ORAL CONTRACEPTIVE STEROIDS: EFFECTS ON FOOD CONSUMPTION, DIGESTIBILITIES OF VARIOUS NUTRIENTS, BODY COMPOSITION, AND LIFE SPAN IN RATS

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ABSTRACT

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By

Kanagavalli Manoharan

Effects of oral contraceptive steroids on food consumption, digestibilities, and retentions of various nutrients, body composition and life span were studied in female rats fed a basal grain ration and the contraceptive steroids norethynodrel, a progestine and mestranol, an estrogen. The results from these rats were compared with those from control rats fed just the basal grain ration. Food consumption and body weights were measured. Urine and feces were collected twice; 22 days and 173 days after steroid treatment to study the digestibility and retention of nutrients. Four groups of rats were killed at different time intervals to study the changes in body composition and to determine whether the changes are reversible. Two groups were sacrificed at 4 weeks and 25 weeks and 3 days

respectively after continuously feeding the steroid mixed diet. The other two groups were sacrificed after refeeding the control diet for 6 weeks following 4 weeks and 25 weeks and 5 days of steroid treatment.

Food consumption and body weights were significantly lower for the steroid treated rats. Analysis of urine and feces revealed that the digestibilities of protein, fat, sodium, and potassium were not affected during the short-term or long-term feeding of the steroids. A significant difference occurred in the retentions of the dietary nitrogen and sodium between the treated and control rats. After 22 days of steroid treatment, the control rats retained significantly ($P < 0.05$) more nitrogen than the treated rats. Dietary sodium was retained significantly more ($P < 0.05$) by the treated rats than by the control rats at this period. At 173 days of steroid treatment the treated rats retained significantly more ($P < 0.01$) dietary nitrogen than the control ones. No difference was noticed in the retention of potassium between the two groups either at 22 days or at 173 days after steroid treatment.

Body composition analyses revealed that percent moisture in the carcasses of the treated rats was higher

in all four groups. However, it was significant ($P < 0.05$) between the treated and the control rats only in the first group which was sacrificed after 4 weeks of steroid treatment. Percent moisture in the lean body masses was almost the same for the treated and the control groups. Absolute amounts of dry body weights were significantly higher for the control rats both after 4 weeks and 25 weeks and 3 days of steroid treatment. Significant difference occurred in lean body masses between the treated and the control groups after 25 weeks and 3 days of steroid treatment. Refeeding the control diet for 6 weeks did not bring the dry body weights and lean body masses comparable to control rats.

Treatment with steroids suppressed the gain in body fat in treated rats. This happened both in short and long-term feeding of steroids. Refeeding the control diet helped the treated rats to gain more fat. Nitrogen as a percent of either wet or dry lean body mass was not significantly different between the treated and control groups throughout the experiment. However, a significant difference was noticed between the two groups in the absolute amounts of nitrogen and protein after 25 weeks and 3 days of steroid treatment. Refeeding the control diet for 6

weeks did not change difference in body nitrogen and protein between the treated and control groups. Sodium and potassium per 100 gm. of wet and dry lean body masses were almost the same for both groups.

Life span study revealed that there were no significant differences in the life spans of the treated and control rats. However an increased incidence of mammary tumors was observed in the treated group.

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INTRODUCTION

During the past ten years, a revolution has occurred in the field of contraception. The revolution was brought about by the discovery of the oral contraceptive pill. In 1960 the oral contraceptive pill was licensed for general use in the United States. Now the use of the pill has become a common practice among women. It was estimated in 1965 that 3.8 million women under 45 and living with husbands were using the oral contraceptive pill in the U.S.A. and an additional 4.7 million indicated that they might use it in the future. The percentages of women now using the oral contraception vary positively and strongly with the amount of education they have. The widespread use of this medication for the control of conception or for various other reasons by healthy fertile women for many years implicates concern for the safety with which these preparations can be used. Clinical data from observations and research on thousands of women pointed out no apparent relationship between contraceptive therapy and any other major abnormalities. This made the FDA panel declare the

pill "not unsafe" for human use. However, there are some women who are very susceptible to changes brought about by oral contraceptive therapy.

Special interest attaches to the physiological actions of the oral contraceptives because these preparations contain two hormones which are the synthetic counterparts of natural estrogen and progesterone. Thus, one could expect these hormones to have some effects on their target organs such as the uterus, ovary, and pituitary. They might also have endocrine effects characteristic of the ovarian estrogen and progesterone. Recently there have been many reports on the oral contraceptive pills and their action on pitutitary and adrenocortical secretions and metabolisms of various nutrients. There have been some controversies whether the pill really changes the secretion rate of adrenal cortex and thyroid and causes liver damage. But there is no doubt that it may cause many changes in carbohydrate, fat, protein, mineral, and various other metabolisms associated with its hormonal action. Seemingly, it brings changes even in cholesterol metabolism which plays a central role in the synthesis of all body steroids.

Many questions have to be answered concerning the long-term and intermediate-term safety of these

preparations. Regarding long-term safety, the most frequent question concerns the possible increased occurrence of malignancies as a result of the use of these endocrine preparations. Concerning the intermediate safety, the questions relate particularly to possible deleterious effects on the liver, adrenals, and pituitary. Additional side chain like 17-alkylation which is not part of the natural estrogen and progesterone but is present in the synthetic counterparts may also bring about some deleterious effects on various organs.

In the view of nutrition, changes in body compositions of various compartments imposed by the changes in various metabolisms are very important. Since contraceptive preparations are taken through oral route, digestibilities of various nutrients may be affected. Estrogen which is one of the component of the pill is known for its action on 'appetite' and 'weight gain' especially in laboratory animals (Meites, 1949; Sullivan and Smith, 1957). Results of the few experiments thus far published clearly demonstrate that the nutritional significance of the oral contraceptives should be studied in greater depth.

The present experiment was undertaken to study the extent and in which compartments of the body these

antiovulatory steroids bring changes, whether digestibilities of various nutrients like fat, proteins, carbohydrate and some minerals are affected and to see whether any changes occur in the balances of various major nutrients. In addition to the above mentioned aspects of the experiments, long-term study was initiated to determine the life span and various other abnormalities, if any, like cancer, tumor, etc. Food intakes and body weight gains were also studied.

REVIEW OF THE LITERATURE

History of Oral Contraceptive Pill

Various types of contraceptive methods were being used for many years. But none of these methods proved to be completely effective. Only in the past few years, there has been a change in the medical attitudes toward contraceptive techniques. In 1960, a new approach was made in the field of contraception with the availability of the contraceptive pill which proved to be nearly 100% effective in controlling conception. The development of the contraceptive pill has really been an interesting research adventure. Synthetic sex hormones, estrogen, and progesterone are the two active components of the oral contraceptive pill. The very idea of controlling conception with progesterone was initiated by Beard (1897) who postulated that the corpus luteum of the ovary which secretes progesterone was responsible for the inhibition of ovulation during pregnancy. Then, when progesterone was isolated, Makepeace *et al*. (1937) administered this hormone into rabbits and

found that this inhibited the ovulation. Later on Pincus (1956) reported that the oral administration of natural progesterone was not suitable for contraceptive purposes probably because of the variable absorption from the gastro-intestinal tract. So research was turned toward synthetic steroid hormones having both the properties of progesterone and high oral activity.

Norethynodrel, the progestin of the first approved oral contraceptive pill was prepared by Frank Colton at the Searle Laboratories. Its contraceptive effectiveness was tested and proved by Rock et al. (1956). Even though norethynodrel proved its effectiveness in the control of conception, the incidence of spotting and breakthrough bleeding was high. Estrogen was added to the progestin to control the occurrence of spotting and it was proved successful. Later on, estrogen was added to other progestins in order to improve endometrial support. In addition to norethynodrel, several other progesterone preparations, all with added estrogen, have been tried including norethindrone, norethindrone acetate, medroxyprogesterone acetate, ethynodiol diacetate and chlormadinone acetate. All have been effective in various dosage combinations.

Thus an effective biochemical control of conception was attained by means of oral contraceptive pills.

Many oral contraceptive pills are now available under various brand names in various countries. Some of them are Enovid, Orthonovum, Anovlar, Norlestrin, Lyndiol, Ovulen, Provest, Ovex, Aconcen, etc. Their composition is presented below.

Product	Progesterone	Estrogen
Enovid	Norethynodrel	Mestranol
Orthonovum	Norethindrone	Mestranol
Anovlar	Norethindrone acetate	Ethynylestradiol
Norlestrin	Norethindrone acetate	Ethynylestradiol
Lyndiol	Lynestrenol	Mestranol
Ovulen	Ethynodiol diacetate	Mestranol
Provest	Medroxyprogesterone acetate	Ethynylestradiol
Ovex	Megestrol acetate	Ethynylestradiol
Aconcen	Chlormadinone acetate	Mestranol

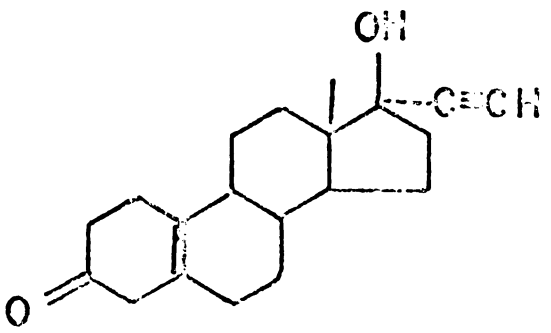
Common preparations currently available commercially consist of a progestin in combination with an estrogen throughout the treatment period or the so-called "combined therapy." In sequential oral contraception, estrogen

alone is given for 15 days of therapy, followed by the combination of estrogen and progesterone for 5 days. C-Quens and Oracon are the first "sequential" oral contraceptives to be marketed. One of the major problems with the use of sequential therapy is the failure of occurrence of menses following the withdrawal of the therapy and, in addition, it is not as effective as the other therapy.

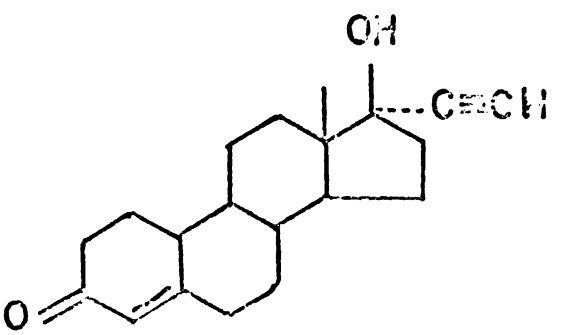
Structure of Progestins and Estrogens

The chemical structures of two of the progestins used in the oral contraceptives are shown in Figure 1. Norethynodrel, norethindrone, and several other compounds have an ethynyl group at the 17 position of the steroid molecule; it is apparently this group with the nor structure that gives these compounds their high oral activity. Compounds in which the 19-methyl group in the steroid molecule has been replaced by a hydrogen atom are referred to as 19-nor compounds. Each of these compounds has a carbon-carbon double bond in the basic steroid molecule. Norethynodrel, a progestin which is used in Enovid, has this double bond at the 5(10) position. This double bond at the 5(10)

PROGESTINS

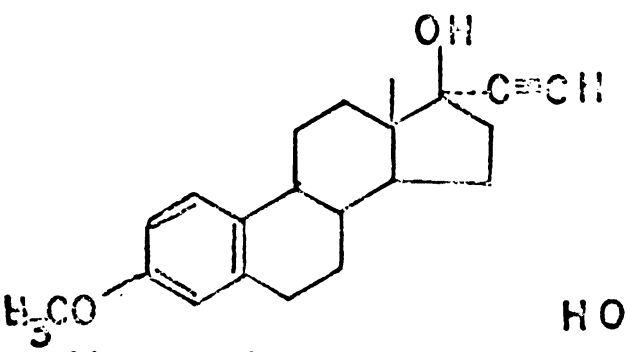


Norethynodrel

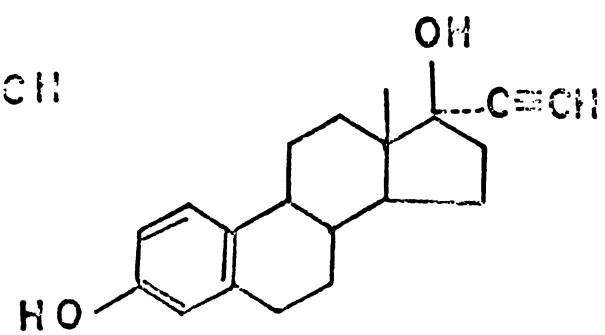


Norethindrone

ESTROGENS



Mestranol



Ethynylestradiol

Fig. 1.--Structures of two of the progestins and the estrogens used in oral contraceptives.

position is biologically significant, for in addition to being progestational, it makes norethynodrel estrogenic and devoid of androgenic effects in both animals and men (Drill, 1966). In other substances like norethindrone, the double bond is at the 4(5) position. Some authors, thus, classify norethisterone as a derivative of 19-nortestosterone. But norethynodrel cannot be classified as a testosterone derivative because the double bond is in the 5(10) position rather than the 4(5) position. The two estrogens presently used in all oral contraceptive preparations are either mestranol or ethynylestradiol. Their structures are also shown in Figure 1. Both compounds contain a 17-ethynyl group which imparts high oral potency.

Mechanism of Action

The mechanism by which contraception is assured has been a subject of some discussion. The high degree of effectiveness has been attributed to inhibition of ovulation. However, two other mechanisms have also been suggested. One is the alteration of the cervical mucous so that the

sperm penetration is inhibited, and the other is the alteration in the endometrium so that it becomes unsuitable for nidation. However, the inhibition of ovulation is undoubtedly the most important mechanism involved. The administration of oral contraceptives, which are the combination of estrogen and progesterone, mimic natural estrogen and progesterone. They prevent ovulation much like the natural hormones which prevent ovulation during pregnancy. By studies on animals, it has been established that oral contraceptives achieve ovulation control by inhibiting the secretion of gonadotropins from the pituitary gland. This was shown by Saunders and Drill (1958) who demonstrated that norethynodrel, the progestin in Enovid, decreases the gonadotropin content of the pituitary gland in the ovariectomized rat. Other studies have also demonstrated the effectiveness of norethynodrel (Saunders, 1964) and various other progestins in inhibiting the secretion of the pituitary gonadotropins in the rat. Estrogens are also quite effective in inhibiting pituitary gonadotropin secretion in the rat and, in terms of dosage, they are much more potent than the progestins (Ibid.).

Venning (1962) found evidence for the suppressed follicle growth as well as ovulation with norethynodrel which would indicate that Follicle Stimulating Hormone and Leutinizing Hormone were decreased. Further evidence that ovulation was suppressed came from the work of Shearman (1965). He demonstrated two cycles in patients aged 26 years. The first was without treatment which showed the normal pattern of excretion observed in ovulatory menstruation with a peak of estrogen excretion at about the 14th day associated with ovulation and a luteal phase rise in estrogen and pregnanediol followed by regression before the start of menstruation. On day 5 of the second cycle, treatment with Anovlar was started. There was a progressive fall in estrogen excretion and no evidence of ovulation or corpus luteum activity. When Anovlar was withdrawn in the next month, the pattern of normal ovulation was produced. These assays were repeated after a further six months treatment and the same results were obtained.

