66.80
MEAN	(1)	135.89	152.63	160.76	149.76
	(2)	69.49	63.15	73.67	68.77

n=4 for each cell
(1) resting (2) post exercise

The results from the three way analysis of variance performed on the glucose data show that among the F ratio for the three main effects exercise, time and length, only the F ratio for the variable exercise was significant at 95% confidence interval. Furthermore, the F ratios for the two way interaction between exercise and time and time and length were not found to be significant but the F ratio for the two way interaction between the two variables exercise and was found to be significant at the 0.05 level. Finally, the F

ratio for the three way interaction effect between the variables exercise time and length was not found to be significant.

Table 8

Results from Tukey's technique following ANOVA for glucose levels

Length of tube		empty	1.5 cm	4.0 cm
	MEAN (1)	122.07	181.32	145.89
empty	122.07	-----	59.25*	23.82*
1.5 cm	181.32	-----	-----	35.43*
4.0 cm	145.89	-----	-----	-----

n=4 for each cell
(*) significant at 0.05 level

Results show that exercise to exhaustion had a significant effect on plasma glucose levels, resulting in a severe decrease in plasma glucose levels in all groups studied. It can also be seen from the results that rats which received 1.5 cm implants exhibited the highest resting plasma glucose levels, averaging 181.32 mg/dL. Rats which received 4.0 cm implants exhibited resting levels of glucose averaging 145.89 mg/dL which are significantly lower than those found in the group receiving 1.5 cm implants but remained signifi-

cantly higher than the levels found in the animals which received empty implants.

As previously stated, exercise to exhaustion caused a severe fall in plasma glucose concentrations in all groups studied. Finally, no significant differences were found between post exercise glucose levels of progesterone treated rats and untreated rats.

A graph of the results obtained for glucose levels following a treatment with progesterone can be found in appendix K.

4.2.3 Glycogen storage and depletion:

The effect of progesterone on glycogen levels were investigated using the soleus, the plantaris and the white vastus lateralis (WVL) muscles. Hepatic glycogen levels were also measured. Results concerning levels of glycogen found in soleus muscle are presented in tables 9 an 10 whereas results concerning plantaris, WVL and liver glycogen are presented in tables 11, 12, 13 and 14.

Results in table 10 show that glycogen levels of soleus muscle were significantly affected by exercise, with all groups showing lower glycogen levels after exercise as compared to resting levels. Furthermore, statistical analysis of the results show that administration of progesterone resulted in significantly different glycogen levels between treatment groups, with the lowest levels being found in the groups which received 4.0 cm implants and the highest glyco-

gen levels being found in groups of rats which received 1.5 cm implants. The glycogen levels in soleus of rats which received empty implants were slightly but not significantly lower than those found in rats implanted with 1.5 cm of Silastic tubing.

The duration of the treatment did not significantly affect the glycogen levels in soleus muscle of the different treatment groups, therefore suggesting that a treatment lasting 7 days is sufficient in order for progesterone to significantly affect glycogen levels in soleus muscle.

Table 9

Means and standard deviations for levels of glycogen in soleus muscle, in mg /g tissue

Length of tube		7 days	14 days	21 days	MEAN
empty	(1)	6.19 +1.85	4.58 +1.65	5.01 +0.55	5.26
	(2)	2.13 +1.33	1.04 +0.56	0.80 +0.20	1.32
1.5 cm	(1)	6.22 +1.29	5.33 +1.88	6.71 +4.92	6.09
	(2)	1.00 +0.61	0.85 +0.58	1.16 +0.38	1.00
4.0 cm	(1)	2.54 +0.92	4.21 +1.30	4.13 +1.66	3.62
	(2)	0.32 +0.36	0.62 +0.18	0.90 +0.36	0.61
MEAN	(1)	4.98	4.71	5.28	4.99
	(2)	1.15	0.84	0.95	0.98

n=4 for each cell
(1) resting (2) post exercise

The results of the three way ANOVA for glycogen levels in soleus muscle revealed that there were significant main effects for a confidence interval of 95%. It was found that the differences between means for the variables exercise and length were significant whereas the F ratio for the variable time was not significant. It was also found that the F ratio for the two way interaction between the variables and the F ratio for the three way interaction were not significant.

Table 10

Results from Tukey's technique following ANOVA on glycogen levels in soleus muscle

Length of tube	MEAN (1)	0 cm	1.5 cm	4.0 cm
		5.26	6.09	3.62
empty	5.26	---	0.83	1.64*
1.5 cm	6.09	0.83	---	2.47*
4.0 cm	3.62	1.64*	2.47*	---

n=4 for each cell
(*) significant at 0.05 level

A graphic representation of the results concerning glycogen levels found in soleus muscle after treatment with progesterone is presented in appendix G.

The results concerning the glycogen levels found in plantaris muscle following progesterone treatment are presented in table 11.

It can be seen from the results that there is a significant difference between levels of glycogen at rest and after a run to exhaustion, i.e. the glycogen levels in the exercised animals of all groups being significantly lower than the resting animals of the same group. As for soleus muscle, resting glycogen levels in plantaris were found to be lowest in the rats which received 4.0 cm implants and highest in rats which received 1.5 cm implants. However, the differences observed in glycogen levels of the different treatments did not prove to be statistically significant.

Table 11

Means and standard deviations for levels of glycogen in plantaris muscle, in mg/ g tissue.

Length of tube		7 days	14 days	21 days	MEAN
empty	(1)	6.04 +0.97	5.31 +0.57	6.04 +0.86	5.80
	(2)	2.99 +1.46	0.99 +0.77	2.99 +1.34	2.32
1.5 cm	(1)	6.65 +1.47	4.48 +1.04	9.05 +8.64	6.73
	(2)	0.85 +0.47	1.46 +0.54	1.83 +2.19	1.38
4.0 cm	(1)	3.48 +0.90	4.78 +1.62	5.60 +0.80	4.62
	(2)	0.21 +0.08	1.48 +1.01	1.17 +0.91	0.95
MEAN	(1)	5.39	4.86	6.90	5.72
	(2)	1.35	1.31	2.00	1.55

n=4 for each cell
(1) resting (2) post-exercise

The results from the three way analysis of variance for levels of glycogen in plantaris muscle shoved that the F ratio for the variable exercise was found to be significant at 0.05 level but that the F ratios for the variables time and length were not found to be significant. Furthermore the F ratios for the two way and the three way interactions were not found to be significant. However, it is interesting to see that the results follow the same trend as in soleus muscle i.e. progesterone admistration resulted in a decrease in glycogen content of the muscle.

Results concerning glycogen levels in white vastus lateralis (WVL) are presented in table 12. As expected, exercise was shown to have a significant effect on glycogen levels in all groups studied. However, treatment with either 1.5 or 4.0 cm of Silastic tubing did not result in any significant changes in resting glycogen levels when compared with the ovariectomized untreated animals.

