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Effect of Ethynyl Estradiol on the Secretion of Hepatic Triglyceride (38768)

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(Introduced by T. H. Maren)

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Elevation of the concentration of serum triglyceride is one of the many biochemical changes induced by oral contraceptive therapy (1–5). The mechanisms by which such drugs elevate the concentration of serum triglyceride remain obscure. Very low density lipoprotein (VLDL), the triglyceride-rich lipoprotein of hepatic origin, is elevated during therapy but not chylomicron triglyceride (1). It has been suggested that the estrogenic component is responsible for the hypertriglyceridemia (5, 6) and that the level of the hypertriglyceridemia produced is proportional to the estrogen content of the estrogen–progestin mixture of the combined pill (7). Various studies have examined the separate influences of the estrogen and progestin components of the oral contraceptive drugs on triglyceride metabolism in humans (6) and rats (8). The elevation of serum triglyceride could be induced by the estrogenic component alone.

The influx from the liver and the efflux from the serum (by extrahepatic utilization) determine the level of serum triglyceride in the fasting state. Thus, it was important to establish the effect of ethynyl estradiol, an active estrogenic component, on rates of triglyceride secretion from the liver and to correlate this effect with the elevation of serum triglyceride in the donor animal.

Materials and Methods. Virgin female, Sprague–Dawley rats weighing 190–240 g served as liver donors. Blood for the perfusions was obtained from retired breeding stock of similar strain and sex. All rats were obtained from Holtzman and Co., Madison, Wisconsin. The liver donors were housed in separate metabolic cages and fed powdered Purina laboratory chow. The food was removed from the cages 12–16 hr prior to sacrifice. Ethynyl estradiol was dissolved in sesame oil. The animals were divided into three groups: the control group received sesame oil alone; two experimental groups received 5 or 15 μg ethynyl estradiol/kg body weight, respectively, daily for 14 days by sc injection. The treatment schedules were chosen to approximate the anovulatory (9, 10) and hypertriglyceridemic (8) doses for the rat.

The livers were obtained from each group of animals as described previously (11). Just prior to the cannulation of the portal vein, a blood sample was removed from the vena cava in order to determine the concentrations of serum triglyceride and cholesterol. The surgery was then completed. The adrenal glands were removed and freed of nonadrenal tissue, extracted, and the corticosterone content was determined (12).

The livers were placed in a recirculating liver perfusion apparatus (13) and perfused with a medium containing 40 ml defibrinated rat blood, 30 ml Krebs–Henseleit bicarbonate buffer, pH 7.4. Bovine serum albumin (BSA), 900 mg, was treated according to the method of Chen (14) and then complexed with 22.5 mg of palmitic acid (11). The BSA–palmitic acid complex was adjusted to 25 ml with 0.9% NaCl and infused into the reservoir at a rate of 7.38 ml/hr. After an equilibration period of 20 min, to establish normal perfusate flow rates through the liver, an infusion of the palmitic acid–BSA complex was begun. During the 3-hr experimental period 88 μmoles of palmitic acid were infused.

Aliquots of erythrocyte-free perfusate and liver samples were treated as described previously (15). Triglyceride (16) and cholesterol (17) were determined colorimetrically. An additional sample of perfusate was taken for the determination of glucose (15). The statistical significance of the differences between experimental groups was evaluated using a two-tailed table of Students distribution of t (18).

Results and Discussion. The effect of ethynyl estradiol on concentration of serum

triglyceride and cholesterol is shown in Table I. Both concentrations of ethynyl estradiol produced a hypertriglyceridemia and hypocholesterolemia. The 15 μg/kg dose of ethynyl estradiol increased the concentration of triglyceride in the serum whereas the concentration of serum cholesterol was not lowered further. At higher doses of ethynyl estradiol both serum triglyceride and cholesterol concentrations were depressed (8).

TABLE I. THE EFFECT OF ETHYNYL ESTRADIOL ON CONCENTRATIONS OF SERUM TRIGLYCERIDE AND AND CHOLESTEROL.[a]

	Triglyceride (μmoles/ml)	Cholesterol (μmoles/ml)
Treatment		
Control (6) (sesame oil)	0.59 ± 0.09	1.62 ± 0.18
Ethynyl estradiol		
1. 5 μg/kg (6) body weight	1.45 ± 0.22[b]	1.04 ± 0.18[c]
2. 15 μg/kg (6) body weight	2.30 ± 0.43[d]	1.02 ± 0.07[e]

[a] The number of observations is shown in parentheses; values are ± SE.

[b] Indicates significance of differences between groups to be $P < 0.01$.

[c] Indicates significance of differences between groups to be $P < 0.05$.

