CARBOHYDRATE METABOLISM IN RATS FED ORAL CONTRACEPTIVE STEROIDS

Thesis for the Degree of M. S.

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ABSTRACT

CARBOHYDRATE METABOLISM IN RATS FED ORAL CONTRACEPTIVE STEROIDS

By

Angela Kung-Mei Young

The effect of oral contraceptive steroids on the metabolism of different sugars (glucose, galactose, fructose and ribose) was studied in 11-week old female rats. One group of rats were fed ad libitum a basal grain diet containing the contraceptive steroids norethynodrel, a progestin and mestranol, an estrogen. A second group of rats, control, were pair-fed with the experimental rats receiving only the basal grain diet. Oral sugar tolerance tests were performed by gavage of sugars and collecting blood samples one week and four weeks of steroid treatment 0, 20, 40 and 120 min. after administering the sugar solution to the animals. Steroid treatment did not have any significant effect on fasting blood glucose level after one or four weeks treatment. After one week of steroid treatment, no effect of steroid on blood glucose level was found. However, after four weeks of treatment, those rats administered glucose ($p < 0.01$) or ribose ($p < 0.05$) had higher blood glucose levels, than the control group.

Urine samples were collected after two weeks of steroid treatment to determine the quantity of urinary sugars after sugar loading. Urine was collected successively for 6 and 18 hours after administering the sugar solution to the animals. No difference in urinary sugar levels was detected at 6 hours, however, at 18 hours, there was a higher urinary glucose level in the treated than the control animals when glucose ($p < 0.05$) or ribose ($p < 0.01$) was administered.

CARBOHYDRATE METABOLISM IN RATS FED ORAL CONTRACEPTIVE STEROIDS

By

Angela Kung-Mei Young

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Human Nutrition and Foods

1970

TO MY PARENTS

THIS IS MORE THEIRS

THAN

MINE

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INTRODUCTION

The availability of various synthetic estrogenic compounds enables a wide use in recent years for different treatment such as, contraception, estrogen deficiency symptoms, osteoporosis, and anovulatory menses. The physiological actions of the oral contraceptives are due to two hormones which are the synthetic counterparts of natural estrogen and progesterone. There are many studies on the effect of oral contraceptive pills and their action on pituitary and adrenocortical secretions and metabolisms of various nutrients. The changes in carbohydrate, fat, protein, mineral, and various other metabolisms associated with their hormonal action have also been studied. In spite of the many studies, the effect of oral contraceptive drugs on carbohydrate metabolism has not been clarified. Some investigators have reported that the estrogenic part of the oral contraceptive drugs has a diabetogenic effect in both man and experimental animals as indicated by impaired glucose tolerance tests.

Several studies have shown that fructose utilization is normal in diabetic man. The rate of fructose disappearance following intravenous infusion is only slightly

prolonged in diabetic human subjects, and changes in blood lactic, pyruvic, and α-ketoglutaric acid are of the same magnitude as in normal persons. Insulin fails to influence fructose utilization. Thus, the general purpose of this study is to gather information which might help in understanding the utilization of other monosaccharides besides glucose in oral contraceptive steroid treated subjects.

Specifically, this work was undertaken to find out whether: (1) contraceptive steroids have a diabetogenic effect on the metabolism of six carbon sugars such as glucose, fructose and galactose, and a five carbon sugar, ribose, in rats by measuring blood glucose and the respective sugar that was gavaged, and (2) urinary excretion rates of these sugars were affected by steroid treatment.

LITERATURE REVIEW

I. Structural Requirement for Activity

The idea of birth control with progesterone was initiated by Beard in 1897 (6), who postulated that the corpus luteum of ovary secretes progesterone, which is responsible for the inhibition of ovulation during pregnancy. In 1937, Makepeace et al. (59) demonstrated that progesterone will inhibit ovulation in rabbits. Later, Pincus and Chang (76) extended these studies and obtained similar antiovulatory activity with a series of synthetic progestins. In the early 1950's the orally active steroids with the biological properties of progesterone was developed by Searle Laboratories and Syntex Laboratories (28). Norethynodrel, the first synthetic oral progestin, was prepared by Frank B. Coltone at the Searle Laboratories in 1952. Later, estrogen was added to the progestin in order to increase the effectiveness of contraception, since estrogen can reduce the incidence of spotting and improve endometrial development (34). In addition to norethynodrel, several other progestational compounds have been used in the oral contraceptive pills. These include: norethindrone,

norethindrone acetate, methroxyprogesterone acetate, ethynodiol diacetate and chlormadinone acetate.

Numerous reports have shown structure-activity relationships for pregnene and estrogen derivatives with regard to glucose and insulin metabolism. The structural features of pregnene or nortestosterone and estrogen, i.e., steroids are shown in Fig. 1. The presence or absence of the C_{19} methyl group or of hydroxyl, ethinyl, acetyl, of acetoxy substitution at the C_{17} position do not appear to alter glucose or insulin metabolism. On the other hand, the presence of a partial positive charge at the C_5 carbon is common to mestranol and all of the pregnene and nortestosterone derivatives which are associated with increased insulin production following glucose stimulation. A double bond at the C_6 position appears to be a convenient way of separating the gluconeogenic or hyperglycemic effects of the steroid nucleus from its ability to stimulate the islet cells of the pancreas or sensitize them to glucose stimulation.

In monkeys, it has been shown that steroid compounds with a relatively positive charge at C_5 possess insulinogenic as well as insulin-resistance activities (Table 1) (7) which generally neutralize each other when measured in terms of the disposal of intravenously administered glucose. These two activities may be separable by the introduction of unsaturation at the C_6 position of the B ring of these steroid compounds.

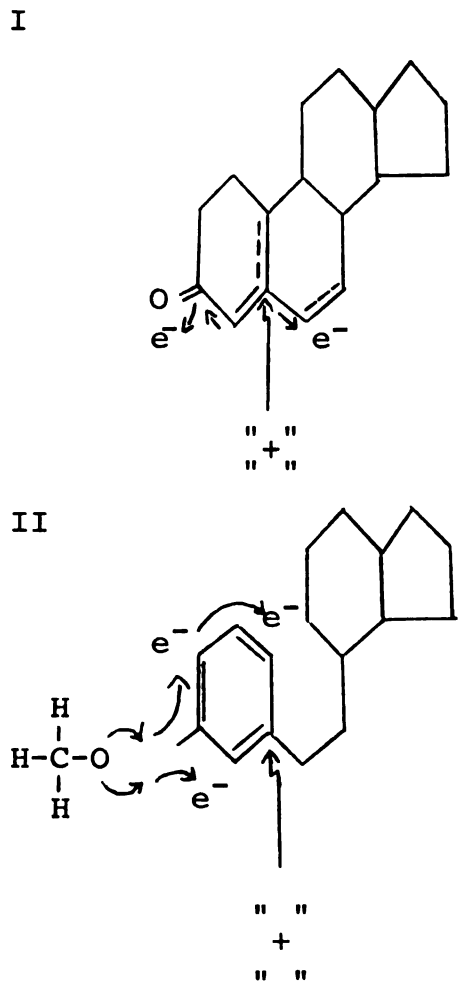

Fig. 1. Structural features of pregnene or nortestosterone (I) and estrogen (II) steroids which are essential for insulinogenic activity. + denotes a partial positive charge; e^-, electrons. Progesterone, and norethindrone are 4-dehydro-, 3-ketopregnene steroids; norethynodrel is a 5(10) dehydro- 3-keto-steroid; chlormadinine, 4-dehydro-, 6-dehydro-, 6 chloro-, 3-keto-steroid. Mestranol is a 3-methy-ether estrogen (7).

TABLE 1. Summary of insulinogenic and insulin antagonistic properties of gonadal and contraceptive steroids in Rhesus monkeys (7).

	Insulin Response	Resistance to Insulin	Glucose Tolerance
A. Insulinogenic, non-antagonistic			
Pregnene derivative			
Chlormadinone	↑	-	↑
B. Insulinogenic, insulin-antagonistic			
Pregnene derivative			
Progesterone	↑	↑	0
Nortestosterone derivatives			
Norethynodrel	↑	↑	0
Norethindrone	↑	-	0
Estrogen derivative			
Mestranol	↑	↑	0
C. Non-insulinogenic			
Nortestosterone derivative			
Ethynodiol diacetate	0	-	0
Estrogens			
Estradiol	0	-	0
Estriol	0	↑	0
Ethinyl estradiol	0	↑	0

↑ = increased or improved
↓ = decreased or worse
0 = no change
- = not measured

The chemical structure of two commonly used progestins in oral contraceptives are shown in Fig. 2. Norethynodrel, a progestin which is used in Enovid,[1] has a double bond at the 5'th position. This double bond at the 5'th position is biologically significant. In addition to being progestational, it makes norethynodrel estrogenic and devoid of androgenic effects in both animals and men (27, 90). The two estrogens presently used in all oral contraceptive preparations are either ethynylestradiol 3-methyl ether, mestranol, or ethynylestradiol (Fig. 2). It has been observed that mestranol behaves in a significantly different way with respect to carbohydrate metabolism than do other estrogens. The administration of mestranol produced a significant increase in the plasma insulin response to glucose and resistance to the hypoglycemic action of exogenous insulin, even though there was no significant alteration in the rate of glucose disposal.

II. Biological Effect of Progesterone and Estrogen on Glucose Metabolism

A. Progesterone

Although the effect of progesterone on the deposition of glycogen and glucose in the endometrial tissue has been established (105), very little is known about the

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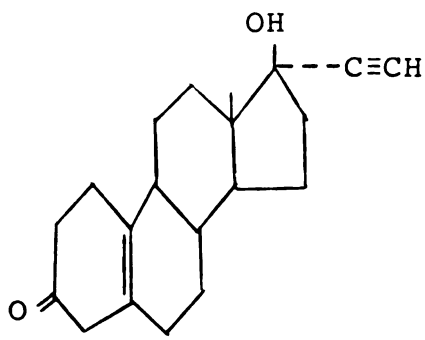
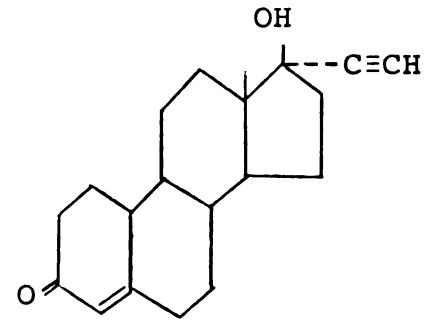
[1]Enovid- norethynodrel 5.0 mg and mestranol (ethynylestradiol 3-methyl ether) 0.075 mg.

PROGESTINS

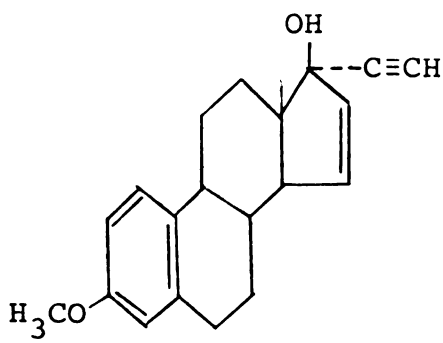


Norethynodrel

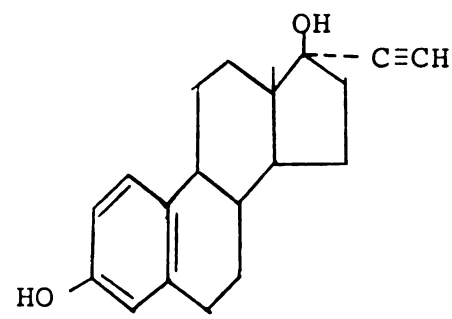


Norethindrone

ESTROGENS



Mestranol



Ethynylestradiol

Fig. 2. Structures of two of the progestins and the estrogens commonly used in oral contraceptives.

systemic effects of progesterone on carbohydrate metabolism (72). Human studies provide good evidence that the progestin components of at least some oral contraceptive agents may alter the glycometabolic effect of synthetic estrogens. Nevertheless, analysis of the data for individual birth control pills shows that not all contraceptive progestins affect carbohydrate tolerance adversely, and the effect may depend on the quantity and chemical structure of the progestin administered and the type of glucose tolerance test employed.

A few studies suggested that the 6-dehydro-derivatives of progesterone may actually improve carbohydrate tolerance by counteracting the diabetogenic effect of the estrogen present in the preparation. Studies by Beck, O'Haver and Bestley (8), found that neither subclinical nor non-diabetic individuals showed consistent changes in oral glucose tolerance over a 2 1/2 month period of treatment with 0.5 mg chlormadinone (6 α-chloro, 6-dehydro, 17-α-acetoxyprogesterone) daily. However, the mean integrated plasma insulin response to glucose was elevated in the non-diabetic subjects, but not in the diabetic subjects, after 2 1/2 months of treatment.