Table 12

Means and standard deviations for levels of glycogen in WVL muscle. in mg/ g tissue

Length of tube		7 days	14 days	21 days	MEAN
empty	(1)	4.84 +2.10	5.10 +0.51	5.10 +1.59	5.02
	(2)	1.44 +0.43	0.39 +1.16	1.35 +1.02	1.06
1.5 cm	(1)	6.09 +0.89	4.22 +1.52	4.24 +0.74	4.85
	(2)	0.75 +0.23	1.85 +1.48	1.53 +1.19	1.38
4.0 cm	(1)	4.69 +1.20	5.61 +1.69	4.86 +0.80	5.05
	(2)	0.18 +0.15	0.61 +0.11	1.09 +1.22	0.63
MEAN	(1)	5.21	4.98	4.73	4.97
	(2)	0.79	0.95	1.32	1.02

n=4 for each cell.
(1) resting (2) post exercise

The results of the three way ANOVA performed on the glycogen data in WVL muscle revealed that there were significant main effects for a confidence interval of 95%. The F ratio for the variable exercise was found to be significant but the F ratios for the variables length and time were not found to be significant. Furthermore, the F ratios for the two way and three way interactions were not found to be significant.

The effects of progesterone treatment on liver glycogen were also investigated and results are presented in tables 13 and 14.

Table 13

Means and standard deviations for levels of glycogen in liver, in mg/ g tissue

Length of tube		7 days	14 days	21 days	MEAN
empty	(1)	50.52+28.40	6.39 +6.95	12.95 + 8.63	23.28
	(2)	11.88+ 7.82	1.05 +0.32	3.18 + 2.31	5.37
1.5 cm	(1)	6.94 +13.74	5.87 +2.07	15.00 +12.67	9.93
	(2)	0.82 +0.19	0.96 +0.26	1.13 +0.16	0.97
4.0 cm	(1)	9.02 +8.18	8.41 +3.55	8.14 +3.60	8.52
	(2)	0.75 +0.23	1.42 +0.70	1.56 +0.29	1.24
MEAN	(1)	22.82	6.89	12.03	13.91
	(2)	4.48	1.14	1.96	2.53

n=4 for each cell
(1) resting (2) post exercise

Results from the three way ANOVA of liver glycogen data reveal that the F ratios for the main effects are all significant in the 95% confidence interval i.e. there were significant differences between means for the variables time, length and exercise. Furthermore, the F ratio for the two way interaction between time and length was found to be significant at a confidence interval of 95%, whereas the F ratios for the two way interaction between exercise and time

and exercise and length were not found to be significant, Finally, the F ratio for the three way interaction between the variables was found to be significant for a confidence interval of 95%.

Table 14

Results of Tukey's technique following ANOVA on liver glycogen results

Length of tube		empty	1.5 cm	4.0 cm
	MEAN (1)	23.28	9.93	8.52
empty	23.28	-----	13.35*	14.76*
1.5 cm	9.93	-----	-----	1.41
4.0 cm	8.52	-----	-----	----

n=4 for each cell
(*) significant at 0.05 level

As in the case for glycogen levels in muscle, exercise resulted in a severe depletion in liver glycogen in all groups studied. Furthermore, the results show that progesterone treatment affects liver glycogen in the same fashion as it does in soleus muscle causing hepatic glycogen levels to be significantly lower in the progesterone treated groups than in the untreated groups. Also, contrary to what was shown for muscle glycogen, the duration of the progesterone

treatment resulted in changes in the hepatic glycogen levels with time. Levels of glycogen in the untreated rats were shown to decrease significantly over time, whereas the rats which received 1.5 cm implants exhibited significantly higher resting glycogen levels after 21 days of treatment as compared with treatments lasting 7 and 14 days. Glycogen levels found in livers of rats treated with 4.0 cm implants were not found to vary significantly over a 21 day period.

Results presented in tables 15 and 16 illustrate the differences in running times between groups. Results show that both level of progesteron e administered and duration of treatment were found to significantly affect performance. It was found that performance was impaired as the duration of the treatment was increased from 7 to 21 days for the groups receiving no progesterone or 1.5 cm implants. However, there was no significant difference between the running times of the 7 days 14 days and 21 days groups receiving 4.0 cm implants. Furthermore, the results show that the rats which received either 1.5 cm or 4.0 cm implants ran significantly longer than the rats which received empty implants. Finally, even though the rats which received 1.5 cm implants exhibited greater running times than the rats which received 4.0 cm implants, the difference was not found to be statistically significant.

Table 15

Means and standard deviations for running times, in minutes

Length of tube	7 days	14 days	21 days	MEAN
0 cm	53.78+18.24	30.24+19.24	27.92+13.32	37.31
1.5 cm	70.74+13.68	53.24+14.86	43.86+11.12	55.85
4.0 cm	49.25+11.72	45.40+11.50	42.25+20.61	45.63
MEAN	57.83	42.95	38.01	46.27

n=4 for each cell

The two way analysis of variance that was done on the results concerning the running times of the exercised animals revealed that the F ratio for the main effects was significant for a confidence interval of 95% for both the length variable and the time variable. Furthermore, it was also found that the F ratio for the two way interaction between the variables length and time was not significant at the 0.05 level.

Table 16

Results of Tukey's technique following ANOVA on running times

Length of tube		empty	1.5 cm	4 cm
	MEAN	37.31	55.85	45.63
empty	37.31	-----	18.54*	8.32*
1.5 cm	55.85	-----	-----	10.22
4.0 cm	45.63	-----	-----	-----

n=4 for each cell
(*) significant at 0,05 level

Finally, when differences in weight gain between groups are studied, it can be seen that rats which received either 1.5 cm or 4.0 cm implants gained significantly more weight over the course of the treatment than the untreated animals. Also, it was found that , as expected, the duration of the treatment had a significant effect on weight gain.

Table 17

Means and standard deviations for weight gains in grams

Length of tube	7 days	14 days	21 days	MEAN
empty	6.63+ 5.10	25.63+ 9.44	24.50+ 7.38	18.92
1.5 cm	28.00+12.56	17.63+11.10	29.50+10.73	25.04
4.0 cm	34.38+ 9.24	29.88+ 6.92	30.13+ 9.73	31.46
MEAN	23.00	24.38	28.04	25.14

n=4 for each cell

The results from the two way analysis of variance of the weight gains show that the F ratio for main effects is significant for a confidence interval of 95% but only for the variable length. In addition, it was found that the F ratio for the two way interaction between the variables time and length was also found to be significant at a 95% confidence interval.

Table 18

Results of Tukey's technique following ANOVA on weight gain results

Length of tube		empty	1.5 cm	4.0 cm
	MEAN	18.92	25.04	31.46
0 cm	18.92	-----	6.12*	12.54*
1.5 cm	25.04	-----	------	6.42*
4.0 cm	31.46	-----	------	------

n=4 for each cell
(*) significant at 0.05 level

4.3 Discussion:

Long term ovariectomies were performed in the animals used in this study in order to better isolate the effects of progesterone administration on glucose homeostasis. The surgical procedure was expected to yield P4 levels in the low physiological range mostly accounted for by the contribution of the adrenal cortex. It has proved difficult however to compare the average circulating P4 levels in ovariectomized rats found in this study with results obtained in other studies since most of the studies that verified the effects of ovariectomy and subsequent hormone replacement on carbohydrate metabolism did not measure the levels of circulating

progesterone after ovariectomy. However, when post ovariectomy P4 levels achieved in the present experiments are compared to those found by Weick et al. (1977) (which averaged 7.6 ng/ml) it can be seen that the levels found in the present study are high by comparision.