Biological Properties of Oral Contraceptives

The Adrenal Cortex and the Pituitary

Pregnancy or the administration of estrogens increases protein-bound cortisol in the plasma and, thereby, the total plasma cortisol. A decreased excretion of 17-keto steroids (17KS) and 17-ketogenic steroids (17KGS) has been observed by Starup et al. (1966), who treated women with 5 mg. of megestrol acetate and 0.1 mg. of mestranol for a period of 3-74 weeks. This suppression might have been caused by an inhibition of the production or release of ACTH. However, the authors found a normal response to metyrapone or ACTH during treatment in all patients studied, indicating a normal adrenal cortical function during treatment with megestrol acetate plus mestranol. The authors concluded that the pituitary-adrenocortical feedback mechanism was undisturbed. Since they found increased plasma protein-bound cortisol during treatment they also concluded that the protein-binding of plasma cortisol made it less available for conjugation by the liver and renal excretion. Even though it has been shown that protein-bound cortisol is physiologically inactive, there is a possibility that

the increased circulating corticosteroids may cause diminished carbohydrate tolerance in certain subjects taking oral contraceptives.

In contrast to the work of Starup et al. (1966), Enovid has been reported to decrease the response to metyrapone (Mestman et al., 1963) but since the response to ACTH is not affected, the changes in the excretion of 17KS and 17KGS appear to be related more to inhibition of pituitary responsiveness than to altered adrenal cortical response (Leach et al., 1965). Reduced ACTH reserves noted in some of the Enovid users with reduced glucose tolerance (Waine et al., 1963) suggests a direct action of steroids especially estrogen on adrenocortical secretion. A direct corticoid action of medroxyprogesterone acetate has been demonstrated in adrenalectomized and hypophysectomized subjects (Cumanni et al., 1963).

Certain oral contraceptives have also been found to change the secretion rate of mineralocorticoid hormones like aldosterone from the adrenocortex (Singer et al., 1963; Laidlaw et al., 1962).

Liver Function

Whether oral contraceptives cause liver damage has been a subject of controversy. Finnish investigators Eisalo et al. (1964) have reported reversible elevations of serum transaminase and Bromosulfonphthalein (BSP) in a series of 12 menopausal women. On the other hand, such contraceptives have not been found to cause liver damage by other investigators (Linthorst, 1964; Tyler, 1964). Linthorst treated 52 women with lynestrenol for a period of 14-43 months. No laboratory or clinical indication of hepatic dysfunction was noticed. Borglin (1965) treated 36 women with lynestrenol or lynestrenol + mestranol for a considerable period. Even though the frequency of abnormal liver function tests was fairly low, in a few cases, the values obtained did deviate from normal--namely an increase in the transaminase activity and a slight increase in the BSP retention. Serum Glutamic Pyruvic Transaminase was also increased in two of the patients treated with lynestrenol. However, the alkaline phosphatase values, bilirubin values, and the thymol turbidity tests were normal. The work of Datta et al. (1965) with rabbits using two anovulatory steroids, noracyclin and orthonovum, showed

that no hepatic damage was associated with these steroids by estimating the serum Glutamic Oxaloacetic Transaminase level in the serum.

Thyroid Function

Measuring the protein bound iodine (PBI) and the red cell uptake of radioactive triiodothyronine are measures of thyroid functions. The free thyroxine factor may also be used to assess thyroid status in women taking oral contraceptives. Goolden et al. (1967) measured PBI and the uptake of ^{131}I-triiodothyronine in a group of women who were taking oral contraceptives and in a control group of healthy women. Free thyroxine factor which is proportional to the free thyroxine in the serum was calculated. The data suggested that thyroid status was normal in the women taking estrogen and progestational compounds. However, in subjects studied before and after 3 months of use of oral contraceptives, there was a tendency to a reduced uptake of radioiodine by the thyroid of the users of 2mg. Ovulen but not by users of Enovid or 1 mg. Ovulen (Pincus, 1965).

Effects of Oral Contraceptives on Metabolisms

Oral contraceptives contain estrogens and progestational compounds and thus are likely to exhibit biological activities characteristic of natural ovarian hormones. In fact many of the metabolic actions of oral contraceptives mimic the effects of estrogen administration. There have been many reports during the past few years which point out the estrogenic or progestational or combined effects of oral contraceptives on carbohydrate, fat, protein, and mineral metabolisms. Various changes in metabolisms may be brought about by alterations in the secretion and functional rates of endocrine glands. In addition they may also alter the binding power of plasma proteins and thus bring changes in metabolism. Since the estrogen and progesterone present in oral contraceptive pills are synthetic and have additional modification like 17-alkylation, these steroids may have some other deleterious effects which are not associated with natural estrogen and progesterone.

Carbohydrate Metabolism

Oral contraceptives seem to produce alterations in glucose metabolism. An impairment of oral glucose tolerance have been noted in a group of women receiving Enovid (Gershberg et al., 1964). Ten percent of 59 women receiving Enovid had elevated fasting levels and 20% had elevated one hour levels and 46% had elevated 2 hour levels. The incidence of abnormal glucose tolerance appeared to be greater in women with a family history of diabetes than those without. The observation of abnormal glucose tolerance, especially with diabetic women, is in agreement with the work of Cochran and Pote (1963). They found fifteen of a group of 30 menopausal women under Enovid therapy for 21 months, to have abnormal glucose tolerances. Wynn and Doar (1966) investigated a number of aspects of carbohydrate metabolism in women taking various oral contraceptives. They found abnormalities of oral and intravenous glucose tolerance, plasma nonesterified free fatty acid (N.E.F.A.) and blood pyruvate values. But the mean fasting-plasma-glucose was not significantly different from that of a control group. A striking metabolic abnormality was an increased fasting-blood-pyruvate level or an increased maximum pyruvate increment following glucose administration or both.

This occurred in about 20% of women in the test group. These abnormalities were similar to those found in steroid diabetes. The elevated plasma N.E.F.A.s, shown in the test group, might be a cause of impaired glucose assimilation and pyruvate oxidation (Randle, et al., 1963). The striking elevation of blood pyruvate level might be due to an excess of glucocorticoid action, even though it has been reported by Plager et al. (1964) that despite elevated levels of bound and unbound plasma cortisol in the estrogen-treated subject, the total amount of cortisol in the tissues may be equivalent to that found in the control subject since there may be a higher level of transcortin present outside the vascular compartment in the estrogen-treated subjects.

Another study was undertaken by Spellacy and Carlson (1966) to explore the area of carbohydrate metabolism in subjects receiving an oral contraceptive pill by measuring both plasma insulin and blood glucose before and after drug therapy. This was done in a fasting state and after an intravenous glucose stimulus. Twenty-five subjects were tested before and after one cycle of oral Enovid treatment. The results of this study showed both the glucose and

insulin levels were higher in the drug-treated group. These authors postulated that the rise in insulin level could be due to the prolonged stimulation of the pancreas by these oral contraceptive drugs.

It has been suggested that the estrogen component of the contraceptive drugs is apparently responsible for the changes in carbohydrate metabolism, because treatment with a progestational agent alone does not alter glucose tolerance (Puchulu et al., 1967). A short-term study of the effect of sequential treatment with ethynylestradiol and megestrol acetate on glucose tolerance revealed that estrogen was more involved in producing abnormal glucose tolerance than progesterone (Pyörälä et al., 1967). The results of this study indicated that glucose tolerance decreased during the estrogen phase of sequential treatment and then was slightly impaired during the following phase of combined estrogen-progesterone treatment. Puchulu et al. (1967) have suggested that ethynylestradiol has less effect on glucose tolerance than mestranol, which has been employed as an estrogen component in contraceptive drugs. Another study by Buchler and Warren (1966) suggests that the estrogen effects are related to changes in delayed absorption

of glucose rather than any diabetogenic effect, since normal intravenous glucose tolerance was observed in patients treated with diethylstilbestrol.

Lipid Metabolism

The involvement of gonadal hormones in fat metabolism has been a subject of study. It has been reported that when estrogen therapy, such as ethynylestradiol or dioxydiethylstilbestrol, is combined with orally active synthetic androgens, such as methyl testosterone, it caused a sharp increase in low density lipoproteins (L.D.L.). This was followed by a decrease in high density lipoproteins (H.D. L.)(Russ et al., 1955). It is possible that the chemical structure of progestin in oral contraceptives can be close to various synthetic androgens. Oral contraceptives can then be expected to cause changes in lipid metabolisms and transport. The work of Aurell et al. (1966) proves this to a certain extent. They studied the effect of the oral contraceptive Anovlar containing 50 μg. of 17-ethynylestradiol and 4 mg. of norethisterone. Serum lipids and serum lipoprotein fractions were measured before administration and at regular intervals during one year. After a one year administration there was a significant

rise in serum-lipids, especially in serum low density lipoproteins. The low density lipoprotein reached levels typical for the post-menopausal women. This effect seems in favor either of an androgen like activity of norethisterone or that the prolonged medication leads to an inhibition of normal ovarian increment of estrogen.

Pincus (1965) studied the effect of Enovid which contains norethynodrel and mestranol. The study covered a whole year to rule out the seasonal influence on the serum lipid levels. In pre-menopausal women, no significant changes were found in either blood cholesterol or β-lipoprotein levels following Enovid therapy.

Elevation of serum triglyceride, cholesterol, and low density and very low density lipoprotein levels have been described in a group of 102 women receiving cyclical oral contraceptives (Wynn et al., 1966). Thirty-one percent of the women had fasting-serum-triglyceride levels above 131 mg. per 100 ml., which is the greatest value observed in a control group of 75 women. The authors attribute the changes in serum lipid and serum lipoprotein patterns to the combined effect of the estrogen with a progestational steroid sharing the metabolic effect of a 17-α-alkylated androgen.

Andrews et al. (1949), testing the effect of stilbestrol and testosterone on growth and fattening of black face wether feeder lambs, showed that implants of stilbestrol increase the rate of gain and feed efficiency. The lambs were divided into groups and were given five different treatments for a period of 68 days. The results of their experiment showed that subcutaneous implantation of 12 or 24 mg. of stilbestrol or 10 mg. of testosterone significantly increased the gains of wether lambs during the 68-day feeding period. All hormone-treated groups required significantly less feed per pound of gain than the control lambs. However, no measurements of skeletal growth were made. Moreover, the data on carcass grades suggest that the lambs which received stilbestrol were characterized by somewhat less finish and more growth than the controls and that the lambs which received testosterone were fatter and less growthy than the stilbestrol groups.

The study of Jordan et al. (1956) revealed the combined effect of an estrogen and a progesterone. They treated lambs with the implants of various hormone combinations. Stilbestrol was implanted in one group.

Testosterone and estradiol combination and progesterone and estradiol combination were implanted in two different groups. The results of this trial showed that implants of stilbestrol and the combinations of testosterone and estradiol significantly increased the average daily gain. The combination of progesterone and estradiol increased the daily gain slightly, but the increase was not sufficient to be significant. The authors suggested that the progesterone inhibited the growth acceleration from the estradiol to a certain extent. The work of Day et al. (1960) is in agreement with the above mentioned work. They used forty-eight Poland China barrows in their experiment to study the influence of subcutaneous progesterone-estradiol (high and low levels) implants on average daily gain and carcass composition. The hormone implants had no significant effect on gain although the high level of progesterone-estradiol combination tended to reduce the growth rate. The most pronounced difference between the control group and the progesterone-estradiol implanted barrows was that the treated animals had a significantly decreased average back fat. Carcasses of the control and treated barrows were similar; however, those from the

barrows implanted with high level of progesterone and estradiol showed a trend toward increased leanness.

Stimulation of body weight gain or increase in food utilization efficiency, which occurred with lambs, treated with estrogens, did not occur with rats. The experiment conducted by Meites (1949) with diethylstilbestrol and by Sullivan and Smith (1957) with estradiol, showed growth depression in rats. However, in both experiments, the pair fed control groups paralled the growth of animals treated with estrogen. But the work of Yang et al. (1969) revealed that even though steroid treated animals and their pair fed controls consumed equal quantities of diet, the control rats had greater body weights. The effect of prolonged treatment with norethynodrel on body weight was found to depend on the age of the animals (Holmes and Mandl, 1962). The treatment reduced the growth of rats aged 55-66 days; and caused a slight reduction in the weight of the fully grown animals aged 148 days. Similar observations were found with animals of intermediate age, 89-102 days treated for a period of up to 86 days.

The study of Bakker and Dightman (1966) showed that women taking norethynodrel did not reveal a significant trend in the direction of weight gain or weight loss.

There was no evidence to support the hypothesis that norethynodrel leads to weight gain due to increased deposition of fat. The variation among the different reports concerning the weight changes in women is quite wide, and so it is difficult to reach a general conclusion regarding the oral contraceptives and changes in body weight.

Protein Metabolism

Not much work has been done on the effects of oral contraceptives on protein metabolism and nitrogen retention. Whitehair et al. (1953) treated lambs with 24 mg. of stilbestrol implanted in the neck region. Marked increases in calcium, phosphorous, and nitrogen retentions in the stilbestrol treated lambs were observed. Landau and Lugibihl (1963) said that protein catabolism was induced by progesterone which was associated with a decline in plasma amino nitrogen. In four studies in normal subjects, all amino acids measurable except phenylalanine participated in the lowering of the concentration of plasma amino acids. Fasting concentration of most of the amino acids were 12-34% lower than control values. Since when protein catabolism was accelerated by progesterone the amino acid levels drop, enhancement of the utilization of

the amino acids and their conversion to urea by liver was the indicated mechanism. Moreover, the authors also mentioned that urinary amino acid nitrogen was unaffected. However, the work of Adams (1966) indicated that 19-nor-progestin or 17-acetoxy progestin caused a gain of lean tissues and a loss of fat without gain in body weight and positive nitrogen, potassium, and sodium balances in women compared to premedication period. The changes in lean body mass and in the amount of protein retained were more uniform with the 17-acetoxy progestins than with the 19-nor progestins. This study was conducted for a short period of three weeks only. It should be pointed out that no genuine anabolic effect can be demonstrated with any of the preparations in current use.

Fluid Retention

Mahan (1962) treated 100 women with 5 mg. of Enovid. He reported that there were minor side effects in approximately 10% of the cases. One of them was weight gain, associated with this medication, due to fluid retention. Ringrose (1963) reported that out of 115 persons who were taking orthonovum, four noticed swelling of ankles in eight of the 31 cycles. In the study of Street (1960) with 10

persons taking 10 mg. Enovid, four complained of edema of the fingers and ankles during the middle to the last part of the menstrual periods. The above mentioned cases of edema were all judged by subjective means.