Recently, Benjamin and Casper (9) found that the carbohydrate tolerance improved in women with endometrial hyperplasia or carcinoma after treatment with 17-hydroxy-progesterone caproate. They also found that there was a

depression in plasma inorganic phosphate level in every case following the glucose administration. They suggested that the hormone may in some way favorably influence total body glucose utilization and metabolism, as reflected by an improvement glucose tolerance curve. Probably, the improvement was not mediated through the adrenal cortex since the urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids was unchanged by the hormone. In contrast, Schreibman (92) has reported that glucose tolerances deteriorated in several diabetic subjects treated with the same compound. In Beck's recent work (7), it has been shown that there were no consistent changes in either the mean fasting serum glucose or insulin concentrations in rhesus monkeys after three weeks of treatment with progesterone. Although he found the mean serum progesterone level (17 mμg/ml) 16 hours after the first progesterone injection was 12 times greater than the control value. However, progesterone treatment enhanced the mean plasma insulin response in monkeys without significant alterations in the glucose disappearance rates following Intravenous (I.V.) glucose administration. Beck (7) also found that progesterone reduced the sensitivity of these animals to the hypoglycemic action of insulin. Thus, it was shown clearly that progesterone enhanced insulin release following glucose stimulation in the rhesus monkey and produce a mild but significant peripheral resistance to the hypoglycemic action of insulin.

And he suggested that the insulin antagonistic effect of progesterone did not appear to be mediated by growth hormone since fasting serum growth hormone concentrations were not altered significantly by progesterone treatment.

Wynn and Doar (126) reported that there was a higher incidence of abnormal oral glucose tolerances in women who received ethynodiol diacetate plus mestranol than mestranol alone. Thus it was indicated that ethynodiol diacetate could enhance the diabetogenic effect of mestranol.

B. Estrogen

It has been suggested that the estrogen component of the contraceptive drugs is apparently responsible for the changes in carbohydrate metabolism, because of the fact that treatment with a progestational agent alone does not alter glucose tolerance (39, 80).

In 1954, Houssay and co-workers (48) clearly described the effect of estrogenic substances on carbohydrate tolerances. They found that during the first month of estrogen treatment the severity of diabetic symptoms was greater in the estrogen-treated animals than in the control animals. Subsequently, the hyperglycemia and glucosuria were attenuated or suppressed completely in the estrogen-treated animals due to the enhancement of peripheral glucose utilization by estrogens. Nevertheless, treatment with estrogens has not always resulted in improvement in glucose metabolism. In some depancreatized diabetic animals,

estrogens either did not affect or increase the severity of diabetes.

Javier and associates (49) were probably first to show that glucose tolerance might deteriorate in human subjects treated with mestranol alone as well as with mestranol in combination with norethynodrel (35). Beck (7) indicated that subclinical diabetic individuals were much more subjected to deterioration of glucose tolerance than non-diabetic individuals following exposure to mestranol. It seems that mestranol produces deterioration of glucose tolerance only if the increased peripheral resistance to the hypoglycemic action of insulin is not compensated by an additional elaboration of insulin (7). Consequently, only those subjects with borderline or limited pancreatic islet insulin reserve will show deterioration of carbohydrate tolerance following exposure to mestranol. This is the reason why the subclinical diabetic individuals are more subjected to deterioration of glucose tolerance following exposure to oral contraceptive agents than non-diabetic individuals (7).

Diddle et al. (18) investigated 525 patients who received norethindrone and norethindrone acetate with or without ethinyl estradiol during 24-77 cycles. They found that norethindrone and norethindrone acetate with or without ethinyl estradiol did not produce deleterious metabolic effects when administered continuously for as long as 6

years. In contrast, Pyorala and Lampinen (82) reported a significant slowing of glucose disappearance rate in 5 subjects treated for 20 days with ethinyl estradiol. They indicated that this estrogen may be an insulin antagonist in man as well as in monkey. However, they also reported that ethinyl estradiol did not appear to produce hyperglycemia as readily as mestranol in man.

Currently, mestranol is extensively employed in estrogen therapy in oral contraceptive because of its marked inhibitory effect on FSH secretion, and its capacity to stimulate the genital tract. DiPaola and co-workers (19) found that only those patients who received mestranol had highly significant percentage of abnormal prednisone glucose tolerance tests (PGTT).[1] The percentage of diabetic type PGTT was higher in women with diabetic family histories and early menarche (before the twelfth year) than non-diabetic and later menarche.

Since it is known that estrogen can cause elevations in plasma protein-bound iodine and plasma cortisol levels without necessarily increasing their functional levels (1, 47, 56, 69), it is possible that these plasma protein elevations may have a short-term effect in decreasing glucose tolerance in both animals and humans. Over a longer time

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[1]Prednisone administration causes an increase in blood glucose levels and in normal subjects an increase in insulin secretion.

period, the effect may act as a stimulus to the beta cells' proliferation, with increased insulin production and improvement in glucose tolerance (2, 98).

C. Progesterone and Estrogen

Carbohydrate and insulin metabolisms have been studied more frequently in women taking norethynodrel with mestranol (Enovid) than any other oral contraceptive agent. Relative impairment of oral and intravenous (I.V.) glucose tolerances has been found in certain women receiving estrogen-progestagen combination (35, 79, 106, 107, 108, 126). There is no uniform agreement for the incidence of altered glucose tolerance after estrogen-progestagen treatment (40).

Peterson, Steel and Coyne (75) found that neither the dosage of the estrogenic agent nor the type of progestin used seemed to affect the incidence of decreased glucose tolerance, when they correlated decreased glucose tolerance with the type of medication the patient received. Posner and co-workers (79) found that the effect of the oral contraceptives does not continue to progress indefinitely in their patients treated with norethynodrel plus mestranol and this might be due to a certain amount of pancreatic reserve which was taken up by the hormonal contraceptives.

Buchler and Warren (12) indicated that administration of diethylstilbestrol or norethynodrel with mestranol may produce within 30 days, an abnormal glucose tolerance

curve that mimics that seen in diabetes mellitus. But there was no change in the intravenous (I.V.) glucose tolerance curve. Thus, they suggested that estrogen might be responsible in delaying gastrointestinal absorption rather than exerting a diabetogenic effect. A similar lack of correlation between I.V. and oral glucose tolerance tests in women during gestation has been reported by Benjamin and Casper (10). Moreover, the work of McIntyre (63) and of Unger (115) clearly demonstrated that oral glucose administration resulted in a release of intestinal factors which might play a part in regulating the rate of insulin secretion. Posner et al. (78) reported that the I.V. glucose tolerance in women treated with Enovid[1] or Ovulen[2] was different, when the observation was done early in the treatment. There was a significant tendency for the K value[3] to decline in women taking Enovid, but not for those taking Ovulen. They suggested that this might be due to the progestin in the drug, since the progestin in Enovid is more estrogenic than progestin in Ovulen. Similarly, Puchulu et al. (74) have shown that 13% to 66.7% of women in their

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[1]Enovid-norethynodrel 5.0 mg and mestranol (ethynyl estradiol 3-methyl ether) 0.075 mg.

[2]Ovulen-ethynodiol diacetate 1.0 mg and mestranol 0.1 mg.

[3]K values--the index of tolerance or the percentage decrease per minute of blood glucose over 10-60 min. following the rapid I.V. injection of 25 gm. of glucose (89).

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study developed an abnormal oral glucose tolerance test (O.G.T.T.) or a cortisone-sensitized O.G.T.T.[1] after treatment with a combination of mestranol and norethisterone acetate for 20 days. The corresponding figures were 6.5% to 11.1% in women treated with a combination of ethinyl estradiol and noresthisterone acetate for the same period. It indicated that the estrogen component of the contraceptive drugs is apparently responsible for the changes in carbohydrate metabolism. Pyorala et al. (82) indicated that glucose tolerance decreased during the estrogen phase of a sequential treatment and then slightly improved during the following phase of combined estrogen-progesterone treatment. Halling, Michals, and Paulsen (42) found that there was a significant impaired carbohydrate tolerance in their patients after a long-term treatment with ethynodiol diacetate-mestranol. The fasting glucose levels during treatment were unaffected, and the impaired carbohydrate tolerance reversed to normal when the treatment was discontinued. Later, Wynn and Doar (127) reported that the mean fasting plasma glucose level was not significantly changed by oral contraceptive therapy both in a group which contained 67 women who were tested before and again while receiving oral contraceptive therapy, and another group

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[1]Cortisone administration causes an increase in blood glucose levels and in normal subjects an increase in insulin secretion.

which contained 24 women who were tested during therapy and after withdrawing the drugs. There was a significant impairment of oral and I.V. glucose tolerance during therapy and it improved after oral contraceptive therapy was discontinued.

Starup et al. (112) treated 27 non-diabetic women with a daily dose of 5 mg of megestrol acetate and 0.1 mg of mestranol for 12 months in their study. They reported that there were no significant changes in the fasting blood sugar, the K-value in the I.V. glucose tolerance test, the fasting serum insulin and the response in serum insulin after an I.V. glucose stimulus, which were tested before treatment started, after 12 months of treatment and one month after withdrawal of treatment. The diabetic woman in their study showed a significant increase in fasting blood glucose and a slight decrease in the K-value during the treatment period. Her insulin level was not changed during treatment. Thus they concluded that treatment with megestrol acetate and mestranol has no effect on the carbohydrate metabolism in normal fertile women with no family history of diabetes.

III. Effect of Progesterone and Estrogen on Insulin Secretion

There have been few studies on plasma insulin levels during oral glucose tolerance tests in subjects receiving oral contraceptives. Most (49, 106, 108, 112), but not all

(107, 112), previous workers have found that the fasting plasma insulin levels are unchanged during oral contraceptive therapy. Spellacy et al. (102, 106, 107, 108) observed higher plasma insulin levels in patients during treatment than pretherapy levels during I.V. glucose tolerance tests. This observation was confirmed by Wynn and Doar in 1969 (127). On the other hand, Starup et al. (112) found that there was no change in insulin level after steroid treatment. Javier et al. (49) found higher plasma levels early in treatment but, with prolonged therapy insulin levels tended to be lower in some subjects as glucose tolerance and insulin levels were elevated soon after contraceptive steroids were used. Thus when the insulin level was high enough it might have brought the blood glucose level back to its pre-treatment range after several months of use. But with prolonged usage the glucose levels in some subjects would rise again, and he suggested that this incidence of decompensation with the resultant abnormal glucose tolerance curve might be due to the alteration in the insulin secretion mechanism, thus delaying the insulin release and perhaps alter the type of insulin that is released (proinsulin) (114). Javier, Gershberg and Hulse (49) found that insulin secretion tended to increase as the blood glucose level increased early in oral contraceptive treatment. They suggested that the failure of the pancreas to response to hyperglycemia might cause the decrease of

insulin secretion in certain women as glucose tolerance decreased with prolonged treatment. They indicated that the estrogen in the contraceptives is the major cause of these metabolic changes, though the progestin might exert an additional effect by being converted in the body to substances having estrogenic activity (73). The pathway by which estrogen impairs glucose tolerance are not clear yet. From what is known of their actions Pyorala et al. (82) and Kitay (54) suggested that it could impair carbohydrate utilization by stimulating growth hormone (GH) and adrenocorticotropic secretion. Estrogens could also act by modifying liver function (55, 68), increase uterine and liver glycogen (51, 117) and influence the enzymes involved in the disposal of glucose (5, 64). Recently, Kalkhoff, Kim and Stoddard (52) indicated that an acquired form of subclinical diabetes mellitus was found among women receiving mestranol-containing oral contraceptive agents. It was also shown that there was an impaired prednisolone glucose tolerance in these subjects and which was associated with a defective plasma insulin response. It was also clearly indicated in their study that 8 1/2 hours of prednisolone administration had a suppressive effect on the plasma insulin response to oral glucose in these subjects. Whether, mestranol has a direct deleterious effect on pancreatic islet secretion of insulin is still unknown, although there was a normal response of insulin secretion by prednisolone administration

in the drug-treated group after the contraceptive agent was withdrawn. However, Gold and his co-workers (38) found that blood glucose rose significantly in overt diabetics treated with Ovulen as well as with ethynodiol diacetate alone for one month. Ovulen-induced hyperglycemia, demonstrable both during fasting and following introduction of glucose loads, was not accompanied by: (a) increased concentrations of endogenous plasma insulin, (b) increased resistance to administered insulin. They interpreted this to mean a loss of beta-cell sensitivity of "indifference" to fasting hyperglycemia while still retaining the capacity to response to acute increments in blood glucose. It seemed that in diabetics the insulin secretory response, once initiated, assumes a fixed, somewhat autonomous pattern no longer related either to the magnitude or the duration of the hyperglycemic stimulus (38).

IV. Hormonal and Metabolic Changes which Affect Carbohydrate Metabolism During Oral Contraceptive Therapy

Elevated circulating levels of plasma cortisol (65, 81), thyroxine (26, 85), GH (110) and pyruvate (128) have been noted during oral contraceptive therapy, and the estrogenic component is thought to be responsible. Some investigations have shown that estrogens have a direct action on the peripheral insulin by: (a) increasing hormones antagonistic to insulin (ACTH, corticoids, or human growth

hormone) (17, 27, 32, 41, 56, 65, 69, 77, 87), (b) increasing in plasma antagonists (synalbumine, nonesterified fatty acids, etc.) (2, 3, 11), and (c) increasing the combination of insulin with plasma protein (30, 46, 57, 65). The possibility exists that one or more of these may be responsible for the observed carbohydrate metabolic changes. The elevated plasma levels of cortisol and thyroxine are due to increased levels of the respective binding proteins, and the free non-protein bound hormone concentrations are probably unchanged. Nevertheless, the possibility exists that the protein bound hormone, in general thought to be biologically inactive (62), may cause diminished carbohydrate tolerance in certain subjects taking oral contraceptives.