The method of implanting Silastic capsules in animals in which long term treatment with a steroid hormone is desired is considered far better than injecting animals subcutaneously once or twice daily with a certain dose of the steroid hormone since it is thought to produce constant ciculating levels of the steroid throughout the treatment period and that the levels achieved by this method can be controlled to a certain degree by manipulating the length and diameter of the capsule. Another assumption that seems to be made in most studies which employ the former method is that levels of circulating hormones are not only constant over time but also are fairly constant between animals of the same group. However the results from the present study would appear to refute this: analyses were performed to measure the levels of P4 achieved through this method, and these show that the plasma levels are highly variable between animals of the same group.

Furthermore it was reported by Legan et al. (1975) that subcutaneous Silastic implants could be left in for up to 76 days with successful results. However it was found in the present experiments that even though the capsules still con-

tained crystallized progesterone, the circulating levels of P4 were consistently lower after 21 days that after 7 or 14 days.

A possible reason for the lower levels of P4 found in animals treated for 14 and 21 days as compared to 7 days may be the weight gain that occurs during the treatment period. Adipose tissue has been shown to be a steroid sensitive tissue and an increased fat mass may cause the circulating levels of progesterone to fall when compared to the leaner rats which received the same size implants.

In addition, results from this study tend to suggest that there is also considerable variation in the plasma P4 levels for the same subjects over time. Results show, that in most cases, there is a decrease in circulating levels of P4 from the time that the animals were fitted with the implants to the time of sacrifice. A possible explanation for this would be that even though the rats were fitted with the implants 3 days after the ovariectomy was performed, there was still some endogenous P4 contributing to the circulating pool i.e. not all of the progesterone that originated from the ovaries had been cleared from the blood at the time of implantation but was completely cleared at the time of sacrifice.

Previous studies concerning the effects of P4 administration on glucose homeostasis have produced conflicting results which may be due in part to the differences in the experimental protocol or to undetected difference in P4 levels between animals of the same group.

Sutter-Dub et al. (1981, 1982) in a series of experiments have consistently shown that P4 administration in rats results in significantly higher levels of circulating glucose than in castrated non treated animals. On the other hand, studies by Ashby et al. (1978) and Matty (1978) found that progesterone treatment resulted in either no change in glucose homeostasis or in a decrease in plasma glucose levels with a concomittant rise in insulin levels. However it should be noted that these authors did not attempt to measure the levels of P4 administered and also, in Ashby's study, the animals used in the experiments were not ovariectomized and therefore were subjected to influences of estrogens as well as progesterone.

The results from this study appear to confirm those reported by Sutter- Dub et al. (1981, 1982): progesterone administration by implatation of either 1.5 cm or 4.0 cm resulted in elevated plasma glucose levels when compared to ovariectomized non treated animals. Therefore, it would appear that the underlying mechanism for detecting glucose is affected by progesterone. Numerous studies have reported that progesterone may act as a diabetogenic hormone, i.e. creating a state of insulin resitance in such tissue as liver, skeletal muscle and adipose tissue (Ashby et al. 1978; Sutter-Dub et Dazey, 1981) and therefore resulting in higher than normal circulating levels of insulin during the P4 treatment. Even though the present study did not investi-

gate levels of insulin in response to a glucose challenge in the P4 treated rats, progesterone induced insulin resistance appears to be the most likely mechanism by which hyperglycemia was achieved in the animals receiving P4. Another fact to support this is that rats which are administered progesterone consistently gain more weight than the non treated animals and increased fat mass has been shown to be associated with a state of insulin resistance (Mendes et al., 1985, Ashby et al., 1978). The results of the present investigation also show that length of treatment does not constitute an important factor in the development of the progestone induced hyperglycemia since plasma glucose levels in the P4 treated rats were elevated after 7 days and did not vary significantly after 14 or 21 days.

Matty (1978) investigated the effects of ovariectomy and P4 replacement therapy on glycogen accumulation in mice. Their results show that long term ovariectomy results in a substantial decrease in glycogen levels in skeletal muscles and liver. Furthermore they showed that P4 administration in ovariectomized mice resulted in a return of the glycogen levels to pre ovariectomy levels.

Matute and Kalkhoff (1973) found that P4 treatment in non ovariectomized rats results in a decreased hepatic gluconeogenesis accompanied by a net liver glycogen storage. Furthermore they showed that while both estrogen and progesterone treatments resulted in hyperinsulinemia and depressed

liver glucose release, estrogens increased peripheral substrate utilization and therefore glycogen storage while progesterone treatment resulted in impaired substrate utilization in extra hepatic tissue such skeletal muscle, probably due to its contra-insulin effect.

The results of the present study are in disagreement with those of Matute and Kalkhoff (1973) and Matty (1978). It was found that ovariectomy followed by a P4 treatment resulted in a decrease in glycogen storage in both skeletal muscle and liver. However, these differences were found to be significant only in soleus muscle and liver, therefore suggesting that progesterone may have a specific muscle fiber type effect, i.e. progesterone treatment may result in a greater insulin resistance in red muscle fibers than in white or in muscles composed of different amounts of red or white fibers. Furthermore, these results lend support to the findings of Sutter Dub et al. (1980, 1981) which show that progesterone by creating a state of insulin resistance decreases the hormone's ability to lower plasma glucose and therefore causing muscle and fat cells to use and store less glucose which in turn results in lower muscle glycogen levels.

Their results further show that P4 treatment results in a decrease in lipogenesis and fatty acid synthesis and stimulated lipolysis. These findings may provide an explanation for the results obtained in the present study. It is a well

known fact that pre-exercise glycogen levels have a significant effect on endurance performance. Subjects (both human and animal) that show the highest pre-exercise muscle and liver glycogen levels exhibit better performances. However, the present study clearly indicates that while progesterone treated rats had significantly lower resting glycogen levels in both liver and muscle, they also ran significantly longer than the ovariectomized non treated rats when exercised to exhaustion. Furthermore, even though the rats that were treated with the progesterone ran significantly longer than the non- treated rats, they did not exhibit significantly lower post exercise glycogen levels when compared to the non treated rats. It would therefore appear that progesterone treatment induces a greater use of non-carbohydrate sources, probably lipids and results in a sparing of carbohydrate reserves.

However, the results found in the present experiments concerning the effects of P4 on carbohydrate metabolism cannot be interpreted as a direct cause and effect relationship between progesterone administration and changes in carbohydrate metabolism because of the questions that have been raised following the verification of the progesterone levels achieved through implantation of Silastic tubing.

Therefore, further studies are needed to explore the possible reasons for obtaining fluctuating levels of progesterone before any additional study of the effects of progesterone on carbohydrate metabolism is undertaken.

Chapter V

SUMMARY, CONCLUSIONS AND RECCOMMENDATIONS

5.1 Summary:

This study was designed to determine the effects of progesterone treatment on carbohydrate metabolism at rest and during exercise. The subjects used in this study were female Wistar rats. All animals were ovariectomized and fitted with an implant of Silastic tubing which was either empty or filled with progesterone and measured either 1.5 or 4.0 cm in length. The progesterone treatment lasted for either 7, 14 or 21 days. The animals were run on a motorized treadmill 5 days a week in order for the rats to get acclimatized to the running protocol. Blood sampling was done after ovariectomy, in order to verify the success of the surgery and again after implantation of the Silastic tubing and at sacrifice in order to verify the levels of progesterone.

