Oral Contraceptives, Norethynodrel and Mestranol: Effects on Glucose Tolerance, Tissue Uptake of Glucose-U-^{14}C and Insulin Sensitivity[1] (36731)

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It has been well documented that impaired glucose tolerance occurs in women using combination-type oral contraceptives (1). However, controversy exists regarding which of the two common steroidal agents used in contraceptive preparations is responsible for the glucose intolerance. Gershberg *et al.* (2) found that medroxy-progesterone acetate, a progestational steroid, produced impaired glucose tolerance within three months of treatment, whereas Javier, Gershberg and Hulse (3) showed that mestranol, a common estrogenic component of oral contraceptives decreased glucose tolerances in women. In contrast, Halling, Michals and Paulsen (4) found that there was an improvement of this disturbance when women switched from an estrogen–progestin combination to mestranol alone. In a recent review, Goldzieher (1) indicated that both steroidal agents affect glucose metabolism, but the effect of the estrogen seems to be minor.

The mechanism(s) by which the oral contraceptives impair glucose tolerance is unknown, but several theories have been proposed. The impairment has been attributed to changes in glucose absorption (5), in gut insulin-releasing factors (6), in liver function (7, 8), to increase insulin resistance of the peripheral tissues (9) and to elevation of plasma glucocorticoids (10) and growth hormone (11).

The present work was done to determine which of the two steroidal agents commonly used in contraceptive preparations is responsible for changes in glucose tolerance. Furthermore, *in vitro* experiments were done to determine the possible role of liver, depot fat and diaphragm muscle.

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Materials and Methods. Eleven-week-old female Sprague-Dawley rats weighing between 250 and 270 g were housed individually in suspended wire cages in a temperature and light regulated room. Mestranol (an estrogen) and norethynodrel (a progestin) were fed either singly or in combination by incorporating 0.027 mg mestranol or 1.83 mg norethynodrel or both into each kilogram of a nutritionally adequate diet.[2] Control rats received the diet without steroids. Each rat in the three groups received an amount of feed equal to that consumed the previous day by the rat fed the diet containing mestranol plus norethynodrel. By this equalized feeding method the intake of steroids approximated 0.1 mg of norethynodrel or 1.5 μg of mestranol/kg of body weight. These levels are comparable, on a body weight basis, to that used by women for contraceptive purposes. Water was given *ad libitum* to all groups. Specific treatment procedures and duration for each experiment are shown in the figure legends and table footnotes. Since the diet intake was equalized, the various groups showed about the same weight gain.

In the first experiment, oral glucose tolerance was performed by force-feeding glucose (300 mg/100 g body wt) in a 50% solution (w/v), to rats that had been fasted for 18 hr, and obtaining blood samples at specified times for glucose determinations. Sodium pentabarbital (5 mg/100 g body wt) was injected subcutaneously to anesthetize the rat for blood sampling by heart puncture. Serum glucose was measured by a glucose oxidase method (13).

In a second experiment, tissue uptake and utilization of glucose were measured using

[2] Basal diet composition: previously described (12).

glucose-U-^{14}C. Glucose (300 mg/100 g body wt) and glucose-U-^{14}C (1.67 μCi/300 mg glucose load) in a 45% glucose solution (w/v) were given by stomach tube to rats fasted for 18 hr. Immediately after gastric intubation the rat was placed in a respiratory chamber (14) and the expired air was collected into Hyamine hydroxide traps (15) to absorb CO_2. The air flow into the chamber was adjusted to maintain the chamber temperature near 22°. The Hyamine traps were changed every 20 min to insure complete absorption of CO_2 (Fig. 4). The method for counting ^{14}C has been described previously (15).

The rate of gastric emptying and of glucose absorption from the gastrointestinal tract were determined by a method similar to that described by Cori (16). For this purpose, rats were removed from the respiratory chamber at different time intervals and decapitated. The alimentary tract was exposed and ligatures were placed at the cardiac, pyloric and ileo-cecal sphincters. The stomach and intestine were removed separately, blotted dried and their contents were washed out and made up to 100 ml with isotonic saline. Portions of liver (150–200 mg) and diaphragm muscle (150–200 mg) were rapidly excised and homogenized in saline. Radioactivities in the gastrointestinal contents, liver and diaphragm homogenates were determined using a method similar to that described by Yang *et al.* (17). Samples of parametrial adipose tissue (100–150 mg) were digested in 2 ml of Hyamine hydroxide. The radioactivity of the digest was counted as described above.