It has been demonstrated by Walass (117) in adult rats that estrogens could affect carbohydrate metabolism by elevating glycogen formation in liver and which in turn may cause the increased blood sugar level. He suggested that this effect may be due to an upset of the hormonal balance in the organism. Fry, Miller and Long (33) have indicated that the estrogenic effect on liver glycogen content is probably transmitted through the pituitary-adrenal system, since they were unable to find any glycogen formation in the liver after estrogen injection in hypophysectomized or adrenalectomized rats. Walass (117), also concluded that the changes in carbohydrate metabolism after estrogen administrations may be explained by an increased anterior pituitary and adrenocortical secretion.

A. Cortisol

It is well known that glucocorticoids promote hyperglycemia by augmenting hepatic glucose production (122) and impairing peripheral glucose utilization (83). And it was found that estrogens greatly potentiate this hormonal action because of the elevation of plasma free cortisol (71, 77) and an increased cortisol secretory rate (53).

Transcortin, a plasma protein with high affinity for cortisol, and corticosterone, had been shown to be elevated during pregnancy and following administration of estrogens. In the same time, there was an increase of plasma cortisol (87, 97, 118). A more clear picture was given by Sandberg et al. (88) in 1963. They found that administration of adequate amounts of estrogens produced increased concentrations of transcortin within 3 to 7 days, followed by a rise in the levels of the 17-hydroxycorticosteroids (17-HOCS). The levels declined to normal within 7 to 10 days after cessation of estrogen treatment. It has been indicated that estrogen is the only steroid which can cause the elevation of plasma transcortin level. Sandberg et al. (88) also postulated two hypotheses from indirect evidences that transcortin-bound cortisol is: (a) biologically inactive, and (b) unavailable for catabolism (15, 66, 67, 74, 119). The first hypothesis was supported by the observation that transcortin prevented cortisol-induced glycogen deposition in adrenalectomized mice treated with cortisol (96). The

second hypothesis was clarified by the findings that less cortisol was metabolized in the presence of plasma from pregnant women or subjects given estrogens, than was observed in the presence of normal plasma or no plasma at all (88). In 1965, Leach and Margulis (57) investigated the inhibition of adrenocortical responsiveness during progestin therapy. They found that there was an increased value of 17-hydroxysteroid excretion which was obtained two months following cessation of therapy. It indicated that there was a definite suppression of responsiveness to metyrapone[1] during treatment with norethindrone acetate, 2.5 mg, and ethynyl estradiol, 0.05 mg. Since the response to administered ACTH was not affected they suggested that the changes due to the estrogenic activity appeared to be related more to inhibition of pituitary responsiveness than to altered adrenal cortical activity. In another study a reduced level of ACTH was noted in some of the Enovid users with reduced glucose tolerance. These findings suggested a direct action of steroids especially estrogen on adrenocortical secretion (116).

Kitay (54) reported that spayed female rats had lower plasma steroid concentrations at rest and after stress or after ACTH injection when compared with intact controls. Also there was an impaired steroid turnover in spayed female

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[1] A compound which can stimulate the secretion of pituitary hormones.

rats with prolonged steroid half-life and decreased liver activity. In his *in vitro* study he demonstrated that estradiol stimulated steroid production by increasing adrenal steroidogenesis. He suggested that ACTH secretion is modified by the gonadal hormones without mediation by the adrenal glands. Mills *et al.* (67) found that the half-time for disappearance of plasma hydrocortisone was considerably prolonged by administration of estrogen. They also found that administration of estrogen had two distinct effects on hydrocortisone metabolism. The effects are: (a) a marked rise in the level of protein-bound steroid, and (b) an increased total pool, out of proportion to the rise in the level of plasma hydrocortisone and simultaneously a decreased excretion of metabolites. They suggested that this probably represents protection from destruction by the liver by means of greater protein bindings.

B. Pyruvate

It has been noted that both the fasting blood pyruvate level and/or the maximum blood pyruvate increment above the fasting level can be increased in certain women receiving oral contraceptives. These changes resemble the changes found in subjects receiving glucocorticoid drug (128). But it is not known whether the increased blood pyruvate levels were due to an increased rate of pyruvate production and/or impaired pyruvate removal by the estrogen and/or progestagen, although some findings indicated that

it is due to increased rates of production rather than impaired removal of this metabolite. It has been suggested that these increased rates of production were mainly due to the estrogen component of the drug which may increase amounts of glucose passing down the glycolytic pathway to pyruvate. In a cross-sectional study by Wynn and Doar in 1966 (128), it was found that there were impaired oral and I.V. glucose tolerances, elevated fasting plasma non-esterified fatty acids levels, and elevated venous blood pyruvate levels, both before and after oral or intravenous glucose administration in women taking oral contraceptives. Their recent study (127) confirmed the majority of these findings with the exception that mean plasma non-esterified fatty acids levels before and after glucose administration were not affected by oral contraceptive therapy. Most of these metabolic changes were reversed after oral contraceptive therapy had been discontinued. Elevation of blood pyruvate levels were found in six women treated with estrogen alone (127). It was not determined with the progestagen treatment. Furthermore, it is not known whether these changes result from increased levels of other circulating hormones such as cortisol, growth hormone and thyroxine.

Some studies suggested that the fasting blood pyruvate level is neither increased in thyrotoxicosis (22), nor in control subjects after L-triiodothyronine administration (111). No elevation of blood pyruvate levels could

be found above control values during oral and I.V. glucose tolerance tests in acromegalic subjects (22), nor during I.V. glucose tolerance tests performed after administration of human growth hormone to control subjects (22). The elevated blood pyruvate levels during oral contraceptive therapy resembled those found during glucocorticoid therapy (44) and also those found in obesity (24, 25). It is possible that the biological inactive protein, transcortin (62), which is thought to be involved in the elevation of plasma cortisol levels during oral contraceptive therapy may have biological activity in certain tissues, such as the liver, and thus may be responsible for the changes of glucose tolerance and blood pyruvate levels found during oral contraceptive treatment.

Since the plasma cortisol levels are normal in obesity and the cortisol production rate is often increased (93), the hepatic clearance of cortisol must therefore be increased and it is possible that this may be responsible for the impaired oral glucose tolerance and elevated blood pyruvate levels commonly found in this condition.

C. Growth Hormone

The effect of estrogens on growth hormone secretion has not been clarified. Early work by Young (129) demonstrated GH has diabetogenic effect. Other investigations have shown that estrogens can cause a marked increase in pituitary secretion and plasma levels of growth hormone in

human. This effect may be due to enhanced sensitivity of the growth hormone releasing mechanisms by estrogens. A similar effect was found when the various endogenous estrogen production associated with differences in sex, age, and time of the menstrual cycle were correlated with plasma GH levels (104). Frantz and Rabkin (32) found that the elevation of plasma human growth hormone (HGH) in response to insulin was not affected by estrogen treatment, but the plasma HGH level of the fasting subject before insulin injection was elevated in the estrogen treated group. They indicated that increased pituitary secretion, rather than decreased peripheral degradation, was responsible for the elevated HGH levels seen after estrogen administration. Increased pituitary secretion may be due to the possibility that estrogen in some way increases intracellular glucose requirement, not entirely reflected by the blood sugar, and that this in turn acted as the stimulus for growth hormone release. Spellacy et al. (102, 109) found that there was a significant elevation of the HGH values in fasting women at rest produced by the oral contraceptive. In other studies, they confirmed that both blood glucose and plasma human growth hormone levels were elevated in drug treated group after three months (110) and twelve months (103) of treatment. They suggested that the estrogenic action of the tablets caused an elevation in the circulating HGH levels. The

HGH can then antagonize insulin action and this results in an elevation of blood glucose level, and a resistance to an insulin-induced hypoglycemia.

MATERIALS AND METHODS

One hundred and four, 10 weeks old female Sprague-Dawley rats (Spartan Research Animals, Inc., Williamston, Mich.), having an average body weight of 220 grams (196 - 255) were used in this experiment. All the rats were housed individually in metabolism cages, and maintained at a constant temperature of 27°C with 12 hours each of light and darkness. All the animals were fed ad libitum a grain diet (14) (Appendix 1) for one week. At this time the rats were paired according to body weight, and one of each pair was fed the grain diet plus norethynodrel and mestranol ad libitum. This diet was prepared by dissolving the two steroids in 70% ethyl alcohol and mixing the solution with the grain diet with a Hobart mixer. The diet contained 0.0275 mg mestranol and 1.83 mg norethynodrel per kg diet and provided approximately similar dosage used by women (0.1 mg norethynodrel and 0.0015 mg mestranol per kg per day). The dosage used in the study was based on several other experiments wherein feed containing the steroids had been measured (61).

The amount of diet containing steroids consumed by individual rats was measured every afternoon at 2:00 o'clock

throughout the experiment. For this study, the average food consumption was 15 g per day (Appendix Fig. 2). Since their average body weight (Appendix Fig. 1) was 250 g, the average mestranol and norethynodrel consumed was 0.0017, 0.11 mg per kg body weight. Diet spillage was measured and recorded, if there were any, and subtracted from the total daily food intake. The data were then used as a basis for the individual pair-feeding of the remaining 52 control rats with the basal diet only.

At the end of the first, second and fourth week of the pair feeding, all rats were weighed and then fasted from 5:00 pm to 8:00 am. The rats were then lightly anesthetized with ether and gavaged with one of four different sugars (300 mg in 1 ml of water/100 g body weight). During the first and fourth week sugar tolerance tests were obtained for four pairs of rats for each sugar at each time interval of 20, 40 and 120 minutes (Appendix II) after gavage by obtaining blood samples with a cardiac puncture technique (13). Another four pairs of rats were bled immediately after fasting, and considered as 0 time interval in the sugar tolerance test.

The other gavage at the end of the second week was done in order to measure urinary excretion rate of the four sugars by collecting urine for 6 and the subsequent 18 hours. Urine was collected by metabolism funnels into flasks containing toluene to prevent bacterial growth.

Similar collection of urine were made for the four pairs of rats used for the zero time blood collection. These rats were not force-fed any sugar.

Serum and urine samples were kept in the freezer until analysis. Before analyzing for the sugars in the serum 1.8% $Ba(OH)_2$ and 2% $ZnSO_4$ were used to deproteinize and neutralize the serum respectively (70, 99, 100). The ratio for the serum, the two reagents, and water for deproteinization was 1:5:5:9. This mixture was centrifuged and the supernatant used for sugar determinations.

Urine was treated by a modified method of Saloman and Janson (86) before analysis. Amberlite (Rohn and Jass Co.) Ion Exchange resins, IR-120 and IR-45 were washed with distilled water, dried in air at room temperature and combined in a 1-1 mixture. Three ml of urine were added to 3.0 g of resin in a small beaker. After standing for 30 min., the moist resin mass was transferred to a glass funnel and the deionized urine was removed by suction into a test tube within a filter flask. The deproteinized sera and urine eluates were analyzed for glucose in addition to the appropriate sugar.

Glucose was determined by the method of Washka et al. (120) using "GLUCOSTAT." Galactose was determined by a similar enzymatic method "GALACTOSTAT" (4). Fructose was determined by using the method of Roe et al. (84). And ribose was determined according to the method of Dische and Borenfreund (20).

All data were analyzed by analysis of variance (21). Since a few rats died during blood collection due to hemorrhage caused by uncontrollable accidental puncturing of major blood vessels around the heart, and in order to have the same number of observations needed for the computer analysis, only half the original number was used for statistical analysis. Data used for statistical analysis were chosen randomly and analysis of variance (21) performed by using the University computer. Dr. John Gill (Dairy Department) and Mr. Bill Allard (Computer Center) helped in programming the data for the statistical analysis.

RESULTS AND DISCUSSION

I. Blood Glucose

Fasting or 0-time blood glucose levels were not significantly higher in the experimental group than in the control group either after one or four weeks of steroid treatment (Table 2). This finding has been reported by many other workers (42, 78, 79, 108, 112, 127) for women receiving oral contraceptives. Other reports however indicated an opposite effect in that patients receiving oral contraceptive steroids had increased fasting blood glucose levels (35, 50). The overall blood glucose level was significantly higher in the treated group than in the control group, taking into consideration all sugars that were force fed and the two time intervals used for the tolerance tests ($p < 0.02$) (Figs. 3, 4). However, after one week of treatment, the statistical analysis showed that there was no significant difference in blood glucose levels between the control group and the experimental group regardless of which sugar solution was force fed to the animals (Table 3). Also there was no significant difference between these two groups for the individual blood glucose level after different sugar tolerance test at this time. After four weeks of

TABLE 2. Fasting[1] blood glucose levels after one or four weeks steroid treatment. (mg/100 ml serum)

No. of Observation	One Week Steroid Treatment		Four Weeks Steroid Treatment	
	Control Rats	Exptl. Rats	Control Rats	Exptl. Rats
1	62.7	94.6	104.2	113.2
2	105.1	121.5	99.5	101.2
3	84.2	90.8	106.4	91.4
4	85.8	86.4	89.1	109.3
Mean	84.4	98.6	99.8	103.8
S.D.	17.3	15.8	7.6	9.4

[1]Animals were fasted 15 hours prior to collection of blood.