Mineral Metabolism

The hormones in the oral contraceptive pills have been reported to change the secretion rate of mineralocorticoids from the adrenal. This change in secretion rate of mineralocorticoids would be expected to change the mineral metabolism of those who are taking these preparations.

It was shown by Ehrlich et al. (1960) that progesterone inhibits the salt retaining effect of aldosterone. Therefore progesterone must be intimately involved in the hormonal control of renal sodium excretion during pregnancy. In contrast to the effect of progesterone described here, it was shown by Eugenia et al. (1961) that progesterone failed to elicit any influence on electrolyte excretion caused by deoxycorticosterone. According to Singer et al. (1963) in intact male rats, and hypophysectomized rats fed a normal diet, the subcutaneous administration of progesterone for 4.5 days and 5.5 days respectively resulted in a significant increase in the aldosterone secretion rate.

This suggested the fact that the mechanism, by which this increase in aldosterone secretion was brought about, probably did not involve the pituitary gland. The authors discussed the possibility that injected progesterone served as a precursor to aldosterone. Another possible mechanism suggested was that the progesterone was inhibiting the effect of endogenous aldosterone on the kidney, which would lead to an increase in the aldosterone production. This mechanism was also suggested by Laidlaw et al. (1962) who explained that the anti-aldosterone action of progesterone was overcome by hypersecretion of aldosterone and the sodium balance was restored in four men who received 50-200 mg. progesterone daily intramuscularly. The effect of estrogen may also account for the altered metabolism of aldosterone, both in pregnancy and in the treatment with oral contraceptives.

While the mechanism of action of progesterone on sodium balance is obscure, many cases of hypertensions have been reported by Woods (1967) and Laragh et al. (1967) in patients who were taking oral contraceptive pills. They also reported marked improvement in most of the patients after the drug treatment was stopped. In the study of

Laragh et al. (1967) nine out of ten patients who were on the pill had very striking and sustained increases in the concentration of renin-substrate in the serum and there was an increase in renin activity in a few patients. However, in none of the patients can the increased serum renin activity be attributed to a state of sodium depletion because the range of urinary sodium excretion together with the absence of clinical edema provided evidence for normal sodium metabolism. The authors thus concluded that the administration of pharmacological doses of estrogen and progesterone required for a contraceptive action were found to produce a number of abnormalities in the renin-angiotensin-aldosterone system.

Only a few balance studies on calcium and phosphorous have been done. Womack et al. (1950) treated a four month old male infant with estrogen who was suffering from severe osteoporosis. The baby was given a weighed milk diet with vitamin supplements considered adequate to meet his fundamental nutritional requirements. This control period comprised of a total of 18 days. Balance studies were done by collecting urine and stool after the patient had been on the experimental diet. In addition one ml. of

Progynon B, an estrogenic substance, was given intramuscularly for 30 days, while the diet and other conditions remaining unaltered. The result showed that the average daily calcium retention was 0.154 gm. for the control period and 0.153 gm. for the estrogen-treated period. Less phosphorous was retained during the estrogen treatment. The average daily retentions were 0.234 gm. and 0.142 gm. respectively for the control and estrogen periods. Roentgenograms revealed no change in the degree of osteoporosis. In another study Ackermann et al. (1954) determined the nitrogen and calcium balances in six elderly women before and during estrogen therapy to reinduce menstruation. No effect was noted in nitrogen balance on any of these subjects, all of whom were initially in positive nitrogen balance. There was little effect on the calcium balance in subjects initially in positive balance, but some increased retention in subjects initially in negative balance. Treatment with progesterone had less effect.

The work of Adams (1966) showed an interesting feature regarding phosphorous balance. Eight female subjects of 21-29 years were initially stabilized on estrogen with mestranol for fourteen days. Then the test progestins

norethynodrel and chlormadinone acetate were added to the mestranol and they were continued for another three weeks. The result showed a rather uniform negative phosphorous balance which ensued when the patients were placed on progestin therapy. There was no significant difference in the route of phosphorous loss from the body for any of the drugs used.

Much less has been done to study the effect of hormones like estrogen and progesterone on other minerals like potassium, magnesium, chloride, etc. A study done by Sellers (1951) with a small number of dairy cows, given large doses of diethylstilbestrol in late pregnancy and later followed over the parturition period, showed no appreciable or consistent changes in blood levels of sodium, potassium, magnesium, or chloride following either estrogen administration or parturition. Few papers have been published recently which are concerned with the effect of estrogen and oral contraceptives on copper metabolism. German et al. (1961) worked with 11 patients with Wilson's disease to whom ethynylestradiol was administered. In four patients the serum copper and ceruloplasmin concentrations and the urinary copper excretion increased. In six

patients there was an increase in the serum copper concentration, three of whom showed cupruresis. The levels of serum copper and ceruloplasmin and the urinary copper excretion showed no change in one patient. One patient improved clinically, four were unchanged, and six deteriorated during the estrogen administration.

The increase in serum concentration of copper has also been demonstrated with the administration of the oral contraceptive pill, Enovid (O'Leary and Spellacy, 1968). Fourteen female subjects, more than six weeks after parturition, were tested with 10 mg. Enovid daily for 21 days. Prior to treatment, the mean concentration of copper in the serum was 142 μg. per 100 ml. After one cycle of administration of oral contraceptive, the mean rose to 241 μg. per 100 ml. The change was statistically significant. The long-term effects of this alteration in serum copper content, especially in those people taking contraceptives for a long period, remain to be determined.

Bone Metabolism

Sex hormones have been shown to have an effect on bone growth. To a certain extent, they regulate the morphogenesis of the skeleton and may in part control the extent

of skeletal growth. It has been shown by Abdul-Karim et al. (1968) that the estrogens are necessary for the orderly process of endochondrial ossification in the fetal rabbit. However, large amounts of estrogen inhibit the growth of cartilage and hence longitudinal osseous growth. It has been shown by Whitelaw et al. (1963, 1967) that estrogens hasten the bone maturation and epiphyseal closure in prepubertal girls. Breibart et al. (1963) have shown that progestational compounds also increase the bone age of the infants when they are given to pregnant mothers.

Gedalia et al. (1964) conducted an experiment with 20-day old female rats. Estradiol benzoate (0.4 mg.) was injected subcutaneously twice a week and the rats were sacrificed after 2, 4, and 6 weeks. The results showed that the mean specific gravity and the breaking strength of the dry defatted femur was significantly higher in rats after two weeks of estrogen treatment as compared with control rats ($p < 0.01$). After six weeks, the differences between the experimental and control groups were no longer present. No significant increase in the mean calcium and fluoride contents of the femur ash of the estrogen-treated rats as compared with the control rats occurred. No

difference was detected between the mean phosphorous content of the femur ash of the estrogen-treated rats and controls.

Parenteral administration of large doses of estrogens to mice result in the deposition of new bone in the marrow cavities. This change is most pronounced in the long bones. Here the new bone is first laid down at both extremities on the trabeculae of the spongiosa and then becoming more compact, it extends along the longitudinal axis of the shaft toward the center of the marrow cavity. Barker and Crossley (1962) conducted research with mice. There were ten male and seven female pairs and each had its litter-mate control. All mice were kept under identical conditions and fed a standard diet. Both mice in each pair were given weekly subcutaneous injections of 0.1 mgm. estradiol monobenzoate. Treatment was begun when the mice were between 21 and 28 days old and continued for periods ranging from 4 to 20 weeks. On completion of the course of the injections the pairs together with their controls were killed. X-rays were then taken of the femurs, which were then decalcified, cut into longitudinal sections, stained with haematoxylin and eosin and mounted. The

radiographs of the femurs of the mice treated with estradiol showed an increase in the amount and density of the bone, first at the distal and later at the proximal epiphysis. This is the characteristic initial response of the mouse skeleton to estrogens. The amount of bone formation increased with the length of the course of the injections.

The effect of an estrogen and progesterone combination on bones of the adult and immature female rats has been shown by Yang et al. (1969). They treated adult rats with 0.1911 and 0.0028 mg. of norethynodrel and mestranol respectively or 0.0967 mg. of norethynodrel and 0.0014 mg. of mestranol per kilogram of body weight per day. Both the levels were effective. Immature rats were treated with 0.128 mg. of norethynodrel and 0.0019 mg. of mestranol per kg. per day. The results of this experiment showed no significant difference in the lengths of the femurs of the adult rats when compared to their pair fed control rats. However, the lengths of the femurs of the immature rats were significantly shorter when compared to their pair fed immature controls. Cross sectional growths of the tibias were significantly different between control and

experimental group of the adult rats, 100 microns and 83 microns respectively. With immature rats there was no difference in the cross section of the tibias between the control and experimental groups. Tetracycline labeling was used to measure the cross section of the bones.

MATERIALS AND METHODS

Animals

One hundred and fifty female Sprague-Dawley rats, 3 weeks old, were obtained from a local supplier. They were fed a grain ration until 11 weeks old. The composition of this diet is shown in Appendix I (Campbell et al., 1966). At that time they were divided into groups. The first group comprised of 20 rats out of which 10 served as control rats were continued on the same grain ration. The remaining 10 were fed the grain ration plus the contraceptive steroids. The average weight of these rats was 257 gms. at the start of the experiment. These 20 rats served as representatives of the entire 150 rats. Food intake, body weight, and digestibility studies were conducted with these rats. The second group consisted of 80 rats, 40 each of control and experimental. These rats were used for the determination of body compositional changes during steroid therapy. Finally the third group of 22 control and 28 experimental rats was used to study

the long-term effect of the steroid therapy. All rats were housed in individual suspended wire cages and were maintained in an air-conditioned room at a constant temperature of 27°C and with 12 hours each of light and darkness. The rats of the long-term study were kept at the Veterinary Barn in contrast to all the rest that were kept at the animal room in the Home Economics building.

Contraceptive Steroids

The contraceptive steroids used in this experiment were norethynodrel and mestranol, the progestin and estrogen of the first oral contraceptive pill Enovid. The dosage used to feed the rats was equivalent to that used by women (0.1 mg. norethynodrel and 0.0015 mg. mestranol per Kilogram per day). The steroids were fed to the rats by first dissolving in 70% ethyl alcohol and then mixing thoroughly with the grain ration by means of a food mixer.

Preparation and Feeding of Diet

The concentration of the steroids in the diet varied slightly and was adjusted according to the changes

in body weights and food intake of the 10 rats which represented the whole experimental group. The experimental diet was adjusted and prepared every week. However, eight weeks after the beginning of the experiment, when the weights of the rats and their food-intakes were stabilized, the diet was prepared once in two weeks. Both the experimental and control diets were fed on an ad libitum basis. Water was also given free choice.

Digestibility and Balance Studies

Digestibility and balance studies were done by collecting urine and feces for a known period of time. Two collections were done. The first collection was done 22 days after feeding the experimental diet with the 20 representative rats. The second collection was started after 24 weeks and 5 days of steroid therapy. For this collection 20 rats of the long-term study, which were housed at the Veterinary Barn, were used since the 20 representative rats, originally used for the first collection, came down with respiratory infection. The collection periods were 4 days in both the trials.

Feces were dried in a force-air oven at 90°C and then ground in a Wiley-Mill to a fine powder. These powdered feces were analyzed for nitrogen, fat, sodium, and potassium. After removing the food spilled in the urine by filtering, urine samples were made into a known volume by adding deionized water. Aliquots were taken and analyzed for nitrogen, sodium, and potassium. The grain ration, portions of which were saved during the collection period, was also analyzed for nitrogen, fat, sodium, and potassium.

The quantities of nitrogen, sodium, and potassium consumed and the total excreted in the feces and the urine were used to calculate the balances and digestibilities. With fat, only digestibility was determined. Percent digestibilities and retentions of various nutrients were calculated using the formulas shown in Appendix II.

Body Composition

After treating the rats with steroids, they were sacrificed at four different intervals for body composition analysis.

First Group

Ten steroid-treated and 10 control rats were killed after they were fed for four weeks. This was immediately after the first urine and feces collection.

Second Group

This group consisted of 10 treated rats, 10 control rats. They were sacrificed 10 weeks after the start of the experiment. The experimental rats were on steroid treatment for the first four weeks and were fed just the control grain ration for the rest of the six weeks. This was to see whether there is any reversibility in the body compositional changes after the steroids were removed from the diet.

Third Group

Eight steroid-treated and eight control rats were killed after the experimental ones were fed with steroid-mixed diet for 25 weeks and 3 days continuously. This sacrifice was made one day after the second collection.

Fourth Group

The treated rats were on the steroid diet for 25 weeks and 5 days and then they were placed on control diet

for six more weeks. After this they and 7 control rats were killed. This was 31 weeks and 5 days after the initiation of the experiment.

All the above mentioned rats were picked at random. The first two groups of rats were killed by a blow on their heads and the next two groups were killed by over etherization.

Life Span Study

For this study, the animals were kept in the Veterinary Barn and were weighed only 3 times to avoid disturbing them unduly. The life span of each one of the treated and control rats was noted when it died. Tumor growths and other incidences were also recorded.

Preparation of Rats for Analysis

The gastro-intestinal tracts of the rats were removed and their contents washed off. The carcasses and the G.I. tracts were placed in tared jars. The jars containing the entire carcasses were then autoclaved at 15

lbs. pressure for 20 minutes. The rats were then homogenized in a Waring blender with water equaling approximately to the body weights of the rats. This homogenate was found to be too soft in consistency. It was discovered by trial and error that 100 ml. for rats that weighed 200-300 gms. and 150 ml. for the rats that weighed above 300 gms. worked best.

Analysis of Various Components

Moisture

Aliquots of carcasses were analyzed for moisture by the method described by Mickelsen and Anderson (1959). Three aliquots were analyzed for each carcass sample.

Nitrogen

Nitrogen in the feces, urine, and the carcasses was determined by Kjeldahl method. Carcass homogenate was weighed in a piece of wax paper and together dropped in a Kjeldahl flask since the consistency of the homogenate was quite thick. Nitrogen was converted into protein by applying a factor of 6.25.

Fat

Fat in feces, grain ration, and carcasses were analyzed by ether extraction in a goldfisch extractor. Three dried aliquots were analyzed for each carcass sample.

Sodium and Potassium

Carcass, feces, and grain ration were ashed for the determination of minerals. Urine was used as such. Sodium and potassium were determined by atomic absorption and flame emission spectroscopic methods respectively. For these determinations, the feces and diet samples were weighed (0.15-0.35 gm.) and ashed in a muffle furnace at 475°C for approximately eight hours. Wet carcass samples were weighed (4-7 gms.) in Vicor crucibles and dried in the force-air oven at 90°C overnight, and then ashed in a muffle furnace for approximately 10 hours. The ashed samples were dissolved in 1 ml. of 50% HCl and made into known volumes. Appropriate dilutions of these original ash solutions were taken in duplicates and analyzed for sodium and potassium by comparing with known standard solutions of sodium and potassium. Urine samples were only diluted with deionized water and the minerals were determined as the ash solutions.