On the last day of the treatment, (i.e. day 7, day 14 or day 21), the animals were either sacrificed at rest or after a run to exhaustion. Muscle samples from the soleus, plantaris, white vastus lateralis and samples from liver were taken at the time of sacrifice in order to determine the resting and post exercise glycogen content of these tissues. Running times ang weight gains were also recorded in order to determine any effect of P4 on these parameters.

The results of the study show that progesterone treatment results in elevated plasma glucose levels when compared to ovariectomized untreated animals. This P4 induced hyperglycemia was present in all treatment groups but was found to be highest in rats receiving Silastic implants of 1.5 cm. It was also found that progesterone treatment results in lower resting glycogen levels in both soleus muscle and liver. The glycogen levels in WVL and plantaris muscles of the P4 treated animals were also found to be lower than those of the ovariectomized untreated rats but the difference was not statistically significant.

Even though the progesterone treated animals exhibited lower resting muscle and liver glycogen concentrations, it was found that these animals ran significantly longer than the ovariectomized untreated animals. Furthermore, it was found that despite these differences in running time there was no significant difference in post exercise glycogen levels between the P4 treated and the untreated animals.

Finally, it was determined that animals which received either a 1.5 cm or a 4.0 cm implant filled with progesterone gained significantly more weight than the animals which received an empty implant.

5.2 Conclusions:

Within the scope of this study, the following conclusions may be drawn:

1. Progesterone treatment by way of Silastic tubing implantation produces circulating levels of P4 that are highly variable between animals of the same group as well as within the same animals over time.

Therefore, within the limitations imposed by the conclusions drawn in 1 it can be said that:

2. Long term progesterone treatment results in a rise in plasma glucose levels when compared to ovariectomized untreated animals.

3. Progesterone treatment also results in a decrease in resting glycogen levels in soleus muscle and in liver.

4. Finally, rats which received the progesterone treatment run significantly longer than the ovariectomized untreated animals during a run to exhaustion on a motorized treadmill, as well as exhibit greater weight gains during the treatment than the ovariectomized untreated animals.

5.3 Reccommendations:

The present structure of this study allowed for the demonstration of the effects of progesterone administration on carbohydrate metabolism. However, after the completion of such a study, a few changes as well as additions are recommended if such a study were to be conducted again. These changes and additions concern the methodology.

First, it would be preferable to allow longer than three days between performing ovariectomies and implantation of the Silastic tubing in order for endogenous progesterone to be completely cleared from the blood.

Second, a greater difference between lengths of tube should be allowed in order to obtain two or more distinct groups with significantly different P4 levels. For example, Silastic tubes measuring 2 cm and 8 cm could be used. However in the light of the results found in this study, it would appear that both 2 cm and 8 cm of Silastic tubing would yield suprapharmacological levels of P4 therefore eliminating the possibility of studying the effects of a physiological dose of P4 on carbohydrate metabolism.

Finally, in order to test the hypothesis that the P4 induced hyperglycemia results from a resistance of muscle and liver tissue to insulin therefore would probably be seen in higher circulating levels of insulin, monitering of insulin levels throughout the treatment period should be undertaken.

Also, resting and especially post exercise free fatty acids levels should be measured in order to determine if the glycogen sparing effect of P4 during exercise results in a greater utilization of free fatty acids as as source of fuel during exhaustive exercise.

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Appendix A

ASSAY PROCEDURES:

A.1 Plasma progesterone radioimmunoassay (RIA)

Reagents:

Phosphate buffered saline (PBS)

gelatin

tracer: 3-H-progesterone

ether

Preparation of solutions:

a) Phosphate bufferd saline-gelatin (PBS-G):

To 90 ml of PBS is added 10 ml of gelatin

b) Tracer preparation:

0.30 ul of tracer is needed per aliquot, to which is added 100 ul of PBS-G The tracer is dried with air before the PBS-G is added, and the tracer preparation has to stand for 10 minutes before it can be used.

c) Antibody preparation:

To 0.2 ml of antibody is added 7.3ml of PBS-G to make a final volume of 7.5ml.

d) Charcoal preparation:

A stock solution of Dextran coated charcoal comprised of 0.25% Dextran T-70 and 2.5% activated Norit A (charcoal) in 0.1 M PBS (pH 7.4) is diluted 1:10 with 0.01 PBS-G before use. The charcoal preparation is stirred on a magnetic stirrer and kept on ice for at least an hour before use. The charcoal solution is prepared 24 hours before use.

Procedure:

1) Label tubes

2) To tubes 10 to 30 100 ul are added in triplicate of 10 pg, 20 pg, 40 pg, 80 pg, 160 pg, 320 apg and 640 pg for the standard curve determination.

3) To tubes 31-36 are added 100 ul of low pool

4) To tubes 37-42 are added 50 ul of high pool

5) To tubes 43 and on are added either 20, 30 or 50 ul of sample

6) Tubes 10 and on are placed in a water bath at 37 C and dried with air

7) To tubes 1-6 are added 200 ul of PBS-G and 100 ul of tracer

8) To tubes 7-42 are added 100 ul of PBS-G, 100 ul of tracer and 100 ul of antibody

9) Tubes are vortexed

10) Tubes are stored at 4 C overnight

11) To tubes 1-3 are added 0.7 ml of PBS

12) To tubes 4-42 0.7 ml of the charcoal preparation is added

13) Tubes are left standing for 15 minutes

14) Tubes are centrifuged at 2000 rpm and 4 C for 15 minutes

15) The upper part of each tube is transferred to scintillation vials.

16) 3.5 ml of RIA cocktail is added to each vial

17) Vials are shaken and left standing in the dark for 3-4 hours

18) The vials are then counted for ten minutes or until 10 000 cpm have accumulated

A computer program which uses logit and log transformations and linear regression analysis is used for automated calculation of the progesterone in each unknown.

Correction for procedural losses is accomplished by calculation of the percentage recovery of tritiated progesterone added to each plasma sample.

Appendix B

B.1 Plasma glucose assay:

Reagents:

PGO enzymes:

contains 500 units of glucose oxidase and 100 Purpurogallin units of peroxidase and buffer salts.

Distilled water

o-Dianisidine dihydrochloride

Glucose standard solution

B-glucose, 100 mg/dl (5.56 mmol/l) in benzoic acid solution 0.1%

Preparation of solutions:

Enzyme solution is prepared by adding contents of one capsule (PGO enzyme capsule) to 100 ml diltilled water. Color reagent solution is prepared by reconstituting 50 mg o-dianisidine dihydrochloride with 20 ml distilled water. Combined enzyme-color reagent solution is prepared by com-

bining 100 ml of enzyme solution and 1.6 ml of color reagent solution. The solution is mixed by inverting several times.

Procedures:

1) Label tubes

2) To blank is added 475 ul distilled water To standard is added 25 ul of standard glucose solution and 475 ul distilled water. To each test is added 25 ul of sample plus 475 ul of distilled water.

3) To each tube is added 5.0 ml of combined enzyme-color reagent solution and mix each tube thoroughly.

4) All tubes are incubated at 37 C for 30 minutes or at room temperature (18-26 C) for 45 minutes. 5) At the end of the incubation period the tubes are removed from the water bath.

