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EFFECT OF ESTROGEN REPLACEMENT ON LIVER FUNCTION IN OVARIECTOMIZED RATS

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Abstract.....The effect of estrogen (17β -estradiol ; E_2) replacement on the liver function was examined in ovariectomized rats.

Ovariectomy decreased serum transaminase activity and physiological dosage levels of E_2 replacement returned the activity up to or above non-ovariectomized levels. Under the conditions of this experiment the elevated serum transaminase levels in the E_2 replacement were not coincident with histological changes suggestive of liver dysfunction. The elevations of both serum glutamic oxaloacetic transaminase (GOT) and serum mitochondrial GOT were accompanied by increased hepatic tissue activity, suggesting that the elevated serum levels can be attributed to the accelerated synthesis of the enzymes. On the other hand, the elevation of serum glutamic pyruvic transaminase was not accompanied by increased tissue activity in the original organs, namely liver, kidney, heart and skeletal muscle. Ovariectomy decreased serum albumin and inhibited development of rough endoplasmic reticulum in the liver, and E_2 replacement returned them up to or above the non-ovariectomized levels.

Key words : Estrogen replacement, liver function, transaminase, protein synthesis, ovariectomized rat.

INTRODUCTION

Estrogen is used for endocrinological therapy in gynaecology, as replacement therapy for the treatment of menopausal symptoms and the prevention of osteoporosis or as contraceptives in combination with progestogen. Although the effects of estrogen on liver function have been long reported, the question of whether estrogen can cause changes in liver which are detectable by light microscopy is open to considerable debate (Heywood and Wadsworth, 1980). Some reports indicate estrogen increases the leakage of liver enzymes into serum (Ishibe, 1975 ; Leis et al., 1978 ; Saoshiro, 1980 ; Shinkawa et al., 1985 ; Smith and Sizto, 1983 ; Wadsworth et al., 1977), but this is disputed by others (Cowan et al., 1982 ; Gopinath et al., 1978 ; Heywood and Wadsworth, 1980 ; Leis et al., 1978). In histopathological investigation, some reports have shown that estrogen can induce liver degeneration or necrosis (Castroal et al., 1941 ; MacBryde et al., 1942), but others could not detect any pathological changes (Gibson et al., 1967 ; Heywood and Wadsworth, 1980 ; Light and Tornaben, 1953 ; Wadsworth et al., 1977). Higher dosage levels in relation to physiological concentrations, or synthetic estrogens such as 17α -substituted forms were used in many of the reports which showed dysfunctional effects of estrogen on liver.

The aim of our present study was to examine the effect of physiological dosage levels of natural estrogen replacement on liver function in ovariectomized rats.

MATERIALS AND METHODS

Experimental animals :

Crj : CD (SD) female rats (Charles River Japan), 13 weeks of age and with a body weight of 234–307 g, were bilaterally ovariectomized under anesthesia with sodium pentobarbital and were used at 18 weeks of age after they were confirmed as being in continuous diestrus. The rats were maintained at a constant temperature and humidity ($23\pm 3^{\circ}\text{C}$, $50\pm 20\%$) with a 12 hr light-dark cycle and were given water and a CE-2 diet (CLEA Japan) ad libitum. Ten animals were used in each group.

Chemicals and administration :

17β -Estradiol (E_2) was continuously infused rats using ALZET mini osmotic pumps (Model No. 2ML4, ALZA Corp.) which have a nominal pumping rate of $2.5\ \mu\text{l/hr}$. The pumps were primed with E_2 dissolved in propylene glycol and were implanted subcutaneously on the dorsum of the rats under ether anesthesia. E_2 was infused at the constant rates of 0.3, 1.0 and $3.0\ \mu\text{g/body/day}$ which maintained serum E_2 concentration of approximately 20, 70 and $200\ \text{pg/ml}$, respectively for a period of 28 days (measured by RIA method). The rats in the control group received the vehicle only in the same manner as the E_2 treatment groups.

Biochemistry :

All rats had individual blood samples collected from the abdominal aorta under

anesthesia with sodium pentobarbital 28 days after implantation. Animals fasted for 19–24 hours before blood collection. Plasma (for lactate dehydrogenase, LDH) and serum were separated, stored at -90°C and were then analyzed for glutamic oxaloacetic transaminase (GOT ; UV method), glutamic pyruvic transaminase (GPT ; UV method), alkaline phosphatase (ALP ; Bessey-Lowry-Brock modified method), creatine phosphokinase (CPK ; Oliver modified method), LDH (UV method), total protein (Biuret method), albumin (bromocresol green method), urea nitrogen (BUN ; urease modified method), creatinine (Jaffé method), glucose (hexokinase method), total cholesterol (enzymic method), phospholipid (enzymic method), triglyceride (enzymic method), total bilirubin (dialysis method) and bile acid (enzymic method). Two isozymes of GOT were immunologically differentiated by the method of Teranishi et al. (1978) using the EIKEN kit, and mitochondrial GOT (mGOT) activity was measured by UV method.