Statistical Analysis

All data were analyzed by analysis of variance (Dixon and Massey, 1957).

RESULTS AND DISCUSSION

Food Consumption and Body Weight Gain

Food consumption and body weight gains were significantly lower for the steroid-treated rats, Figure 2. The average food consumption and body weights of the steroid-treated and control rats for 26 weeks are presented in Table 1 (Appendix). The results of the present studies are in agreement with the works of Meites (1949) and Sullivan and Smith (1957) who showed that estrogen treatment caused a reduction in food intake and body weight gain in rats. Depression in body growth and appetite has also been noted by Husain and Pincus (1965) who treated rats with the same steroids used in the present experiment. They fed female rats Enovid and noted that the treated rats had decreased food intake and body weight gains throughout a six-month period. Water consumption was also lower for the treated rats near the end of the treatment.

In the present experiments food intake during the first 19 weeks was measured with the 20 representative

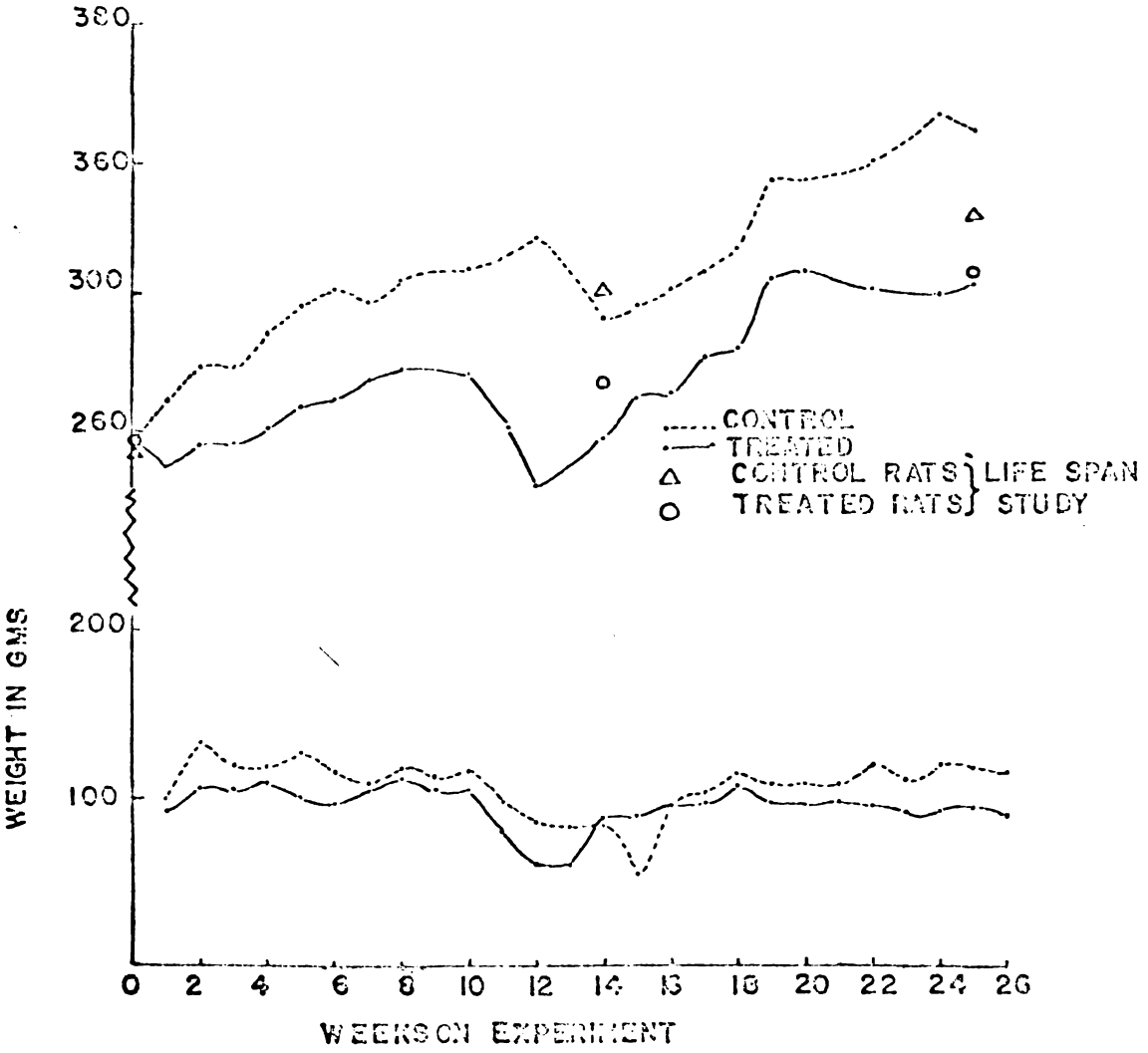


Fig. 2.--Body weights (upper curves) and weekly food consumption (lower curves) of the control and treated rats.

rats. After this period it was not possible to continue the food intake measurement without the addition of a few more control and treated rats. The addition came from the group which was to be used for body composition analysis. This was necessary because some of the rats died from respiratory infections. Thus, from the 20 to 26 weeks of the experiment, the measurement was continued with rats from the representative group as well as those from the groups originally planned for the determination of body compositional changes. For about 7 weeks the control rats continued on an average of 2-2.5 gms. more food per day than the treated rats, but consumption was the same for both groups from the 7th to 9th weeks. This was the time when the infection occurred. After this time, the control rats began to consume a little more food but again the food consumption of both groups went down far below normal until the 15th week. During the 12th and 13th weeks, the average food intake of the treated rats was only 8.6 gms. per day and during the 15th week the control rats' average food consumption was 8 gms. per day. From the 16th week onwards the food consumption of the control rats gradually went up and remained between 15 and 17 gms. while

the consumption of the treated rats remained at 13-15 gms. throughout the experiment.

From the growth curves it is obvious that the growth rates of the treated rats were always below the growth rates of the control rats. The treated rats also lost weight during the first week of the treatment itself. It took another week for them to regain the lost weight and then they stayed in the same average weight for one more week; after that they were gaining weight at a slower rate than the control rats. Food consumption and body weight went down for both groups when they became sick at the 7th week. It should be pointed out that the body weights went down very drastically for both groups when they consumed only an average of 8 gms. of food per day. The treated rats were consuming an average of 8.6 gms. of food per day during the 12th week, which is far below the average intake of 13 to 15 gms. per day. For that particular week, the average body weight went down from 276 gms. to 242 gms. The control rats consumed about 9.5 gms. of food per day during the 14th and 15th weeks, at which period they lost weight from 316 gms. to 292 gms. The treated rats lost more weight; however, they recovered

faster. Though the sickness came into picture for a while, it did not change the usual pattern of food consumption and body weight gain between the control and treated rats, since they continued to exist throughout the experimental period. In order to show that the growths of the two groups were not appreciably changed subsequent to the infection and that they were similar to those of rats not affected by the infection, the body weights of the life-span-study rats have also been included in Figure 2. However, these rats have been weighed only three times: at the beginning, at 14 weeks, and at 25 weeks of steroid treatment.

It has been shown by Meites (1949) that the growth-inhibiting effect of estrogen was due mainly to its ability to depress appetite. Sullivan and Smith (1957) also proved that the restriction of food intake in the control rats equal the quantity consumed by the treated animals duplicated the effects of estrogen on depression of body weight. However, Glasser (1954) showed that the daily injection of 0.1 mg. of stilbestrol for 21 days to adult male rats receiving a diet containing 18% casein resulted in a marked loss of body weight which exceeded that of the pair fed

controls. The author claimed that the difference was sufficient to suggest some direct effect of the hormone on body weight. Since the rats of the present experiment were fed on an ad libitum basis, the exact effect of estrogen and progesterone could not be determined. The mechanism by which these hormones depress body weight, apart from the restriction of food intake, has never been clarified. It has been suggested that the estrogen may reduce the secretion of pituitary growth hormone (Richards and Kueter, 1941) and thyrotropic hormone (Meites and Turner, 1948) or increase in adrenocorticotropin (Baker, 1949), any one of which would depress growth. Another hypothesis suggested by Meites and Turner (1948) was that deficiency of B vitamins might be created by the administration of artificial estrogen like hexestrol, which would result in growth depression.

Digestibility and Nutrient Balance

The digestibilities of protein, fat, sodium and potassium were not significantly different between the steroid-treated and control rats either after 22 or nearly

173 days after feeding the steroids. Table 1 shows the percent digestibilities of nitrogen and fat for the first collection. During this period the percent digestibility of nitrogen was not significantly different between the treated and control rats; however, the control rats retained significantly ($P < 0.05$), more dietary nitrogen in their body than the treated rats, Table 2. This was due to a lesser quantity of urinary nitrogen expressed as a percent of what was absorbed. Until the first collection period, the food intake of the treated rats was 2-2.5 gms. less per day as compared to the control rats. The decreased food intake might have resulted in increased digestion and absorption of nitrogen. However, in spite of the fact the treated rats ate less food and digested it efficiently, they excreted as much urinary nitrogen as did the control rats. Urinary nitrogens excreted in the four day period for the treated and control rats were 1.53 and 1.57 gms. respectively. The difference was in the amount of food consumed. The voluntary restriction of food intake without conserving body nitrogen of the treated animals may be one possible reason for the decreased nitrogen retention of the treated animals.

Table 1

Nitrogen and Fat Digestibilities after 22 Days of Steroid Treatment

	% Digestibility of Nitrogen		% Digestibility of Fat	
	Treated	Control*	Treated	Control
	82.2	77.5	85.8	87.7
	82.1	82.3	96.4	83.2
	81.3	81.1	78.3	88.9
	81.4	80.8	88.5	88.1
	83.8	83.2	76.8	87.1
	82.1	79.8		
	85.1	81.3		
	80.8	82.8		
	83.1	80.0		
	82.0			
Mean	82.4	81.0	85.2	87.0
S.D.	1.3	1.8	8.0	2.2

*Only 9 rats are included in control group for nitrogen digestibility since one rat was sick at the time of collection.

Table 2

Percent Retention of Dietary-Nitrogen in Rats after 22 Days of Steroid Treatment†

	Treated			Control**		
	Fecal Nitrogen (gms.)	Urinary Nitrogen (gms.)	Nitrogen Retention* (%)	Fecal Nitrogen (gms.)	Urinary Nitrogen (gms.)	Nitrogen Retention* (%)
	0.34	1.5	5.5	0.38	1.2	6.0
	0.40	1.7	4.1	0.50	1.8	18.0
	0.34	1.2	10.8	0.42	1.5	14.5
	0.33	1.5	5.3	0.41	1.6	6.7
	0.35	1.6	8.6	0.41	1.6	17.5
	0.41	1.7	7.9	0.44	1.6	7.9
	0.18	0.9	10.7	0.36	1.3	11.3
	0.39	1.5	8.7	0.46	1.9	12.0
	0.47	2.0	9.6	0.48	1.7	10.1
	0.43	1.7	10.3			
Mean	0.36	1.53	8.2	0.38	1.57	11.5
S.D.	0.08	0.30	2.4	0.14	0.20	4.4

*The mean 11.5% nitrogen retention with control rats during the four days collection period is significantly ($P < 0.05$) more than that of 8.2% with the treated rats.

**Only 9 rats are included for the control group, since 1 out of 10 was sick during the collection.

†Values are for 4 days per rat.

For the second collection also, there were no significant differences in the digestibilities of nitrogen between the treated and control groups, Table 3. However the nitrogen balance study during the second period revealed that the treated rats retained significantly ($P < 0.01$) more dietary nitrogen than the control rats, Table 4. The average quantity of urinary nitrogen of the control rats was not much altered in the second time as compared to the first time. They were 1.502 and 1.57 gms. respectively. But the total urinary nitrogen and the urinary nitrogen as a percentage of what was absorbed were decreased with treated rats in the second collection period. The average total urinary nitrogen was 1.35 gm. during the second collection compared to 1.53 gm. average during the first time. There was not much difference in the food consumption between the two groups during the second collection period. The averages were 15.0 gms. per day for the controls and 14.5 gms. per day for the treated rats.

The rats used in the second collection were placed in metabolism cages three days before the collection was started. For this reason, both the treated and control rats lost an average of 14 gms. of body weight. However,

Table 3

Nitrogen and Fat Digestibilities after 24 Weeks and 5 Days of Steroid Treatment

	% Digestibility of Nitrogen		% Digestibility of Fat	
	Treated	Control	Treated	Control
	79.6	82.5	84.3	82.8
	82.0	82.1	77.7	70.3
	82.1	80.1	88.0	75.2
	82.2	81.1	86.1	85.1
	83.0	84.0	63.2	79.0
	84.5	83.1		
	84.1	81.8		
	86.6	82.5		
	86.9	82.6		
	85.7	82.6		
Mean	83.7	82.2	80.0	78.5
S.D.	2.3	1.0	10.0	6.0

Table 4

Percent Nitrogen Retention in Rats Calculated on the Basis of Four Days of Dietary Nitrogen and Four Days of Urinary and Fecal Nitrogen after 24 Weeks and 5 Days of Steroid Treatment

	Treated			Control		
	Fecal Nitrogen (gms.)	Urinary Nitrogen (gms.)	Nitrogen** Retention (%)	Fecal Nitrogen (gms.)	Urinary Nitrogen (gms.)	Nitrogen** Retention (%)
	0.45	1.4	17.2	0.34	1.5	4.1
	0.34	1.2	18.9	0.37	1.5	9.4
	0.36	1.3	17.4	0.33	1.4	-2.3
	0.40	1.5	14.8	0.43	1.5	15.1
	0.36	1.5	11.1	0.31	1.5	7.1
	0.34	1.4	19.8	0.32	1.5	2.8
	0.23	1.2	5.1	0.37	1.5	10.5
	0.22	1.3	10.7	0.42	1.7	11.3
	0.33	1.3	18.0	0.37	1.5	10.2
	0.27	1.2	19.3	0.35	1.5	9.6
Mean	0.33	1.35	15.0	0.36	1.5	7.8
S.D.	0.07	.11	4.8	0.04	0.07	5.0

**The mean 15% nitrogen retention with the treated rats is significantly higher ($P < 0.01$) than the 7.8% retention of the control rats.

when the collection was started they were beginning to gain weight. During the 4 days of the first collection period the average weight gain of the control rats was 5 gms. and that of the treated ones was 3.4 gms. But during the second collection the average weight gain of the treated rats for 4 days was 6.2 gms. versus 3.8 gms. for the control rats. This slow gain in body weight and decreased food consumption of the control rats at the second collection revealed that the rats of the 2 groups might have reacted differntially to the new environment. It could also be true that the treated rats had adapted to the steroid in the long range and thus begun to conserve more nitrogen in order to compensate for the loss at the beginning of the steroid treatment. It has been pointed out by Leathem (1956) that more urinary nitrogen is excreted by the animal with well-filled protein stores than by the depleted animals. Even though the treated rats were not depleted, they had less protein in their body as compared to the control ones according to analysis of body composition at this time. This is another possible explanation for the increased nitrogen-retention of the treated rats.