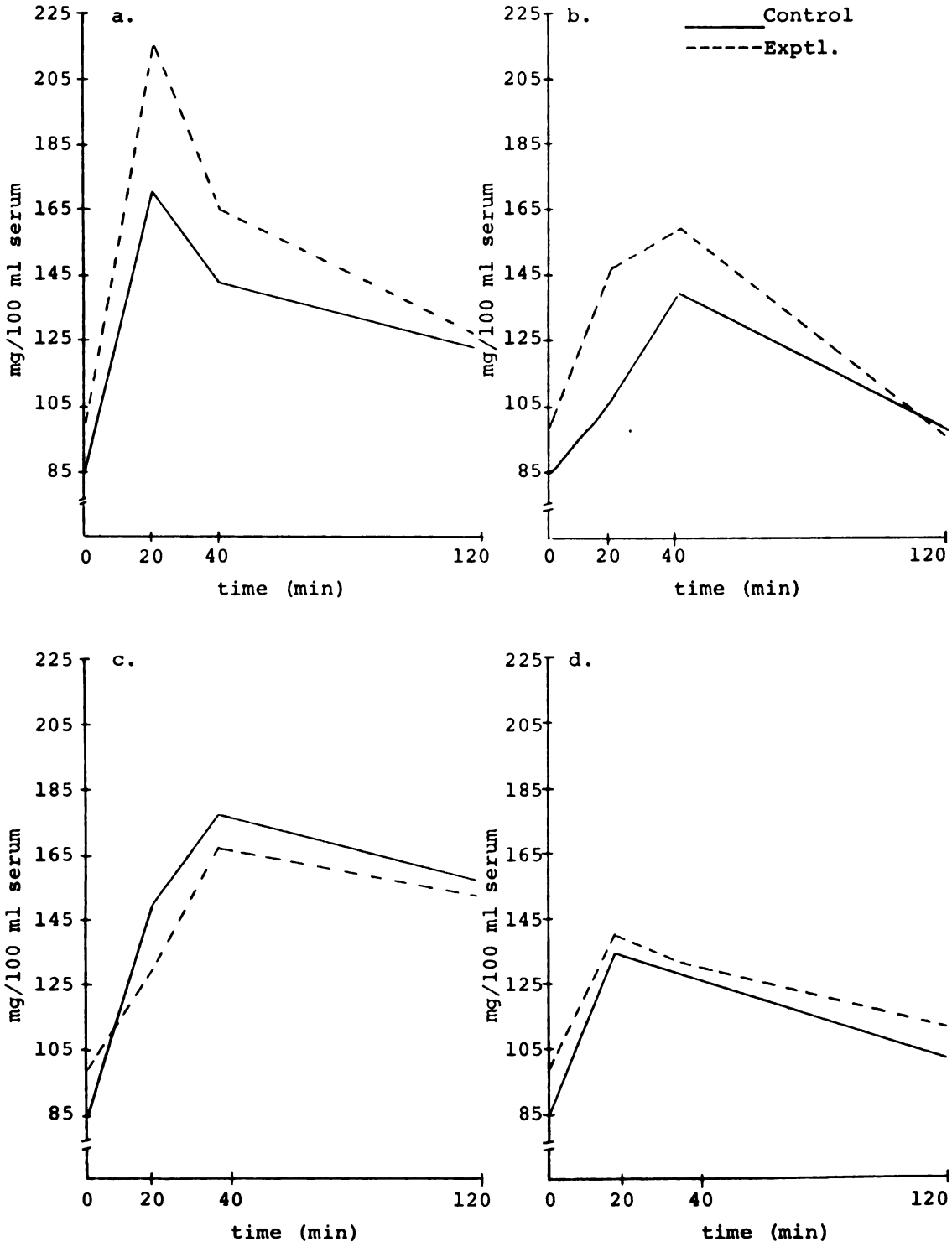

Fig. 3. Blood glucose concentration after force-feeding (a) glucose, (b) galactose, (c) fructose, and (d) ribose (after one week of steroid treatment).

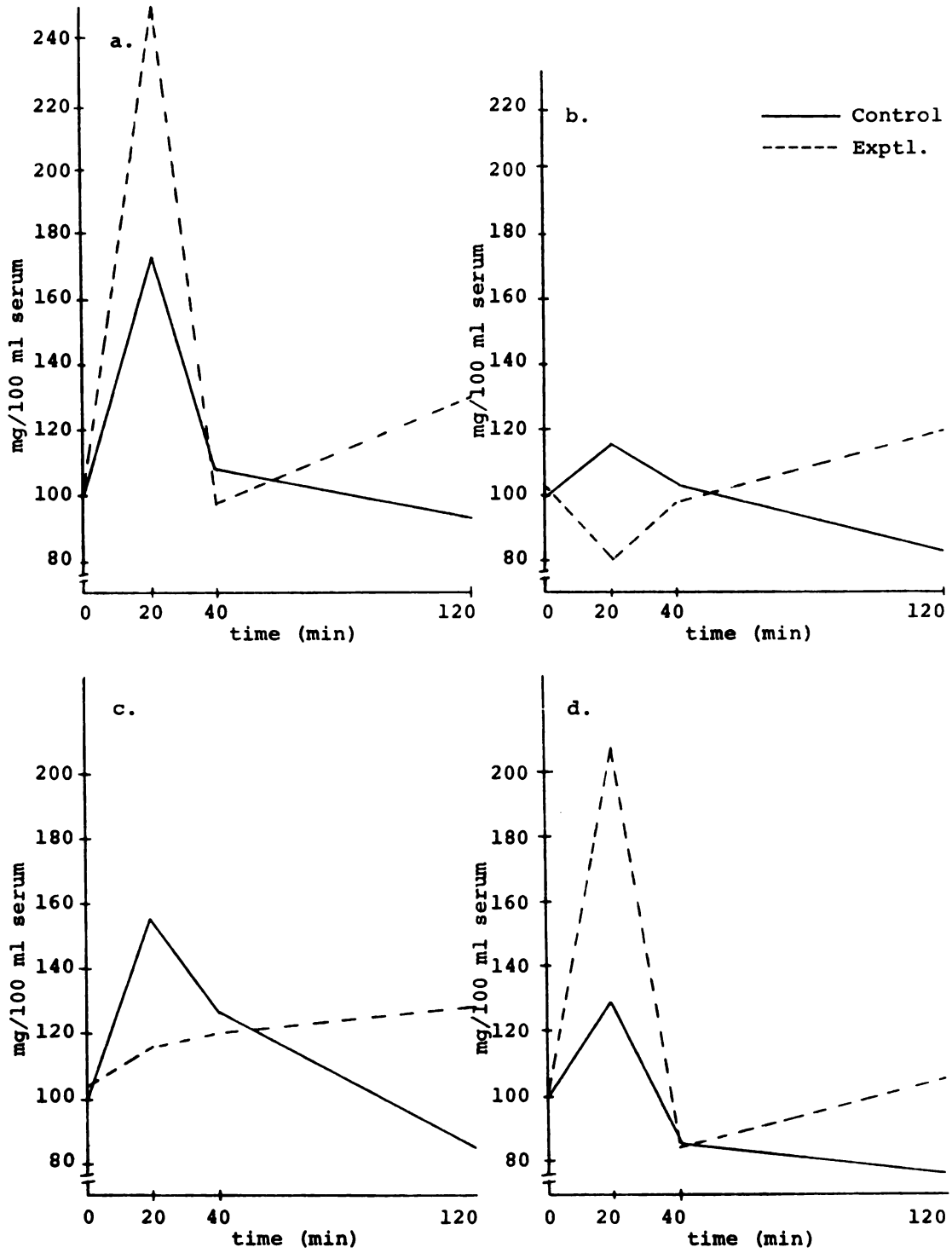

Fig. 4. Blood glucose concentration after force-feeding (a) glucose, (b) galactose, (c) fructose, and (d) ribose (after four weeks of steroid treatment).

TABLE 3. Blood glucose levels after force feeding glucose, galactose, fructose and ribose,[1] one week or four weeks after steroid treatment. (mg/100 ml serum, averages ± Standard Deviation)

	Sugar Force-Feeding	Control Rats			Exptl. Rats		
		Min. After Sugar Force Feeding					
		20	40	120	20	40	120
ONE WEEK	Glucose	170.7 ±24.8	143.4 ±20.8	122.2 ±25.4	215.8 ±85.2	165.6 ±14.4	125.7 ±17.1
	Galactose	108.8 ±28.8	139.8 ±36.4	101.3 ±29.2	148.1 ±15.2	159.4 ±66.0	96.8 ±21.3
	Fructose	149.5 ±20.2	177.0 ±24.8	156.6 ±15.1	130.2 ±22.0	166.5 +28.4	152.4 ± 9.2
	Ribose	134.8 ±53.0	127.5 ±20.7	102.4 ±34.7	140.5 ±20.4	131.9 ±39.2	111.6 ±21.0
FOUR WEEK	Glucose[2]	173.9 ±49.4	107.3 ±27.0	92.6 ±20.6	249.1 ±175.9	97.4 ±28.2	130.8 ± 3.5
	Galactose	115.9 ±36.9	103.6 ±14.1	82.1 ±25.0	80.0 ±21.6	97.3 ± 6.1	118.6 ±34.8
	Fructose	155.9 ±51.4	126.5 ±17.9	83.6 ±32.3	116.0 ±53.6	119.9 ±38.2	127.9 ±21.6
	Ribose[3]	128.2 ±18.7	84.8 ±27.1	75.4 ±21.4	207.6 ±80.5	85.4 ±20.0	104.6 + 8.1

[1] 300 mg of each sugar in 1 ml of water solution/100 g body weight.
[2] Mean for control is significantly lower than experimental ($p < 0.01$).
[3] Mean for control is significantly lower than for experimental ($p < 0.05$).

steroid treatment, the blood glucose levels in those animals which were force fed glucose ($p < 0.01$) or ribose ($p < 0.05$) were significantly higher in the treated group than in the control group. There was also a significantly higher overall blood glucose level in the treated group than the control group after four weeks of treatment ($p < 0.05$). In the present study, therefore, the effect of steroids on glucose and ribose metabolism can only be found after four weeks of steroid treatment (Table 3).

The impaired glucose tolerance in rats after four weeks of steroid treatment supports many previous studies using human subjects (35, 79, 106, 107, 108, 121). Results of this study showed that oral contraceptive steroids fed for one or four weeks did not have a significant effect on galactose and fructose metabolisms, in terms of blood glucose level (Fig. 3b,c and Fig. 4b,c). The effect of steroids on ribose metabolism, in terms of blood glucose level, was obvious only after four weeks of steroid treatment (Fig. 4d), since there was a significantly higher blood glucose level ($p < 0.05$) in the experimental group compared to the control group. The blood glucose level of those animals treated with steroids for one week and force fed fructose was lower than that found in control rats (Fig. 3e). But after four weeks of steroid treatment, the condition was reversed (Fig. 4c). The blood glucose level of those animals treated for one week with steroids and force fed

galactose solution was higher than that of control (Fig. 3b). After four weeks of steroid treatment, the blood glucose level rose even higher in the experimental group (Fig. 4b); the mean difference was larger at this time compared to the mean difference after one week steroid treatment (Table 3). Thus it is clear that four weeks of steroid treatment has a significant effect on glucose and ribose metabolism by raising the blood glucose level after the administration of these sugars (Fig. 4a,d). But it can be concluded that contraceptive steroids might have an effect on galactose and fructose metabolism if treatment were extended. This possibility will be discussed later.

Elevated circulating levels of plasma cortisol, thyroxine, growth hormone, pyruvate or hormones antagonistic to insulin, increased in plasma antagonists and insulin binding protein (2, 3, 11, 17, 27, 30, 32, 41, 46, 56, 57, 65, 69, 77, 81, 85, 87, 110, 128) have been suggested as possible mechanisms impairing glucose tolerances after contraceptive steroid treatment. That estrogens may cause a decrease in the response of the beta-cell of the pancreas to glucose loads by the formation of an insulin-binding protein, transcortin, has been suggested by Slaunwhite, Sandberg and Wallace (87, 88, 89, 96, 97, 118, 119), as the most likely mechanism of all those proposed for the impaired tolerance.

Ribose administration or infusion, as well as glucose caused secretions of insulin according to Goetz et al. (37, 43) and Steinberg et al. (103). It is possible that estrogen may have the same effect on ribose as on glucose by decreasing insulin availability or activity. Mechanisms for the increased insulin secretion after administration or infusion of glucose were suggested to be a direct stimulation of the beta-cell, specified gastrointestinal factor, and through the mediation of the liver (11, 37, 60). However, for ribose only the latter mechanism could be involved (37). On the other hand, some workers have suggested that estrogen can modify liver function (55, 68). This may help explain why there was a higher glucose level in steroid treated group after ribose loading.

It has been reported (37) that galactose infusion can cause insulin secretion as glucose does. Thus, it is possible that oral contraceptive steroids may have the same effect on galactose metabolism as on ribose metabolism after a longer period of steroid treatment.

That fructose infusion can not cause insulin secretion as galactose, glucose and ribose has also been reported by Goetz et al. (37). This might apply to the finding in this study since there was a lower blood glucose level in treated group than in control group after fructose loading, and an opposite result when the other sugars were used.

The rise in blood glucose level following oral administration of fructose, galactose or ribose indicated that part of the sugars was converted to glucose (Fig. 3 and 4). Blood glucose levels are significantly different among the four sugars tested ($p < 0.01$). This might be due to the different metabolic conversion rates of these sugars to glucose. Fructose is easily converted to glucose both in intestinal mucosa and liver through the reversed glycolytic pathway (123). The conversion of galactose to glucose is through the pathway of uridine diphosphate galactose to uridine diphosphate glucose (124). The conversion of ribose to glucose is through the reversed phosphogluconate oxidative pathway (125). It can be interpreted that the oral contraceptive steroids may have the same effect on galactose, fructose and ribose metabolism as on glucose metabolism in terms of the effect on blood glucose level, since these three sugars can be converted to glucose through the above mentioned metabolic pathways.

