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# INFLUENCE OF HORMONAL CONTRACEPTIVES ON CARBOHYDRATE AND LIPID METABOLISM IN THE RAT

EKKEHARD SCHILLINGER and ERICH GERHARDS

Research Laboratories of Schering AG, Berlin/Bergkamen, Germany*

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**Abstract**—The effect of low, physiological doses of hormonal contraceptives on carbohydrate and lipid metabolism in the female rat was investigated. Animals were treated, by subcutaneous injection, for 14 days with a combination of 5 μg/kg ethynyl estradiol + 50 μg/kg *dl*-norgestrel, 5 μg/kg ethynyl estradiol + 400 μg/kg norethindrone acetate and with the single components only. Blood glucose in fasted animals was not altered. There was a dose-dependent increase in glycogen deposition in liver. This effect was confined to the estrogenic component and was dependent on the presence of the adrenal gland. The sensitivity of the organism towards exogenous insulin was not changed. In an insulin sensitive system, e.g. in isolated fat cells, the conversion of glucose-1-$^{14}$C to $^{14}CO_2$ was not significantly altered, neither in the presence nor in the absence of insulin. Treatment of rats with ethynyl estradiol was associated with a highly significant increase in serum triglycerides, a transient reduction in serum total cholesterol and a decrease in total lipids in liver. Similar effects were observed after administration of the combination of ethynyl estradiol + *dl*-norgestrel. The estrogen-induced increase in serum triglycerides was, however, abolished by simultaneous treatment with norethindrone acetate. Liver lipids remained reduced. Neither gestagen substantially altered lipid metabolism. Free fatty acids in serum of fasted and fed rats were unaltered after ethynyl estradiol. There was no increased lipolytic rate with epinephrine, as measured in isolated fat cells. The antilipolytic effect of insulin was not significantly changed in animals treated with ethynyl estradiol and ethynyl estradiol + *dl*-norgestrel. The experiments demonstrate that the estrogenic component of hormonal contraceptives is mainly responsible for the metabolic changes seen in the rat. It is suggested that the gestagens may play an important role in modifying the effects of estrogens on carbohydrate and lipid metabolism.

GREAT interest has been focused in recent years on the development of new sex steroids for oral contraception in women. The broad use of synthetic estrogens and gestagens made it necessary to investigate carefully possible alterations in metabolism. A considerable amount of evidence has accumulated which demonstrates that contraceptive steroids not only inhibit the secretion of pituitary hormones such as FSH and LH, but also increase ACTH and HGH in plasma which in turn may contribute to considerable alterations in carbohydrate and lipid metabolism. The present experiments in rats were designed to contribute to an understanding of metabolic effects of hormonal contraceptives on human metabolism.

## MATERIALS AND METHODS

*Chemicals and reagents.* Biochemicals and enzymes were obtained from Boehringer GmbH, Mannheim, crude collagenase (158 U/mg) from Worthington, Freehold, N.J., insulin (40 U/ml) from Farbwerke Hoechst AG, Frankfurt/Main, bovine serum

* Schering AG, 1 Berlin 65, Müllerstrasse 170–2.

albumin from Behring Werke, Marburg, L-epinephrine from E. Merck AG, Darmstadt. Steroids were obtained from the Department of Steroid Chemistry, Schering AG, Berlin. All other chemicals were of analytical grade. Radioisotopes were purchased from the Radiochemical Centre, Amersham. In the present paper the generic names of the steroids were used. Norethindrone acetate, 17α-ethynyl-19-nortestosterone acetate; *dl*-norgestrel, *dl*-17β-hydroxy-18β-methyl-17α-ethynyl-4-estrene-3-one; ethynyl estradiol, 17α-ethynyl-estra-3,17β-diol.

*Animals and dosage.* Adult female Wistar rats (obtained from Dr. Hagemann, Bösingfeld), weighing approx. 160–180 g, were maintained on a commercial diet (Altromin). Tap water was allowed *ad lib.* The steroids were dissolved in benzyl benzoate–castor oil (1:9, v/v) and administered subcutaneously once daily for 14 days. Control rats were treated with the vehicle only. The doses of steroids given were 5 μg/kg ethynyl estradiol, 50 μg/kg *dl*-norgestrel and 400 μg/kg norethindrone acetate. The number of animals in each group was ten. The ratio of ethynyl estradiol–*dl*-norgestrel (1:10) and ethynyl estradiol–norethindrone acetate (1:80) was the same as used in oral contraceptive drugs.* The minimal subcutaneous dose of the compound was chosen which inhibits ovulation or implantation in the rat. This corresponds to an approximate 6-fold human dose. Treatment of adrenalectomized rats for 14 days with 5 μg/kg ethynyl estradiol was started 3 days after bilateral adrenalectomy performed under ether anesthesia.