In a third experiment, a control and a norethynodrel plus mestranol group of rats were pair fed. The rats were fasted for 12 hr, decapitated and various tissues rapidly excised. Pieces of parametrial adipose tissue weighing about 100 mg, slices of the left lateral lobe of the liver weighing about 200 mg and pieces of diaphragm muscle weighing approximately 100 mg were used to determine the incorporation of ^{14}C from glucose-U-^{14}C into various metabolic products. Various levels of insulin[3] were added to the incubation medium to determine the insulin sensitivity of the tissue. The concentrations of substrate and of insulin in the Krebs–Ringer bicarbonate buffer (pH 7.4) are indicated in the figure legends and table footnotes. The incubation procedure and methods used for isolating radioactive metabolites were similar to those reported by Leveille (18). However, Hyamine hydroxide was used instead of NaOH for the collection of $^{14}CO_2$ evolved during incubation. The radioactivity was determined in a Nuclear-Chicago Unilux I.

All data were analyzed statistically by analysis of variance, as indicated in the figure legends. Tests of significance were made at the 5% probability level for Expts. 1 and 2 and 10% for Expt. 3.

Results. First experiment. After 2 wk of steroid treatment, there were no significant differences in the glucose tolerance curves among the four groups of rats (Fig. 1A). However, an additional 2 wk of treatment resulted in higher glucose levels for the steroid-treated than for control rats (Fig. 1B). Analysis of variance confirmed that norethynodrel was responsible for the overall increase in serum glucose levels in steroid-treated rats. These elevations of serum glucose in the norethynodrel-treated rats were most prominent at 40, 80 and 160 min. Thus, these data revealed that norethynodrel was the major determinant of the elevated serum glucose and the impaired glucose tolerance. Mestranol alone did not modify the glucose tolerance curve significantly, however, the addition of this estrogenic compound seemed to partially reverse the effect of norethynodrel.

Second experiment. Gastric emptying and gastrointestinal absorption of an oral glucose load in the combination and in the mestranol group were significantly lower than in the norethynodrel and in the control group (Fig. 2A and B). Mestranol appeared to be the main factor in decreasing gastric emptying and gastrointestinal absorption of glucose.

There were no significant differences in the

[3] Porcine insulin, free from glucagon, obtained from Eli Lilly and Co.

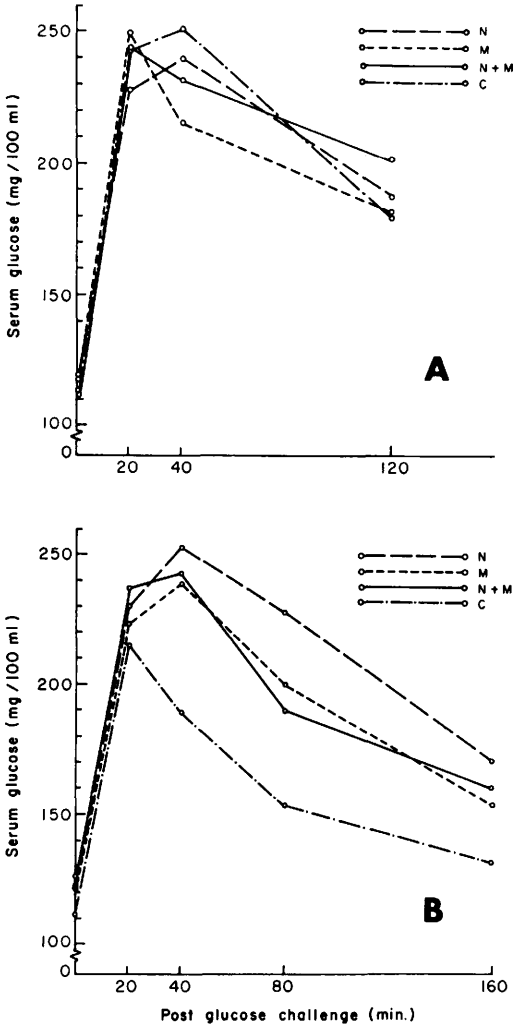

FIG. 1. Blood glucose levels during oral glucose tolerance test: (A) 2 wk, (B) 4 wk of norethynodrel (N) and/or mestranol (M) treatments (8 animals/group). Analysis of variance by split plot design.