Feed efficiency was calculated by dividing the average body weight gain per rat per day in grams by the average food consumption per day in grams, Table 2 (Appendix). During the first collection it was 0.05 gm. weight gain per gram of food per day for the treated rats and 0.1 gm. of weight gain for the control rats. During the second collection they were respectively .12 gm. and 0.06 gm.

Percent digestibilities of fat for the first and second collections were not significantly different between the treated and control groups, Tables 1 and 3. During the first collection the average percent of fat digestibilities were 85 and 87 respectively with the treated and control rats. At the time of the second collection these values were 80% and 79% respectively.

Percent digestibilities of sodium and potassium for the treated and control rats at both collections were not significantly different between the treated and control rats, Table 5. The percent retention of dietary sodium was significantly higher ($P < 0.05$) with the treated rats than with the control rats during the first collection period; however, there was no difference in potassium

Table 5

Percent Digestibilities of Sodium and Potassium During the Two Collection Periods

	At 22 Days of Steroid Treatment		At 24 Weeks and 5 Days of Steroid Treatment	
	Treated	Control	Treated	Control
	% Digestibility of Sodium			
	96.5	89.8	96.8	98.1
	93.6	92.0	82.4	97.1
	94.8	94.4	97.1	98.5
	96.9	95.1	97.1	92.0
	98.1	96.2	97.0	98.7
	97.4	98.1	98.5	97.7
	95.4	97.2	99.5	93.6
	97.3	96.5	99.1	95.6
	97.7	95.5	92.8	97.4
	94.1	94.8	99.0	93.4
Mean	96.2	95.0	95.9	96.2
S.D.	11.6	2.5	5.1	2.4
	% Digestibility of Potassium			
	95.1	82.7	96.6	97.0
	91.3	89.8	87.2	96.0
	86.8	90.9	97.5	97.4
	94.3	92.1	96.8	94.1
	97.0	94.8	94.5	96.9
	95.7	95.6	96.6	96.0
	94.4	94.0	99.0	92.6
	94.8	92.1	98.3	95.4
	95.5	95.0	87.4	95.8
	89.4	92.4	97.4	89.4
Mean	93.4	91.9	95.1	95.0
S.D.	3.2	3.7	4.3	2.5

retention between the two groups, Table 6. The percent retentions of dietary sodium and potassium, during the second collection were not significantly different between the two groups, Table 6. It seems that the steroids may have their effect on sodium retention during the initial stages of treatment. Later on, the rats may become adapted to the steroids and they exert no more effect on sodium excretion. The steroids do not seem to have any significant effect on potassium retentions.

Body Composition

The present study on body composition of rats revealed some interesting changes that occurred in various components such as water, dry body weight, fat, and nitrogen.

First Group

During steroid feeding, the treated rats did not gain as much weight as the control rats (Table 7). The average weight gain of the treated rats during the 4 weeks was 12 gms. while the control rats gained an average of 32 gms. during that period. Analysis of the carcasses

Table 6

Percent Retentions of Sodium and Potassium During Two Collection Periods

	% Rentention of Sodium		% Retention of Potassium	
	Treated*	Control*	Treated	Control
	At 22 Days of Steroid Treatment			
	24.1	17.1	29.5	16.1
	28.9	26.8	17.1	24.6
	19.9	30.9	22.5	24.7
	28.3	26.2	26.7	20.2
	39.4	23.9	26.5	19.4
	32.7	27.5	21.5	33.7
	42.5	17.2	43.5	26.7
	35.6	26.9	21.8	24.3
	41.5	31.2	26.8	31.0
	27.9	23.2	21.5	21.1
Mean	32.1	25.1	25.7	24.2
S.D.	7.6	4.9	7.2	5.3
	At 24 Weeks and 5 Days of Steroid Treatment			
	-45.6	56.3	39.1	31.0
	3.0	18.7	27.1	16.0
	-11.0	30.9	31.8	27.9
	45.5	-10.1	31.8	33.8
	62.2	59.0	27.5	34.4
	59.4	54.3	39.1	31.9
	83.2	37.2	27.8	32.0
	60.2	35.1	33.1	30.7
	67.6	54.8	34.1	32.8
	81.7	62.2	38.1	26.8
Mean	40.6	39.8	32.9	29.7
S.D.	43.4	22.7	4.7	5.4

*The mean 32.1% sodium retention of the treated rats is significantly higher ($P < 0.05$) than the mean of 25.1% sodium retention with control rats.

Table 7

Body Weights of Rats Killed at Different Lengths of Time of Feeding Steroids (first and third parts of table) and after Refeeding of Control Diet for 6 Weeks (second and fourth parts of table) (Values are in gms.)

		4 Weeks			
	Start	1 wk	2 wks	3 wks	4 wks
Treated	261	252	258	261	273
Control	263	272	283	292	295

		10 Weeks				
	Start	5 wks	6 wks	7 wks	8 wks	10 wks
Treated	239	258	260	258	269	275
Control	254	287	291	290	302	303

		25 Weeks + 3 Days	
	Start	24 Weeks	25 Weeks
Treated	260	296	299
Control	254	335	331

		31 Weeks + 5 Days								
	Start	14	15	16	17	18	25	28	29	31
Treated	252	266	276	279	287	292	296	308	307	310
Control	255	305	312	317	320	331	339	343	344	353

revealed that the control rats had significantly more dry body weight ($P < 0.05$) when compared to the treated rats, Table 8. The treated rats had significantly more ($P < 0.05$) water expressed as % moisture of the body than the control rats, Table 8. Even though the treated rats weighed less, they had almost equal quantity of water as the control rats at the end of 4 weeks of steroid treatment. It would be appropriate to mention here that the sodium balance study done by collecting urine and feces after 22 days of steroid treatment revealed that the treated rats retained significantly more sodium ($P < 0.05$) than the control rats. This result is in accord with the phenomenon of an increased water retention by the treated rats during this particular time. The increased water retention by the treated rats at the initial stages of steroid treatment was not significant after 25 weeks of therapy. Complaints about "fluid retention" in women receiving contraceptive steroids may be true during the initial periods but for later periods, the occurrence of fluid retention may be doubtful.

Since there are reports (Adams, 1966) that indicate a gain in the lean body mass in women during the treatment with contraceptive steroids, lean body masses were

Table 8

Moisture in the Whole and Lean Body Masses and Absolute Amounts of Dry Body Weight and Lean Body Masses after Four Weeks of Steroid Treatment

Rat No.	Moisture in Whole Body*(%)	Moisture in Lean Body Mass(%)	Absolute Amount of Water(gms.)	Absolute Amt. of Dry Wt.**(gms.)	Wet Lean Body Mass (gms.)	Dry Lean Body Mass (gms.)
			Treated Rats			
61	58.4	70.6	146.5	104.3	207.5	61.0
62	68.7	79.1	171.4	98.0	216.5	65.1
63	61.7	70.1	151.5	91.7	216.9	63.0
64	60.4	71.3	182.9	119.7	256.7	73.7
65	62.5	70.8	170.2	102.0	240.6	70.4
66	63.9	70.4	145.4	82.2	206.7	61.3
67	62.5	70.9	155.4	93.4	221.2	65.8
68	62.4	70.5	148.1	89.3	210.1	62.0
69	59.7	70.4	181.9	122.9	258.3	76.4
70	61.2	70.5	168.3	106.8	238.8	70.5
Mean	62.1	71.4	162.2	101.0	227.3	67.0
S.D.	2.8	2.7	14.5	13.0	19.8	5.5

Table 8.--Cont.

			Control Rats			
71	59.5	70.2	161.9	110.1	230.6	68.7
72	60.0	70.8	138.9	91.6	196.3	56.6
73	59.8	70.9	158.3	122.4	256.3	74.6
74	61.6	71.2	163.3	101.6	229.4	66.1
75	59.3	71.2	158.5	108.8	222.5	64.0
76	59.8	71.0	182.8	122.8	257.5	74.7
77	59.5	70.8	165.6	113.0	234.0	68.3
78	61.0	69.4	174.6	111.8	251.5	76.9
79	57.5	70.7	164.7	121.8	232.9	68.4
80	59.5	70.4	171.6	116.8	243.6	72.1
Mean	59.8	70.7	164.0	112.1	235.5	69.0
S.D.	1.1	0.5	11.7	10.0	18.3	6.0

*The mean 62.1% moisture in treated rats is significantly ($P < 0.05$) higher than the mean 59.8% moisture in control rats.

**The mean 112.1 gms. absolute dry weight in control rats is significantly higher ($P < 0.05$) than the mean 101.0 gms. in treated rats.

determined for both control and treated rats, Table 8. There were no significant differences between these two groups in the absolute amount of wet and dry lean body masses after 4 weeks of steroid treatment. The average wet lean body mass of control rats was 235.5 gms. whereas that of the treated rats was 227.3 gms. The average total amount of dry lean body masses were 69 and 67 gms. respectively for the control and treated rats. The percent moisture in the lean body mass was also not significantly different between the treated and control groups. The averages were 71.4% and 70.7% for the treated and controls respectively, Table 8.

Percent nitrogen in wet and dry lean body masses was not significantly different between the two groups, Table 9. Absolute amounts of nitrogen and protein were also not significantly different between treated and control rats after 4 weeks of steroid treatment. The control rats had an average of 0.6 gm. nitrogen and an average of 4 gms. protein more than the treated rats; however, they were not statistically significant.

Body fat as a percent of either wet or dry body weight was significantly more ($P < 0.05$) for the control

Table 9

Percent and Absolute Amounts of Nitrogen and Fat in Rats after 4 Weeks of Steroid Treatment

Rat No.	Nitrogen in Wet Lean Body Mass (%)	Nitrogen in Dry Lean Body Mass (%)	Abs. Amt. of Nitrogen (gms.)	Abs. Amt. of Protein (gms.)	Fat in Wet Body Weight* (%)	Fat in Dry Body Weight** (%)	Fat Absolute Amt.*** (gms.)
				Treated Rats			
61	3.8	13.0	7.9	49.4	17.3	41.5	43.3
62	3.8	12.8	8.3	52.0	13.2	33.6	32.9
63	3.8	13.1	8.3	51.7	11.7	31.3	28.7
64	3.6	12.5	9.2	57.5	15.9	38.4	46.0
65	3.6	12.2	8.6	53.5	11.6	31.0	31.6
66	3.6	12.2	7.5	46.6	9.2	25.4	20.9
67	3.7	12.4	8.2	51.0	11.1	29.5	27.5
68	3.7	12.5	7.8	48.6	11.5	30.6	27.3
69	3.7	12.5	9.6	59.8	15.2	37.8	46.5
70	2.7	9.1	6.4	40.1	13.2	34.0	36.3
Mean	3.6	12.2	8.16	51.0	13.0	33.3	34.1
S.D.	0.3	1.1	0.90	5.5	2.5	4.8	8.7
				Control Rats			
71	3.9	12.9	8.9	55.5	15.2	37.6	41.4
72	3.7	12.8	7.2	45.2	15.4	38.3	35.0
73	3.7	12.8	9.6	59.9	15.7	39.0	47.8
74	3.7	12.9	8.5	53.4	13.4	34.9	35.4
75	3.7	12.9	8.3	51.7	16.7	41.2	44.8
76	3.6	12.5	9.4	58.5	15.7	39.2	48.1
77	3.7	12.5	8.6	53.5	16.0	39.5	44.7
78	3.9	12.7	9.7	60.9	12.2	31.2	34.9
79	3.8	12.8	8.8	54.7	18.6	43.8	53.4
80	3.6	12.3	8.9	55.6	15.5	38.3	44.7
Mean	3.7	12.7	8.78	54.9	15.5	38.3	43.0
S.D.	0.1	0.2	0.70	4.5	1.7	3.4	6.3

*The mean value 15.5% of the control group is significantly higher than the mean value 13.0% of the treated rats ($P < 0.05$).

**The mean value of 38.3% in control rats is higher ($P < 0.05$) than the mean value of 33.3% in treated rats.

***The mean 43 gms. of fat in control rats is higher ($P < 0.05$) than the mean value of 34 gms. in treated rats.

rats than for the treated ones, Table 9. Absolute amount of fat was also significantly more ($P < 0.05$) in control rats than in treated rats. There were no significant differences in the amount of body fat per gram of body nitrogen between the treated and control groups, Table 3 (Appendix). However, when the amount of fat per gram of food eaten was calculated, the treated rats were less efficient. The marked loss of body weight and decreased body weight gain that occurred in estrogen treated rats (Meites, 1949; Sullivan and Smith, 1957; and Glasser, 1954), and in estrogen and progesterone treated rats (Husain and Pincus, 1965) are in agreement with the present work. Furthermore, the voluntary restriction of food intake may be another reason for the decreased amount of body fat in the treated rats. The control rats consumed more food and thus could have converted "extra" calories into body fat. It has been shown by many investigators that women who were treated with contraceptive steroids had decreased glucose tolerance (Gershberg et al., 1964; Pyörälä, et al., 1967; and Wynn and Doar, 1966). It has also been shown by Wynn and Doar that the plasma nonesterified fatty acid was elevated in patients taking oral

contraceptive pills. This increased release of fatty acids has been suggested to be because of impaired glucose utilization (Randle et al., 1963) and, thus, may also be a cause for the decreased amount of body fat observed in the treated rats.

This work and the work of Bakker and Dightman (1966) with women using norethynodrel do not support the hypothesis that these steroids lead to weight gains due to increased deposition of fat despite the complaints of some women. However, according to Pincus (1966) exceptional weight gain has been attributed to some but not all oral contraceptives. The percentage of women who were losing weight was more than the percentage of women who gained weight, taking the oral contraceptive pills Enovid and Ovulen.

Analyses of carcasses of treated and control rats for sodium and potassium after 4 weeks of steroid treatment revealed that there were no significant differences in the amount of sodium and potassium in the body between the treated and control group rats. The absolute amounts of sodium and potassium in the whole body of rats and the amounts present per 100 gms. of wet and dry lean body

masses are presented in Table 10. The treated rats had an average of 288 mgm. of sodium and the control 303 mgm. These values were not significantly different. Since there were no significant differences in the amount of wet and dry lean body masses between the two groups of rats, this sort of result is expected for sodium. The average absolute amounts of potassium were 850 mgm. and 864 mgm. for treated and control respectively. They were not significantly different from each other. Sodium and potassium per 100 gms. of wet and dry lean body masses were also not significantly different for the treated and control groups. Since from the time the experiment was started the treated rats were eating less food per day than the control rats, but have the same amount of sodium in their bodies, the treated rats must have significantly retained more of the dietary sodium than did the control rats. This was revealed during the first collection period.

