EFFECT OF OESTRADIOL BENZOATE ON BILIARY PHOSPHOLIPIDS IN THE RAT*

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Abstract

Bile was collected from conscious, ovariectomized, young female rats which had been treated with either oestradiol benzoate or peanut oil for 3 weeks. L-[*Me*-^{14}C]-Methionine was given intravenously immediately before collection of bile was started. The bile flow increased and the concentration of biliary phospholipids decreased as a result of the oestrogen treatment, but increases occurred in the output of radioactivity in bile and in the specific activity of biliary phospholipids. It seems most likely that oestrogen treatment increased the proportion of biliary phospholipids formed by methylation of phosphatidylethanolamine.

Biliary phospholipids, most of which are phosphatidylcholine (Phillips 1960; Spitzer *et al.* 1964), are important in helping to render cholesterol soluble in bile (Admirand and Small 1968). The concentration of phospholipids in the bile has been reported to be lower in women than in men (Van Der Linden and Norman 1967), and this may be important in explaining the higher incidence of cholesterol gallstones in women (see Glenn and McSherry 1969; Grundy *et al.* 1972).

In the liver, phosphatidylcholine may be formed either by addition of choline to 2,3-diglyceride by CDP-choline (see Kennedy and Weiss 1956), or by stepwise methylation of phosphatidylethanolamine by methyl groups from adenosylmethionine (Bremer *et al.* 1960; Bjornstad and Bremer 1966). This methylation process has also been shown to be involved in the formation of biliary phospholipids (Balint *et al.* 1967), which are believed to be produced in a small pool separate from the majority of liver phospholipids (Balint *et al* 1965; Schersten *et al.* 1967). The administration of oestradiol benzoate to castrated male rats has been shown to increase the methylation of phosphatidylethanolamine in the whole liver (Lyman *et al.* 1968), but the effects of oestrogens on this process in the biliary phospholipid pool are not known.

The effects of oestradiol benzoate on the incorporation of methyl groups in the formation of biliary phosphatidylcholine, and on the excretion of bile salts and lipids, were examined in this study.

Materials and Methods

Twelve 6-week-old female rats, which were each ovariectomized 3 weeks previously through a midline incision, were divided into three equal groups and given subcutaneous injections (0·1 ml) three times a week of:

Peanut oil	Group I
16·6 μg (50 μg/week) oestradiol benzoate in peanut oil	Group II
167 μg (500 μg/week) oestradiol benzoate in peanut oil	Group III

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After 3 weeks of hormone treatment, each rat was anaesthetized with i.p. pentobarbitone sodium (Sagatal, May and Baker Ltd., Footscray, Vic.) at 40 mg/kg, and cannulas placed in the common bile duct (see Shaw and Heath 1972) and external jugular vein. Each rat was placed in a restraining cage (Bollman 1948) and given 0·9% sodium chloride to drink, and bile was drained to the exterior. Next morning, an infusion of 4 μ-equiv/hr of taurocholate (Sigma Chemical Company, St. Louis, Mo.) was begun into the jugular cannula to compensate for interruption to the enterohepatic circulation of bile salts, and was continued for 31 hr. As enterohepatic circulation of phospholipids is most unlikely (Nilsson and Schersten 1969) no effort was made to replace phospholipid lost in the bile. At the end of the first hour, 3 μCi of L-[*Me*-^{14}C]methionine (Radiochemical Centre, Amersham, England) in 1 ml 0·9% sodium chloride was injected through the jugular cannula, and bile samples collected for 30 hr. Each rat was then killed with an overdose of pentobarbitone, and the liver and uterus removed and weighed.

