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Effects of Ethynyloestradiol on the Metabolism of [1-^{14}C]Oleate by Perfused Livers and Hepatocytes from Female Rats

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Normal female rats were given 15μg of ethynyloestradiol/kg body wt. for 14 days and were killed on day 15 after starvation for 12–14h. The livers were isolated and were perfused with a medium containing washed bovine erythrocytes, bovine serum albumin, glucose and [1-^{14}C]oleic acid; 414μmol of oleate were infused/h during a 3h experimental period. The output of bile and the flow of perfusate/g of liver were decreased in livers from animals pretreated with ethynyloestradiol, whereas the liver weight was increased slightly. The rates of uptake and of utilization of [1-^{14}C]oleate were measured when the concentration of unesterified fatty acid in the perfusate plasma was constant. The uptake of unesterified fatty acid was unaffected by pretreatment of the animal with oestrogen; however, the rate of incorporation of [1-^{14}C]oleate into hepatic and perfusate triacylglycerol was stimulated, whereas the rate of conversion into ketone bodies was impaired by treatment of the rat with ethynyloestradiol. Pretreatment of the rat with ethynyloestradiol increased the output of very-low-density lipoprotein triacylglycerol, cholesterol, phospholipid and protein. The production of $^{14}CO_2$ and the incorporation of radioactivity into phospholipid, cholesteryl ester and diacylglycerol was unaffected by treatment with the steroid. The net output of glucose by livers from oestrogen-treated rats was impaired despite the apparent increased quantities of glycogen in the liver. The overall effect of pretreatment with oestrogen on hepatic metabolism of fatty acids is the channeling of [1-^{14}C]oleate into synthesis and increased output of triacylglycerol as a moiety of the very-low-density lipoprotein, whereas ketogenesis is decreased. The effect of ethynyloestradiol on the liver is apparently independent of the nutritional state of the animal from which the liver was obtained. It is pertinent that hepatocytes prepared from livers of fed rats that had been treated with ethynyloestradiol produced fewer ketone bodies and secreted more triacylglycerol than did hepatocytes prepared from control animals. In these respects, the effects of the steroid were similar in livers from fed or starved (12–14h) rats. Oestrogens may possibly inhibit hepatic oxidation of fatty acid, making more fatty acid available for the synthesis of triacylglycerol, or may stimulate the biosynthesis of triacylglycerol, or may be active on both metabolic pathways.

Hypertriglyceridaemia is often an undesirable side effect of therapy with oral contraceptives containing oestrogens (Aurell *et al.*, 1966; Wynn *et al.*, 1966; Gershberg *et al.*, 1968; Hazzard *et al.*, 1969). Indeed, the increase in serum triacylglycerol was observed to be proportional to the oestrogen content of the oral contraceptive drug (Stokes & Wynn, 1971). Subsequently, several laboratories reported that the administration of oestrogen component alone to humans (Stokes & Wynn, 1971; Kissbah *et al.*, 1973), rats (Schillinger & Gerhards, 1973; Weinstein *et al.*, 1975) and birds (Kudzma *et al.*, 1975) resulted in hypertriglyceridaemia. It was concluded that livers of oestrogen-treated chickens converted a greater proportion of fatty acid into triacylglycerol than did controls (Kudzma *et al.*, 1975). Furthermore, perfused livers from rats treated with oestrogen secreted more triacylglycerol than did livers from control animals (Weinstein *et al.*, 1975). In that study, however, non-radioactive palmitic acid was used and the effects of ethynyloestradiol on the disposition of fatty acid could, therefore, not be completely evaluated. Previous reports from our laboratory (Watkins *et al.*, 1972; Weinstein *et al.*, 1974; Wilcox *et al.*, 1974; Soler-Argilaga *et al.*, 1975; Soler-Argilaga & Heimberg, 1976) and by Otway & Robinson (1967) have demonstrated that the role of oestrogens may show important sex differences and suggested to us a

Abbreviation used: VLD lipoprotein, very-low-density lipoprotein.