Second Group

The body composition of the second group of rats which were fed the control diet for 6 weeks after 4 weeks of steroid treatment revealed that the sickness which has already been mentioned came into picture when these rats

Table 10

Amounts of Sodium and Potassium in the Whole Body and Per 100 gms. of Wet and Dry Lean Body Masses of Rats Killed after 4 Weeks of Steroid Treatment

Rat No.	Absolute Amt. of Na in the Whole Rat (mgm.)	Absolute Amt. of Na Per 100 Gm. Wet Lean Body Mass (mgm.)	Absolute Amt. of Na Per 100 Gm. Dry Lean Body Mass (mgm.)	Absolute Amt. of K in the Whole Rat (mgm.)	Absolute Amt. of K Per 100 Gm. Wet Lean Body Mass (mgm.)	Absolute Amt. of K Per 100 Gm. Dry Lean Body Mass (mgm.)
			Treated Rats			
61	290.8	140.2	476.7	938.8	452.5	1539.1
62	275.7	127.3	423.4	820.9	379.1	1260.5
63	275.9	127.2	438.0	800.2	369.0	1270.3
64	316.7	123.4	429.4	882.8	343.9	1193.9
65	283.5	117.8	402.9	846.9	352.0	1203.7
66	265.0	128.2	432.4	790.1	382.3	1289.2
67	279.4	126.3	424.4	829.1	374.8	1200.2
68	269.6	128.3	434.8	773.1	367.9	1246.5
69	337.8	130.8	442.0	963.4	372.9	1260.4
70	288.4	120.8	409.3	857.4	359.1	1216.7
Mean	288.3	127.0	431.3	850.3	375.4	1268.1
S.D.	22.5	6.0	20.1	62.5	29.7	100.8
			Control Rats			
71	302.7	131.2	440.5	825.6	357.9	1201.3
72	251.9	128.3	439.0	761.4	387.9	1326.9
73	336.7	131.4	451.4	940.4	366.9	1260.7
74	287.0	125.1	433.9	865.7	377.3	1309.0
75	271.4	122.0	424.0	831.0	373.4	1298.2
76	327.5	127.2	438.5	932.3	362.1	1248.3
77	310.0	132.5	453.7	757.4	323.8	1108.5
78	314.8	125.2	409.6	917.7	365.0	1194.1
79	315.6	135.3	461.5	975.3	418.2	1426.1
80	312.4	128.2	433.0	833.1	342.0	1155.2
Mean	303.0	128.6	438.5	864.0	367.5	1252.8
S.D.	25.7	4.0	15.0	75.4	25.4	92.7

were going on their control diets for the second week. However, the sickness affected the control and treated rats to the same extent. This could be seen from the weight gain data of these rats, Table 7. The carcass analyses for moisture, dry body weight, and lean body masses of refed and control rats are presented in Table 11. Even though the average % moisutre in the body is slightly higher with the refed rats, it was not significantly different from that of the control group. The calculated absolute amount of water was higher ($P < 0.05$) with control rats since they weighed more. Total absolute amount of dry body weights were also significantly more ($P < 0.05$) in control rats than in treated rats. The average % moisture in the lean body mass of the refed rats was 71 as compared to 69.7 of the control rats. Even though the mean % moisture in the lean body mass of the refed rats was significantly higher ($P < 0.05$) than that in control rats, both the 69.7% moisture in control rats and 71% moisture in refed rats were within the normal range of moisture in the lean body mass. The absolute amounts of wet and dry lean body masses were significantly higher in control rats than in refed rats. There was essentially

Table 11

Moisture in the Whole and Lean Body Masses and Absolute Amount of Dry Weight and Lean Body Masses in Control and Refed Rats Placed on Control Diet for 6 Weeks after 4 Weeks of Steroid Treatment

Rat No.	Moisture in Whole Body (%)	Moisture in Lean Body Mass* (%)	Absolute Amount of Water**(gms.)	Absolute Dry wt. in Body***(gms.)	Wet Lean Body Mass# (gms.)	Dry Lean Body Mass† (gms.)
			Refed Rats			
1	61.1	72.5	159.0	101.4	219.3	60.3
2	63.0	71.5	163.1	95.8	228.2	65.1
3	59.4	70.3	152.7	104.5	217.3	64.6
4	64.3	71.8	158.4	88.1	220.8	62.4
5	60.0	71.1	159.9	106.7	225.0	65.1
6	61.0	70.2	147.2	94.2	209.9	62.6
7	61.9	71.8	147.2	90.8	205.1	57.8
8	59.0	70.2	150.8	104.7	214.9	64.1
9	54.4	69.7	142.4	119.3	204.3	61.9
10	60.8	70.8	174.4	112.3	246.5	72.1
Mean	60.5	71.0	155.5	101.8	219.1	63.6
S.D.	2.7	0.9	9.4	9.8	12.4	3.8

Table 11.--Cont.

			Control Rats			
32	57.3	70.0	169.0	125.9	241.5	72.5
33	62.0	71.0	145.1	89.0	204.3	59.2
34	59.2	69.8	172.6	119.2	247.5	74.8
35	61.1	70.1	151.4	96.4	216.0	64.6
36	60.0	69.3	156.2	104.2	225.2	69.0
37	55.5	65.7	174.3	107.6	265.1	58.9
38	56.8	70.7	189.6	144.5	268.3	78.8
39	58.2	70.8	193.2	138.9	273.1	79.9
41	58.2	70.1	175.3	125.8	250.1	74.8
Mean	58.7	69.7	169.6	116.8	243.5	70.3
S.D.	2.1	1.6	16.3	18.9	24.1	7.9

*The mean 71% moisture in the lean body mass of the rats refed the control diet is significantly higher ($P < 0.05$) than the mean of 69.7% in control rats.

**The mean absolute amount of water in control rats is significantly higher ($P < 0.05$) than that in treated rats.

***Absolute amt. of dry wt. with control rats is significantly higher ($P < 0.05$) than that in treated rats.

#Control rats had more wet lean body mass. It is significant at ($P < 0.05$) level.

†Control rats had more dry lean body mass which is significant at ($P < 0.05$) level.

no increase in the dry weight of the refed rats during the 6 weeks period of refeeding of the control diet compared to the treated rats of the first group. But at this period, the control rats gained an average of 4.7 gms. of dry weight. The refed rats of the second group lost about 8 gms. of wet lean body mass when compared to the treated rats of the first group whereas during this same period the control rats gained 8 gms. In the same way, the refed rats lost 3 gms. of dry lean body mass due to sickness while the control gained an average of 1 gm. of dry lean body mass. It seemed that the treated rats were more severely affected by the infection than were the control rats. Neither the body nitrogen nor the protein was responsible for the differences that occurred in the total absolute amounts of wet and dry lean body masses between the control and the refed groups since the % nitrogen in the wet and dry lean masses and absolute amount of nitrogen and protein in the total carcasses, were not significantly different between the refed and control groups, Table 12. Differences in the lean body masses were caused by some changes which were not identified at this time.

Table 12

Percent and Absolute Amounts of Nitrogen and Fat in Control Rats and in Rats Refed the Control Diet for 6 Weeks after 4 Weeks of Steroid Treatment

Rat No.	Nitrogen in Wet Lean Mass (%)	Nitrogen in Dry Lean Mass (%)	Absolute Amt. of Nitrogen (gm.)	Absolute Amt. of Protein (gm.)	Fat in Wet Body Weight (%)	Fat in Dry Body Weight (%)	Absolute Amt. of Fat (gm.)
				Refed Rats			
1	3.7	13.3	8.0	50.2	15.8	40.6	41.2
2	3.8	13.3	8.7	54.2	11.9	32.1	30.7
3	3.7	12.6	8.1	50.9	15.5	38.2	39.9
4	3.7	12.9	8.1	50.4	10.5	29.3	25.8
5	3.6	12.3	8.0	50.2	15.6	38.5	41.6
6	3.8	12.8	8.0	50.1	13.1	33.5	31.5
7	3.8	13.6	7.9	49.1	13.9	36.3	33.0
8	3.7	12.6	8.0	50.3	15.9	38.8	40.6
9	3.9	12.7	7.9	49.2	21.9	48.1	57.5
10	3.7	12.8	9.2	57.6	14.0	35.8	40.2
Mean	3.7	12.9	8.19	51.2	14.8	37.1	38.2
S.D.	0.1	0.4	0.4	2.6	3.1	5.2	8.7
				Control Rats			
32	3.8	12.5	9.1	56.6	18.1	42.4	53.4
33	3.7	12.8	7.6	47.3	12.8	33.6	29.9
34	3.8	12.6	9.4	59.0	15.2	37.2	44.4
35	3.7	12.4	8.0	50.0	12.8	33.0	31.8
36	3.7	12.1	8.4	52.3	13.5	33.7	35.1
37	2.9	12.9	7.6	47.3	20.2	45.3	48.8
38	4.0	13.7	10.8	67.4	19.7	45.5	65.8
39	3.6	12.4	9.9	62.0	17.8	42.5	59.0
41	3.7	12.3	9.2	57.4	17.0	40.6	51.1
Mean	3.7	12.6	8.82	55.5	16.3	39.3	46.6
S.D.	0.3	0.5	1.1	6.9	2.9	5.1	12.4

Neither the percentage nor the absolute amount of fat was significantly different between the control and treated groups, Table 12. From the body weight data (Table 7), it was obvious that the refeeding of the control diet for 6 weeks helped in promoting faster weight gains even though the gain was not comparable to the control rats. Despite the facts that the body weights were different, the absolute amounts of fat in the body of the control and refed rats were not significantly different. Two possible reasons for this can be suggested. One is that the withdrawal of the steroids resulted in corrected fat metabolism. The other one is that the appetite might have increased and thus indirectly affected body fat by increasing it. The amount of body fat per gram of nitrogen in the body was calculated for this 2nd group of rats and has been presented in Table 3 (Appendix). There were no significant differences between the refed and control rats in the amount of body fat per gm. of nitrogen in the body.

The average absolute amount of sodium in the carcass was 272 mgm. in refed rats and 326 mgm. in control rats. The control rats had significantly more sodium ($P < 0.01$) than the treated ones, Table 13. The average

Table 13

Amounts of Sodium and Potassium Present in the Whole Body and Per 100 gm. of Wet and Dry Lean Body Masses in Refed Rats which were Placed on Control Diet for 6 Weeks and Control Rats after 4 Weeks of Steroid Treatment

Rat No.	Total Amt. of Na in the Whole Body* (mgm.)	Amt. of Na Per 100 gm. of Wet Lean Mass*** (mgm.)	Amt. of Na Per 100 gm. of Dry Lean Mass (mgm.)	Total Amt. of K in the Whole Body** (mgm.)	Amt. of K Per 100 gm. of Wet Lean Mass (mgm.)	Amt. of K Per 100gm. of Dry Lean (mgm.)
			Refed Rats			
1	262.8	119.8	435.9	753.3	343.5	1249.5
2	275.1	120.6	422.7	894.0	391.8	1373.4
3	263.8	121.4	408.2	847.2	389.9	1311.1
4	272.2	123.3	436.6	810.2	367.0	1358.8
5	281.6	125.2	432.7	834.6	371.0	1282.4
6	259.5	123.7	414.4	824.0	392.6	1315.6
7	240.0	117.0	415.0	793.0	386.7	1371.5
8	292.1	135.9	455.7	841.2	391.4	1312.3
9	280.3	137.2	452.8	735.4	359.9	1188.3
10	295.1	119.7	409.4	933.0	378.5	1294.5
Mean	272.2	124.4	428.3	826.6	377.2	1305.7
S.D.	16.5	6.8	17.2	59.3	16.6	57.3

Table 13.--Cont.

			Control Rats			
32	380.7	157.7	525.2	916.6	379.5	1264.4
33	285.0	139.5	481.8	740.5	362.5	1251.9
34	310.5	125.5	414.9	960.4	388.1	1283.4
35	280.1	129.7	433.5	780.7	361.4	1208.5
36	307.8	136.7	445.8	820.9	364.5	1189.0
37	353.3	133.3	600.2	953.3	359.6	1619.7
38	339.5	126.6	430.9	1006.0	375.0	1276.9
39	357.0	130.7	442.2	1015.8	371.9	1271.6
41	323.0	129.1	431.8	968.3	387.2	1294.7
Mean	326.3	134.3	465.1	906.9	372.2	1295.6
S.D.	34.0	9.9	61.4	101.0	11.0	126.5

*The mean total amount of sodium 326.3 mgm. in control rats is significantly higher ($P < 0.01$) than the mean of 272.2 mgm. of sodium in refed rats.

**The mean total amount of 906.9 mgm. of Potassium in control rats is significantly higher ($P < 0.05$) than the mean of 826.6 mgm. of Potassium in refed rats.

***The mean amount of 134.3 mgm. per 100 gm. of wet lean body mass in control rats is significantly higher ($P < 0.05$) than the mean of 124.4 mgm. present in rats refed the control diet for 6 weeks.

absolute amounts of potassium in control and refed rats were 827 and 907 mgms. respectively. Control rats had significantly more potassium ($P < 0.05$) than the refed ones. Increased amounts of sodium and potassium in the carcasses of the control rats are expected since they had significantly more lean body masses. However, the calculated amounts of potassium per 100 gms. of wet and dry lean body masses were not different for the refed and control groups. Similarly, the amounts of sodium per 100 gms. of dry lean body mass for the refed and control groups were not significantly different, but the amounts per 100 gms. of wet lean body mass was significantly more ($P < 0.05$) in control rats than in refed rats. However, the difference was only 10 mgm.

Comparisons of the first group which was sacrificed after 4 weeks of steroid treatment and the second group which was sacrificed following 6 weeks of refeeding the control diet after 4 weeks of steroid treatment revealed that there were no significant difference in body nitrogen. The increase in nitrogen between the treated rats of the first group and the refed rats of the second group during 6 weeks period was 0.03 gm. The increase in nitrogen

between the control rats of the first and second group was 0.04 gm. at this period. There was a marked increase in the amount of body fat in rats refed the control diet. The average increase was 4 gms. of fat between the treated rats of the first group and the refed rats of the second group during this six weeks period. At this period the average increase of fat was only 3.6 gms. between the control rats of the two groups.