*Serum determinations.* The animals were killed after fasting for 16 hr by a blow on the head and exsanguinated. Blood was collected and glucose determined enzymatically with glucose oxidase.[1] Total serum cholesterol was estimated colorimetrically with acetic anhydride and concentrated $H_2SO_4$ according to Watson,[2] total glycerol enzymatically[3], and phospholipids as phospho–molybdate complex after precipitation with trichloroacetic acid.[4] Free fatty acids were extracted with chloroform and measured colorimetrically according to Duncombe.[5]

*Glycogen and lipids in liver.* Livers of fasted animals were rapidly excised and aliquots frozen in liquid nitrogen. Glycogen was determined using the method of Balzer and Palm.[6] After digestion of the tissue with 30 per cent KOH, glycogen was precipitated with ethanol and hydrolyzed with 1 N HCl. Glucose was subsequently determined with glucose oxidase and glycogen content expressed in glucose equivalents/100 mg tissue wet wt. Total lipids were extracted from a liver homogenate with chloroform–methanol (2:1, v/v) and estimated gravimetrically. Liver cholesterol was determined from the chloroform–methanol extract.

*Lipolysis and glucose oxidation in fat cells.* Lipolytic studies *in vitro* were performed with isolated fat cells from fed rats. The cells were liberated from the parametrial fat body by collagenase digestion according to Rodbell[7] with minor modifications. Adipocytes (approx. 20–40 mg dry wt) were incubated in 2 ml Krebs–Ringer–bicarbonate buffer, pH 7·4, containing 0·54 mg/ml glucose and 20 mg/ml bovine serum albumin (freed of fatty acids)[8]. Insulin and L-epinephrine were added in concentrations as indicated in the tables. After 2 hr of incubation in a metabolic shaker at 37°, free fatty acids were determined in the medium. The figures in the tables represent the mean of six determinations of two independent experiments. Oxidation of [1-$^{14}$C] glucose to $^{14}CO_2$ was carried out under the same conditions as the lipolytic studies

* Eugynon and Anovlar (reg. trade marks) contain 0·05 mg ethynyl estradiol + 0·5 mg *dl*-norgestrel and 0·05 mg ethynyl estradiol + 4 mg norethindrone acetate, respectively.

except that nonlabeled glucose was replaced by $10^{-3}$ M [1-$^{14}$C]glucose (sp. act. 0·02 mCi/mmole). After the incubation, $^{14}CO_2$ was liberated with 0·3 ml 2 N $H_2SO_4$ and absorbed in 0·3 ml 1 M hyamine. Radioactivity was determined in a Packard liquid scintillation counter.

*Statistical analysis.* The results are given as mean ± S.E.M. Student's *t*-test was employed for statistical evaluation.

## RESULTS

*Carbohydrate metabolism.* Blood glucose of the fasted female rat was not changed after subcutaneous administration for 14 days of 5 μg/kg ethynyl estradiol, 50 μg/kg *dl*-norgestrel, 400 μg/kg norethindrone acetate or a combination thereof. However, the combined administration of estrogen and gestagen produced a significant increase in liver glycogen. The administration of the single steroids revealed that liver glycogen

TABLE 1.

| | Control | Ethynyl estradiol (5 μg/kg) | *dl*-Norgestrel (50 μg/kg) | Norethindrone acetate (400 μg/kg) |
|---|---|---|---|---|
| Blood glucose (mg/100 ml) | 63·9 ± 1·5 | 66·5 ± 1·9 | 61·2 ± 1·6 | 59·6 ± 1·4 |
| Liver glycogen (μg/100 mg wet wt) | 141 ± 32 | 441 ± 62<br>P ⩽ 0·01 | 93 ± 17 | 97 ± 15 |

| | Control | 5 μg/kg Ethynyl estradiol + 50 μg/kg *dl*-norgestrel | 5μg/kg Ethynyl estradiol + 400 μg/kg norethindrone acetate |
|---|---|---|---|
| Blood glucose (mg/100 ml) | 67·1 ± 1·4 | 70·4 ± 2·8 | 68·3 ± 1·5 |
| Liver glycogen (μg/100 mg wet wt) | 470 ± 58 | 716 ± 118<br>P ⩽ 0·02 | 618 ± 61<br>P ⩽ 0·02 |

