Effect of Mestranol upon Hepatic Lipid Metabolism in the Isolated Perfused Rat Liver (36449)

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The precise mechanisms whereby gonadal hormones regulate and influence lipid metabolism in the animal body are obscure. Conditions which produce changes in lipid metabolism coincident with the alteration of estrogen levels in the female species, are well established. For example, in pregnancy, levels of total lipid increase in the serum of animals (1) and human beings (2). During menopause, serum cholesterol:serum phospholipid ratio increases along with a relative rise in low density lipoproteins (LDL) and the cholesterol associated with it (3). The recent increased use of oral contraceptives and in particular, the estrogenic component mestranol, which many of them contain, have been implicated in the hypertriglyceridemia observed in the sera of many patients on these drugs (4). The triglyceride of the serum is associated with the very low density lipoproteins (VLDL) fraction (5, 6). The VLDL, which is supplied to the blood by the liver, may be the major vehicle for transport of plasma triglyceride (6). Mestranol might act to increase serum triglyceride by acting directly upon the liver to stimulate its release. Therefore, experiments were initiated utilizing the isolated perfused rat liver system, which would determine if mestranol produces its effect by acting directly upon the liver, or indirectly, by affecting other hormonal mechanisms operative in the intact animal.

Methods. Female rats obtained from Holtzman & Co., Madison, WI, maintained on water and food *ad libitum* served as liver, blood and serum donors. One group of animals were ovariectomized 28 days prior to sacrifice. Details of the surgical technique and perfusion apparatus have appeared elsewhere (7, 8). The perfusion medium consisted of 40 ml defibrinated rat blood, 15 ml of rat serum and 15 ml of Krebs–Henseleit bicarbonate buffer (pH 7.4) (9). Eighty micromoles of palmitic acid, as a complex with rat serum (7), were infused at a rate of 26.7 μmoles/hr in a volume of 7.38 ml/hr. The mestranol was solubilized in 0.2 ml of acetone (sp gr 0.792). In one series of experiments, mestranol was solubilized in 0.1 ml of propylene glycol (sp gr 1.036) in order to compare the effects of different solubilization agents. In the experiments in which acetone was used 0, 5, 50, 100, and 200 μg of mestranol were dissolved in acetone and added directly to the palmitic acid–serum complex. Assuming the steroid was not metabolized during the 3 hr experimental period, the final concentrations of mestranol in the perfusate would be 2×10^{-7}, 2×10^{-6}, 4×10^{-6}, and 8×10^{-6} *M*. In those experiments in which propylene glycol was used only 100 μg of mestranol was employed, which gave a final concentration of 4×10^{-6} *M*. Estrogens are rapidly taken up and converted to their metabolites by the liver (10). Although the rate of the metabolism of mestranol by the isolated perfused rat liver system to its metabolites is unknown, it was assumed that in the following discussion that mestranol would be metabolized at rates comparable to studies reported with estrogens. Therefore, the concentrations in the experiments were chosen to hasten and emphasize the appearance of any effects. The lipids were isolated from aliquots of perfusate by procedures described previously (11). At the end of the experiment the livers were flushed with ice-cold 0.9% NaCl; nonhepatic tissues were removed; the livers were blotted and weighed. Cholesterol,

TABLE I. The Effect of Mestranol upon Bile Flow and Perfusate Flow Rate.[a]

	Conc of mestranol (M)	Bile flow (ml/g of liver)	Flow rate (ml/g/min of liver)
A. Normal (acetone)	0 (5)	0.129 ± 0.028	2.4 ± 0.1
	2×10^{-7} (4)	0.125 ± 0.015	2.3 ± 0.4
	2×10^{-6} (4)	0.125 ± 0.023	2.4 ± 0.3
	4×10^{-6} (6)	0.147 ± 0.017	2.5 ± 0.2
	8×10^{-6} (5)	0.152 ± 0.016	1.5 ± 0.2[b]
B. Control	0 (6)	0.141 ± 0.025	2.3 ± 0.2

[a] All values $\pm$ SEM, after 3 hr of perfusion. All perfusions in part A contain 0.2 ml acetone including the group in which no mestranol was infused. In part B these perfusions did not contain acetone. The number of experiments is shown in parentheses.

[b] $p < .05$ when compared to its appropriate control.

triglyceride and fatty acids were determined by the colorimetric methods of Zak *et al.* (12), Van Handel and Zilversmit (13) and Duncombe (14), respectively. Urea and glucose were determined on separate aliquots of perfusate by the method of Friedman (15) and Schmidt (16), respectively. The statistical significance of the differences between experimental groups was evaluated using a two-tailed table of Student's distribution of t (17).

Results and Discussion. Indices used as evidence of liver function are appearance, perfusate flow through the liver, and bile production. Bile production was unaffected by any of the concentrations of mestranol infused (Table I). Flow rate, however, was reduced at the highest concentration of steroid. These livers also appeared mottled and hypoxic probably from decreased O_2 being presented to the liver tissue.