role in the regulation of hepatic secretion of triacylglycerol. To obtain further insight into the mechanism of action of oestrogen on the hepatic metabolism of fatty acids, we investigated the incorporation of [1-^{14}C]oleate ($C_{18:1}$) into products of esterification (triacylglycerol and other lipids) and into products of oxidation (CO_2 and ketone bodies) by perfused livers isolated from female rats treated with ethynyloestradiol. It was observed that, although the uptake of unesterified fatty acid by the perfused liver was not affected by treatment with ethynyloestradiol, more fatty acid was esterified into triacylglycerol and less was oxidized to ketone bodies than by livers from control rats. The administration of the oestrogen to female animals profoundly stimulated the synthesis and the release of triacylglycerol by the liver and may explain in part the hypertriglyceridaemia that often accompanies therapy with oestrogens. Preliminary reports of this work have been published (Weinstein *et al.*, 1977*a*,*b*).

Materials and Methods

Animals

Female rats of the Sprague–Dawley strain were obtained from the Charles River Breeding Laboratories, Wilmington, MA, U.S.A. Between 1 and 2 weeks after arrival (which allowed adjustment of the rats to the animal quarters) the rats were separated and housed in metabolic cages and were provided with water and 15g of ground Purina laboratory chow/day. This amount of food maintained the body weights of the control rats in the same range as those of the treated rats (Table 1). The animals were kept in rooms illuminated from 08:00 to 18:00h. Food was removed 12–14h before the animals were used in an experiment. In experiments with isolated hepatocytes, the animals were allowed to feed *ad libitum* for 72h before the preparation of the cells. The body weights of control and of ethynyloestradiol-treated rats, from which hepatocytes were prepared, were similar after feeding.

The animals received sesame oil alone or 15μg of ethynyloestradiol/kg body wt. dissolved in sesame oil. The animals were injected daily for 14 days at alternate sites by the subcutaneous route. This dose was chosen because it is anovulatory for the rat (Yang *et al.*, 1969; Banik *et al.*, 1969).

Chemicals

The chemicals and solvents used were obtained from Fisher Scientific Co., St. Louis, MO, U.S.A., and were of reagent grade. All organic solvents were redistilled from glass before use. Oleic acid (99% purity) was obtained from Schwarz–Mann, Orangeburg, NJ, U.S.A., and Nu-Chek Preparations, Elysian, MN, U.S.A. Bovine serum albumin (fraction V, powder) obtained from Pentex Inc., Kankakee, IL, U.S.A., was purified by a modification (Wilcox *et al.*, 1975) of the method of Goodman (1957). [1-^{14}C]Oleic acid (sp. radioactivity 55mCi/mmol), [3-^{14}C]ethyl acetoacetate (sp. radioactivity 11.3mCi/mmol) and sodium DL-β-hydroxy[3-^{14}C]butyrate (sp. radioactivity 11.8mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A., or Amersham/Searle, Arlington Heights, IL, U.S.A. Ethynyloestradiol was generously provided by Dr. E. Forchielli, Syntex Corp., Palo Alto, CA, U.S.A.

Perfusion and hepatocyte studies

The animals were anaesthetized lightly with diethyl ether, the livers were removed surgically (Kohout *et al.*, 1971) and perfused in a recycling system described by Heimberg *et al.* (1964). The initial perfusion medium consisted of washed bovine erythrocytes (Exton *et al.*, 1969) suspended in Krebs–Henseleit bicarbonate buffer, pH7.4 (Krebs & Henseleit, 1932), containing 3g of purified bovine serum albumin and 100mg of glucose/100ml of buffer. The bovine erythrocytes were collected from living animals maintained for this purpose. The initial perfusion volume was 90ml and the packed-cell volume was 30%. A fatty acid complex containing 3.0g of purified bovine serum albumin and 150mg of oleic acid (532μmol, containing 10μCi of [1-^{14}C]-oleic acid) was prepared as described by Heimberg *et al.* (1962), and the solution adjusted to 45ml with 0.9% NaCl. After a 20min period of equilibration, 5ml (containing 60μmol of oleic acid) was added and 11.5ml was infused/h at a constant rate during the 3h experimental period (138μmol/h). The concentration of unesterified fatty acid in the perfusate plasma after 1h was 0.60 ± 0.08 (mean $\pm$ S.E.M.) mM; this concentration was similar after the additional 2h of perfusion. Portions of perfusate were obtained for analysis after 1 and 3h of perfusion. The collection of $^{14}CO_2$ was begun when the infusion of the fatty acid complex was started. Radioactive CO_2 generated during the experiment was trapped in 10% (w/v) KOH as described by Soler-Argilaga & Heimberg (1976). At the end of the experiment, livers were washed with 20ml of ice-cold 0.9% NaCl to remove residual perfusate. The livers were then freed of non-hepatic tissue, blotted, weighed and homogenized in 0.9% NaCl (6.0ml/g of liver wet wt.) and the lipids were then extracted.