[d] Indicates significance of differences between groups to be $P < 0.005$.

[e] Indicates significance of differences between groups to be $P < 0.02$.

The elevation of serum triglyceride could result from increased secretion of hepatic triglyceride, decreased peripheral utilization, or both of these processes. The release of triglyceride was elevated in the perfusate of livers obtained from animals which were hypertriglyceridemic (Table II). The secretion of cholesterol was not affected; it appeared to increase but the increase was not significant because of the variability within the experimental groups. Schillinger and Gerhards observed liver glycogen to increase in female rats treated with ethynyl estradiol (8). Glycogen was not measured in our experiments but glucose release was impaired from livers of both groups of animals receiving the steroid. The level of serum cholesterol is determined by diet, endogenous synthesis, and peripheral utilization. In our experiments, no differences were observed in the rate of secretion of cholesterol and the animals were fasted prior to the experiment consequently, the concentration of cholesterol in the diet was not a factor. Therefore, the hypocholesterolemia produced in the intact animal with ethynyl estradiol is probably the result of increased peripheral utilization of this lipid by nonhepatic tissues. It has been proposed that the hypertriglyceridemia induced by oral contraceptive therapy may be mediated through hyperactivity of the adrenal glands (19). The corticosterone levels in the adrenal glands of the liver donor animals decreased at both concentrations of

TABLE II. GLUCOSE PRODUCTION, TRIGLYCERIDE AND CHOLESTEROL SECRETION FROM LIVERS OBTAINED FROM FEMALE RATS TREATED WITH ETHYNYL ESTRADIOL.[a]

	Liver (g wet weight)	Glucose[b] (mg/g liver wet weight)	Triglyceride[b] (μmoles/g liver wet weight)	Cholesterol[b] (μmoles/g liver wet weight)
Treatment				
Control (6) (sesame oil)	7.64 ± 0.38	7.02 ± 0.56	2.37 ± 0.44	0.17 ± 0.36
Ethynyl estradiol				
1. 5 μg/kg (6) body weight	7.39 ± 0.26	3.67 ± 0.92[c]	3.94 ± 0.25[d]	1.08 ± 0.38
2. 15 μg/kg (6) body weight	7.19 ± 0.57	4.08 ± 1.24[c]	4.10 ± 0.52[c]	1.34 ± 0.74

[a] The number of observations is shown in parentheses. All values ± SE.

[b] The values represent net production or release after 3 hr of perfusion.

[c] Indicates significance of differences between groups to be $P < 0.05$.

[d] Indicates significance of differences between groups to be $P < 0.02$.

TABLE III. WEIGHT AND CORTICOSTERONE CONTENT OF ADRENAL GLANDS OBTAINED FROM FEMALE RATS TREATED WITH ETHYNYL ESTRADIOL.[a]

	Adrenal weight (mg/2 adrenals)	Corticosterone (μg/100 mg adrenal)
Treatment		
Control (6) (sesame oil)	47.5 ± 4.9	12.5 ± 1.9
Ethynyl estradiol		
1. 5 μg/kg (6) body weight	44.5 ± 3.8	7.9 ± 1.2[b]
2. 15 μg/kg (6) body weight	62.8 ± 6.0	5.7 ± 1.4[c]

[a] The number of observations is shown in parentheses. Values are ± SE.

[b] Indicates significance of differences between groups to be $P < 0.05$.

[c] Indicates significance of differences between groups to be $P < 0.02$.

ethynyl estradiol without affecting the weight of the glands (Table III).

The increased level of free fatty acids in the serum as a consequence of oral contraceptive therapy (20) can be associated, in part, with an elevation of serum cortisol (21). The adrenal glands from rats treated with norethynodrel and mestranol exhibited an elevation of the rate of incorporation of acetate [1-^{14}C]acetate into adrenal cholesterol, indicating perhaps, an increased requirement of this lipid for steroid biosynthesis (22). The decreased concentration of corticosterone observed in these experiments could be taken as an indication of adrenal hyperfunction. However, adrenal hypofunction has been reported in women (23) and rats (24) ingesting estrogenic agents. Furthermore, hypertriglyceridemia was observed in women on oral contraceptive drugs in which free fatty acid concentrations in the serum were not elevated (6). Other hormones (e.g., growth hormone and insulin (25, 26) are increased in the serum as a consequence of oral contraceptive therapy and could contribute to the hypertriglyceridemia by increasing substrate availability for the synthesis and secretion of VLDL. The effects on these hormone levels were obtained with the use of the combined pills and perhaps should be reexamined by studying the action of the individual components.