Tissue transaminase activity :

Approximately 100 mg of fresh samples of the liver from the median lobe, left kidney samples, the heart from the left ventricle and the skeletal muscle from the femoral muscle were briefly rinsed in ice-cold physiological saline, blotted with filter paper and weighed, after which they were rapidly frozen and stored at -90°C until analyzed. The frozen samples were homogenized in 20 times the volume of ice-cold 5 mM Tris-HCl Buffer (pH 8) for 15 strokes in 25 seconds using POLYTRON homogenizer, and sonicated in the condition of 20 kHz, 20 W for 90 seconds (the liver and kidney) or 20 kHz, 40 W for 30 seconds (the heart and skeletal muscle). After centrifugation at 1000 g for 10 minutes, the resulting supernatant was used for GOT, mGOT and GPT estimations.

Tissue protein :

Tissue protein levels from the liver, kidney and heart was measured by the method of Lowry et al. (1951) using each sample prepared for estimation of tissue transaminase activities.

Histopathology :

Formalin fixed samples of the liver, kidney, heart, femoral muscle and adrenal were processed as 3 μm thick paraffin sections and stained by hematoxylin and eosin. The sections were histopathologically investigated under a light microscope.

Ultrastructural histology :

One mm³ blocks of liver samples were fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate to pH 7.4, post-fixed in 1% osmium tetroxide buffered with 0.1 M phosphate to pH 7.4, dehydrated in alcohol and embedded in epoxy resin. One μm survey sections were cut and stained with 1% toluidine blue for determination of intralobular area with a light microscope. Silver/gold ultra-thin sections were mounted on copper grids and subsequently stained with lead acetate and uranyl acetate. These sections of both centri-and peri-lobular area from each liver were examined using a Hitachi H-300 electron microscope.

Statistical analysis :

Biochemistry, organ weights, tissue transaminase activity and tissue protein values were statistically analyzed by F-t test.

RESULTS

Biochemistry :

In comparison of serum transaminase and albumin levels between non-ovariectomized and ovariectomized rats, it was suggested that bilateral ovariectomy significantly decreased their serum levels (Table 1).

Biochemical values of the ovariectomized rats which were replaced by E₂ treatment are shown in Table 2.

Serum enzymes which mainly originated from liver, kidney, heart and skeletal muscle, (i.e., GOT, mGOT, GPT and LDH) were increased by E₂ treatment. Although the increases were dose-related up to 1.0 μ g/body/day, the values obtained with 3.0 μ g/body/day were lower than those at 1.0 μ g/body/day. Serum ALP whose source was principally from bone, intestines and liver was dose-relatedly decreased by E₂ treatment. The muscle-originated enzyme, CPK, was also dose-relatedly decreased by E₂ treatment. Total protein and albumin were dose-relatedly increased by E₂ treatment, while BUN and creatinine were decreased. Of the three lipids, only phospholipid was affected (increased) by E₂ treatment. Total bilirubin and bile acid, which are known to be increased in cases of highly dosed estrogen-induced cholestasis (Saoshiro, 1980), were not significantly altered compared with the control group.

Organ weights :

At the end of the experiment, the liver, kidney, heart and adrenal were weighed in all rats. E₂ treatment increased liver and adrenal weights, while weights of the kidney and heart were not significantly different from the control group (Table 3).

Tissue transaminase activity :

Both GOT and mGOT activity in the liver was elevated at all doses. Increases were dose-related up to 1.0 μ g/body/day, but at 3.0 μ g/body/day the values were lower than those of 1.0 μ g/body/day. In the kidney and heart both GOT and mGOT activity was dose-relatedly decreased by E₂ treatment (Table 4, 5). GPT activity in both the liver and kidney was decreased at the doses above 1.0 μ g/body/day, and also at all doses in the heart (Table 6). Femoral muscle showed no significant change in any of transaminases compared with the control group (Table 4, 5, 6).

Tissue protein :

E₂ induced no significant changes in liver protein both per g tissue and per total liver, but a tendency to increase was observed in protein per total liver at all doses (Table 7). Heart tissue protein was not affected by E₂ treatment, while in the kidney 3.0 μ g/body/day of E₂ significantly decreased the tissue protein (Table 8).