The absorbance (A) of the standard and the test is read at 440-450 nm using the blank as a reference.

N.B. Readings should be completed within 30 minutes.

All readings were done on a LKB Biochrom Ultraspec 4050 spectrophotometer

The equation used for the calculation of the plasma glucose levels is:

$$\text{plasma glucose (mg/dl)} = \frac{A \text{ test} \times 100}{A \text{ standard}}$$

Appendix C

C.1 Glycogen assay:

Reagents:

30% KOH solution saturated with sodium sulfate (300 gm KOH pellets are dissolved in distilled water to one liter and saturated with sodium sulfate).

95% ethanol

98% H_2SO_4

5% phenol

50 mg glycogen powder is dissolved in distilled water to 10 ml (stock solution)

sodium sulfate saturated aqueous solution

Standard solutions: 0.5, 1.5, 3.0, 4.0, 5.0 and 6.0 mls of the stock solution were diluted to 10 ml in volumetric flasks, to obtain standard solutions of 10, 30, 60, 80, 100 and 120 ug/ml glycogen.

Procedure:

1) After removal from frozen storage, the muscle samples are kept on solid CO_2 until they are weighed on a Metler H balance.

2) Samples are transfered to stoppered tubes containing 0.5 ml of 30% KOH saturated with sodium sulfate to 1 ml of glycogen recovery solution is added 0.5 ml NA_2SQ_4 saturated 30% KOH

3) Tubes are capped and placed in a boiling water bath for 30 minutes.

4) After removal from the boiling water bath, tubes are cooled on ice for 10 minutes.

5) 2-3 drops of aqueous NA2SO4 (sat'd) solution to muscle samples 3-4 drops of of the same solution is added to the glycogen recovery solutions

6) To the tubes containing the muscle samples is added 0.8 ml of 95% ethanol and to recovery solutions is added 1.8 ml of 95% ethanol. Samples are mixed thoroughly (vortex).

7) Tubes are cooled on ice for 60 minutes (or are refrigirated overnight after cooling on ice)

8) Tubes are centrifuged at 4 C and 3000 g for 60 minutes (tubes are balanced using an equal amount of ethanol)

9) The supernatant is aspirated using a disposable Pasteur pipette

10) The tubes are inverted and drained by gravity for 15 minutes the excess fluid at the mouth of the tube is drained using a Pasteur pipette

11) The glycogen is redissolved in 3 mls of distilled water and mixed thoroughly

12) To the following aliquots is added 1 ml of 5% phenol:

resting liver: 0.1 ml

resting muscle: 0.5 ml

glycogen standard: 1.0 ml

depleted muscle: 1.0 ml

depleted liver: 0.1 ml

13) To the aliquots are quickly added 5 ml H_2SO_4

14) Solutions are cooled and then mixed

15) Tubes are placed in a water bath at 25-30 C for 30 minutes

16) Tubes are left standing at room temperature for 10 minutes

17) Blanks are prepared by mixing 1 ml H_2O (distilled) 1 ml 5% phenol and 5 ml H_2SO_4

18) The absorbance is read in a LKB Biochrom Ultrospec 4050 spectrophotometer at 490 nm

The equation used for the calculation of the glycogen content of the tissue is:

$$\text{mg glycogen/g wet tissue weight} = \frac{(A490-A) \times V}{k \times v \times w \times r}$$