Hepatocytes were prepared by using collagenase by the method of Berry & Friend (1969) as modified by Berry & Werner (1973) from livers of animals treated with either sesame oil or ethynyloestradiol. Approx. 5×10^6 cells were added to each flask. Cells were incubated for 1 and 2h in a medium containing Krebs–Henseleit bicarbonate buffer (pH7.4), 2g of

bovine serum albumin/100ml, and 100mg of glucose/100ml. Oleic acid was added as the complex with bovine serum albumin to give an initial concentration of 1.2μmol/ml. At the end of the incubation the contents of the flasks were centrifuged and a portion of the medium was taken for the determination of triacylglycerol, ketone bodies and unesterified fatty acid. The cell suspension was washed 3 times with cold (4°C) 0.9% NaCl and the lipids were extracted.

Analytical methods

Lipids were extracted from the cell-free perfusate and from the VLD lipoprotein and from the incubation medium and hepatocytes with chloroform/methanol (2:1, v/v; Folch *et al.*, 1957). Lipids were obtained from a portion of the liver homogenate as described by Weinstein *et al.* (1974). The extracts were washed, fractionated and analysed as described by Soler-Argilaga *et al.* (1976). VLD lipoprotein was obtained from the cell-free perfusate at 1 and 3h of perfusion by the method of Havel *et al.* (1955). The VLD lipoprotein was mixed with 0.9% NaCl and recentrifuged. Total protein was determined by the method of Lowry *et al.* (1951) on the washed VLD lipoprotein. Triacylglycerol was analysed by the modification (Newman *et al.*, 1961) of the Van Handel & Zilversmit (1957) procedure. Phospholipid (Bartlett, 1959), cholesterol (Leffler, 1959) and fatty acid (Duncombe, 1963) were determined by colorimetric methods.

The radioactivity incorporated into the various lipid classes was estimated directly in the bands scraped from thin-layer plates (Spector & Steinberg, 1965; Soler-Argilaga & Heimberg, 1976). The total recovery of ^{14}C from ketone bodies (Bieberdorf *et al.*, 1970), CO_2 (Soler-Argilaga & Heimberg, 1976), perfusate lipids and hepatic lipids was calculated. The uptake of unesterified fatty acid and the output of metabolites was calculated as described by Soler-Argilaga & Heimberg (1976), except that calculations were based on a 2h experimental period.

Samples of the perfusate were haemolysed and deproteinized with $Ba(OH)_2/ZnSO_4$ (Van Harken *et al.*, 1969) and portions of the protein-free supernatant were analysed for ketone bodies (Soler-Argilaga & Heimberg, 1976) and for glucose (Weinstein *et al.*, 1974).

The statistical significance of the differences between control and experimental groups was evaluated by using a two-tailed table of Student's distribution of *t* (Diem, 1962).

Results

The effect of treatment with ethynyloestradiol on various characteristics of the isolated perfused liver is shown in Table 1. Treatment with oestrogen increased the wet weight of the liver slightly and the ratio of liver weight to body weight. The flow of perfusate through the liver and the output of bile were decreased by pretreatment of the animal with ethynyloestradiol.

Table 1. *The effect of treatment with ethynyloestradiol on characteristics of the perfused liver*

Results are expressed as means ± S.E.M. The numbers of observations are shown in parentheses.