Blood glucose and liver glycogen in fasted female rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol, 50 μg/kg *dl*-norgestrel and 400 μg/kg norethindrone acetate and the combination thereof (mean ± S.E.M., $n = 10$).

was only elevated after treatment with ethynyl estradiol, whereas both gestagens were without effect (Table 1). The differences in glycogen levels in control animals are due to the great variation between experiments. The increase in glycogen after ethynyl estradiol was shown to be dose-dependent (Fig. 1). As little as 0·5 μg/kg ethynyl estradiol doubled the amount in the liver. Considering the known effects of glucocorticoids on glycogen synthesis, experiments were performed with adrenalectomized rats in order to find out whether the adrenals were necessary for the increase in glycogen after ethynyl estradiol administration. As seen in Fig. 2, only intact animals showed an increase in liver glycogen. Thus, the stimulation of glycogen synthesis by ethynyl estradiol depends on the presence of the adrenal gland.

Alterations in the glucose disappearance rate after insulin injections have been shown after treatment with progesterone, norethyndrel, estriol and mestranol in rhesus monkeys.[9] In our experiments the intravenous injection of 0·3 U insulin/kg provoked a pronounced decrease in blood glucose after 10 and 20 min. Treatment of the animals

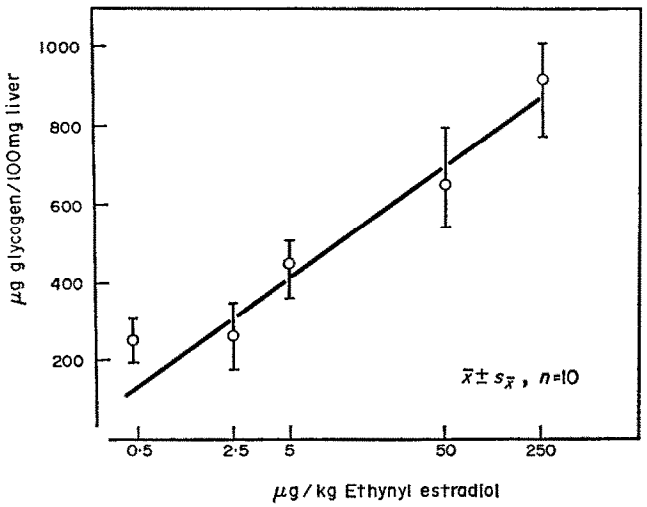


FIG. 1. Liver glycogen in fasted female rats after s.c. treatment for 14 days with ethynyl estradiol (mean ± S.E.M.).

for 10 days with 50 μg/kg norgestrel or with 5 μg/kg ethynyl estradiol did not significantly alter the blood sugar lowering effect of insulin (Fig. 3).

In addition, the utilization of glucose was investigated in isolated fat cells. The net conversion of glucose to $CO_2$ without insulin showed a rather broad variation after the treatment with both steroid combinations. However, the stimulatory effect of 250 μU/ml insulin on glucose entry did not appear to be significantly altered (Table 2).

*Lipid metabolism.* Total cholesterol, phospholipids, total glycerol (as a measure for triglycerides) and free fatty acids were determined as the main constituents of serum

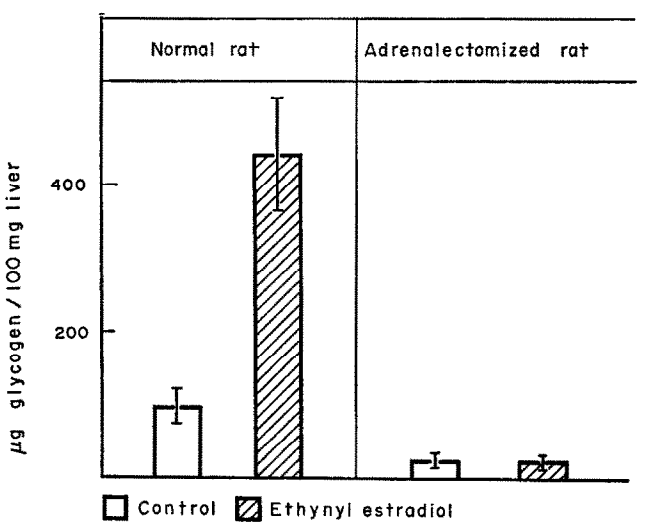


FIG. 2. Liver glycogen in fasted normal and adrenalectomized rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol (mean ± S.E.M., $n = 10$).