Third Group

The third group of 16 rats was killed after 25 weeks and 3 days of steroid treatment. Their body weight data are shown in Table 7. Moisture content in the body and lean body, absolute amounts of dry body weights, wet and dry lean body masses are presented in Table 14. This group of 16 rats weighted almost the same during the commencement of the experiment, but they had different weights when they finished their 25 weeks and 3 days of steroid treatment. The mean average body weights were 260 and 254 gms. respectively for the treated and control rats at the start of the experiment. However, they respectively weighed 299 and 331 gms. at the time of the sacrifice. The treated rats had a slightly higher percentage of moisture in their

Table 14

Percent Moisture in the Whole and Lean Body Masses and Absolute Amount of Water, Dry Weight, Wet and Dry Lean Body Masses in Rats after 25 Weeks and 3 Days of Steroid Treatment

Rat No.	Moisture in Whole Body (%)	Moisture in Lean Body Mass (%)	Absolute# Amt. of Water (gms.)	Absolute* Amt. of Dry Wt. (gms.)	Wet Lean@ Body Mass (gms.)	Dry Lean Body Mass (gms.)
			Treated Rats			
01	60.0	69.6	169.7	113.3	243.9	74.3
03	61.7	68.4	168.5	104.5	246.3	77.9
04	63.0	70.4	186.7	109.6	265.1	78.4
05	58.5	69.6	168.5	119.6	242.3	73.8
2	64.0	69.2	162.5	91.3	235.0	72.4
4	58.3	70.1	170.1	121.7	242.5	72.4
5	62.3	68.9	160.3	97.2	232.7	72.4
7	57.5	70.4	169.8	125.3	241.3	71.5
Mean	60.7	69.6	169.5	110.3	243.6	74.1
S.D.	2.4	0.7	7.9	12.1	9.8	2.6

Table 14.--Cont.

			Control Rats			
013	60.0	68.8	186.3	124.3	270.6	84.3
014	58.3	69.7	186.1	133.0	267.0	80.9
015	59.7	69.4	161.4	108.8	232.4	71.0
016	61.4	69.7	181.1	114.0	259.8	78.8
11	52.9	69.3	163.7	145.7	236.1	72.4
17	57.7	73.2	193.1	141.7	263.8	70.7
19	61.0	70.2	211.3	135.2	301.1	89.8
20	58.4	71.1	187.9	134.0	264.5	76.5
Mean	58.7	70.2	183.9	129.6	261.9	78.1
S.D.	2.7	1.4	16.0	12.9	21.3	6.8

#The mean absolute amount of water 183.9 gms. in control rats is significantly higher ($P < 0.05$) than the mean of 169.5 gms. in the treated rats.

*The mean value of 129.6 gms. of dry weight with control rats is significantly higher ($P < 0.01$) than the mean value of 110.3 gms. of dry weight with treated rats.

@The mean value of 261.9 gms. of wet lean mass with control rats is significantly higher ($P < 0.05$) than the mean of 243.6 gms. with treated rats.

whole body than the control rats, but it was not statistically significant. There was no significant difference between the control and treated groups in the percent moistures of the lean body masses. However, the average absolute amount of water in the body of the control rats was significantly higher ($P < 0.05$) than that of the treated rats since the former had more body weight. Absolute amount of dry body weight was also significantly higher in control rats ($P < 0.01$). Control rats had significantly more wet lean body mass ($P < 0.05$). There was no significant difference in the dry lean body masses of the control and experimental groups. Averages were 74 gms. in the treated rats and 78 gms. in control group. Percentages of nitrogen in wet and dry lean body masses were not significantly different for the treated and control groups. Percent nitrogen of the lean body masses, absolute amounts of nitrogen and protein, percent fat of wet and dry carcasses and absolute amounts of fat are presented in Table 15. It is obvious from this table that even though the dry lean body masses between the two groups were not significantly different at this period, absolute amounts of nitrogen and protein in the body were significantly different for these

Table 15

Percent and Absolute Amounts of Nitrogen and Fat in Rats after 25 Weeks and 3 Days of Steroid Treatment

Rat No.	Nitrogen in Wet Lean Body Mass (%)	Nitrogen in Dry Lean Body Mass (%)	Absolute Amt. of Nitrogen# (gms.)	Absolute Amt. of Protein# (gms.)	Fat in Wet Body Weight (%)	Fat in Dry Body Weight (%)	Absolute Amount of Fat@ (gms.)
			Treated Rats				
01	3.8	12.4	9.2	57.7	13.8	34.5	39.1
03	3.8	12.0	9.4	58.6	9.7	25.4	26.6
04	3.8	12.7	9.9	62.2	10.5	28.5	31.2
05	3.7	12.0	8.9	55.7	15.9	38.3	45.8
2	3.8	12.4	9.0	56.1	7.4	20.7	18.9
4	3.7	12.5	9.1	56.6	16.9	40.5	49.3
5	3.7	11.8	8.6	53.5	9.6	25.5	24.7
7	3.8	12.7	9.1	56.7	18.3	43.0	53.9
Mean	3.8	12.3	9.2	57.1	12.8	32.0	36.2
S.D.	0.1	0.3	0.3	2.5	4.0	8.2	12.7
			Control Rats				
013	3.7	12.0	10.1	63.3	12.9	32.2	40.1
014	3.8	12.7	10.3	64.1	16.3	39.2	52.1
015	3.8	12.4	8.8	55.2	14.0	34.7	37.8
016	3.8	12.4	9.8	61.0	11.9	30.9	35.2
11	3.8	12.6	9.1	56.8	24.2	50.3	73.3
17	3.9	14.5	10.3	64.3	21.2	50.1	71.0
19	3.6	12.1	10.8	67.8	13.1	33.6	45.4
20	3.9	13.5	10.3	64.4	17.8	42.9	57.5
Mean	3.8	12.8	9.9	62.1	16.4	39.2	51.5
S.D.	0.1	0.8	0.7	4.2	4.4	7.8	14.7

#Absolute amount of nitrogen and protein in the carcasses of the control rats are significantly ($P < 0.05$) higher than the treated rats of the same age.

@The average quantity of 51.5 gm. of fat present in control rats is significantly higher than the amount of 36.2 gms. of fat present in the treated rats ($P < 0.05$).

two groups. The control rats had mean values of 9.9 gms. of nitrogen and 62.1 gms. of protein. These values are significantly higher than the mean values for the treated rats which were 9.2 gms. of nitrogen and 57.1 gms. of protein. Expressing fat as a percentage of wet body weight or as a percentage of dry body weight revealed that there was no significant difference between the control and treated groups. This is thus a significant improvement in this group of treated rats sacrificed at 25 weeks and 3 days of steroid treatment as compared to the treated rats sacrificed at 4 weeks of steroid treatment which had a significantly lesser percentage of fat ($P < 0.05$) in both wet and dry carcasses. However, a significant difference was found in the absolute amounts of fat in the control and treated groups. The control rats had a mean value of 52 gms. of fat which was significantly higher ($P < 0.05$) than the mean value of 36 gms. of fat in the treated rats. Table 16, presents the total amounts of sodium and potassium present in the carcasses and the amounts of sodium and potassium present in 100 gms. of wet and dry lean body masses. The treated rats had an average of 355 mgm. of sodium and the control rats had an

Table 16

Amounts of Sodium and Potassium Present in the Whole Rats and the Amount Present Per 100 gm. Wet and Dry Lean Body Masses after 25 Weeks and 3 Days of Steroid Treatment

Rat No.	Total Amt. of Na in the Body (mgm.)	Amt. of Na per 100 gm. of Wet Lean Body Mass (mgm.)	Amt. of Na per 100 gm. of Dry Lean Body Mass (mgm.)	Total Amt. of K in the Body (mgm.)	Amt. of K per 100 gm. of Wet Lean Body Mass (mgm.)	Amt. of K per 100 gm. of Dry Lean Body Mass (mgm.)
			Treated Rats			
01	354.1	145.1	476.7	1056.5	433.1	1422.5
03	363.0	153.9	486.5	1129.4	425.0	1344.0
04	379.0	146.0	493.5	1047.0	420.4	1421.4
05	359.2	159.7	524.6	1077.5	408.3	1341.1
2	387.0	154.7	501.9	1114.6	480.6	1558.9
4	257.1	148.1	495.8	773.2	444.3	1487.4
5	378.6	110.5	354.9	989.3	332.3	1067.5
7	364.7	151.2	510.2	964.7	399.9	1349.6
Mean	355.3	146.1	480.5	1019.0	418.0	1374.1
S.D.	41.3	15.2	52.8	114.1	42.5	145.5
			Control Rats			
013	401.7	148.5	476.6	1124.9	415.8	1334.5
014	354.3	132.7	438.0	1098.5	411.5	1357.9
015	319.1	137.3	449.2	967.1	416.1	1361.3
016	392.9	151.2	498.6	1068.3	411.1	1355.8
11	320.4	135.7	442.7	952.3	403.4	1315.8
17	383.1	145.2	541.8	1134.0	429.8	1603.8
19	412.0	136.8	458.8	1265.7	420.3	1409.6
20	380.5	143.9	497.3	1130.1	427.6	1477.1
Mean	370.5	141.4	475.4	1092.6	417.0	1402.0
S.D.	35.5	6.7	35.6	100.1	8.8	95.7

average of 371 mgm. in the whole carcasses. These values are not significantly different from each other though the control rats had significantly more wet lean body masses. Quantities of sodium per 100 gms. of wet and dry lean body masses were almost the same for the control and treated rats. Similarly, the total amounts of potassium present in the whole carcasses of treated and control groups did not show any significant difference. Furthermore, there was no difference in the amounts of potassium per 100 gms. of wet and dry lean body masses between the treated and control groups. Results on the amounts of sodium in the carcasses of the treated rats indicate support for the reports of Woods (1967) and Laragh et al. (1967) about hypertension in women taking oral contraceptive pills.

We can compare the first and third groups of rats which were killed at 4 weeks or 25 weeks and 3 days of steroid treatment in order to know the long-term effect of the pill. If we take the treated rats of the first and third groups and compare them with each other for their absolute amounts of dry weight, it is seen that the increase in dry body weight from the 4th to the 25th week

is only 9.1 gms. while the corresponding increase for the control groups for the same interval was 17.5 gms. The increase in the wet lean body mass was 16 gms. between the two treated groups while it was 26 gms. for the control groups. However, the increase in the dry lean body masses was approximately the same during this 21 weeks interval. The differences were 7 gms. for the treated groups and 9 gms. between the control groups. Similarly there was not much difference in the gain of nitrogen between the treated and control groups. This difference between the two treated groups was 1.04 gms. and between the control groups, it was 1.12 gms. Less improvement occurred in the fat content of the treated rats belonging to the third group in 21 weeks interval. The difference in the absolute amount of fat between the first and third group treated rats was only 2 gms. while at this period the control rats had a mean increase of 8.5 gms. Body fat per gram of food was still lower in the treated rats than in the control rats. However there was no difference between the control and treated groups in the amount of fat present per gram of nitrogen in the body, Table 3 (Appendix). From the above mentioned results, it is clear that the steroid

treatment definitely exerts some effects on many components of the body.

Fourth Group

The last or the 4th group of 14 rats were killed immediately following 6 weeks of refeeding the control diet after 25 weeks and 5 days of steroid treatment. These rats were placed on the control diet two days after the 3rd group of rats were sacrificed. Their body weight data are presented in Table 7. Their percent moisture in the whole body and the lean body and the absolute amounts of water, dry body weight, wet and dry lean body masses are presented in Table 17. Refeeding the control diet again did not bring the percent moisture of the whole body of treated rats to the value of the control rats. Percent moisture in the lean body masses of the treated and control rats were almost the same. The absolute amount of water in the treated rats was 174.5 gms. and in the control rats 190 gms. This was because the control rats weighed more. However, the absolute amounts of water in the refed and control rats were not statistically different. But it was approaching statistical significance. The absolute dry body weight was still higher ($P < 0.05$) with control rats

Table 17

Percent Moisture in the Whole and Lean Body Masses and Absolute Amounts of Water, Dry Weight, Wet and Dry Lean Body Masses in Control Rats and in Rats Refed the Control Diet for 6 Weeks after 25 Weeks and 5 Days of Steroid Treatment

Rat No.	Moisture in Whole Body (%)	Moisture in Lean Body Mass (%)	Absolute Amt. of Water(gms.)	Absolute Dry Weight (gms.)#	Wet Lean Body Mass (gms.)@	Dry Lean Body Mass (gms.)@
			Refed Rats			
11	57.4	70.4	175.7	130.5	249.6	73.9
12	57.6	69.9	171.0	126.2	244.6	73.5
14	60.4	70.7	206.8	135.8	292.4	85.6
15	55.6	68.9	173.6	138.7	251.9	78.3
16	63.5	69.8	167.7	96.4	240.4	72.7
18	64.5	69.5	164.4	90.4	236.4	72.1
19	58.6	70.1	162.0	114.3	231.2	69.2
Mean	59.7	69.9	174.5	118.9	249.5	75.0
S.D.	3.3	0.6	15.1	19.2	20.3	5.4
			Control Rats			
113	53.7	69.3	192.0	165.4	276.9	84.9
43	59.1	69.7	176.4	122.1	253.2	76.8
56	55.3	70.3	206.1	166.3	293.2	87.1
60	59.4	69.0	197.8	135.4	286.7	89.0
50	61.6	69.6	201.9	126.4	290.1	88.2
119	53.1	69.2	187.2	165.4	270.6	83.4
58	58.5	69.5	170.6	121.0	245.3	74.7
Mean	57.2	69.5	190.3	143.1	273.7	83.5
S.D.	3.2	0.4	13.1	21.6	18.5	5.6

#The mean absolute value 143.1 gm. of dry weight of control rats is significantly higher ($P < 0.05$) than that 118.9 gms. of the refed rats.