From Figs. 3 and 4, it can be seen that the highest blood glucose level when glucose or ribose was force fed to the animals was 20 min. after administration. This occurred both in control and experimental groups, after one or four weeks of steroid treatment (Figs. 3a,d; 4a,d). When galactose or fructose was force fed to the animals, the highest blood glucose level was after 40 min (Fig. 3b,e), both in control and experimental groups but only after one week of

steroid treatment. After four weeks of steroid treatment, the highest blood glucose level was after 20 min. in the control group and 120 min. in the experimental group (Fig. 4b,c). Based on blood glucose peak time, it seems that oral contraceptive steroids have an effect on the conversion rate of galactose or fructose to glucose.

Weinstein and Roe (121) have reported that there was a maximum of 47% rise in the total blood sugar at the end of fructose infusion. They pointed out that there was a rapid metabolic conversion of fructose to glucose. In the present experiment, the blood glucose level was higher when measured 120 min. after fructose force feeding than after force feeding galactose or ribose (Figs. 3 and 4). It seems that after a prolonged steroid treatment the effect of this drug on fructose metabolism in terms of raising blood glucose level may be due to decreasing conversion rate from fructose to glucose. The possible mechanism involved might be the binding of estrogen with enzymes which are involved in the conversion reaction of fructose to glucose. This assumption might also apply to galactose, since a similar blood sugar picture has been found in those animals force fed galactose.

II. Blood Galactose

Although galactosemia has been observed only in man, analogous physiologic changes can be induced in animals by feeding diet high in galactose. It has been shown

that when rats are placed on 30% galactose diets, cataracts can be developed within a period of 14 to 21 days (94). This is due to the accumulation of galactose-1-P, which inhibits phosphoglucomutase and causes a decrease in glucose-1,6-P_2 (95, 36). The physiologic significance of this interference is uncertain but it may be a factor contributing to the hypoglycemia which occurs in galactosemic patients when they ingest galactose or lactose. The experimental observations of Foa (34) suggested that the hypoglycemia in the galactosemic patients when they ingest galactose may be due to the stimulation of insulin release from the pancreas by the elevated blood galactose. In his experiment, 1.75 g galactose/kg body weight given orally to the galactosemic patient produced on an average 225 mg galactose/100 ml blood two hours after the dosing, at this time, the blood glucose concentration was 48 mg/100 ml blood. In the present experiment, the amount of galactose given orally to the animals was almost two times that used by Foe (31), but the average blood galactose level was 260 mg and the blood glucose level was almost near fasting level two hours after dosing compare to his values. This may indicate that the animals used in the present work were able to utilize galactose efficiently whether or not the possible effect of steroid treatment was excluded.

From the present data, there appear to be no impaired galactose tolerance with steroid treated. The

experimental group has a lower blood galactose level, but a higher blood glucose level, than in the control group, after one or four weeks of steroid treatment (Table 4). This suggested that the steroids may not have an effect on galactose metabolism directly. But it has the effect on blood glucose level since galactose can be converted to glucose. Present results further indicated that after four weeks of steroid treatment, there was probably a decreased conversion rate of galactose to glucose (Fig. 5), since there was an increased blood galactose level, and a decreased blood glucose level (Tables 3 and 4). It can not be determined from the present experiment how long a period of steroid treatment is required before galactose tolerance is impaired. It may be that a longer period of steroid treatment is required before galactose tolerance is affected than is required before glucose tolerance is impaired.

III. Blood Fructose

The disturbances of fructose utilization have been observed. The possible causes may be a deficiency of aldolase in the liver (45) and the benign or idiopathic fructosuria, which involves deficiency of fructokinase in the liver (91). Two pathways are recognized by which fructose can be converted to glucose. One begins with fructose-1-P, formed by the specific fructokinase and proceeds through the triose phosphates to fructose-1,6-P_2,

TABLE 4. Blood galactose, fructose, and ribose levels after force feeding galactose, fructose, and ribose,[1] respectively, one week or four weeks after steroid treatment. (mg/100 ml serum, averages ± Standard Deviation)

	Sugar Force Feeding	Control Rats				Exptl. Rats			
		Min. After Sugar Force Feeding							
		Fasting	20	40	120	Fasting	20	40	120
ONE WEEK	Galactose	0	217.0 ±99.0	435.3 ±76.5	319.7 ±153.6	0	217.6 ±117.1	273.8 ±60.0	247.5 ±54.8
	Fructose	0	13.2 ±5.3	16.1 ±2.0	10.9 ±2.9	0	11.2 ±1.0	12.5 ±2.2	14.8 ±1.6
	Ribose	0	0	0	0	0	0	0	0
FOUR WEEK	Galactose	0	220.0 ±118.4	607.0 ±235.4	203.1 ±121.8	0	231.9 ±106.7	417.3 ±105.8	242.7 ±168.2
	Fructose	0	16.4 ±3.3	12.5 ±4.1	13.5 ±4.2	0	13.3 ±3.2	13.8 ±1.2	13.1 ±2.5
	Ribose	0	0	0	0	0	0	0	0

[1] 300 mg of each sugar in 1 ml of water solution/100 g body weight.

fructose-6-P and glucose-6-P. The other begins with fructose-6-P formed directly by hexokinase. After giving a large amount of fructose to rats it was shown that fructose-1-P aldolase activity was inadequate to keep up with fructokinase and may represent a limitation to the first pathway (58). The abnormal high level of fructose-1-P was found and which may lead to a profound decrease in ATP and UTP, and thus may indirectly influence other physiological reactions (58).

In the study of Lowry et al. (58), an accumulation of 100-fold of fructose-1-P was found 60 min. after giving intraperitonealy 40 μmoles/kg of fructose to male rats averaging 100 g in body weight. The amount of fructose force fed to the animals in the present work was equal to 17 μmoles/kg of body weight, which was less than half of the amount used in Lowry's work. Thus, the level of fructose-1-P formed from fructose may not be high enough to cause an impaired fructose tolerance.

From present results, it seems that the steroids did not affect fructose metabolism appreciably. There was no significant difference in blood fructose level between the control and the experimental groups after one or four weeks of steroid treatment (Table 4). However, blood glucose level after force feeding fructose was lower in the experimental group than the control group after one week of steroid treatment (Fig. 3c). There was an increase in mean

difference of blood glucose level of these two groups after four weeks of steroid treatment (Table 3). And there seems to be a delay in the conversion of fructose to glucose in the experimental group after four weeks of steroid treatment (Fig. 4c). This indicates that the contraceptive steroids may affect fructose metabolism, in terms of blood glucose level after a prolonged treatment. This effect might be due to the fact that estrogen may bind enzymes which are involved in the conversion reactions of fructose to glucose which might lead to an increased blood glucose level and a decreased blood fructose level in the steroid treated animals.

IV. Blood Ribose

No ribose was found in any blood sample. This indicates that all the ribose force fed was converted to other metabolites within 20 min. A significant mean difference of blood glucose level was found between the control and the experimental groups after four weeks of steroid treatment, but not after one week of steroid treatment (Tables 3 and 4). It is clear then that contraceptive steroids did not have any effect on ribose metabolism. But an indirect effect has been shown by a significant rise in blood glucose level in the experimental group.

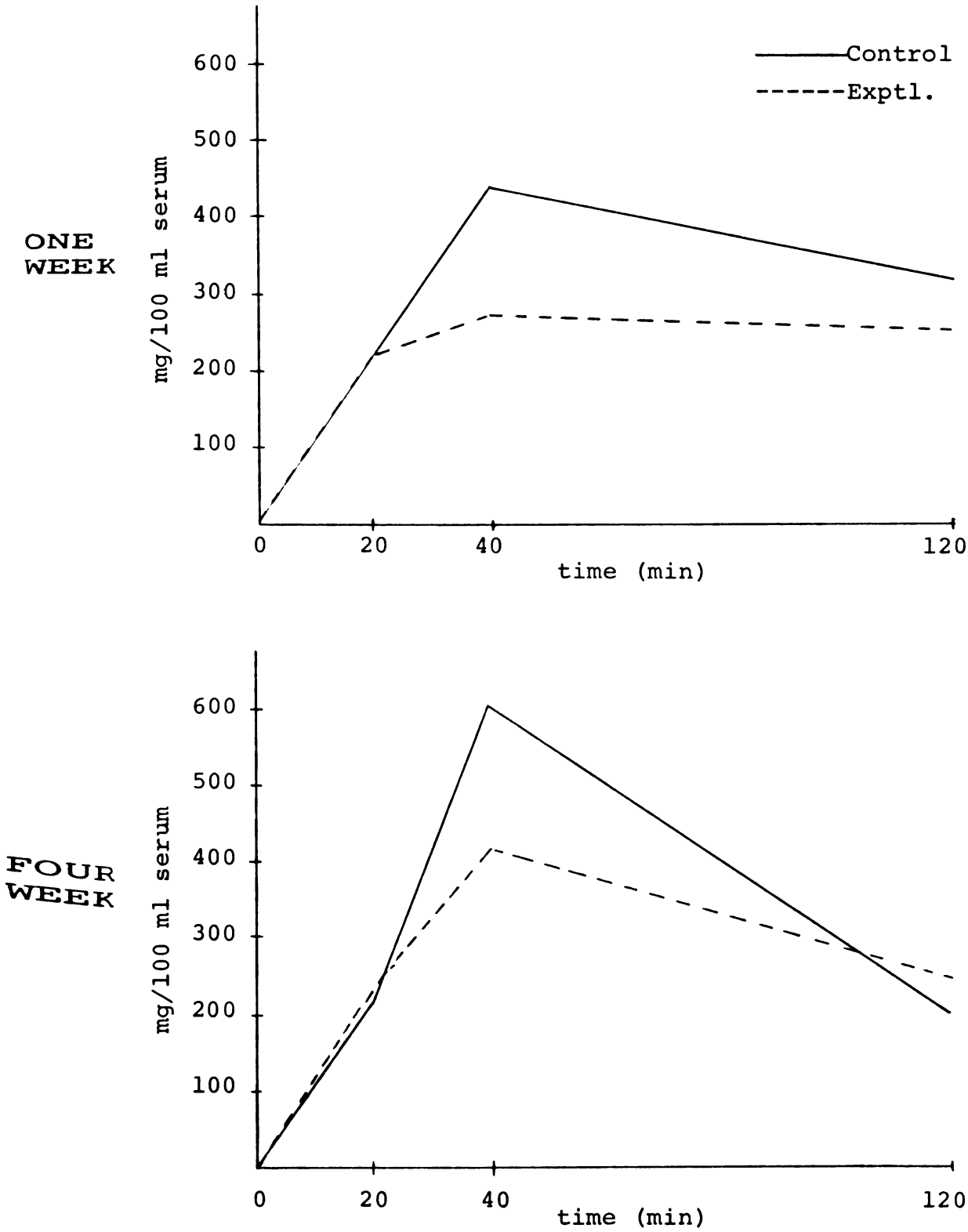

Fig. 5. Blood galactose concentration after galactose force-feeding, after one or four weeks of steroid treatment.

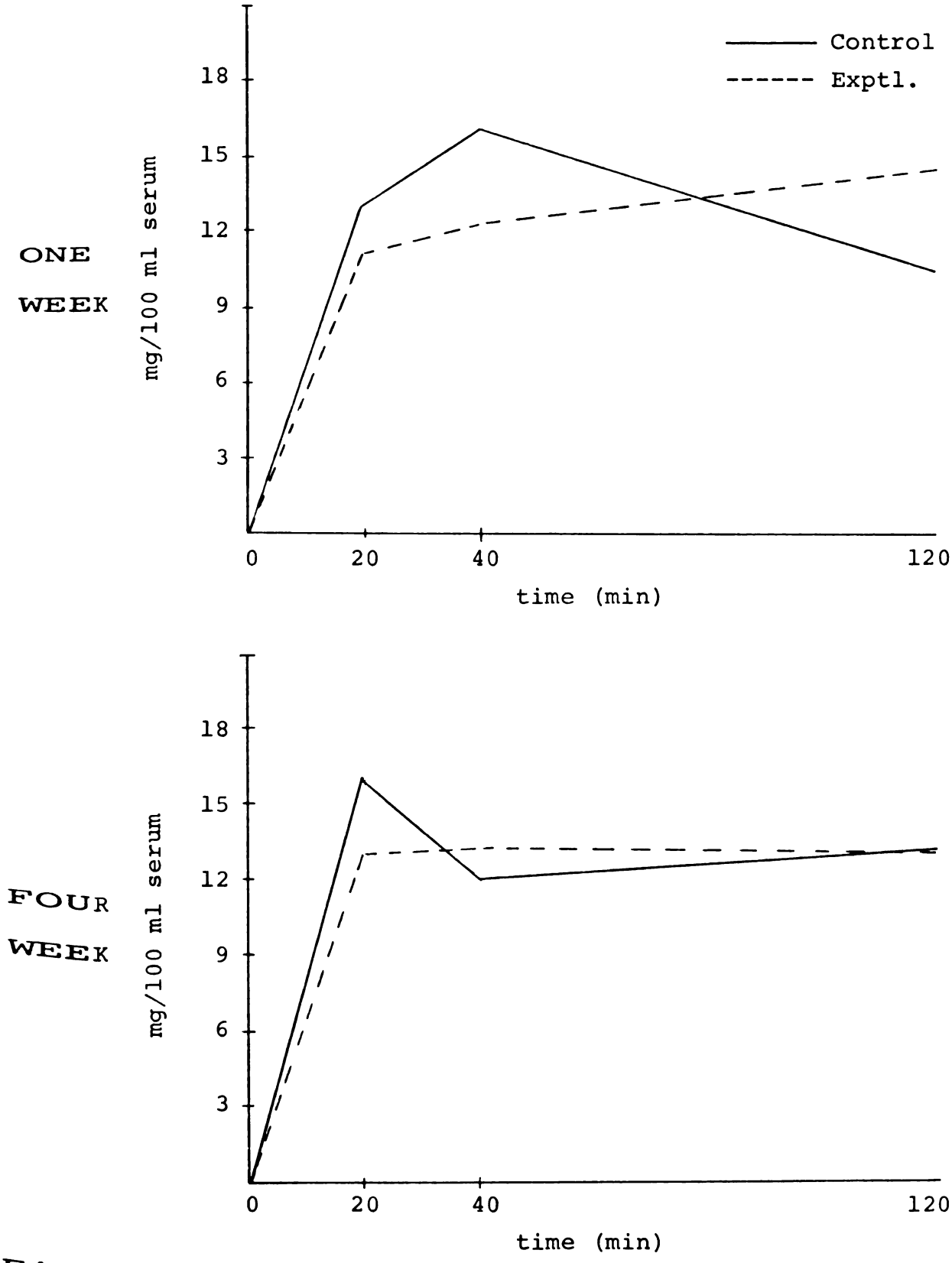

Fig. 6. Blood fructose concentration after fructose force-feeding, after one or four weeks of steroid treatment.