uptake of radioactivity from the glucose load by liver (Fig. 3A) and diaphragm muscle (Fig. 3B), between rats fed the control diet or a diet containing one or both steroids. However, the ability of adipose tissue (Fig. 3C), to take up radioactivity was significantly lower in the combination and in the norethynodrel group than in the control and in the mestranol group. Norethynodrel was again the major causative agent in reducing uptake of radioactivity from glucose-U-^{14}C. There were no significant differences in the conversion of glucose into CO_2 (Fig. 4) among rats fed the control diet or diets containing the two hormones or the combination of hormones. In all cases, as expected, the uptake of radioactivity by tissues and the conversion of glucose into CO_2 increased with time after oral loading (Figs. 3 and 4).

Third experiment. The conversion of glucose into CO_2, nonsaponifiable lipid, fatty acids and glyceride-glycerol of the control adipose tissue was increased significantly with each level of insulin added into the incubation medium (Fig. 5). On the other hand, the conversion in the tissue from the steroid-treated rat plateaued near the lowest level of insulin used in the medium. Therefore, the conversion into nonsaponifiable lipid

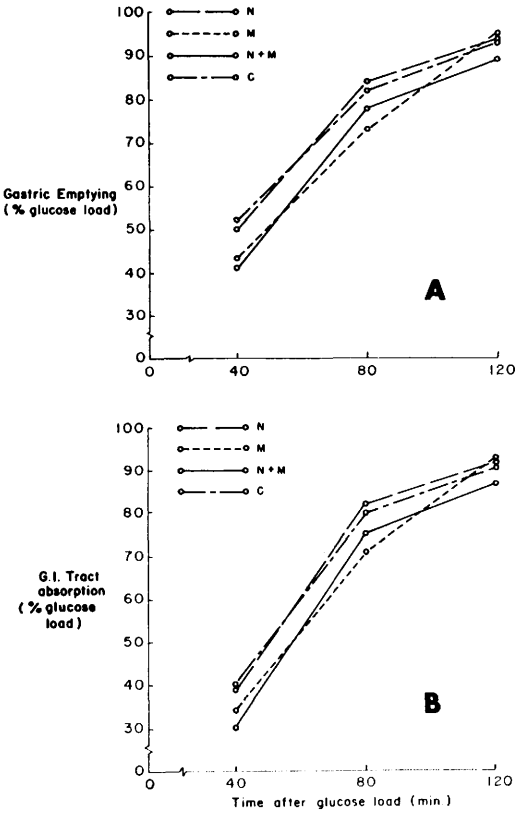

FIG. 2. Gastric emptying and intestinal absorption of a glucose-U-^{14}C load after 6 wk of norethynodrel (N) and/or mestranol (M) treatment (5 animals/group/time interval). Analysis of variance by a completely randomized design.

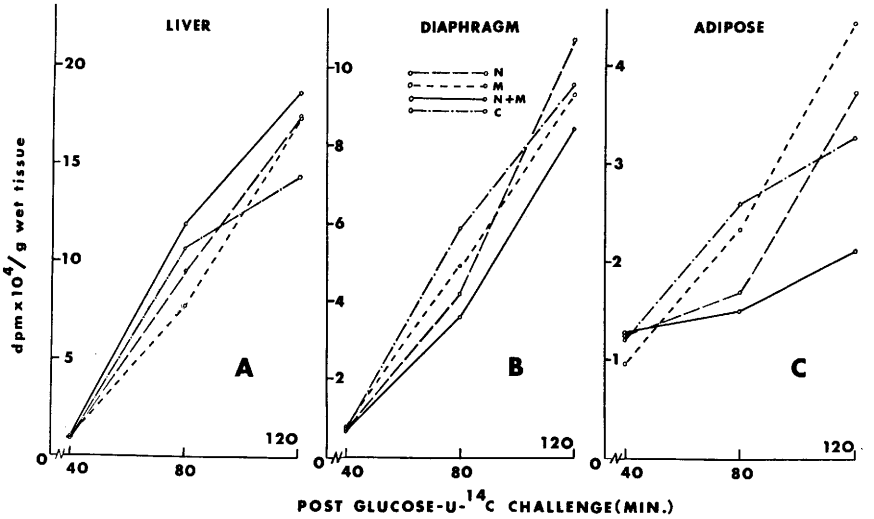

FIG. 3. Radioactivities in liver, diaphragm and adipose tissues at various time intervals after a glucose-U-^{14}C load. Six weeks of norethynodrel (N) and/or mestranol (M) treatments were employed (5 rats/group/time interval). Analysis of variance by a completely randomized design.

and fatty acids in the control tissue was significantly higher than in the treated tissue, when either 100 or 200 μU of insulin were added to 1 ml of incubation medium ($p < 0.1$). These two levels of insulin caused a progressive response in the control but had only a slight effect on the steroid treated tissue (Figs. 5B and C). Fatty acids and nonsaponifiable lipid, but not CO_2 and glyceride–glycerol, syntheses from glucose were significantly ($p < 0.1$) lower in the steroid treated than in the control tissue.