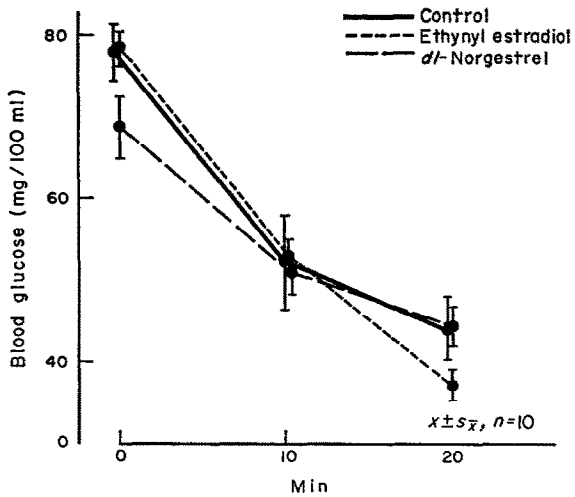


FIG. 3. Blood glucose after i.v. injection of of 0·3 U insulin/kg after s.c. treatment with 5 μg/kg ethynyl estradiol and 50 μg/kg dl-norgestrel for 10 days.

TABLE 2.

| | nmole $^{14}CO_2$/100 mg cell dry wt × 2 hr | | |
|---|---|---|---|
| | Control | 5 μg/kg Ethynyl estradiol + 50 μg/kg *dl*-norgestrel | 5 μg/kg Ethynyl estradiol + 400 μg/kg norethindrone acetate |
| No addition | 49·1 ± 5·1 | 67·2 ± 3·5 | 75·0 ± 8·2 |
| 250 μU/ml insulin | 122·2 ± 8·4 | 132·2 ± 6·2 | 153·2 ± 13·6 |
| | P ⩽ 0·05 | P ⩽ 0·05 | P ⩽ 0·05 |
| Stimulation (%) | 249 | 197 | 204 |

Oxidation of [1-$^{14}$C]glucose to $^{14}CO_2$ with and without 250 μU/ml insulin in isolated fat cells of fed female rats after s.c. treatment for 14 days with ethynyl estradiol + *dl*-norgestrel and ethynyl estradiol + norethindrone acetate. Each value represents six determinations of two independent experiments (mean ± S.E.M., $n = 10$).

lipids. Free fatty acids in serum of fasted and fed animals after ethynyl estradiol were not altered (Table 3). No changes in the levels of cholesterol and phospholipids were observed under the combined steroid treatment. Total glycerol remained unchanged after treatment with 5 μg/kg ethynyl estradiol + 400 μg/kg norethindrone acetate,

TABLE 3.

| Free fatty acids (FFA) | Control ($n = 8$) | 5 μg/kg Ethynyl estradiol ($n = 7$) |
|---|---|---|
| Fed animals | 0·45 ± 0·04 | 0·46 ± 0·06 |
| Starved animals | 1·07 ± 0·08 | 0·99 ± 0·06 |

Free fatty acids in serum of fed and starved female rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol (mean ± S.E.M.).

TABLE 4.

| | Control | Ethynyl estradiol *dl*-norgestrel | Ethynyl estradiol + norethindrone acetate |
|---|---|---|---|
| Serum | | | |
| Total cholesterol (mg/100 ml) | 60·9 ± 1·9 | 58·4 ± 3·4 | 62·9 ± 3·4 |
| Total glycerol (mg/100 ml) | 7·78 ± 0·47 | 12·53 ± 1·52 P ⩽ 0·01 | 7·93 ± 0·55 |
| Phospholipids (mg/100 ml) | 112·8 ± 2·9 | 106·0 ± 5·1 | 101·9 ± 5·3 |
| Liver | | | |
| Total lipids (mg/g liver) | 59·1 ± 1·5 | 54·2 ± 1·1 P ⩽ 0·01 | 54·4 ± 0·9 P ⩽ 0·01 |
| Total cholesterol (mg/g total lipids) | 80·4 ± 3·0 | 76·3 ± 1·6 N.S. | 73·2 ± 1·5 N.S. |
| (mg/g liver) | 4·80 ± 0·15 | 4·14 ± 0·13 P ⩽ 0·01 | 3·98 ± 0·10 P ⩽ 0·01 |