	Control (4)	Ethynyloestradiol treatment (5)
Animal weight (g)	229±7	212±5
Liver weight (g)	7.41±0.21	8.20±0.21*
$\frac{\text{Liver weight}}{\text{Body weight}} \times 100$	3.23±0.05	3.88±0.15*
Perfusate flow rate (ml/g of liver per min)	3.78±0.36	2.51±0.39*
Bile output (μl/g of liver per min)	52±9	37±3*

* $P<0.05$, for control against ethynyloestradiol treatment.

Treatment with ethynyloestradiol did not affect the uptake of unesterified fatty acid by the perfused liver. The uptake and specific radioactivity of the unesterified fatty acids were calculated as reported by Soler-Argilaga & Heimberg (1976). Uptake of unesterified fatty acid was 19.7±1.9 and 18.8±2.0 (mean±S.E.M.) μmol/g of liver per h for control and ethynyloestradiol-treatment groups respectively. Specific radioactivity of unesterified fatty acid taken up by the liver was approx. 3.1×10^4 d.p.m./μmol in both groups. The total disposition of [1-^{14}C]oleate by perfused livers from control female rats and from animals treated with ethynyloestradiol, however, was affected (Table 2). The administration of oestrogen to the rat increased the incorporation of [1-^{14}C]oleate into hepatic and perfusate triacylglycerol, but did not affect incorporation into diacylglycerol, phospholipid and cholesteryl esters. By contrast, the incorporation of [1-^{14}C]oleate into ketone bodies was inhibited, whereas the incorporation into CO_2 was unaffected.

In the control, the fraction derived from infused (exogenous) fatty acid was about 60% of the mass of triacylglycerol fatty acids secreted, indicating dilution by fatty acids from the pool of esterified endogenous fatty acids (Table 3). In agreement with data reported by Weinstein *et al.* (1975), the secretion of triacylglycerol was stimulated by treatment of the rat with ethynyloestradiol. In experiments with ethynyloestradiol, the output of triacylglycerol fatty acid calculated from chemical data without regard to amount of labelled oleate in the triacylglycerol molecule or from the incorporation of oleic acid was

Table 2. *The effect of ethynyloestradiol on the disposition of [1-^{14}C]oleate by the perfused liver*

Results are expressed as means ± S.E.M. for the percentage of oleic acid taken up/g of liver per h and incorporated into the various metabolites. The calculations were as reported by Soler-Argilaga & Heimberg (1976). The numbers of observations are shown in parentheses.

	Oleic acid taken up (%)	
	Control (4)	Ethynyloestradiol treatment (5)
Products of esterification		
In perfusate		
Triacylglycerol	13.8 ± 1.8	36.1 ± 1.4*
Diacylglycerol	0.5 ± 0.2	0.9 ± 0.2
Phospholipid	0.5 ± 0.1	0.4 ± 0.1
Cholesteryl ester	0.1 ± 0.1	0.2 ± 0.05
In liver		
Triacylglycerol	14.9 ± 0.4	32.9 ± 6.0*
Diacylglycerol	2.9 ± 0.3	3.0 ± 0.3
Phospholipid	6.5 ± 0.6	7.7 ± 0.9
Cholesteryl ester	0.3 ± 0.03	0.4 ± 0.1
Products of oxidation		
CO_2	28.9 ± 2.7	25.4 ± 3.8
Ketone bodies	25.7 ± 2.6	4.0 ± 1.0*
Total recovery (%)	93.8 ± 2.0	111 ± 3.3

* $P < 0.001$, for control against ethynyloestradiol treatment.

identical. This indicated that the primary source of fatty acid in the secreted triacylglycerol fatty acids was infused fatty acid.

Even though ketogenesis was decreased by treatment with ethynyloestradiol, the fraction of the total output of ketone bodies derived from exogenous fatty acid was similar to that of the control (Table 4).

It was known from previous experiments that starvation decreased the output of triacylglycerol and increased ketogenesis by perfused rat livers (Heimberg *et al.*, 1962). The overnight period of food deprivation in the present study (12–14h) may have exaggerated the differences between the control and the oestrogen-treated groups. It was therefore of interest to evaluate the effects of oestrogen pretreatment in livers from animals fed *ad libitum*. For these experiments, isolated hepatocytes were used instead of the perfused liver. The data obtained with the hepatocytes were in essential agreement with those observed with the perfused liver. The output of triacylglycerol was stimulated and ketogenesis diminished in hepatocytes from ethynyloestradiol-treated rats, in comparison with cells prepared from the livers of control animals (Table 5).