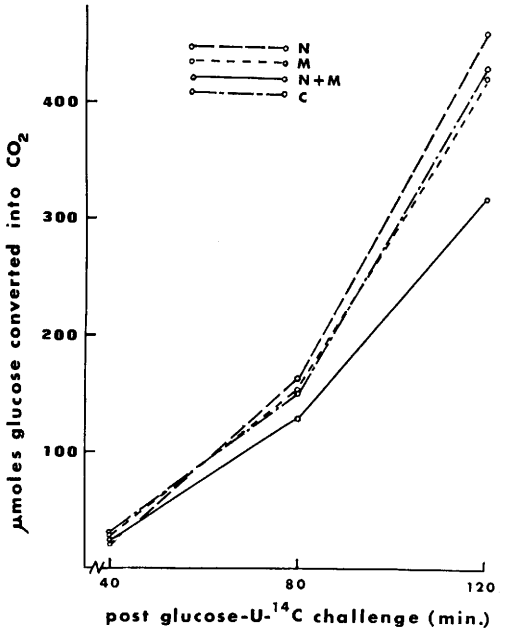

FIG. 4. Glucose-U-^{14}C incorporation into exhaled $^{14}CO_2$ after 6 wk of norethynodrel (N) and/or mestranol (M) treatments (5 animals/group/time interval). Analysis of variance by a completely randomized design.

The conversion of glucose to CO_2 and glycogen (Fig. 6) by the control and steroid-treated diaphragm muscle was significantly increased by insulin. Maximum effect in the control and in the treated group was obtained with 300 and with 500 μU of insulin/ml of incubation medium, respectively. Thus the control tissue was more sensitive to insulin than the steroid treated one.

No significant differences were observed between the conversion of glucose to CO_2, glycogen, nonsaponifiable lipid, fatty acids or glyceride–glycerol, by liver slices of control and treated rats (Table I).

Discussion. In the present experiments, norethynodrel appeared to be responsible for the decrease in the oral glucose tolerance (Fig. 1) and in tissue utilization of glucose (Fig. 3C). This conclusion is strengthened by the finding that norethynodrel decreased also the rate of glucose uptake by adipose