where A490= mean absorbance of 2 aliquots

k= slope of standard curve; units= 1 per ug glycogen

V= total volume of glycogen solution

v= aliquot volume of solution used for color reaction

w= weight in grams of tissue sample

A= y intercept

r= recovery

Appendix D

D.1 Figure 1.Glycogen levels in soleus muscle

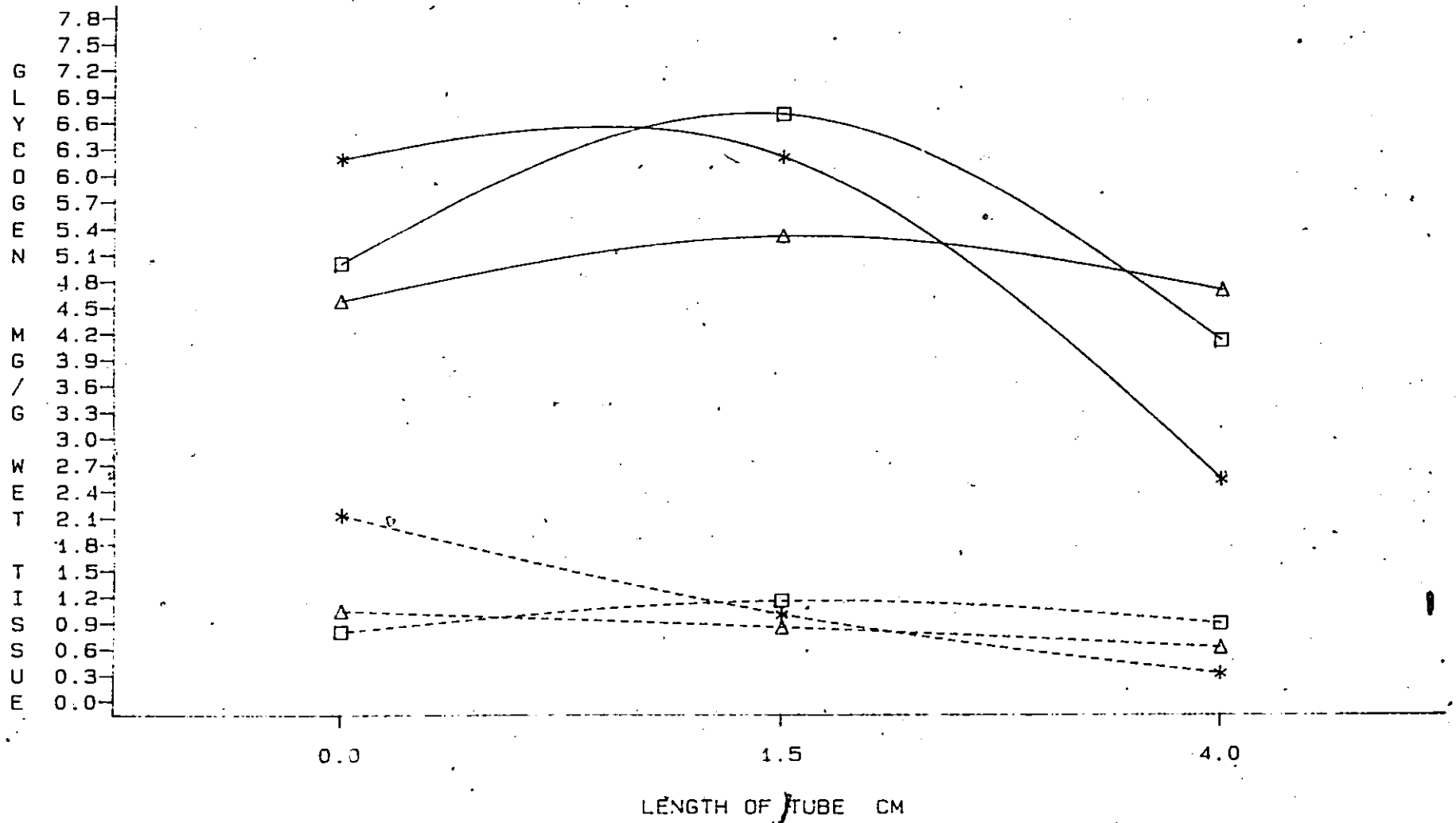


FIG. 1 GLYCOGEN LEVELS IN SOLEUS MUSCLE OF PROGESTERONE TREATED AND UNTREATED RATS

SOLID–STAR=REST7 TRIANGLE=REST14 SQUARE=REST21
DOTTED–STAR=EX7 TRIANGLE=EX14 SQUARE=EX21

Appendix E

E.1 Figure 2.Glycogen levels in plantaris muscle

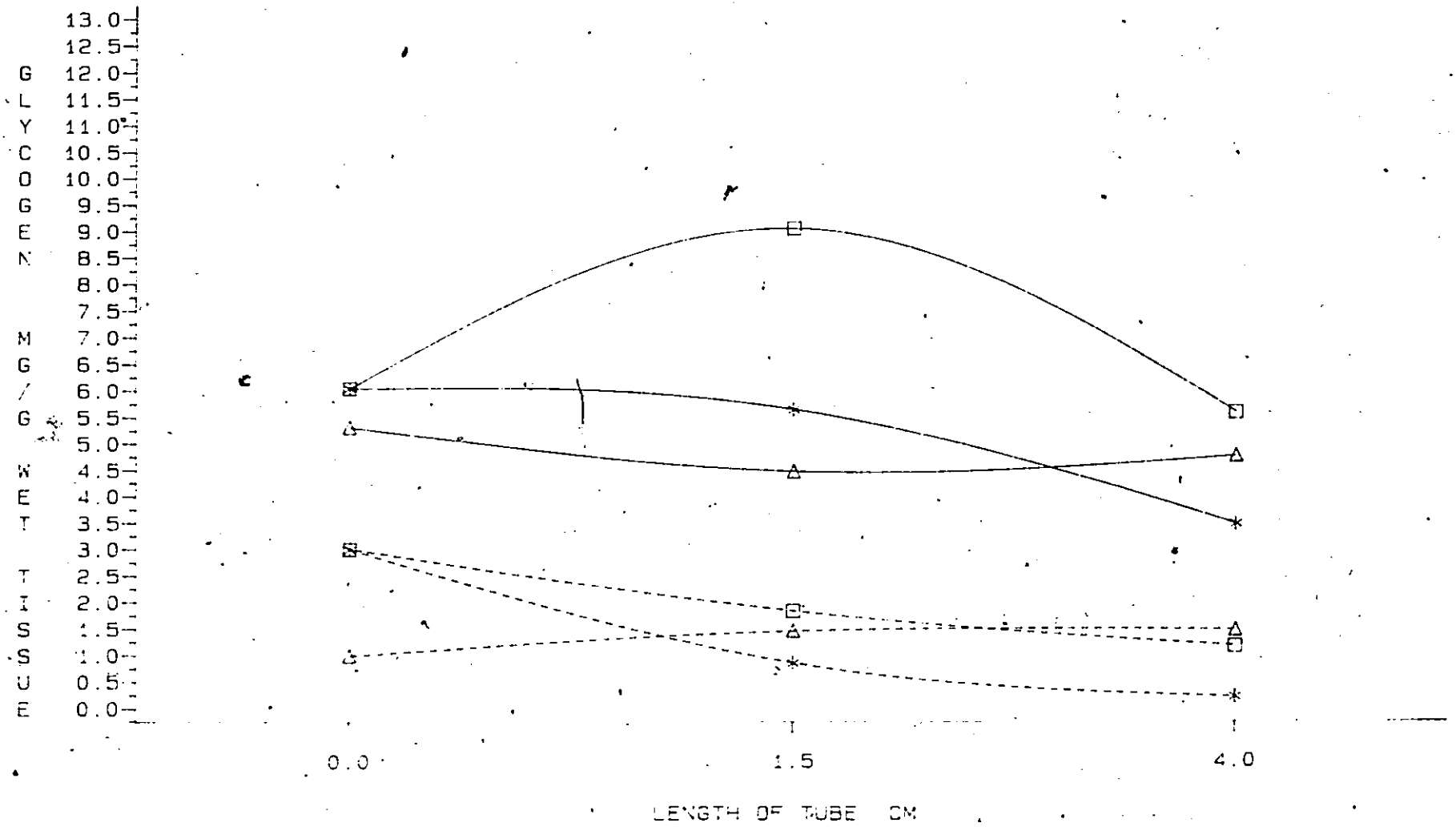
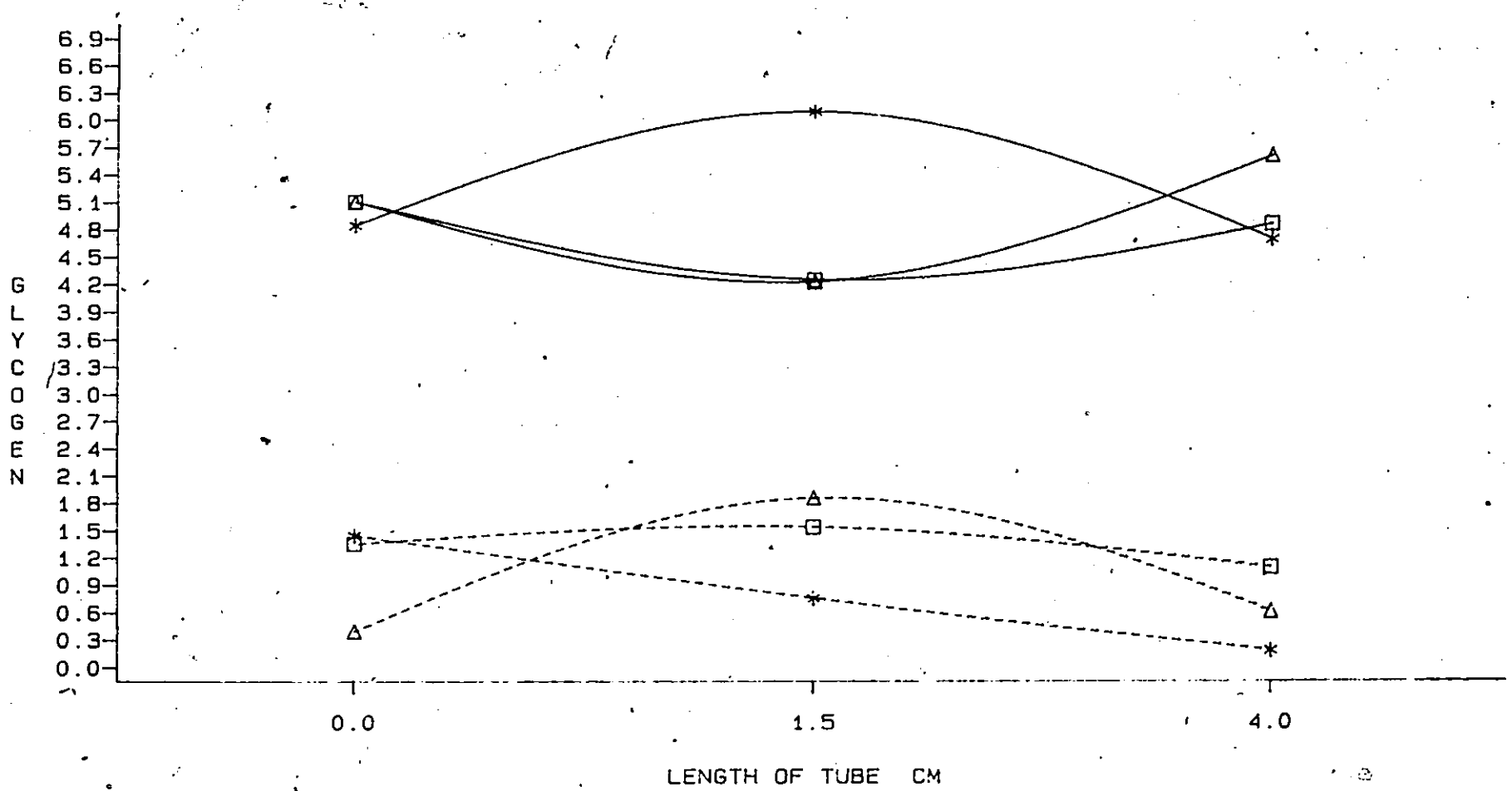