Table 3. *Source of fatty acid in triacylglycerol secreted by the perfused liver*

Results are expressed as means ± S.E.M. The numbers of observations are shown in parentheses.

	Control (4)	Ethynyloestradiol treatment (5)
(A) Net output of triacylglycerol fatty acid (μmol/g of liver per h)*	2.52 ± 0.61	4.24 ± 0.25‡
(B) Secretion of triacylglycerol fatty acid derived from [1-^{14}C]oleate (μmol/g of liver per h)†	1.54 ± 0.43	4.72 ± 0.45‡
(B/A) × 100	59 ± 8	111 ± 7‡

* This includes unesterified fatty acids of endogenous and exogenous sources.

† Calculated from the amount of [1-^{14}C]oleate taken up and incorporated into the triacylglycerol released into the perfusate; unesterified fatty acid of exogenous source only.

‡ $P < 0.001$, for control against ethynyloestradiol.

Table 4. *Effect of ethynyloestradiol on ketogenesis by the perfused liver*

Results are expressed as means ± S.E.M. The numbers of observations are shown in parentheses.

	Control (4)	Ethynyloestradiol treatment (5)
(A) Total ketone bodies produced*	42.6 ± 4.8	6.9 ± 1.2‡
(B) Ketone bodies† derived from [1-^{14}C]oleate	22.3 ± 1.2	3.4 ± 0.8‡
(B/A) × 100	52.9 ± 4.0	47.1 ± 8.2

* μmol of ketone bodies, expressed as μmol of acetone (4.5 μmol of acetone/μmol of oleic acid) produced from both exogenous and endogenous fatty acid/g of liver per h.

† μmol of ketone bodies/g of liver per h expressed as in *, but derived from unesterified fatty acids taken up from the perfusate.

‡ $P < 0.001$, control against ethynyloestradiol.

Since triacylglycerol is secreted as a moiety of the VLD lipoprotein, conditions that increase the secretion of VLD lipoprotein also concomitantly increase the secretion of phospholipid and of cholesterol (Heimberg *et al.*, 1965; Soler-Argilaga *et al.*, 1975, 1976). Ethynyloestradiol clearly increased the secretion of VLD lipoprotein triacylglycerol, phospholipid, and cholesterol (Table 6). In addition, the output of VLD lipoprotein protein was also increased by prior treatment of the animal with the oestrogen.

Table 5. *Effects of ethynyloestradiol on formation and output of triacylglycerol and on ketogenesis by isolated hepatocytes*
Values are averages of two preparations in duplicate and represent net production of triacylglycerol or ketone bodies after 60 and 120min of incubation. Animals were fed as described in the Materials and Methods section.

	Triacylglycerol (nmol/mg of DNA)				Ketone bodies in the medium (μmol/mg of DNA)	
	Cells		Medium			
Incubation period (min) ...	60	120	60	120	60	120
Control	908	750	145	486	15.9	26.0
Ethynyloestradiol treatment	2015	2036	346	938	12.4	11.1

Table 6. *Effect of ethynyloestradiol on the output of lipids and protein in the VLD lipoprotein*
Results are expressed as means ± S.E.M. The numbers of observations are shown in parentheses.

Group	Triacylglycerol (μmol/g of liver per h)	Phospholipid (μmol/g of liver per h)	Cholesterol (μmol/g of liver per h)	Protein (μg/g of liver per 3 h)
Control (4)	0.32 ± 0.09	0.32 ± 0.05	0.13 ± 0.05	7.4 ± 0.7
Ethynyloestradiol treatment (5)*	0.79 ± 0.07	0.51 ± 0.05	0.29 ± 0.04	12.9 ± 0.9

* $P < 0.05$, for control against ethynyloestradiol treatment.