Lipids in serum and liver in fasted female rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol + 50 μg/kg *dl*-norgestrel and ethynyl estradiol + 400 μg/kg norethindrone acetate (mean ± S.E.M., $n = 10$). N.S., not significant.

however, a highly significant increase was observed after 5 μg/kg ethynyl estradiol + 50 μg/kg *dl*-norgestrel (Table 4). Administration of the single components revealed that only the treatment with ethynyl estradiol led to an elevation of total glycerol, whereas *dl*-norgestrel and norethindrone acetate were without effect (Table 5). It

TABLE 5.

| | Control | Ethynyl estradiol | *dl*-Norgestrel | Norethindrone acetate |
|---|---|---|---|---|
| Serum | | | | |
| Total cholesterol (mg/100 ml) | 78·4 ± 3·3 | 64·0 ± 4·4 P ⩽ 0·05 | 72·4 ± 3·4 N.S. | 69·7 ± 2·2 N.S. |
| Total glycerol (mg/100 ml) | 8·64 ± 0·61 | 13·08 ± 0·68 P ⩽ 0·01 | 9·31 ± 0·34 N.S. | 7·94 ± 0·64 N.S. |
| Phospholipids (mg/100 ml) | 123·3 ± 3·4 | 127·0 ± 7·0 N.S. | 121·3 ± 5·4 N.S. | 112·2 ± 3·4 P ⩽ 0·05 |
| Liver | | | | |
| Total lipids (mg/g liver) | 59·1 ± 1·2 | 54·8 ± 1·0 P ⩽ 0·02 | 63·7 ± 1·1 P ⩽ 0·02 | 57·5 ± 1·2 N.S. |
| Total cholesterol (mg/g total lipids) | 74·9 ± 1·7 | 77·4 ± 2·1 | 74·3 ± 1·2 | 82·0 ± 2·5 P ⩽ 0·05 |
| (mg/g liver) | 4·41 ± 0·11 | 4·22 ± 0·13 | 4·72 ± 0·05 | 4·76 ± 0·10 P ⩽ 0·05 |

Lipids in serum and liver in fasted female rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol, 50 μg/kg *dl*-norgestrel and 400 μg/kg norethindrone acetate (mean ± S.E.M., $n = 10$). N.S., not significant.

remains unclear why the combination of ethynyl estradiol + norethindrone acetate failed to increase the glycerol levels in serum. Likewise, total cholesterol was only depressed under estrogen treatment, both gestagens had no effect. A slight drop in phospholipids was encountered after norethindrone acetate.

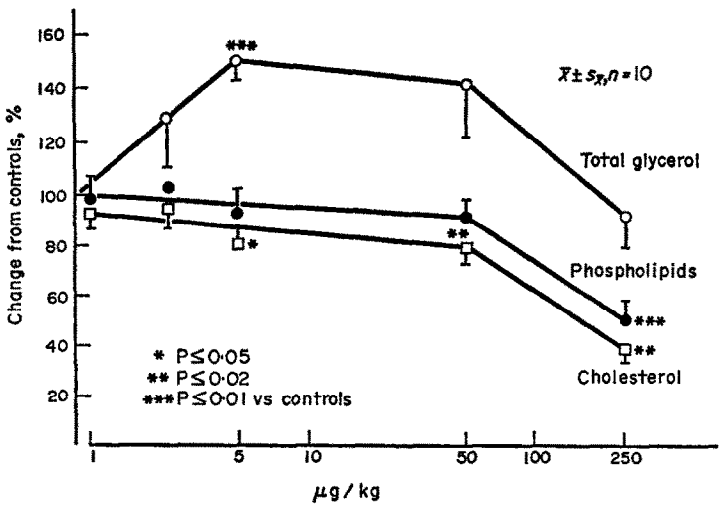


FIG. 4. Serum lipids in the fasted female rat after subcutaneous treatment with various doses of ethynyl estradiol for 14 days.