V. Urinary Glucose, Galactose, Fructose and Ribose

There was no significant difference in urinary glucose content between the control and the experimental groups in the six hour urine collection regardless of which one of the four sugars was force fed. However, the eighteen hour urine of the treated animals force fed glucose ($p < 0.05$) or ribose ($p < 0.01$) had a significantly higher quantity of glucose than control (Table 5), but not for those force fed fructose or galactose (Table 5). The treated animals having a high urinary excretion of glucose, also have a high blood glucose level. This indicates that the high blood glucose, and the high urinary glucose excretion are probably correlated.

There was no galactose, fructose or ribose found in any of the urine samples of those animals which have not been force fed these sugars. Unfortunately, because of the spillage of feed and the contamination of the urine samples with feces in this group of rats, the level of glucose in the urine were higher than the force-fed rats (Table 5).

At the present time, there was no sufficient data for making further discussions concerning the effect of steroid treatment on urinary glucose excretion *per se*. However, indications are that an impaired blood sugar tolerance might be in existence two weeks after treatment, since a significantly higher urinary glucose was found in

TABLE 5. Total urinary glucose excretion after glucose, galactose, fructose and ribose[1] force feeding, after two weeks of steroid treatment. (mg, averages ± Standard Deviation)

Sugar Force Feeding	Control Rats			Exptl. Rats		
	Duration of Collection					
	1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
None[5]	0.1 ± 0.2	4.3 ± 5.8	4.4	4.3 ± 2.1	7.8 ± 9.7	12.1
Glucose	0.1 ± 0.1	2.8 ± 3.2[6]	2.9	0.3 ± 0.4	4.9 ± 5.6[6]	5.2
Galactose	2.7 ± 3.0	5.3 ± 6.6	8.0	2.6 ± 2.7	5.8 ± 6.7	8.4
Fructose	0.1 ± 0.1	3.3 ± 4.2	3.4	0.5 ± 0.7	4.3 ± 1.7	4.8
Ribose	0.2 ± 0.2	4.4 ± 5.2[7]	4.6	0.2 ± 0.2	7.3 ±10.4[7]	7.5

[1]300 mg of each sugar in 1 ml of water solution/100 g body weight.

[2]Six hours after force feeding.

[3]Eighteen hours after force feeding.

[4]Twenty-four hours after force feeding.

[5]Animals were fasted 15 hours prior to collection.

[6]Mean for control is significantly lower than experimental ($p < 0.05$).

[7]Mean for control is significantly lower than experimental ($p < 0.01$).

the treated rats than the control at this time. Furthermore, Fenichel et al. (29) reported that normal intact female rats treated with norgestrel, ethynyl oestradiol or their combinations had a higher blood glucose than control after two weeks of treatment.

Present results did not show any significant difference of urinary galactose, fructose or ribose content between the control and the experimental groups (Table 6). This indicates that oral contraceptive steroids do not enhance galactose, fructose and ribose excretion in the urine.

TABLE 6. Total urinary galactose, fructose and ribose excretion after force feeding galactose, fructose or ribose[1] respectively, after two weeks of steroid treatment. (mg, averages ± Standard Deviation)

Sugar Force Feeding	Control Rats			Exptl. Rats		
	Duration of Collection					
	1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
Galactose	1.3 ±1.4	1.8 ±0.6	3.1	1.3 ±0.4	2.1 ±2.4	3.4
Fructose	1.5 ±1.8	2.5 ±3.0	4.0	1.2 ±1.4	3.1 ±3.5	4.3
Ribose	3.3 ±6.3	2.5 ±3.2	5.8	2.6 ±4.2	1.9 ±3.3	4.5

[1] 300 mg of each sugar in 1 ml of water solution/100 g body weight.

[2] Six hours after force feeding.

[3] Eighteen hours after force feeding.

[4] Twenty-four hours after force feeding.

SUMMARY

After feeding the contraceptive steroids norethynodrel and mestranol to 11 week old female rats, there was a significantly higher ($p < 0.05$) urinary glucose excretion (24 hours) in the experimental group after two weeks of steroid treatment and a significantly higher ($p < 0.05$) blood glucose level after four weeks of treatment in those rats administered glucose or ribose solution. No effect on blood glucose level was noticed after one week of steroid treatment. Glucose in urine collected for 6 hours after force-feeding glucose, galactose, fructose and ribose was not significantly different between the control and the experimental groups after two weeks of steroid treatment. No significant effects of steroids on galactose or fructose metabolism was found in this study. The galactose and fructose metabolism could be affected after a longer steroid treatment, since present results show this tendency. It is suggested that the oral contraceptive should be fed for a longer period in order to give more detailed information on their effect on galactose and fructose metabolism.

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APPENDICES

APPENDIX I

COMPOSITION OF BASAL GRAIN DIET (in %)

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COMPOSITION OF BASAL GRAIN DIET (in %)

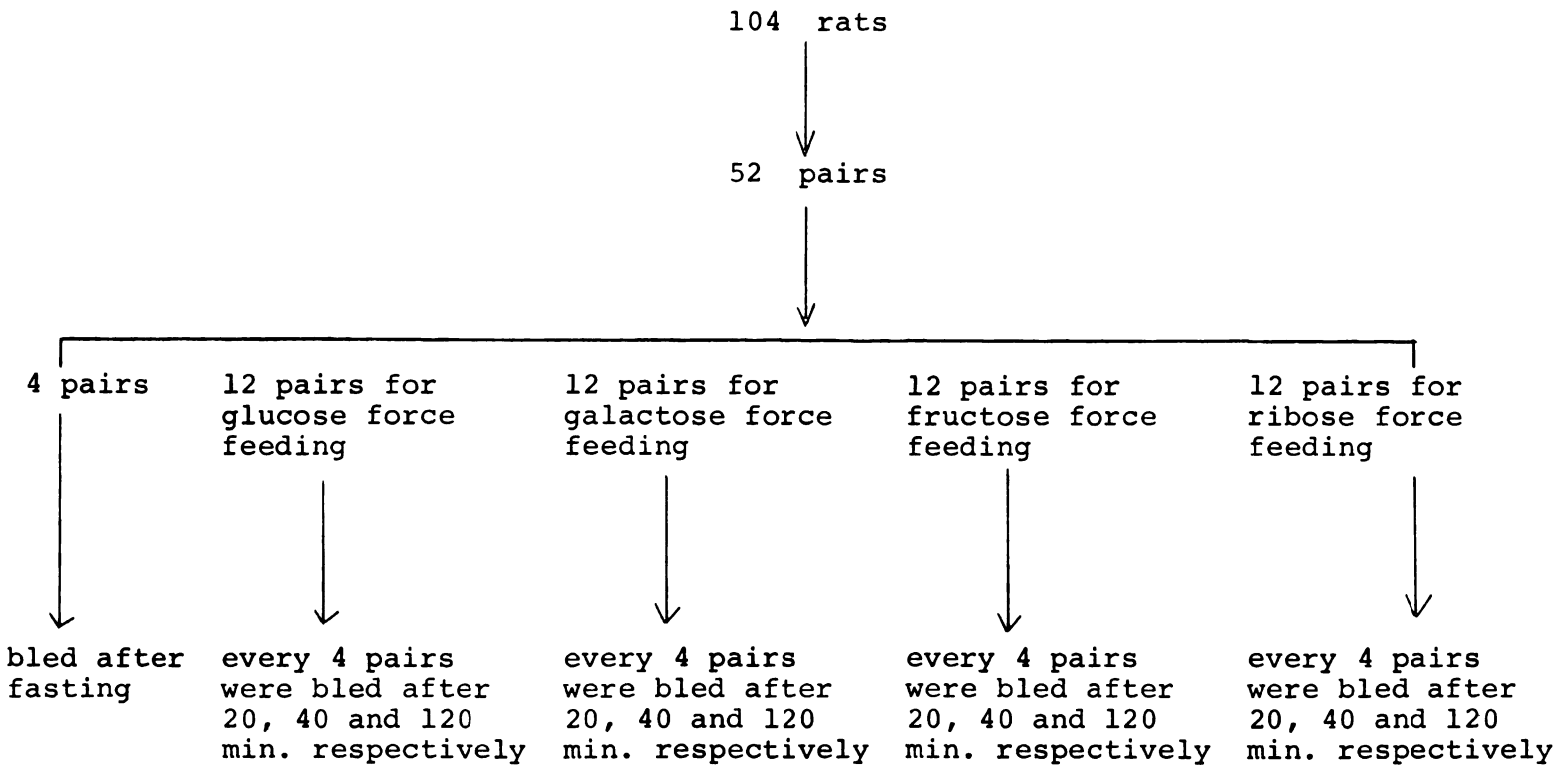
Ground corn 60.7; soybean meal (50% protein), 28.0; alfalfa meal (17% protein), 2.0; fish meal (60% protein), 2.5; dried whey (67% lactose), 2.5; limestone (38% Ca), 1.6; dicalcium phosphate (18.5% P, 22-25% Ca), 1.75; iodized salt, 0.5. Supplementary minerals and vitamins were added to provide per kg of diet: (in mg.) Mn, 121; Fe, 95; Cu, 7; Zn, 4; I_2, 4; Co, 2; Choline chloride, 400; Ca pantothenate, 6; riboflavin, 3; niacin, 33; menadione, 2; DL-methionine, 500; (in microgram) vitamin B_{12}, 7; (in I.U.) vitamin A, 8010; vitamin D_2, 750; vitamin E, 5.

APPENDIX II

EXPERIMENTAL DESIGN

APPENDIX II

EXPERIMENTAL DESIGN



APPENDIX III

FIGURES

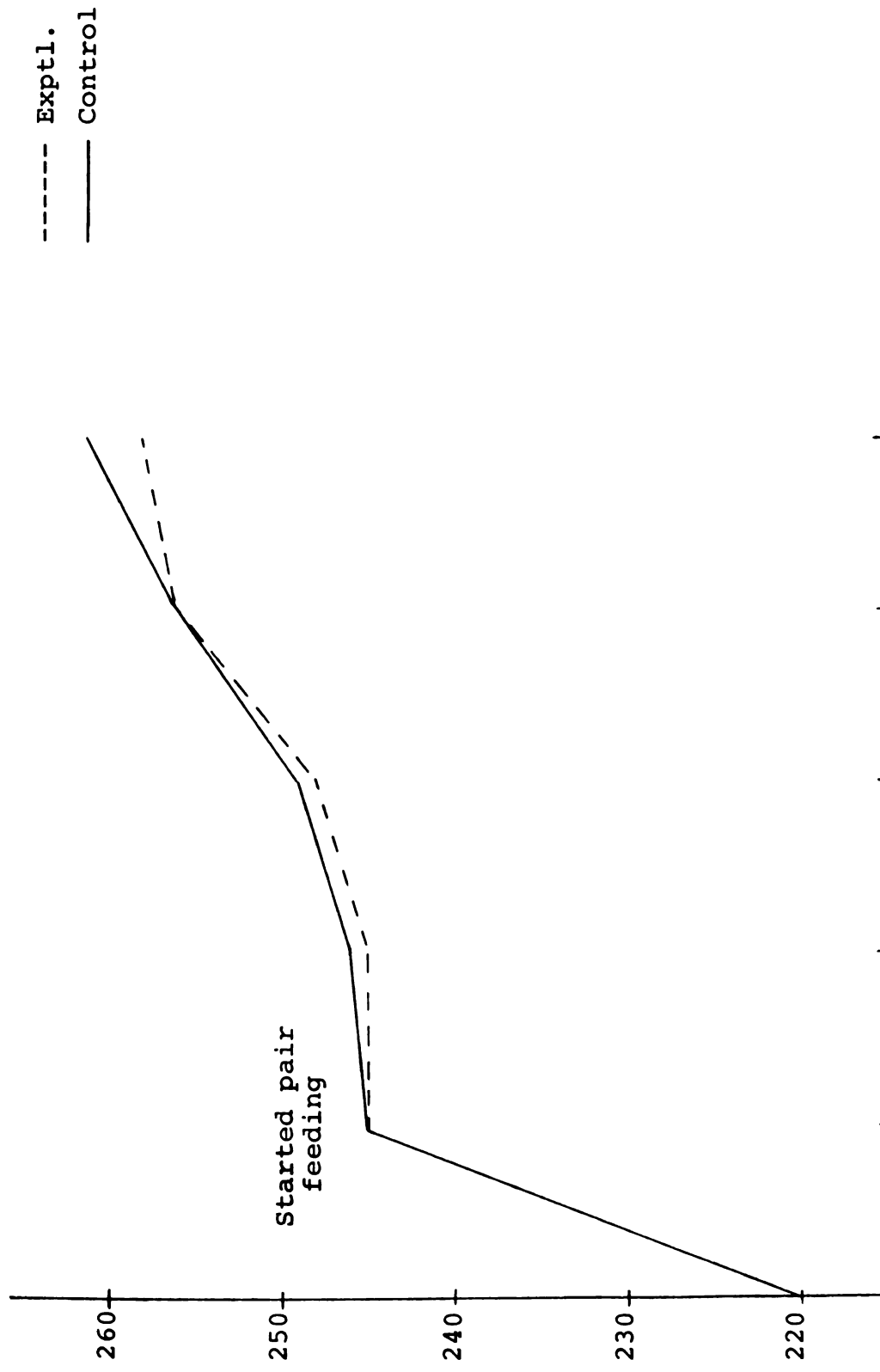


Fig. A-1. Body weight records of the control and the treated rats.