Discussion

Treatment of female rats with ethynyloestradiol altered the metabolism of [1-^{14}C]oleate by the isolated perfused liver. Treatment with the oestrogen channeled the oleate preferentially into the synthesis and secretion of triacylglycerol, and ketogenesis was decreased. This was not the result of the differences in perfusate flow rate (ml/g of liver per min) due to the oestrogen treatment, since uptake of unesterified fatty acid was similar in controls even though the livers of oestrogen-treated rats were larger. Furthermore, the values for μmol of triacylglycerol secreted/μmol of unesterified fatty acid taken up by the liver were 0.13 ± 0.03 (mean ± S.E.M.) for the control against 0.23 ± 0.02 (mean ± S.E.M.; $P < 0.02$) for livers from ethynyloestradiol-treated rats. These data lead us to conclude that the effects of ethynyloestradiol on the output of triacylglycerol by the liver (Table 3) are independent of any differences in the perfusate flow rate or of liver weight. The metabolic changes probably reflect the altered intrahepatic compartmentalization of fatty acid by treatment with oestrogen. The oestrogen may act directly on the liver, or indirectly through modulation of secretion of other hormones to decrease ketogenesis and to stimulate the biosynthesis of triacylglycerol. Ethynyloestradiol may stimulate the synthesis of triacylglycerol, or of formation and secretion of the VLD lipoprotein or all of these. The incorporation of [1-^{14}C]oleate into perfusate and into hepatic triacylglycerol was clearly stimulated, as was the secretion of the VLD lipoprotein. This could result from increased availability of long-chain fatty acyl-CoA to the microsomal fraction, since mitochondrial ketogenesis is decreased, but might also result from direct effects of oestrogen on the synthesis of triacylglycerol and protein; and the fatty acid or fatty acyl-CoA derivative might signal increased VLD lipoprotein apoprotein synthesis. Chan *et al.* (1976) reported that in the cockerel diethylstilboestrol induced an increase in VLD lipoprotein apoprotein synthesis by the liver, which was mediated at the level of transcription. The oestrogen may act to stimulate the microsomal enzymes mediating triacylglycerol synthesis. To test this, we recently measured the incorporation of *sn*-[U-^{14}C]glycerol 3-phosphate into phosphatidic acid, diacylglycerol and triacylglycerol by a microsomal fraction from livers of control female rats and from animals treated with 15μg of ethynyloestradiol/kg body wt. daily for 14 days. The microsomal assay system is described in detail by Soler-Argilaga *et al.* (1978). Treatment with ethynyloestradiol did not affect rates of glycerolipid synthesis when calculated as nmol of glycerol 3-phosphate incorporated/mg of microsomal protein (mean ± S.E.M.) (control values of 29.2 ± 1.2, 9.3 ± 0.3 and 5.2 ± 0.3 against ethynyloestradiol-treatment values of 30.0 ± 2.1, 11.0 ± 1.1 and 5.7 ± 1.5 for phosphatidate, diacylglycerol and triacylglycerol respectively). Oestrogen induces hepatic cellular hypertrophy, and hypertrophy of the endoplasmic reticulum and cellular hyperplasia in rats (Song *et al.*, 1969) and chicks (Schjeide & Lai, 1970). It is therefore probable that the total activity of the enzymes mediating the synthesis of triacylglycerol and the VLD lipoprotein are increased. This suggestion is supported by the observations of Coleman *et al.* (1977) that the total activities of

microsomal enzymes common to the triacylglycerol and phospholipid biosynthetic pathways in the chick increased approx. 2.5–3.6-fold after treatment with diethylstilboestrol, although little change in specific activities of these enzymes was observed.

It seems unlikely that treatment with ethynyloestradiol affected the hepatic mechanisms for mitochondrial inward transport of fatty acids or the β-oxidation of fatty acids since $^{14}CO_2$ production was unchanged, although ketogenesis was decreased. It is possible that treatment with this oestrogen may have had a specific effect on the enzymes catalysing the synthesis of ketone bodies.