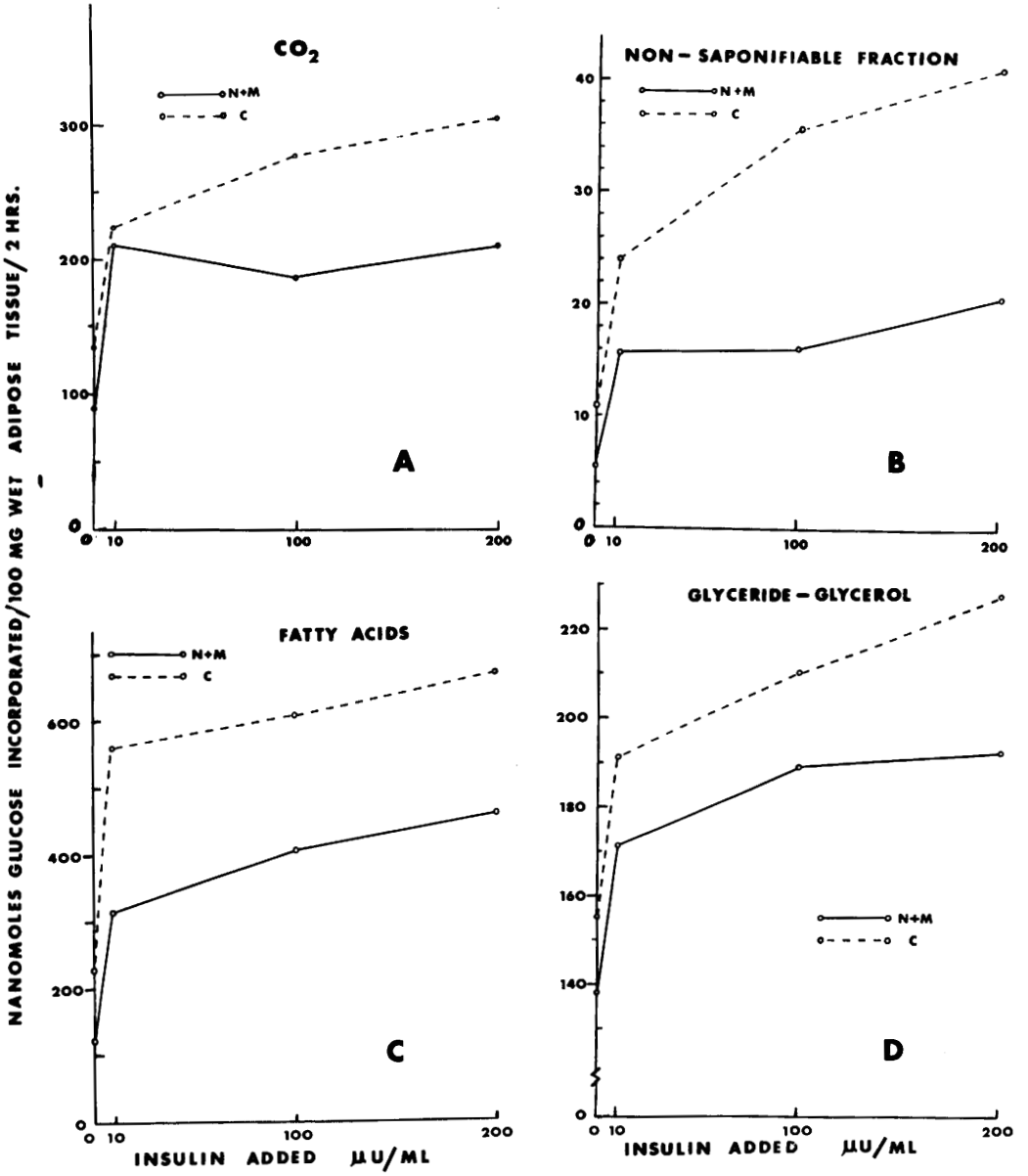

FIG. 5. *In vitro* glucose-U-^{14}C incorporation into (A) carbon dioxide, (B) non-saponifiable fraction, (C) fatty acids, (D) glyceride–glycerol by adipose tissue of control (- - -) and steroid-treated (—) rats (10 animals/group). All buffers contained (per ml): glucose, 10 μmoles; glucose-U-^{14}C, 0.2 μCi; and insulin 0, 10, 100 or 200 μU. Analysis of variance by split plot design.

tissue in the *in vivo* experiment (Fig. 3). This effect required at least 4 wk of steroid treatment. A similar glucose intolerance has also been observed in some, but not in all rats when norgestrel was given alone or with ethynyl estradiol for 2 wk (19). Thus, about 4 wk of treatment appears to be necessary for the development of glucose intolerance. This is suggested also by the experiments of Young and Yang (20).

The slower rate of gastric emptying and gastrointestinal absorption observed in rats

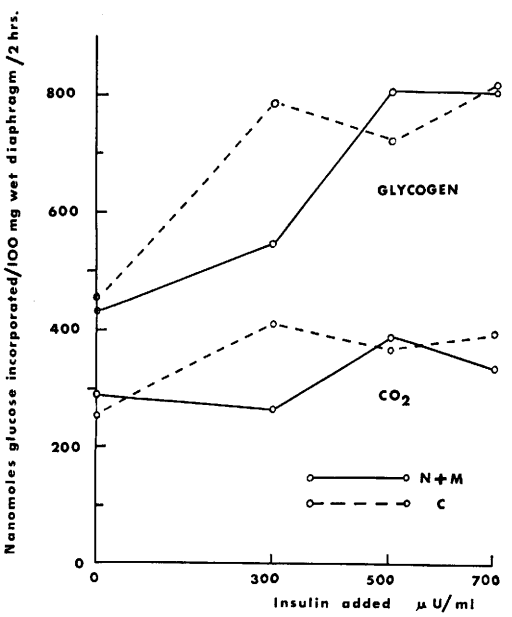

FIG. 6. *In vitro* glucose-U-^{14}C incorporation into carbon dioxide and glycogen by diaphragm of control (- - -) and steroid-treated (—) rats (10 animals/group). All buffers contained (per ml): glucose 10 μmoles; glucose-U-^{14}C, 0.2 μCi; and insulin 0, 300, 500 or 700 μU. Analysis of variance by split plot design.