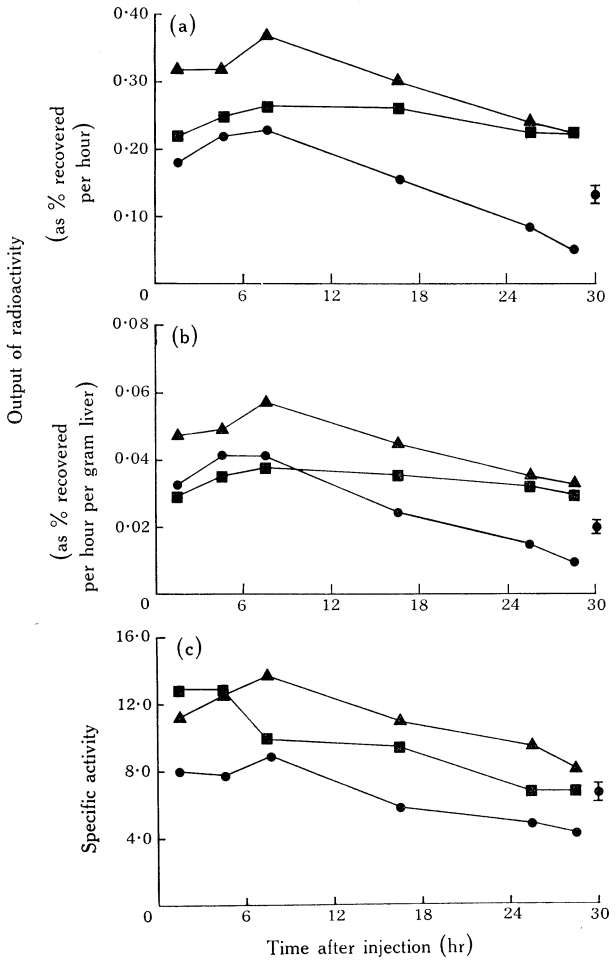

Fig. 1.—Effect of time after injection of [*Me*-^{14}C]methionine on the mean specific activity (expressed as defined in Table 1) (*c*), mean output of radioactivity per gram of liver (*b*), and mean total output of radioactivity (*a*) in the bile of groups of ovariectomized rats given peanut oil (●), 50 μg oestradiol benzoate per week (▲), and 500 μg oestradiol benzoate per week (■). Vertical bars at right represent ± S.E.M. for a single time interval.

Radioactivity in biliary lipids was estimated by liquid scintillation counting: the lipids were extracted into a chloroform phase (Folch *et al.* 1957) which was evaporated, then 0·5% diphenyloxazole was added and the radioactivity counted before and after addition of a high activity standard used to correct for quenching.

Total biliary fatty acids were used as an indication of phospholipid concentration, and were estimated after hydrolysis of fatty acid esters in alkaline ethanol (Heath and Hill 1969). Radioactive

nickel soaps were formed from the fatty acids with ^{63}Ni, and liquid scintillation counting used to estimate the level of radioactivity in the soaps (M. W. Simpson-Morgan, personal communication).

Bile salts were estimated by the method of Irvin *et al.* (1944) for total cholates.

Student's *t*-tests, coupled with analyses of variance, were used to estimate the significance of differences between means. The standard error of the mean (S.E.M.) was estimated from the error mean square (E.M.S.) and the sample number (n) as follows:

$$\text{S.E.M.} = (\text{E.M.S.}/n)^{\frac{1}{2}}.$$

Results and Discussion

An indication of the effectiveness of hormone treatment was provided by the uterine weights, which were greater in treated than in control groups ($P < 0{\cdot}01$; Table 1). The treatments did not have significant effects on liver weights (Table 1), but, because of the variations observed in liver weight and the possible influence of liver size on bile secretion, the outputs of radioactivity, bile salts, and total fatty acids were also expressed in terms of liver weight (Table 1).