The results of the effects of mestranol upon the release of glucose, urea, cholesterol and triglyceride from livers of normal and ovariectomized rats are shown in Table II. The release of glucose was inhibited by mestranol, only when the steroid was solubilized in acetone. In the series of perfusions in which mestranol was solubilized in propylene glycol, no inhibition of glucose release was observed. This effect of acetone is unexplained, but appears to be an effect upon the metabolism of glucose by the liver rather than upon glycolysis by red blood cells (RBC). In one series of experiments, in which the liver was omitted from the perfusion system, glucose disappeared from the perfusate as might be predicted, as a result of glycolysis by RBC. The disappearance of glucose was unaffected by the presence or absence of steroid. Mestranol is believed to be responsible for the impaired tolerance to glucose observed in some women on oral contraceptives. Perhaps, its effect on glucose might be exerted by potentiating some agent which produces an elevation in blood glucose (18), *e.g.*, glucagon. Mestranol, 4×10^{-6} M (solubilized in acetone) infused simultaneously with glucagon (at submaximal concentrations, 1×10^{-9} M), neither potentiated nor inhibited glucagon-induced glucose release (19). In the present study, no effect upon urea production could be detected even with the highest concentration of mestranol.

The release of cholesterol is stimulated by both acetone and propylene glycol. At concentrations of mestranol, 4×10^{-6} M and 8×10^{-6} M, the release of cholesterol returned to values approaching those obtained when no steroid was perfused. Recently, female rats receiving high doses of Enovid E (norethynodrel, 1.0 mg; and mestranol, 0.04 mg) by gastric intubation exhibited inhibition of hepatic cholesterol synthesis and decreased levels of cholesterol in the plasma (20). Our data confirm this observation and indicate that the effect is probably a direct one. In contrast, the release of triglyceride was unaffected by any of the concentrations of mestranol. In the absence of mestranol, an

TABLE II. The Effect of Mestranol upon the Release of Glucose Urea, Cholesterol and Triglyceride Into the Perfusate of Livers from Normal and Ovariectomized Rats.[a,b]

	Conc of mestranol (M)	(mg/g of liver)		(μmoles/g of liver)	
		Glucose	Urea	Cholesterol	Triglyceride
A. Normal (acetone)	0 (5)	6.85 ± 1.30	1.93 ± 0.39	5.99 ± 0.42[c]	4.37 ± 0.29[c]
	2×10^{-7} (4)	1.63 ± 1.14[c]	1.95 ± 0.14	5.53 ± 0.44	3.59 ± 0.44
	2×10^{-6} (4)	0.96 ± 0.35[c]	2.22 ± 0.25	4.21 ± 0.60	3.41 ± 0.32
	4×10^{-6} (6)	−1.26 ± 0.61[c]	1.73 ± 0.16	4.07 ± 0.40[c]	4.09 ± 0.51
	8×10^{-6} (5)	0.32 ± 0.51[c]	1.98 ± 0.20	3.61 ± 0.64[c]	3.70 ± 0.56
B. Ovariectomized (acetone)	0 (5)	2.94 ± 1.83	1.75 ± 0.16	1.71 ± 0.45	1.99 ± 0.22[c]
	4×10^{-6} (6)	−0.12 ± 1.03	1.93 ± 0.06	1.84 ± 0.65	2.30 ± 0.22
C. Normal (propylene glycol)	0 (4)	5.14 ± 1.83	1.32 ± 0.19	5.71 ± 0.99[c]	2.27 ± 1.10
	4×10^{-6} (4)	4.46 ± 1.25	1.66 ± 0.33	2.01 ± 0.51[c]	2.77 ± 0.86
D. Control	0 (6)	4.09 ± 1.02	2.08 ± 0.19	3.33 ± 0.92	3.05 ± 0.47

[a] Number of perfusions shown in parentheses ± SEM, length of perfusion 3 hr. Negative values indicate net disappearance of glucose from the perfusate.

[b] Liver weight, wet normal 7.44 ± 0.13 g, ovariectomized 9.12[c] ± 0.29 g.

[c] These values were significant ($p < .05$) when compared to its appropriate control.

as yet unexplained finding occurred: in the acetone control, at the end of the third hour, more triglyceride was released from these livers than livers perfused with a medium devoid of acetone.

It is known that triglyceride release from the liver is reduced significantly by ovariectomy (M. Heimberg, personal communication). Livers from ovariectomized animals were perfused with a medium containing mestranol (4×10^{-6} M, solubilized in acetone), in an effort to demonstrate if the steroid could restore triglyceride release to normal. We have confirmed that release of triglyceride, as well as glucose and cholesterol release, are markedly depressed by oophorectomy. Triglyceride release was not stimulated by the concentration of mestranol employed. In addition, oophorectomy abolished the effect of acetone to increase perfusate cholesterol. Even though mestranol appears to inhibit the release of glucose, this inhibition is not significant. It would be extremely important to assess the relationship which exists between acetone and other ketone bodies upon hepatic lipid and carbohydrate metabolism, especially in the diabetic animal.

Under the experimental conditions employed, these observations would indicate that the effect of mestranol in producing an elevation of plasma triglyceride in the intact animal probably does not result from a direct effect upon the liver. This observation might be explained by considering the changes imposed in other endocrine glands. For example, it is known that elevation in cortisol (21) and thyroxine (22) do occur and perhaps, these lipolytic hormones (23) may increase the flow of free fatty acids to the liver where increased synthesis of triglyceride and release into the VLDL can occur. Mestranol, like estradiol, does depress cholesterol release and this was evident in perfusions containing acetone and propylene glycol. The influence of mestranol on cholesterol metabolism in the liver is currently under investigation.

Summary. Mestranol in a wide range of concentrations has no direct effect upon triglyceride release from livers obtained from normal rats. The hypertriglyceridemia seen with mestranol, probably results from a stimulation by the steroid of the release of various endocrine factors which in turn may stimulate the liver to release more trigly-

ceride.

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