It is clear from our studies that the effect on secretion of triglyceride form the liver is an important and perhaps major component producing the hypertriglyceridemia observed in therapy with oral contraceptives. This increased secretion of hepatic triglyceride and the increased turnover of triglyceride, attributable to the estrogenic component of oral contraceptives (6), would contribute to the elevation of serum triglyceride. Peripheral utilization was not studied; therefore, we cannot conclude from our observations that triglyceride uptake by tissues other than liver was not affected. Ethynyl estradiol acts either directly on the liver to increase triglyceride secretion or by interfering with the normal hormonal milieu regulating triglyceride metabolism which results in turn in an increase in secretion of hepatic triglyceride.

Summary. The concentrations of triglyceride in the blood of female rats increased 2- and 4-fold during treatment with 5 and 15 μg/kg of ethynyl estradiol, respectively. The rate of secretion of triglyceride increased 66% over controls with livers obtained from the rats administered ethynyl estradiol. Ethynyl estradiol induced a hypocholesterolemia in the donor animals but the secretion of cholesterol into the perfusate from livers obtained from these animals was not affected. Adrenal corticosterone levels were depressed 48% in animals receiving ethynyl estradiol. The hypertriglyceridemia is produced either through a direct effect of ethynyl estradiol on the liver or secondary to other hormonal changes.

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1. Wynn, V., Doar, J. W. H., and Mills, G. L., Lancet, **1**, 720 (1966).
2. Aurell, M., Cramer, K., Rybo, G., Lancet **1**, 291 (1966).
3. Gershberg, H., Hulse, M., and Javier, Z., Obstet. Gynecol. **31**, 186 (1968).
4. Hazzard, W. R., Spiger, M. J., Bagdade, J. D.,

and Bierman, E. L., N. Engl. J. Med. **280,** 471 (1969).
5. Bierman, E. L., *in* "Metabolic Effects of Gonadal Hormones and Contraceptive Steroids" (H. A. Salhanick, D. M. Kipnis, and R. L. Vande Wiele, eds.), pp. 207–218. Plenum Press, New York (1969)
6. Kissbah, A. H., Harrigan, P., and Wynn, V., Horm. Metab. Res. **5,** 184 (1973).
7. Stokes, T., and Wynn, V., Lancet **2,** 677 (1971).
8. Schillinger, R. E., and Gerhards, E., Biochem. Pharmacol. **22,** 1835 (1973).
9. Yang, M. G., Sanger, V. L., and Michelson, O., Proc. Soc. Exp. Biol. Med. **130,** 1146 (1969).
10. Banik, U. K., Revesz, C. and Herr, F. J., Reprod. Fert. **18,** 509 (1969).
11. Heimberg, M., Weinstein, I., Klausner, H., and Watkins, M. L., Amer. J. Physiol. **202,** 353 (1962).
12. Thatcher, W. W. and Gwazdauskas, F. C., J. Dairy Sci. **56,** 873 (1973).
13. Heimberg, M., Fizette, N., and Klausner, H., J. Amer. Oil Chem. Soc. **41,** 774 (1964).
14. Chen, R. F., J. Biol. Chem. **242,** 173 (1967).
15. Weinstein, I., Seltzer, M., and Belitsky, R. Biochem. Biophys. Acta **374,** 14 (1974).
16. Van Handel, E., and Zilversmit, D. B., J. Lab. Clin. Med. **50,** 152 (1957).
17. Leffler, H. H. Amer. J. Clin. Pathol. **31,** 310 (1959).
18. Diem, K., ed., Documenta Geigy Scientific Tables, 6th ed., pp. 32–33. Geigy Pharmaceutics, Ardsley, New York, (1962).
19. Alfin-Slater, R. B., and Aftergood, L., Lipids **6,** 693 (1971).
20. Wynn, V., and Doar, J. W. H., Lancet **2,** 715 (1966).
21. Haller, J., Contraception **1,** 233 (1970).
22. Aftergood, L., and Alfin-Slater, R. B., *in* "Metabolic Effects of Gonadal Hormones and Contraceptive Steroids" (H. A. Salhanick, D. M. Kipnis, and R. L. Vande Wiele, eds.), pp. 265–274. Plenum Press, New York (1969).
23. Peterson, R. E., Nokes, G., Chen, P. S., Jr., and Black, R. L., J. Clin. Endocrinol. Metab. **20,** 495 (1960).
24. Vogt, M., J. Physiol. **130,** 601 (1955).
25. Spellacy, W. N., and Carlson, K. L., Amer. J. Obstet. Gynecol. **95,** 474 (1970).
26. Spellacy, W. N., and Carlson, K. L., Kirk, S. A., and Schode, S. L., Metabolism **17,** 496 (1968).

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