More detailed studies were performed with ethynyl estradiol as this compound appeared to be mainly responsible for the observed alterations in serum lipids. Figure 4 demonstrates the changes in the lipid fractions observed after treatment with increasing doses of ethynyl estradiol. Significant differences were obtained with 5 μg/kg in total glycerol and cholesterol levels. Toxic doses were apparently reached with 250 μg/kg. Cholesterol and phospholipids dropped to very low levels and even total glycerol was depressed.

TABLE 6.

| | μmole FFA/100 mg cell dry wt × 2 hr | | | |
|---|---|---|---|---|
| | Control | Ethynyl estradiol | *dl*-Norgestrel | Norethindrone acetate |
| No addition | 0·13 ± 0·02 | 0·15 ± 0·02 | 0·13 ± 0·01 | 0·13 ± 0·01 |
| Epinephrine, 0·30 μg/ml | 15·95 ± 1·20 | 14·17 ± 1·49 | 11·36 ± 1·36 | 14·38 ± 0·81 |
| Epinephrine, 0·30 μg/ml + insulin, 100 μU/ml | 8·50 ± 1·37 | 10·37 ± 1·24 | 7·48 ± 0·77 $n = 5$ | 6·29 ± 0·36 |
| Epinephrine value (%) | 53·3 | 73·2 | 65·8 | 43·8 |

Lipolysis in isolated fat cells of fed female rats after s.c. treatment for 14 days with 5 μg/kg ethynyl estradiol, 50 μg/kg *dl*-norgestrel and 400 μg/kg norethindrone acetate. Each value represents six determinations of two independent experiments (mean ± S.E.M.).

In liver, the lipid content decreased after the combined administration of the steroids (Table 4) which was paralleled by a drop in cholesterol levels. A similar depression in total lipids was observed after ethynyl estradiol (Table 5). *dl*-Norgestrel caused an increase in liver weight and in total lipids. Cholesterol was slightly elevated after treatment with norethindrone acetate. It therefore seems reasonable to assume that the reduction in liver lipids is due to the action of ethynyl estradiol. The changes observed after treatment with *dl*-norgestrel need further investigation.

The question was raised whether the treatment with estrogens, which results presumably in increased levels of ACTH, growth hormone and glucocorticoids, leads to enhanced lipolytic activity in adipose tissue. In order to test this possibility in adipocytes, basal lipolysis was stimulated with L-epinephrine and free fatty acids were determined in the medium. The treatment of the rats with the steroids under investigation did not produce any significant changes neither in basal lipolysis nor in the responsiveness of the fat cells towards epinephrine. The inhibitory effect of insulin on lipolysis was also not significantly altered after steroid treatment (Tables 6 and 7).

TABLE 7.

| | μmole FFA/100 mg cell dry wt × 2 hr | | |
|---|---|---|---|
| | Control | Ethynyl estradiol + *dl*-norgestrel | Ethynyl estradiol + norethindrone acetate |
| No addition | 0·10 ± 0·02 | 0·09 ± 0·01 | 0·10 ± 0·01 |
| | $n = 5$ | | |
| Epinephrine, 0·30 μg/ml | 6·94 ± 0·88 | 6·99 ± 1·08 | 6·20 ± 0·83 |
| Epinephrine, 0·15 μg/ml | 1·51 ± 0·49 | 2·15 ± 0·37 | 1·45 ± 0·30 |
| | | $n = 5$ | |
| Epinephrine, 0·15 μg/ml + insulin, 100 μU/ml | 0·47 ± 0·02 | 1·33 ± 0·39 | 0·61 ± 0·04 |
| Epinephrine value (%) | 31 | 62 | 42 |

Lipolysis in isolated fat cells of fed female rats after s.c. treatment for 14 days with a combination of 5 μg/kg ethynyl estradiol + 50 μg/kg *dl*-norgestrel and 5 μg/kg ethynyl estradiol + 400 μg/kg norethindrone acetate. Each value represents six determinations of two independent experiments (mean ± S.E.M.).