The stimulation of hepatic secretion of triacylglycerol and the VLD lipoprotein by oestrogens, specifically by ethynyloestradiol, has been confirmed repeatedly in our laboratory. It is apparent, however, that the effects of ethynyloestradiol are dose-dependent. When rats were treated with low doses of ethynyloestradiol (5 or 15 μg/kg body wt. per day for 14 days), hypertriglyceridaemia (Weinstein *et al.*, 1978*b*) and stimulation of hepatic secretion of triacylglycerol was observed (Weinstein *et al.*, 1975). When the dose of drug was increased (up to 50 μg/kg body wt. per day for 14 days), as reported by Schillinger & Gerhards (1973), hypertriglyceridaemia was observed; when 250 μg/kg body wt. was administered, plasma concentration of triacylglycerol was equal to, or less than, that of the untreated animals. Davis *et al.* (1978) reported recently that ethynyloestradiol was hypotriglyceridaemic in the male rat. These workers administered 5 mg of ethynyloestradiol/kg body wt. per day for 4 days. We would suggest that the action of the drug at the dose used by Davis *et al.* (1978) was hepatotoxic, whereas the lower doses used in the experiments reported in the present paper represent an extension of the physiological actions of oestrogens.

It should be considered that oestrogen may not act directly on the liver to affect hepatic triacylglycerol synthesis and secretion, but may do so by some indirect mechanism, as through another hormone. It is known that treatment with oestrogen increases the serum concentration of insulin (Spellacy & Carlson, 1966) and of cortisol (Pulkkinen & Pekkarinen, 1967). The administration of dexamethasone to rats (T. Cole & M. Heimberg, unpublished work) stimulates the secretion of triacylglycerol by the perfused liver. Insulin deficiency produced by the administration of alloxan (Heimberg *et al.*, 1966) or of anti-insulin serum (Woodside & Heimberg, 1976) decreased the output of triacylglycerol and accelerated ketogenesis, whereas esterification of unesterified fatty acid was increased and the oxidation into CO_2 was decreased by livers perfused with blood containing an increased concentration of serum insulin (Topping & Mayes, 1972). Our failure to observe a decrease in $^{14}CO_2$ production with pretreatment with ethynyloestradiol may, perhaps, mean that insulin is not an intermediary of oestrogen action, although, clearly, this possibility should be explored further. It has been reported that women who were given ethynyloestradiol showed significant decreases in the secretion of pancreatic glucagon (Beck *et al.*, 1975). Glucagon and dibutyryl cyclic AMP are known to decrease the synthesis and output of triacylglycerol and to stimulate ketogenesis in the perfused liver (Heimberg *et al.*, 1969) and in intact rats (Eaton, 1973) and in man (Schade & Eaton, 1975). In response to treatment with glucagon, the production of cyclic AMP by isolated hepatocytes and by perfused livers from female rats treated with ethynyloestradiol was lower than controls (Weinstein *et al.*, 1978*a*). Furthermore, more glucagon was needed to decrease the secretion of triacylglycerol by livers from normal female rats than from ovariectomized rats (Weinstein *et al.*, 1974). These data suggest that oestrogens, directly or by some indirect mechanism, diminish the response of the liver to stimuli that ordinarily increase the concentration of cyclic AMP in the liver. Further support for this hypothesis can also be inferred from the following data. The output of glucose by perfused livers from rats treated with ethynyloestradiol was less than in the controls [25.0 ± 4.0 for controls against 3.5 ± 3.5 (mean $\pm$ S.E.M.) μmol/g of liver per h for the treated animals respectively; $P < 0.02$], despite increased concentrations of glycogen that have been reported in the livers of oestrogen-treated rats (Schillinger & Gerhards, 1973; Matute & Kalkhoff, 1973). Since glycogenolysis and ketogenesis are stimulated and the output of triacylglycerol is inhibited by cyclic AMP, oestrogens may act by diminishing the response to factors that increase the intracellular concentration of the cyclic nucleotide.

The actions of oestrogen on the liver are complex. Ethynyloestradiol may act by stimulating the total synthesis of triacylglycerol from fatty acids and fatty acid precursors (e.g. glucose), which then becomes a signal for the synthesis and secretion of VLD lipoprotein. The preferential channeling of [1-^{14}C]oleate into the synthesis of triacylglycerol and the increased output of triacylglycerol as a moiety of the VLD lipoprotein, as shown in the present study, is evidence for the stimulation of triacylglycerol synthesis by oestrogen, in part, at the expense of ketogenesis.

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