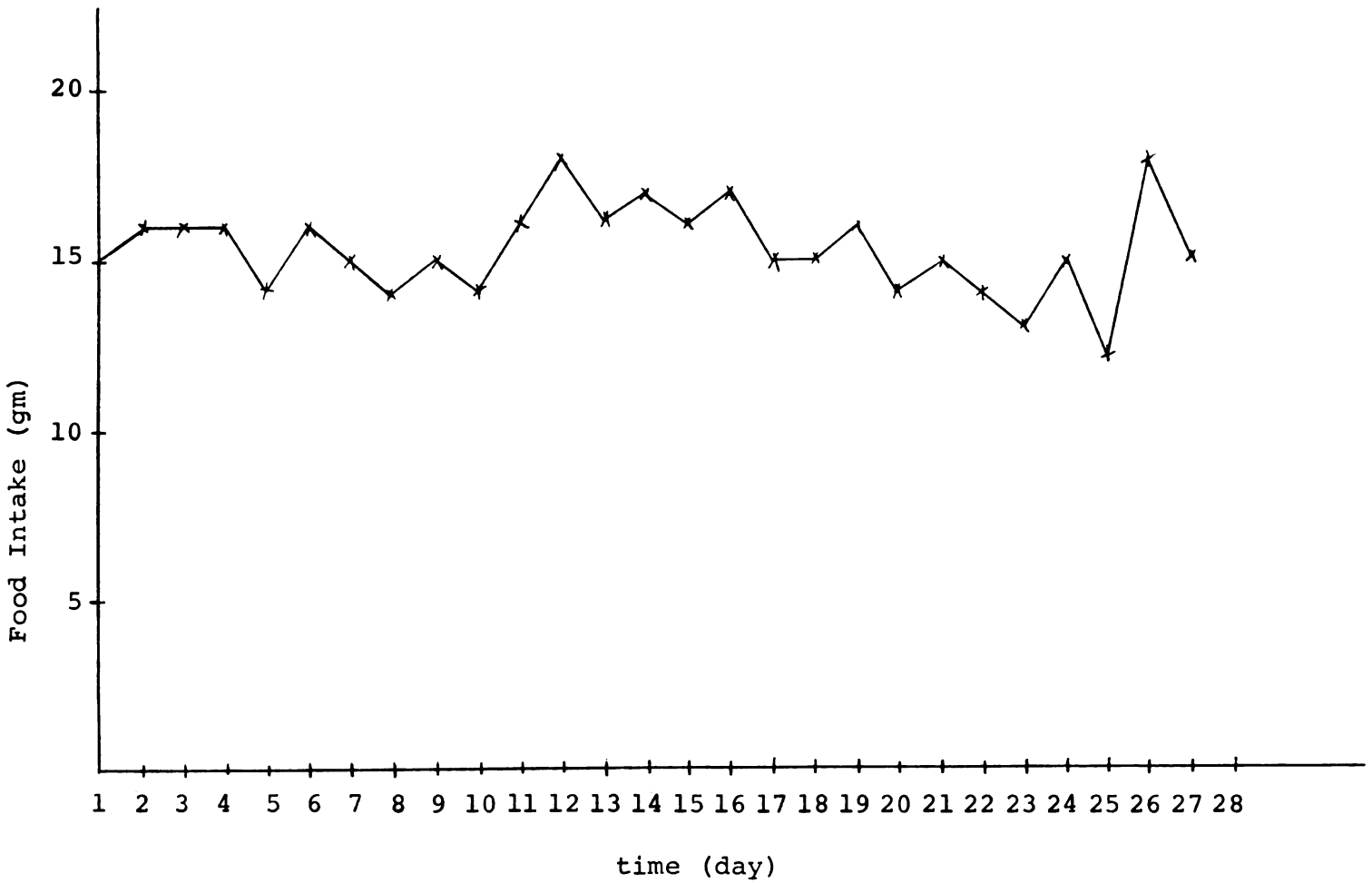


Fig. A-2. Daily food consumption of the treated rats.

APPENDIX IV

TABLES

TABLE A-1. Blood glucose levels after force feeding glucose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Glucose Force Feeding					
	20	40	120	20	40	120
1	167.9	171.1	141.4	256.3	182.1	121.0
2	137.0	154.0	139.2	238.7	149.1	108.9
3	192.2[3]	114.4[3]	125.4[3]	90.2[3]	159.5[3]	123.3[3]
4	185.9[3]	134.2[3]	83.1[3]	277.8[3]	171.6[3]	149.6[3]
Mean	170.7	143.4	122.2	215.8	165.6	125.7
S.D.	24.8	20.8	25.4	85.2	14.4	17.1

[1]300 mg of glucose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-2. Blood glucose levels after force feeding glucose,[1] four weeks after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Glucose Force Feeding					
	20	40	120	20	40	120
1	111.6	--- [3]	111.6	187.4	--- [3]	127.7
2	188.6	80.6	--- [3]	130.6	69.0	--- [3]
3	165.6[4]	106.7[4]	95.5[4]	510.6[4]	125.4[4]	134.6[4]
4	230.0[4]	134.6[4]	70.7[4]	167.6[4]	97.8[4]	130.3[4]
Mean[5]	173.9	107.3	92.6	249.1	97.4	130.8
S.D.	49.4	27.0	20.6	175.9	28.2	3.5

[1] 300 mg of glucose in 1 ml of water solution/100 g body weight.

[2] Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

[4] Used for statistical analysis.

[5] Mean for control is significantly lower than experimental ($p < 0.01$).

TABLE A-3. Blood glucose levels after force feeding galactose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Galactose Force Feeding					
	20	40	120	20	40	120
1	128.6	184.8	140.5[3]	166.1	194.2	65.5[3]
2	132.3	150.2[3]	73.2[3]	155.1	132.6[3]	102.3[3]
3	69.7[3]	124.9	86.4	136.4[3]	229.9	107.2
4	104.5[3]	99.6[3]	105.1	134.7[3]	80.9[3]	112.2
Mean	108.8	139.8	101.3	148.1	159.4	96.8
S.D.	28.8	36.4	29.2	15.2	66.0	21.3

[1]300 mg of galactose in 1 ml H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-4. Blood glucose levels after force feeding galactose,[1] four weeks after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Galactose Force Feeding					
	20	40	120	20	40	120
1	142.6	116.3	72.5[3]	52.9	96.6	83.4[3]
2	63.3	86.3[3]	63.3[3]	91.0	100.6[3]	119.6[3]
3	117.3[3]	97.8	110.4	102.4[3]	89.1	153.0
4	140.3[3]	113.9[3]	--- [4]	73.6[3]	102.9[3]	--- [4]
Mean	115.9	103.6	82.1	80.0	97.3	118.6
S.D.	36.9	14.1	25.0	21.6	6.1	34.8

[1] 300 mg of galactose in 1 ml of H_2O solution/100 g body weight.

[2] Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3] Used for statistical analysis.

[4] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-5. Blood glucose levels after force feeding fructose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Fructose Force Feeding					
	20	40	120	20	40	120
1	149.6[3]	174.9[3]	152.4	152.9[3]	174.9[3]	143.0
2	150.2	143.0	140.3	127.0	125.4	147.4
3	124.3	198.0[3]	157.3[3]	139.7	190.9[3]	155.1[3]
4	173.8[3]	192.5	176.6[3]	101.2[3]	174.9	163.9[3]
Mean	149.5	177.0	156.6	130.2	166.5	152.4
S.D.	20.2	24.8	15.1	22.0	28.4	9.2

[1]300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-6. Blood glucose levels after force feeding fructose,[1] four weeks after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Fructose Force Feeding					
	20	40	120	20	40	120
1	138.9[3]	139.2[3]	44.9	144.9[3]	140.9[3]	131.1
2	156.4	--- [4]	96.6	82.7	--- [4]	105.8
3	102.9	113.9[3]	120.2[3]	176.0	151.8[3]	156.4[3]
4	225.4[3]	--- [4]	72.5[3]	60.4[3]	--- [4]	118.5[3]
Mean	155.9	126.5	83.6	116.0	119.9	127.9
S.D.	51.4	17.9	32.3	53.6	38.2	21.6

[1]300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

[4]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-7. Blood glucose levels after force feeding ribose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Ribose Force Feeding					
	20	40	120	20	40	120
1	79.2	157.6[3]	58.9	140.8	81.9[3]	84.7
2	205.7	112.2	143.0[3]	152.9	174.9	117.7[3]
3	117.2[3]	124.3	97.9[3]	111.6[3]	124.3	108.9[3]
4	137.5[3]	116.1[3]	109.9	156.8[3]	146.3[3]	135.2
Mean	134.8	127.5	102.4	140.5	131.9	111.6
S.D.	53.0	20.7	34.7	20.4	39.2	21.0

[1]300 mg of ribose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-8. Blood glucose levels after force feeding ribose,[1] four weeks after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats			Exptl. Rats		
	Min. After Ribose Force Feeding					
	20	40	120	20	40	120
1	---[3]	103.5[4]	---[3]	---[3]	94.5[4]	---[3]
2	---[3]	103.5	49.7[4]	---[3]	95.5	96.6[4]
3	115.0[4]	86.3	109.8[4]	150.7[4]	95.5	104.4[4]
4	141.5[4]	46.0[4]	66.7	264.5[4]	55.2[4]	112.7
Mean[5]	128.2	84.8	75.4	207.6	85.4	104.6
S.D.	18.7	27.1	21.4	80.5	20.0	8.1

[1]300 mg of ribose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

[4]Used for statistical analysis.

[5]Mean for control is significantly lower than for experimental ($0.025<p<0.05$).

TABLE A-9. Blood galactose levels after force feeding galactose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats				Exptl. Rats			
	Min. After Galactose Force Feeding							
	Fasting	20	40	120	Fasting	20	40	120
1	0.0	149.4	463.1[3]	303.3[3]	0.0	330.6	327.8[3]	226.1[3]
2	0.0	209.8[3]	448.2[3]	141.9	0.0	153.7[3]	220.0[3]	182.6
3	0.0	359.2[3]	326.2	517.0[3]	0.0	325.1[3]	223.9	273.4[3]
4	0.0	149.6	503.8	316.5	0.0	330.6	323.4	308.0
Mean	0.0	217.0	435.3	319.7	0.0	217.6	273.8	247.5
S.D.		99.0	76.5	153.6		117.1	60.0	54.8

[1]300 mg of galactose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-10. Blood galactose levels after force feeding galactose,[1] four weeks after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats				Exptl. Rats			
	Min. After Galactose Force Feeding							
	Fasting	20	40	120	Fasting	20	40	120
1	0.0	97.5	335.2[3]	93.7[3]	0.0	79.4	567.3[3]	139.2[3]
2	0.0	188.3[3]	738.3[3]	181.4	0.0	325.5[3]	134.7[3]	152.2
3	0.0	380.9[3]	---[4]	334.4[3]	0.0	248.4[3]	---[4]	436.7[3]
4	0.0	231.4	747.5	---[4]	0.0	274.3	528.1	---[4]
Mean	0.0	220.0	607.0	203.1	0.0	231.9	417.3	242.7
S.D.		118.4	235.4	121.8		106.7	195.8	168.2

[1]300 mg of galactose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

[4]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-11. Blood fructose levels after force feeding fructose,[1] one week after steroid treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats				Exptl. Rats			
	Min. After Fructose Force Feeding							
	Fasting	20	40	120	Fasting	20	40	120
1	0.0	10.5	14.3[3]	7.5	0.0	10.9	10.5[3]	16.5
2	0.0	15.4[3]	17.4	14.5[3]	0.0	9.9[3]	12.1	12.7[3]
3	0.0	7.5	18.2[3]	10.5	0.0	11.8	15.6[3]	14.5
4	0.0	19.5[3]	14.5	11.3[3]	0.0	12.2[3]	12.1	15.6[3]
Mean	0.0	13.2	16.1	10.9	0.0	11.2	12.5	14.8
S.D.		5.3	2.0	2.9		1.0	2.2	1.6

[1]300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2]Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3]Used for statistical analysis.