FIG. 12 GLYCOGEN LEVELS IN PLANTARIS MUSCLE OF PROGESTERONE TREATED AND UNTREATED RATS

SOLID-STAR=REST1 TRIANGLE=REST14 SQUARE=REST21
DOTTED-STAR=EX1 TRIANGLE=EX14 SQUARE=EX21

Appendix F

F.1 Figure 3.Glycogen levels in white vastus lateralis



SOLID–STAR=REST7 TRIANGLE=REST14 SQUARE=REST21
DOTTED–STAR=EX7 TRIANGLE=EX14 SQUARE=EX21

FIG. 3 GLYCOGEN LEVELS IN WHITE VASTUS LATERALIS OF PROGESTERONE TREATED AND UNTREATED RATS

Appendix G

G.1 Figure 4.Glycogen levels in liver

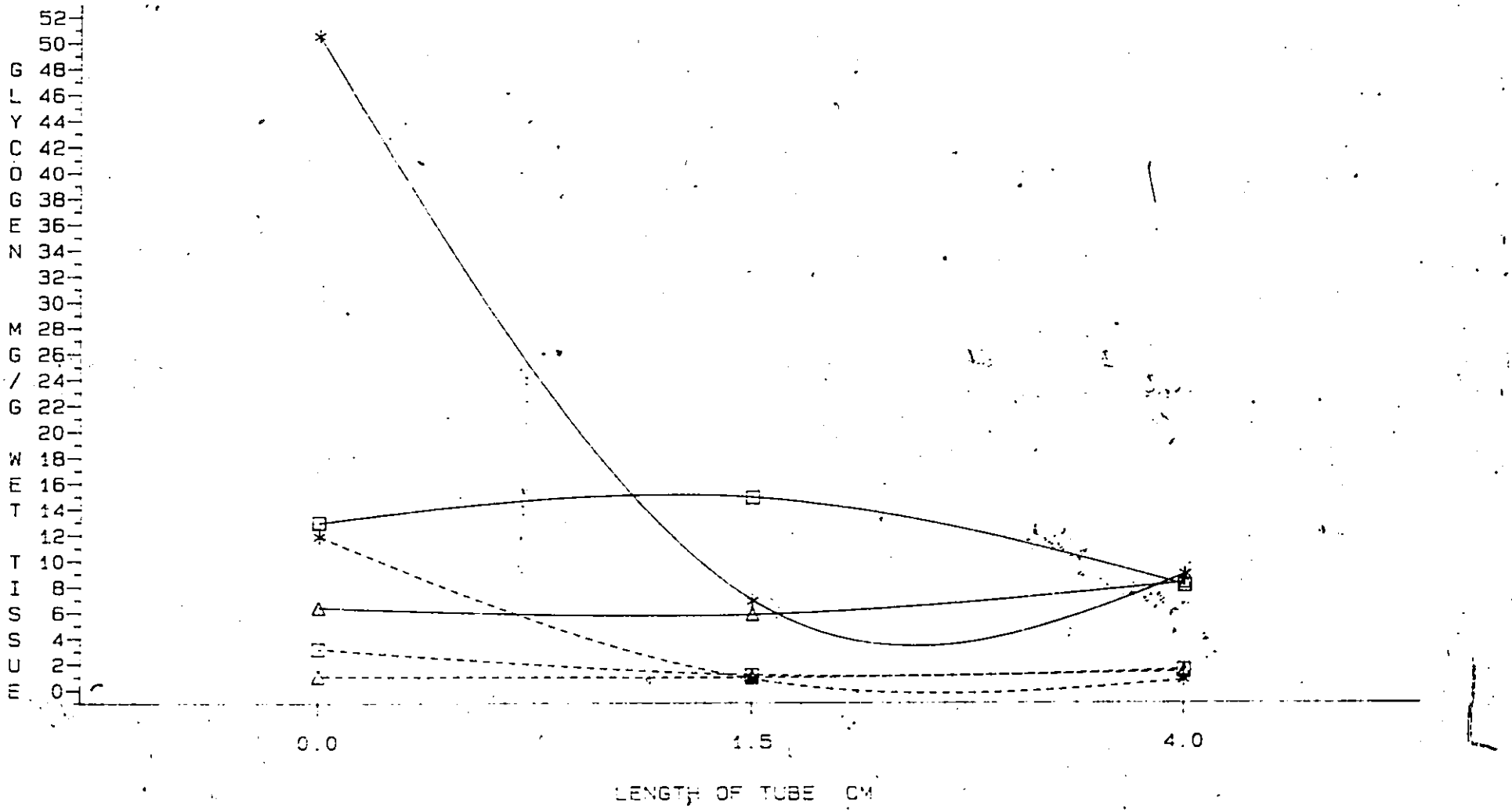


FIG. 4 GLYCOGEN LEVELS IN LIVER OF PROGESTERONE TREATED AND UNTREATED RATS