treated with mestranol and with mestranol and norethynodrel, although statistically significant, appears to be too small to have appreciable effects on the level of blood glucose and on the rate of glucose metabolism in various tissues (Fig. 2). Buchler and Warren (5) found that the oral administration of diethylstilbestrol or norethynodrel plus mestranol in women decreased their tolerance for oral glucose, but not that for intravenous glucose. Thus, they postulated that estrogen might impair the gastrointestinal absorption of glucose rather than exerting a diabetogenic effect.

No statistically significant differences between the rate of conversion of glucose into respiratory CO_2 by the four groups of rats in the second experiment were noted (Fig. 4). This finding is not in accord with the results of Young and Yang (20). In the latter experiments, however, the rats were treated for a much longer period of time and the radioactive glucose was injected intravenously rather than orally. Norethynodrel and mestranol had little adverse effect on the oxidation of glucose to CO_2 in short-term feeding trials, confirming the importance of the length of treatment.

The results of our *in vitro* experiments (Figs. 5 and 6) indicating that less insulin was required to stimulate maximum glycogenesis by diaphragm tissue from control than from steroid treated animals, suggest that muscle and adipose tissue may play a significant role in the impairment of glucose metabolism in steroid-treated rats.

Steroid treatment depressed also lipogenesis and conversion of glucose into nonsaponifiable lipid of adipose tissue (Fig. 5). It is of interest that there was a twofold increase in lipogenesis in the control tissue when 10 μU of insulin were added to the medium and that further addition of insulin produced only a slight increment of lipogenesis. A similar type of dose–response relationship was observed by Crofford (21) using fat cells isolated from rat epididymal tissues. Thus our results suggest that the im-

TABLE I. *In vitro* Glucose-U-^{14}C Utilization by Liver Slices of Control and Steroid-Treated Rats.[a]

	Incorporation of labeled substrate into				
Treatment[b]	Carbon dioxide	Glycogen	Nonsaponifiable lipid	Fatty acids	Glyceride–glycerol
	Substrate incorporated (nmoles/100 mg tissue/2 hr)				
Control	545[c] ± 41	752 ± 121	14.0 ± 3.4	239 ± 33	177 ± 14
N + M	565 ± 45	727 ± 152	12.3 ± 2.7	193 ± 33	181 ± 10

[a] The buffer contained (per ml): glucose, 100 μmoles; glucose-U-^{14}C, 1 μCi.
[b] Ten animals per treatment. N + M = norethynodrel plus mestranol.
[c] Mean for 10 observations ± SEM.

pairment of glucose tolerance following oral treatment with contraceptive steroids could be partly the results of decreased insulin sensitivity of the adipose and muscle tissues. A mild peripheral resistance to the hypoglycemic action of exogenous insulin and an enhancement of plasma insulin response to intravenous glucose have been observed also in progesterone- (22) and mestranol- (9) treated female rhesus monkeys. Furthermore, fasting hyperglycemia, decreased glucose tolerance and increased insulin level have been reported in women receiving oral contraceptives (23). In these women, peripheral insulin resistance may have caused persistent hyperglycemia and hyperinsulinism. Since insulin may increase the synthesis of glycogen (24) and of fatty acids from glucose independently of its effect on glucose transport, further work is contemplated to determine whether the resistance to insulin occurs at the point of glucose entry into the cells or at subsequent steps in glycogenesis or lipogenesis.

Summary. Mestranol and norethynodrel were fed singly or in combination to 11-week-old female Sprague-Dawley rats to determine their effects on glucose tolerance, *in vivo* tissue uptake and utilization of glucose-U-^{14}C and *in vitro* insulin sensitivity. The results indicate that norethynodrel impaired oral glucose tolerance, and reduced the levels of radioactivity in adipose tissues at various time intervals after an oral glucose-U-^{14}C load. Mestranol slightly depressed gastric emptying and intestinal absorption of the radioactive glucose load. *In vitro* incubation of diaphragm muscle and adipose tissue revealed that the impairment of glucose tolerance may have resulted from reduced insulin sensitivity in these tissues.

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