@Control rats have significantly more wet and dry ($P < 0.05$) lean body masses as compared to the refed rats which were placed on control diet for 6 weeks.

than with the treated ones. Six weeks of refeeding the control diet did not have much effect in increasing the dry body weights of the treated rats compared to the control rats. The mean difference in the increase in dry body weight was 8.6 gms. between the treated rats of the 3rd and the refed rats of the 4th group while the corresponding difference was 13.5 gms. between the control rats of the 3rd group and the 4th group. Calculated amounts of wet and dry lean body masses were also significantly higher ($P < 0.05$) with the control rats than with the treated rats. On comparing the wet and dry lean body masses of the 3rd and 4th group, we can see that the mean wet lean body mass of the treated rats of the 3rd group was 244 gms. and it was 262 gms. with the control rats. The mean dry lean body mass of the treated rats belonging to the 3rd group was 74 gms. and control rats 78 gms. After refeeding the control diet for 6 weeks to the fourth group of rats, the mean wet lean body mass increased to 250 gms. with the treated rats and it was 274 gms. with the control rats. The difference was 6 and 12 gms. for the treated-refed and control-control rats respectively. Similarly the increase in mean dry lean body mass was 1 gm. between

the treated-refed rats and 6 gms. between the control-control rats. Refeeding the control diet for 6 weeks did not increase the lean body masses of the treated rats as it did with the rats fed continuously the control diet. Percent nitrogen in the lean body masses, absolute amounts of nitrogen and protein in the whole carcasses, percent fat of the wet and dry weight of the carcasses, and the absolute amount of fat appear in Table 18. Percent nitrogen in the wet and dry lean body masses of the control and treated rats were almost the same. However, the total absolute amounts of nitrogen and protein in the carcasses of the control rats were higher ($P < 0.05$) than the treated rats. In essence, there was not much change in the absolute amounts of nitrogen and protein among the treated rats of the 3rd group and the refed rats of the 4th group which were refed with control diet for 6 weeks subsequent to feeding on the experimental diet. But during this 6 weeks period, the difference in nitrogen and protein were 0.5 and 3.1 gms. respectively between the control rats of the 3rd and 4th groups. Feeding the control diet for 6 weeks did help the treated rats to gain more fat. During this 6 weeks period of feeding the control diet, the mean

Table 18

Percent and Absolute Amounts of Nitrogen and Fat in Control Rats and in Rats Which Were Placed on Control Diet for 6 Weeks after 25 Weeks and 5 Days of Steroid Treatment

Rat No.	Nitrogen in the Wet Lean Body Mass (%)	Nitrogen in the Dry Lean Body Mass (%)	Absolute Amt. of Nitrogen# (gm.)	Absolute Amt. of Protein# (gm.)	Fat in Wet Body Weight (%)	Fat in Dry Body Weight (%)	Absolute Amt. of Fat(gms.)
			Refed Rats				
11	3.8	12.8	9.5	59.1	18.5	43.3	56.5
12	3.8	12.5	9.2	57.5	17.7	41.7	52.6
14	3.7	12.7	10.9	67.9	14.6	36.9	50.1
15	3.8	12.4	9.7	60.9	19.3	43.5	60.4
16	3.7	12.3	8.9	55.7	8.9	24.5	23.6
18	3.7	12.3	8.8	55.3	7.2	20.3	18.4
19	3.8	12.8	8.9	55.3	16.3	39.5	45.1
Mean	3.8	12.5	9.4	58.8	14.7	35.7	43.8
S.D.	0.05	0.22	0.72	4.50	4.80	9.40	16.40
			Control Rats				
113	3.9	12.8	10.9	67.8	22.5	48.7	80.5
43	4.8	12.5	9.6	59.9	15.2	37.0	45.2
56	3.7	12.4	10.8	67.6	21.3	47.7	79.2
60	3.8	12.1	10.8	67.5	13.9	34.3	46.4
50	3.8	12.5	11.0	68.8	11.6	30.2	38.2
119	3.9	12.6	10.5	65.9	23.3	49.6	82.0
58	3.8	12.6	9.4	58.8	15.7	38.2	46.3
Mean	3.8	12.5	10.4	65.2	17.6	40.8	59.7
S.D.	0.07	0.20	0.66	4.10	4.60	7.80	19.80

#Absolute amounts of nitrogen and protein are higher in control rats ($P < 0.05$) than in refed rats which were placed on control diet for 6 weeks.

increase in fat of the refed rats was 7.6 gms. which is much higher than the 2 gms. fat gained by the treated rats of the 3rd group in 21 weeks of eating the steroid diet. Among the 4th group of rats, there was no significant differences between the refed and control groups in the percentages of fat of the wet and dry carcasses and in the absolute amount of fat in the carcasses.

The total amounts of sodium and potassium in the whole carcasses and per 100 gms. of wet and dry lean body masses were calculated and presented in Table 19. None of the above values differed significantly between the refed and control rats. There was not much difference in the amounts of sodium and potassium both in the whole and lean body masses after refeeding the control diet for 6 weeks. It is important to note that the treated rats had significantly lesser ($P < 0.05$) wet and dry lean body masses but still had sodium and potassium close to the control values.

Life Span Study

Finally, mention must be made of the rats which were kept for studying the long-term effect of the pill.

Table 19

Amounts of Sodium and Potassium in the Whole Body and Per 100 Gm. of Wet and Dry Lean Body Masses in Refed Rats which were Placed on Control Diet for 6 Weeks and in Control Rats after 25 Weeks and 5 Days of Steroid Treatment

Rat No.	Total Amt. of Na in Body (mgm.)	Amt. in 100 Gm. of Wet Lean Mass (mgm.)	Amt. in 100 Gm. of Dry Lean Mass (mgm.)	Total Amt. of K in Body (mgm.)	Amt. in 100 Gm. of Wet Lean Mass (mgm.)	Amt. in 100 Gm. of Dry Lean Mass (mgm.)
			Refed Rats			
11	339.0	135.8	458.7	1034.2	414.3	1399.3
12	335.4	137.1	456.2	991.7	405.5	1348.6
14	422.1	144.3	492.9	1226.2	419.3	1432.1
15	364.3	144.6	465.2	1009.7	400.8	1289.3
16	339.1	141.0	466.2	1043.4	433.9	1434.4
18	327.9	138.7	455.0	1017.3	430.3	1411.6
19	322.8	139.6	466.9	934.2	404.1	1350.8
Mean	350.1	140.2	465.9	1036.7	415.5	1380.9
S.D.	34.4	3.4	12.9	90.9	13.0	53.4
			Control Rats			
113	444.0	160.3	522.8	1091.6	394.2	1285.6
43	357.2	141.1	464.9	1027.1	405.6	1336.6
56	401.1	136.8	460.5	1191.3	406.4	1367.8
60	397.6	138.7	446.9	1217.3	424.5	1368.1
50	441.4	152.2	500.5	1202.4	414.5	1363.4
119	317.4	117.3	380.5	904.6	334.3	1084.5
58	333.6	136.0	446.4	1103.5	449.9	1476.6
Mean	384.6	140.3	460.4	1105.4	404.2	1326.1
S.D.	50.1	13.6	45.2	112.5	35.6	120.9

Their growth rates were similar to the rest of the animals used for the other studies, Figure 2. After they had eaten diet containing steroids for about nine months, two out of these 28 rats developed rapidly growing mammary gland tumors. The tumors were examined at the Veterinary Diagnostic Laboratories of Michigan State University by Dr. V. Sanger. The tumor in one of them was in the inguinal region and measured 3 x 2 x 1.5 inches. The tissue was composed entirely of connective tissue stroma and small discrete ducts. Most ducts were intact and lined with small epithelial cells. A few groups of these cells were present which were not confined to the arrangement of a duct but were bunched in the stroma. Mitotic figures were scarce. Evidence for malignancy was sparse. The tumor in the second animal was in the axillary region. This tumor tissue was composed of a dense arrangement of mammary gland alveoli and cells which had escaped from the alveolar arrangement or had never formed a structure. The stroma was sparse. Much of the tissue appeared as well-organized alveoli with epithelial cells lining the alveolus and secretion filling the lumen. Some of the structure was large and open. Others were small and partially collapsed

because of crowding. The epithelial cells were large, hyperchromic, and crowded. Large numbers of cells were present in the stroma and had not formed alveoli, Many mitotic figures were present. Most cells appeared to be relatively immature although some had secretory materials in the cytoplasm. This tissue presented the appearance of early malignancy.

After 74 weeks of steroid treatment, two more treated rats developed mammary gland tumors. One control also had a small mass in the axillary region, and at the time of this report was still alive. However, during the 1 1/2 year period of the experiment, the occurrence of tumors in four out of 28 treated rats is more significant than in one out of 22 control rats. This result agrees with the finding that the prolonged administration of estrogen to rats caused a high incidence of mammary tumors (Dunning and Curtis, 1952, 1954). According to the present experiment, estrogen and progesterone treatment resulted in a 4-fold incidence of tumors. Unfortunately, the total number of rats used may not be sufficient for a proper statistical evaluation, though the incidence is high enough to warrant further investigation of this aspect of oral contraceptives.

Life span of the treated and control rats during the 1 1/2 year period was calculated. Twelve rats in each group were included for the statistical analysis of the life span. These groups did not include those which had tumors and those which were still alive. The life spans of the 12 control and 12 treated rats are given in Table 4 (Appendix) and their mean values were 60.9 weeks and 61.6 weeks respectively. Statistical analysis proved that these values were not significantly different.

SUMMARY

Contraceptive steroids norethynodrel and mestranol fed to 11 weeks old female rats on a body weight basis caused a reduction in food consumption and body weight gain. Treatment with steroids for a short or a long period did not affect the digestibilities of protein, fat, sodium, and potassium. However, feeding the steroids for 4 weeks resulted in different retentions of nitrogen and sodium between the control and treated groups. Control rats retained significantly more dietary nitrogen ($P < 0.05$) than the treated rats and the treated rats retained more dietary sodium ($P < 0.05$) than the control rats. Feeding the steroids for 24 weeks and 5 days resulted in a higher retention ($P < 0.01$) of nitrogen by treated rats when compared to the control rats. No effect of steroids was observed in the retention of potassium either at short or long term feeding of steroids.

Many changes occurred in the body composition of rats due to steroid treatment. Treated rats had less dry body weight throughout the experiment. Significant

differences occurred in the lean body masses of the treated and control rats. Treated rats had less lean body mass after 25 weeks and 3 days of steroid treatment. Refeeding the control diet for 6 weeks did not alter the situation. Percent nitrogen in wet and dry lean body masses was not altered between the treated and control groups. However, significantly less ($P < 0.05$) absolute amounts of body nitrogen and protein were observed with the treated rats after 25 weeks and 3 days of steroid treatment than the control rats. Refeeding of the control diet did not bring the values any closer to control rats. Significantly more ($P < 0.05$) percentage and absolute amounts of fat were observed with the control rats compared to the treated ones after 4 weeks of steroid treatment. Also, absolute amounts of body fat were significantly higher ($P < 0.05$) with the control rats than the treated rats after feeding the steroids for 25 weeks and 3 days. Refeeding the control diet for six weeks after each treatment helped the treated rats to gain more fat. Not much difference was observed in the amounts of sodium and potassium in the carcasses of the treated and control groups.

Life span was not affected due to steroid treatment. An increased incidence of mammary tumors was observed in the steroid treated group.

Since there was not much difference in the percent digestibilities of various nutrients and in the percentages of various components of the body except the fat component between steroid treated and control groups, it may be concluded that the contraceptive steroids have no marked effect on the body composition of adult female rats.

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APPENDICES

APPENDIX I

COMPOSITION OF GRAIN RATION (in %)

Ground corn 60.7; soybean meal (50% protein), 28.0; alfalfa meal (17% protein), 2.0; fish meal (12.5% protein), 2.5; dried whey (67% lacose), 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; rifoflavin, 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

% NITROGEN* DIGESTIBILITY

$$\text{\% Nitrogen* Digestibility} = \frac{\text{Dietary Nitrogen - Nitrogen in the feces}}{\text{Dietary Nitrogen}} \times 100$$

% NITROGEN* RETENTION

$$\text{\% Nitrogen* Retention} = \frac{\text{Dietary Nitrogen - Nitrogen in urine + feces}}{\text{Dietary Nitrogen}} \times 100$$

*Other nutrients were substituted in the place of nitrogen in order to find out their digestibilities and retentions.

Table 1

Average Food Consumption and Body Weights of Rats

Food Consumption*			Body Weight*		
Weeks	Treated (gms.)	Control (gms.)	Weeks	Treated (gms.)	Control (gms.)
1	92	99	Start	257	257
2	106	133	1	248	268
3	105	120	2	255	278
4	109	118	3	255	278
5	101	126	4	260	288
6	96	115	5	266	296
7	105	107	6	268	301
8	111	111	7	274	297
9	105	111	8	277	304
10	106	116	10	276	307
11	79	95	12	242	316
12	61	85	14	257	292
13	60	82	15	269	296
14	87	84	16	270	301
15	89	50	17	281	306
16	96	97	18	283	313
17	96	102	19	304	333
18	107	114	20	305	333
19	97	108	22	301	339
20	96	107	24	299	352
21	97	108	25	302	347
22	95	115			
23	88	110			
24	92	119			
25	94	117			
26	89	114			
Mean	94.6	106.3		273.8	304.9
S.D.	12.7	16.9		19.4	25.2

*The food consumption and the body weights of control rats are significantly higher ($P < 0.01$) when compared to the treated rats.

Table 2

Feed Efficiency* of the Treated and Control Rats

	Treated	Control
At 22 Days of Steroid Treatment		
	0.23	0.08
	0.06	0.17
	0.06	0.13
	-0.01	0.05
	-0.01	
	0.08	0.14
	-0.18	0.04
	0.03	0.05
	0.20	0.18
	0.01	0.03
Mean	0.05	0.10
S.D.	0.12	0.06
At 173 Days of Steroid Treatment		
	0.14	0.00
	-0.02	0.04
	0.12	-0.16
	0.12	0.05
	0.13	0.14
	0.19	0.19
	0.21	0.00
	0.25	0.13
	0.06	0.18
	0.02	0.08
Mean	0.12	0.06
S.D.	0.08	0.10

*Weight gain in gms./gm. of food/day.

(-) Negative value indicates that rat had a negative weight gain.

Table 3

Absolute Amount of Fat Per Gram of Nitrogen in the Body of the Rats Killed at Various Time Intervals

First Group (Treated)		Second Group (Refed)		Third Group (Treated)		Fourth Group (Refed)	
Rat No.	Amount (gm.)	No.	Amount (gm.)	No.	Amount (gm.)	No.	Amount (gm.)
61	5.48	1	5.12	01	4.23	11	5.98
62	3.96	2	3.54	03	2.83	12	5.72
63	3.47	3	4.90	04	3.14	14	4.61
64	5.37	4	3.20	05	5.14	15	6.20
65	3.69	5	5.18	2	2.10	16	2.65
66	2.80	6	3.93	4	5.44	18	2.08
67	3.38	7	4.19	5	2.89	19	4.80
68	3.51	8	5.04	7	5.94		
69	4.86	9	7.29				
70	5.66	10	4.36				
Mean	4.22		4.67		3.96		3.69
S.D.	1.02		1.19		1.42		3.35
(Control)		(Control)		(Control)		(Control)	
71	4.66	32	5.90	013	3.95	113	7.41
72	4.84	33	3.95	014	5.08	43	4.99
73	4.99	34	4.70	015	4.27	56	7.33
74	4.15	35	3.97	016	3.60	60	4.30
75	5.41	36	4.19	11	8.07	50	3.47
76	5.13	37	6.45	17	6.90	119	7.78
77	5.22	38	6.10	19	4.19	58	4.92
78	3.58	39	5.95	20	5.57		
79	6.10	41	5.56				
80	5.02						
Mean	4.91		5.20		5.21		5.74
S.D.	0.69		0.98		1.57		1.73

Table 4

Life Span of Rats Treated with Steroid Mixed Diet and Rats Fed the Control Diet*

	Weeks the Rats Lived		
Rat No.	Treated	Rat No.	Control
9	38	49	44.5
7	43.5	32	45.0
27	44.5	40	49
19	48	42	51.5
16	64	35	52
14	64.5	36	53
25	66	30	53
3	71	39	67.5
20	71.5	43	74
12	72	37	74
5	77.5	50	80.5
4	79	29	87
Average	61.6		60.9
S.D.	14.3		14.8

*Remaining rats are still alive and will be allowed to continue on the trial.