## DISCUSSION

The alterations in carbohydrate metabolism in the rat suggest that attention must be given to the estrogenic component in hormonal contraceptives of the combination type. Conflicting results have been reported previously. It has been suggested that the peripheral utilization of glucose is inhibited by estrogen treatment and data have been put forward that elevated plasma corticoid and growth hormone levels or an increased lipolytic rate or impairment of liver function might be responsible for the inhibition. A discussion of some of the aspects has been presented earlier.[10]

As early as 1952, Gemzell[11] had shown that treatment with estrogens lead to a marked stimulation of the biosynthesis and the secretion of ACTH. The increased plasma level of the hormone stimulates the secretory function of the adrenals which causes an increase in both free and protein-bound corticoids.[12] It is of interest to

note that the higher levels of corticoids, provoked by estrogen treatment, are obviously not able to interfere with the pituitary–adrenal regulatory system so as to suppress ACTH secretion to such an extend that normal corticoid values in plasma are achieved. It has also been suggested that hepatic enzyme systems dealing with corticoid metabolism are affected which may result in a decreased removal of the hormone(s) from the circulation.[12,13] It is apparent from the present experiments that ethynyl estradiol has no direct corticoid properties as glycogen deposition does not occur in adrenalectomized animals. Higher levels of blood glucose in fasted rats after large doses of ethynyl estradiol (unpublished results) can be attributed, in addition to increased gluconeogenesis, to the inhibitory action of glucocorticoids on glucose uptake. The application of a combination of both estrogen and gestagen, in general, results in changes in carbohydrate metabolism as seen under treatment with estrogens only. Liver glycogen is increased and the oral glucose tolerance deteriorates in a similar way as seen in humans.[14] Beck observed in women and rhesus monkeys that treatment with some estrogens or gestagens considerably reduces the sensitivity towards exogenous insulin as measured by blood glucose depression.[15,16] Similar studies were performed during pregnancy and after progestogen treatment in humans and rats by Kalkhoff *et al.*,[17] Costrini and Kalkhoff[18] and others.[19–21] These findings are in agreement with the hypothesis of an apparently decreased efficacy of insulin.

In our experiments treatment with low doses of steroids, e.g. ethynyl estradiol or norgestrel, did not alter the sensitivity of the organism towards added insulin in metabolizing glucose. In addition, ethynyl estradiol and the gestagens *dl*-norgestrel and norethindrone acetate do not appear to change carbohydrate metabolism in isolated fat cells of the rat.

The changes in lipid metabolism can only be properly evaluated in connection with the results in carbohydrate metabolism. Estrogens appear to play an outstanding role. Borden *et al.*[22] suggested that, as in humans, treatment of rats with estrogens led to characteristic changes in the distribution of lipoproteins. The high levels in serum triglycerides and the slight depression of serum cholesterol seen after treatment with ethynyl estradiol may be explained by the increase of "very low density lipoproteins" (VLDL) plus "high density lipoproteins" (HDL) at the cost of the "low density lipoproteins" (LDL). Similar results with higher doses of estradiol and ethynyl estradiol in female rats have been reported by Hill and Dvornik.[23] Administration of low doses of Enovid E* (a combination of norethynodrel and mestranol) to female rats was shown to decrease esterified cholesterol in serum and adrenals while increasing it in the liver.[24]

In addition to the changes in the lipoprotein pattern, the decreased activity of lipoprotein lipase after treatment with estrogens or after reduced insulin levels in serum may also contribute to an increase in circulating triglycerides.

No changes in lipid metabolism of the rat could be observed after treatment with *dl*-norgestrel. The combined administration of ethynyl estradiol and *dl*-norgestrel resulted in a pattern similar to that seen after estrogen treatment alone. It is, however, interesting to note that the increase in serum triglycerides and the decrease in serum cholesterol, due to the action of ethynyl estradiol, could be prevented by the combination of the estrogen with norethindrone acetate. Similar findings were obtained in women by Brody *et al.*[25] who found that serum triglycerides were not altered after

* Registered trade mark.

medication with an oral contraceptive, consisting of a combination of ethynyl estradiol and norethindrone acetate. A marked increase in triglycerides was observed after treatment with the combination of mestranol plus ethynodiol diacetate and ethynyl estradiol plus megestrol acetate.

In experiments with animals, norethindrone acetate and *dl*-norgestrel exhibit androgenic as well as antiestrogenic activity. As norethindrone acetate was given in a relatively higher dose than *dl*-norgestrel in the combination with ethynyl estradiol, we may assume that the antiestrogenic activity of norethindrone acetate overcame the estrogenic properties of ethynyl estradiol on carbohydrate and lipid metabolism.

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