TABLE A-12. Blood fructose levels after force feeding fructose,[1] four weeks after treatment. (mg/100 ml serum)

No. of Observation[2]	Control Rats				Exptl. Rats			
	Min. After Fructose Force Feeding							
	Fasting	20	40	120	Fasting	20	40	120
1	0.0	12.3	15.4[3]	17.8	0.0	9.4	14.7[3]	11.4
2	0.0	15.3[3]	---[4]	14.4[3]	0.0	12.3[3]	---[4]	16.9[3]
3	0.0	18.4	9.7[3]	14.2	0.0	16.8	12.8[3]	12.0
4	0.0	19.6[3]	---[4]	7.7[3]	0.0	14.9[3]	---[4]	12.0[3]
Mean	0.0	16.4	12.5	13.5	0.0	13.3	13.8	13.1
S.D.		3.3	4.1	4.2		3.2	1.2	2.5

[1] 300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2] Each sample was collected from a different rat so that each rat was sampled only once during the sugar tolerance test.

[3] Used for statistical analysis.

[4] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-13. Total urinary glucose excretion after glucose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	0.20	2.86	3.06	1	0.09	1.60	1.60
2	-- [5]	-- [5]		2	-- [5]	-- [5]	
3	0.07	2.47	2.54	3	0.21	5.04	5.25
4	0.14	3.18	3.32	4	0.36	9.15	9.51
5	0.18	4.22	4.40	5	0.39	8.05	8.44
6	-- [5]	-- [5]		6	-- [5]	-- [5]	
7	0.02[6]	1.68[6]	1.70[6]	7	0.23[6]	3.48[6]	3.71[6]
8	0.10[6]	4.79[6]	4.89[6]	8	0.40[6]	3.56[6]	3.96[6]
9	0.05[6]	1.61[6]	1.66[6]	9	0.13[6]	5.69[6]	5.82[6]
10	0.11[6]	4.14[6]	4.25[6]	10	0.28[6]	4.58[6]	4.86[6]
11	0.16[6]	1.40[6]	1.56[6]	11	0.12[6]	3.53[6]	3.65[6]
12	0.22[6]	1.99[6]	2.21[6]	12	0.99[6]	3.82[6]	4.81[6]
Mean	0.13	2.83[7]	2.96	Mean	0.32	4.85[7]	5.17
S.D.	0.1	3.2		S.D.	0.4	5.6	

[1] 300 mg of glucose in 1 ml of H_2O solution/100 g body weight.

[2] Six hours after force-feeding.

[3] Eighteen hours after force-feeding.

[4] Twenty-four hours after force-feeding.

[5] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

[6] Used for statistical analysis.

[7] Mean for control is significantly lower than experimental ($p < 0.05$).

TABLE A-14. Total urinary glucose excretion after galactose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	2.77	7.16	9.93	1	2.30	12.21	14.51
2	5.82	6.24	12.06	2	2.26	4.21	6.47
3	1.96[5]	3.90[5]	5.85[5]	3	2.34[5]	1.38[5]	3.72[5]
4	3.30	2.08	5.38	4	1.32	3.62	4.94
5	1.59[5]	7.69[5]	9.28[5]	5	1.67[5]	7.51[5]	9.18[5]
6	2.76[5]	6.41[5]	9.17[5]	6	4.33[5]	2.45[5]	6.78[5]
7	2.27[5]	1.45[5]	3.72[5]	7	1.15[5]	9.89[5]	11.04[5]
8	1.64	1.64	3.28	8	2.21	9.45	11.66
9	1.81	15.44	17.25	9	3.99	5.62	9.61
10	1.81[5]	2.32[5]	4.13[5]	10	3.42[5]	3.31[5]	6.73[5]
11	4.06[5]	3.90[5]	7.96[5]	11	3.12[5]	4.68[5]	7.80[5]
12	--[6]	--[6]		12	--[6]	--[6]	--[6]
Mean	2.70	5.29	7.99	Mean	2.55	5.84	8.39
S.D.	3.0	6.6		S.D.	2.7	6.7	

[1]300 mg of galactose in 1 ml of H_2O solution/100 g body weight.

[2]Six hours after force-feeding.

[3]Eighteen hours after force-feeding.

[4]Twenty-four hours after force feeding.

[5]Used for statistical analysis.

[6]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-15. Total urinary glucose excretion after fructose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection: 1st 6 hrs.[2]	Duration of Collection: Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection: 1st 6 hrs.[2]	Duration of Collection: Next 18 hrs.[3]	Total[4]
1	0.09[5]	0.81[5]	0.90[5]	1	0.63[5]	4.69[5]	5.32[5]
2	0.07[5]	3.11[5]	3.18[5]	2	0.85[5]	0.94[5]	1.79[5]
3	0.15[5]	1.00[5]	1.15[5]	3	1.61[5]	5.56[5]	7.17[5]
4	0.09	1.17	1.26	4	0.25	6.14	6.39
5	--[6]	--[6]		5	--[6]	--[6]	
6	0.09	1.35	1.44	6	0.43	4.19	4.62
7	0.09[5]	5.54[5]	5.63[5]	7	0.33[5]	3.53[5]	3.86[5]
8	0.05[5]	5.77[5]	5.82[5]	8	0.14[5]	4.99[5]	5.13[5]
9	0.24[5]	3.79[5]	4.03[5]	9	0.35[5]	2.45[5]	2.80[5]
10	0.11	4.12	4.23	10	0.23	5.13	5.36
11	--[6]	--[6]		11	--[6]	--[6]	
12	0.13	6.76	6.89	12	0.30	4.95	5.25
Mean	0.11	3.34	3.45	Mean	0.51	4.26	4.77
S.D.	0.1	4.2		S.D.	0.7	1.7	

[1]300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2]Six hours after force-feeding.

[3]Eighteen hours after force-feeding.

[4]Twenty-four hours after force-feeding.

[5]Used for statistical analysis.

[6]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-16. Total urinary glucose excretion after ribose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection: 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection: 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	-- [5]	-- [5]		1	-- [5]	-- [5]	
2	0.09[6]	2.81[6]	2.90[6]	2	0.15[6]	2.62[6]	2.77[6]
3	-- [5]	-- [5]		3	-- [5]	-- [5]	
4	-- [5]	-- [5]		4	-- [5]	-- [5]	
5	0.15	1.92	2.07	5	0.08	0.98	1.06
6	0.04[6]	0.98[6]	1.02[6]	6	0.11[6]	0.62[6]	0.73[6]
7	0.13[6]	3.60[6]	3.73[6]	7	0.08[6]	5.13[6]	5.21[6]
8	0.14	5.32	5.46	8	0.10	14.87	14.97
9	0.30[6]	5.45[6]	5.75[6]	9	0.20[6]	3.16[6]	3.36[6]
10	0.26[6]	6.72[6]	6.97[6]	10	0.16[6]	4.91[6]	5.07[6]
11	0.10[6]	4.84[6]	4.94[6]	111	0.13[6]	17.97[6]	18.10[6]
12	0.14	7.82	7.96	12	0.37	15.80	16.17
Mean	0.15	4.38[7]	4.53	Mean	0.15	7.34[7]	7.49
S.D.	0.2	5.2		S.D.	0.2	10.4	

[1] 300 mg of ribose in 1 ml of H_2O solution/100 g body weight.

[2] Six hours after force-feeding.

[3] Eighteen hours after force-feeding.

[4] Twenty-four hours after force-feeding.

[5] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

[6] Used for statistical analysis.

[7] Mean for control is significantly lower than experimental ($p < 0.01$).

TABLE A-17. Total urinary galactose excretion after galactose force-feeding, following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	1.12	3.02	4.14	1	1.11	3.61	4.72
2	1.20	1.57	2.77	2	1.37	3.00	4.37
3	1.52[5]	1.65[5]	3.17[5]	3	1.15[5]	0.00[5]	1.15[5]
4	1.91	1.88	3.79	4	1.53	2.39	3.92
5	1.42[5]	2.50[5]	3.92[5]	5	1.37[5]	1.97[5]	3.34[5]
6	1.01[5]	1.45[5]	2.46[5]	6	0.92[5]	3.01[5]	2.98[5]
7	0.86[5]	1.67[5]	2.53[5]	7	1.30[5]	1.68[5]	2.98[5]
8	1.22	1.30	2.52	8	1.15	1.50	2.65
9	1.18	2.22	3.40	9	1.78	1.77	3.55
10	1.45[5]	1.17[5]	2.62[5]	10	1.22[5]	2.04[5]	3.26[5]
11	1.33[5]	1.73[5]	3.06[5]	11	1.35[5]	1.71[5]	3.06[5]
12	--[6]	--[6]		12	--[6]	--[6]	
Mean	1.29	1.83	3.12	Mean	1.30	2.06	3.36
S.D.	13.8	0.55	1.93	S.D.	0.43	2.37	2.80

[1]300 mg of galactose in 1 ml of H_2O solution/100 g body weight.

[2]Six hours after force-feeding.

[3]Eighteen hours after force-feeding.

[4]Twenty-four hours after force-feeding.

[5]Used for statistical analysis.

[6]One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-18. Total urinary fructose excretion after fructose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection: lst 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection: lst 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	1.14[5]	1.19[5]	2.33[5]	1	2.21[5]	2.36[5]	4.57[5]
2	1.24[5]	2.06[5]	3.30[5]	2	1.19[5]	1.63[5]	2.82[5]
3	3.48[5]	2.33[5]	5.85[5]	3	0.90[5]	5.76[5]	6.66[5]
4	1.52	1.55	3.07	4	0.71	4.68	5.39
5	--[6]	--[6]		5	--[6]	--[6]	
6	0.93	1.31	2.24	6	1.40	2.29	3.69
7	2.53[5]	1.69[5]	4.22[5]	7	0.83[5]	1.66[5]	2.49[5]
8	0.44[5]	6.90[5]	7.34[5]	8	1.25[5]	4.24[5]	5.49[5]
9	1.30[5]	2.16[5]	3.46[5]	9	1.78[5]	2.13[5]	3.91[5]
10	1.06	3.09	4.15	10	0.59	3.17	3.76
11	--[6]	--[6]		11	--[6]	--[6]	
12	1.28	3.06	4.34	12	1.30	2.79	4.09
Mean	1.49	2.53	4.02	Mean	1.22	3.07	4.29
S.D.	1.8	3.0		S.D.	1.4	3.5	

[1] 300 mg of fructose in 1 ml of H_2O solution/100 g body weight.

[2] Six hours after force-feeding.

[3] Eighteen hours after force-feeding.

[4] Twenty-four hours after force-feeding.

[5] Used for statistical analysis.

[6] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

TABLE A-19. Total urinary ribose excretion after ribose force-feeding,[1] following two weeks of steroid treatment. (mg)

Control Rats	Duration of Collection: 1st 6 hrs.[2]	Duration of Collection: Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection: 1st 6 hrs.[2]	Duration of Collection: Next 18 hrs.[3]	Total[4]
1	-- [5]	-- [5]		1	-- [5]	-- [5]	
2	0.00[6]	0.79[6]	0.79[6]	2	0.00[6]	1.24[6]	1.24[6]
3	-- [5]	-- [5]		3	-- [5]	-- [5]	
4	-- [5]	-- [5]		4	-- [5]	-- [5]	
5	13.45	5.62	19.16	5	0.51	7.29	7.80
6	0.00[6]	0.53[6]	0.53[6]	6	0.00[6]	5.70[6]	5.70[6]
7	9.13[6]	1.07[6]	10.20[6]	7	2.10[6]	0.54[6]	2.65[6]
8	0.00	2.81	2.81	8	9.17	0.59	9.76
9	0.00[6]	5.00[6]	5.00[6]	9	2.23[6]	0.43[6]	2.65[6]
10	0.00[6]	2.53[6]	2.53[6]	10	6.09[6]	1.04[6]	7.13[6]
11	7.11[6]	2.25[6]	9.36[6]	11	3.14[6]	0.00[6]	3.14[6]
12	0.14	1.50	1.64	12	0.00	0.00	0.00
Mean	3.32	2.46	5.78	Mean	2.58	1.87	4.45
S.D.	6.3	3.2		S.D.	4.2	3.3	

[1] 300 mg of ribose in 1 ml of H_2O solution/100 g body weight.

[2] Six hours after force-feeding.

[3] Eighteen hours after force-feeding.

[4] Twenty-four hours after force-feeding.

[5] One of the pair-mates died during sampling. Since data were based on paired rats, only data for completed pairs are included in this table.

[6] Used for statistical analysis.

TABLE A-20. Total urinary glucose excretion after two weeks of steroid treatment.[1] (mg)

Control Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]	Exptl. Rats	Duration of Collection 1st 6 hrs.[2]	Next 18 hrs.[3]	Total[4]
1	0.15	1.02	1.17	1	0.14	5.90	6.04
2	0.14	2.56	2.70	2	0.12	14.93	15.05
3	0.15	3.21	3.36	3	0.07	3.55	3.62
4	0.10	10.34	10.44	4	0.03	6.83	6.86
Mean	0.13	4.28	4.41	Mean	4.28	7.80	12.08
S.D.	0.2	5.8		S.D.	2.1	9.7	

[1]Animals were fasted 15 hours prior to collection.

[2]First six hours of collection.

[3]Next eighteen hours of collection.

[4]Total urine collection.

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