SOLID-STAR=REST7 TRIANGLE=REST14 SQUARE=REST21
DOTTED-STAR=EX7 TRIANGLE=EX14 SQUARE=EX21

Appendix H

H.1 Figure 5.Glucose levels

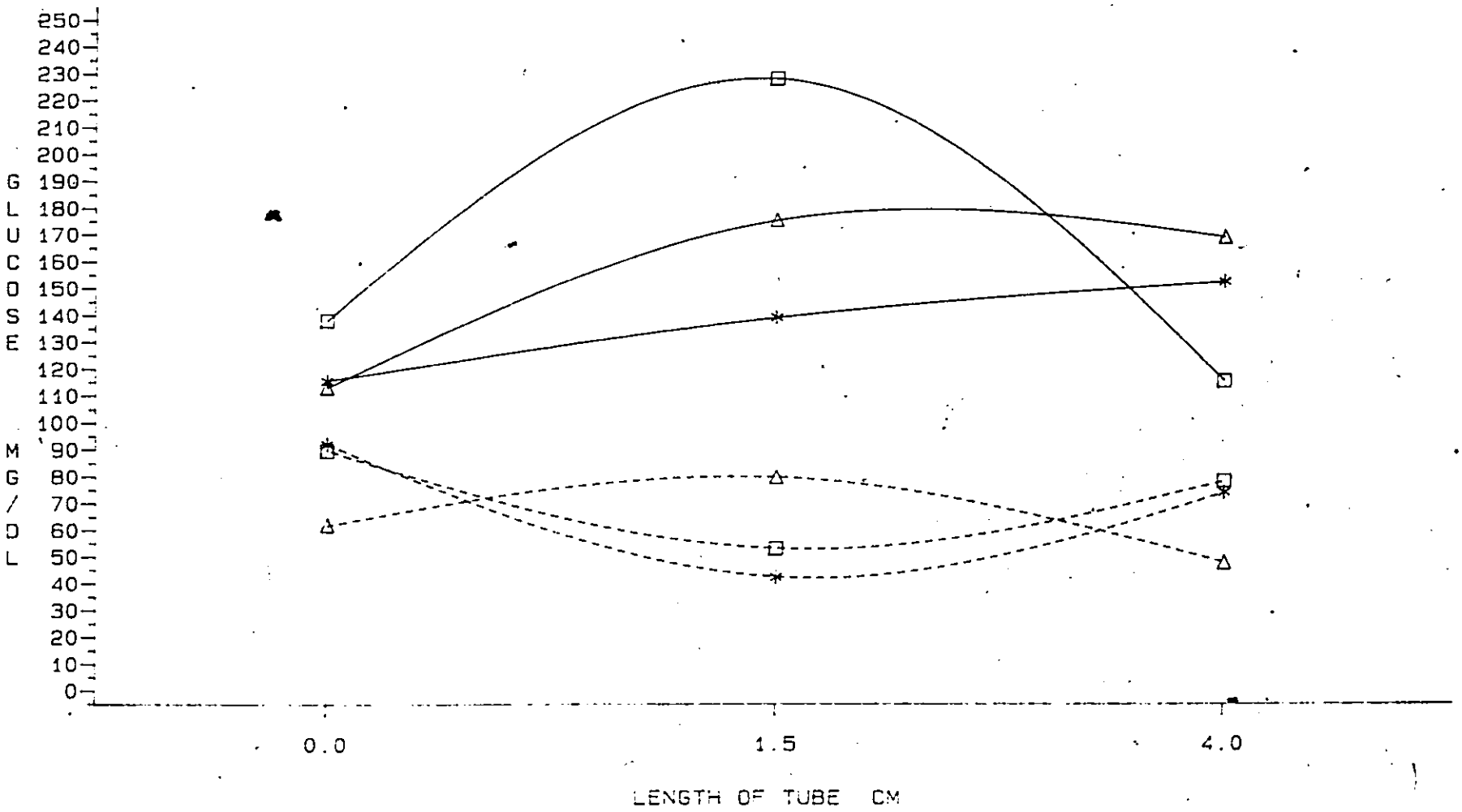


FIG. 5 PLASMA GLUCOSE LEVELS IN PROGESTERONE TREATED AND UNTREATED RATS

SOLID-STAR=REST7 TRIANGLE=REST14 SQUARE=REST21
DOTTED-STAR=EX7 TRIANGLE=EX14 SQUARE=EX21

Appendix I

I.1 Figure 6.Running times

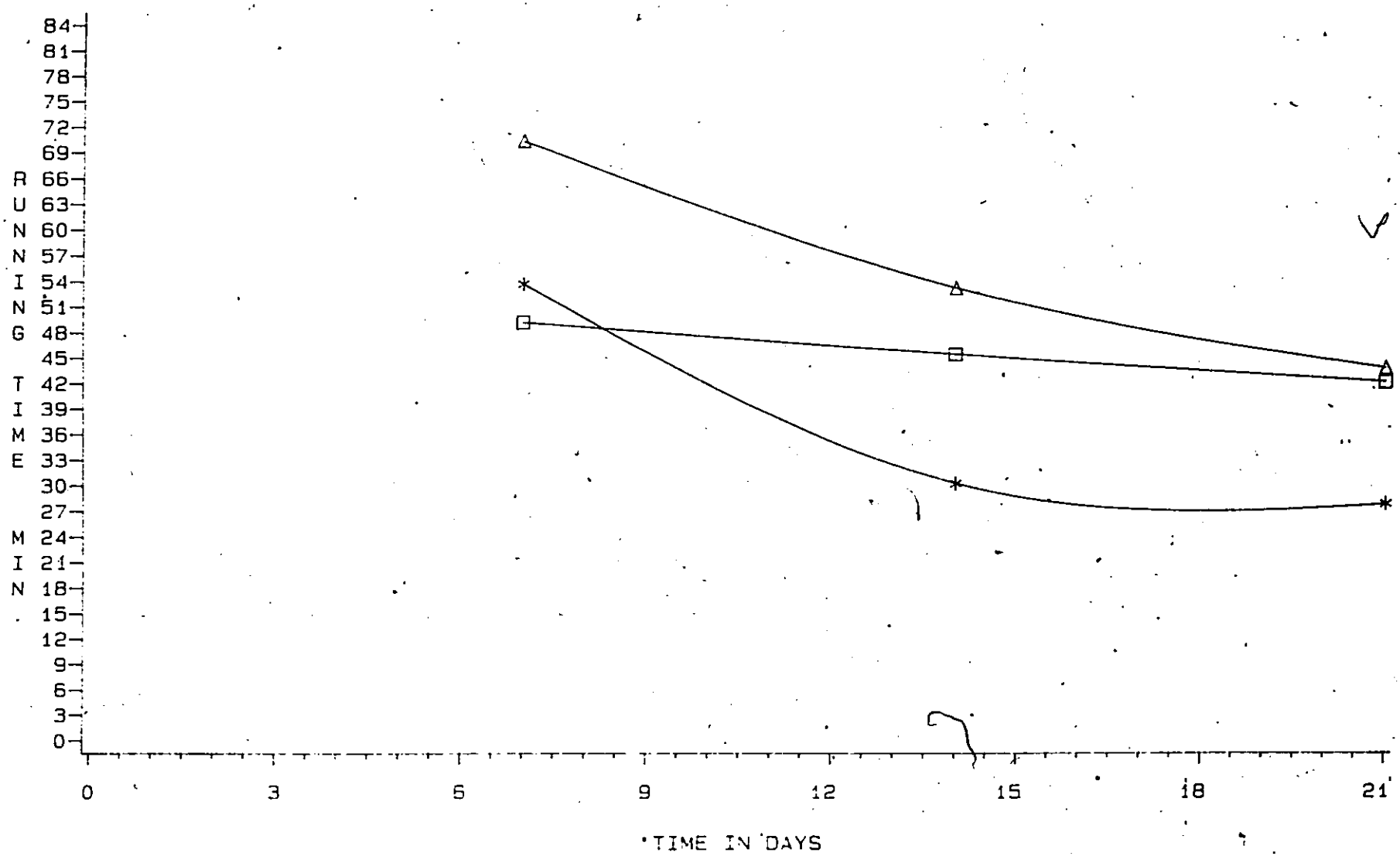


FIG. 6 RUNNING TIMES OF UNTREATED AND PROGESTERONE TREATED RATS

STAR=UNTREATED TRIANGLE=1.5CM TUBE SQUARE=4.0CM TUBE