TABLE 1

EFFECT OF OESTRADIOL BENZOATE ON THE ORGAN WEIGHT, THE OUTPUT AND CONCENTRATION OF BILIARY CONSTITUENTS, AND THE OUTPUT OF RADIOACTIVITY IN THE BILE

Significant differences between control values and those obtained after oestrogen treatment are indicated

Quantity measured	Control	Oestradiol (μg/week)		S.E.M.†
		50	500	
Uterine weight (g)	0·07	0·20**	0·26***	0·022
Liver weight (g)	5·3	6·9	7·2	0·81
Bile flow (ml/hr)	0·33	0·47***	0·48***	0·016
Bile salts				
(μ-equiv/ml)	14·2	11·2**	11·6**	0·63
(μ-equiv/hr)	3·9	4·7**	4·8**	0·21
(μ-equiv. per hr per g liver)	0·75	0·69	0·66	0·034
Total fatty acids				
(μ-equiv/ml)	8·1	6·5*	6·4*	0·47
(μ-equiv/hr)	2·17	2·68*	2·63	0·18
(μ-equiv. per hr per g liver)	0·42	0·40	0·38	0·031
Radioactivity recovered				
(% per hr)	0·15	0·29***	0·24***	0·013
(% per hr per g liver)	0·028	0·044***	0·033	0·0023
Total percentage recovered	3·1	8·8**	7·1*	0·99
Total percentage per gram liver	0·83	1·30**	1·03*	0·18
Specific activity ‡	6·7	11·0***	9·8***	0·39

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

† Degrees of freedom for all biliary constituents except total percentage recovered (3) = 18.

‡ Expressed as ($10^4 \times$ c.p.m./c.p.m. injected) ÷ μ-equiv. total fatty acid.

Radioactivity appeared in the bile during the first 3-hr collection period and remained relatively constant for the next two 3-hr periods, then slowly decreased (Fig. 1). Previous experiments on control rats had revealed that more than 90% of

the radioactivity in bile after injection of L-[*Me*-^{14}C]methionine is in the phosphatidylcholine fraction (Holloway 1971).

The mean output of radioactivity for the 30-hr period of the experiment was higher from oestrogen-treated rats than from controls ($P < 0{\cdot}001$; Table 1). When the radioactivity was expressed in terms of liver weight, there was still a higher output from the rats given 50 μg oestrogen/week ($P < 0{\cdot}001$; Table 1), but there was no significant difference between the controls and the rats given 500 μg oestrogen/week (Table 1). The total amount of radioactivity collected during the 30-hr period from the treated rats was higher than from controls ($P < 0{\cdot}01$ for 50 μg/week; $P < 0{\cdot}05$ for 500 μg/week), and a similar relationship existed after correction for liver weight (Table 1).

The specific activity of biliary fatty acids was higher in each group of treated rats than in controls ($P < 0{\cdot}001$; Fig. 1, Table 1), but was higher in the rats that received 50 μg oestrogen per week than in those that received 500 μg/week ($P < 0{\cdot}05$).

It appears that the oestrogen, particularly at the lower rate used, increased the proportion of biliary phosphatidylcholine being formed by methylation of phosphatidylethanolamine, while not affecting the total rate of phospholipid excretion from each gram of liver. It has been shown that female rats synthesize more phosphatidylethanolamine and in turn convert more of this into liver phosphatidylcholine than do male rats, and that this is due to the effects of oestrogens (Lyman *et al.* 1968). It is possible, therefore, that the biliary phospholipid pool may be affected in the same way.

The output of bile salts ($P < 0{\cdot}01$) and the bile flow ($P < 0{\cdot}001$) were higher in treated rats than in controls but no important differences existed in the output of total fatty acids (Table 1). In addition there were no significant differences between the outputs expressed in terms of liver weight.

However, the concentration of total fatty acids in bile from treated rats was less than for control rats ($P < 0{\cdot}01$; Table 1). This observation is consistent with that of Van Der Linden and Norman (1967) that the concentration of biliary phospholipids is lower in women than in men.

It would appear from these experiments that oestrogens exert two seemingly separate but possibly related effects on biliary phospholipids in rats. Firstly, they decrease phospholipid concentration in bile and secondly, they affect the synthetic pathways and increase the proportion of biliary phosphatidylcholine formed by methylation of phosphatidylethanolamine.

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