Estrogen replacement on liver function

Table 1. Effect of ovariectomy on serum GOT, GPT and albumin in Crj : CD (SD) female rats.

	No. of animals	Serum GOT (IU/l)	Serum GPT (IU/l)	Serum albumin (g/dl)
Non-ovariectomized	20	130.4±49.77	55.9±28.31	3.91±0.359
Ovariectomized ¹⁾	29	91.2±20.06**	28.1±13.88**	3.32±0.158**

1) Ovariectomy was performed at 10–13 weeks of age.

Blood samples were collected from all animals at 22–25 weeks of age.

Each value represents group mean ± standard deviation.

** Significantly different from the intact female rats, P<0.01.

Table 2. Effect of E₂ replacement on biochemical values in ovariectomized rats.

Item (unit)	Doses (μg/body/day)			
	0 (n=10)	0.3 (n=10)	1.0 (n=10)	3.0 (n=10)
GOT (IU/l)	90.9 ± 13.15	109.9 ± 54.58	433.8 ± 457.70*	292.9 ± 353.68
mGOT(IU/l)	20.7 ± 4.64	28.3 ± 17.55	161.3 ± 149.70*	63.9 ± 63.66
GPT (IU/l)	24.0 ± 3.50	44.3 ± 26.08*	177.8 ± 179.59*	154.0 ± 207.29
ALP (IU/l)	58.5 ± 16.90	30.1 ± 14.91**	24.8 ± 4.59**	20.0 ± 5.27**
LDH (IU/l)	74.1 ± 22.86	86.5 ± 27.34	199.9 ± 113.44**	128.8 ± 37.18**
CPK (IU/l)	353.5 ± 102.78	311.1 ± 129.53	243.7 ± 62.32**	195.9 ± 46.79**
TP (g/dl)	5.77± 0.298	6.41± 0.621*	7.16± 0.135**	7.31± 0.296**
Alb (g/dl)	3.23± 0.183	3.82± 0.377**	4.32± 0.092**	4.38± 0.175**
A/G	1.29± 0.189	1.48± 0.106*	1.53± 0.102**	1.50± 0.072*
BUN (mg/dl)	22.8 ± 4.91	15.7 ± 1.78**	19.6 ± 2.52	16.8 ± 1.60**
Cre (mg/dl)	0.69± 0.041	0.64± 0.057*	0.68± 0.060	0.63± 0.054**
Glu (mg/dl)	145.7 ± 18.69	146.7 ± 7.34	148.7 ± 14.30	117.2 ± 8.59**
Cho (mg/dl)	86.4 ± 12.50	90.6 ± 19.46	93.4 ± 12.75	96.9 ± 19.84
PL (mg/dl)	116.5 ± 19.05	138.8 ± 24.43*	157.1 ± 14.60**	161.4 ± 24.64**
TG (mg/dl)	34.8 ± 13.55	38.1 ± 14.64	36.2 ± 9.59	37.9 ± 15.91
TB (mg/dl)	0.32± 0.180	0.24± 0.049	0.23± 0.063	0.23± 0.051
BA (μM)	22.4 ± 12.85	15.9 ± 11.49	37.4 ± 24.15	23.9 ± 18.37

The ovariectomized rats were continuously administered E₂ by subcutaneous implantation of mini osmotic pumps for 28 days.

TP : Total protein

Alb : Albumin

Cre : Creatinine

Glu : Glucose

Cho : Total cholesterol

PL : Phospholipid

TG : Triglyceride

TB : Total bilirubin

BA : Bile acid

Each value represents group mean ± standard deviation.

* and ** Significantly different from control (0 μg/body/day), P<0.05 and P<0.01, respectively.

Table 3. Effect of E₂ replacement on organ weights in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Terminal body weight(g)	Liver (g)	Kidneys (g)	Heart (g)	Adrenals (mg)
0	10	353.4 $\pm$ 25.85	7.83 $\pm$ 0.797	1.842 $\pm$ 0.1349	1.012 $\pm$ 0.0744	63.6 $\pm$ 7.35
0.3	10	325.2 $\pm$ 26.21*	7.94 $\pm$ 0.657	1.932 $\pm$ 0.2463	1.001 $\pm$ 0.0895	83.0 $\pm$ 11.20**
1.0	10	307.6 $\pm$ 17.49**	8.44 $\pm$ 0.593	1.846 $\pm$ 0.1409	0.981 $\pm$ 0.0799	80.2 $\pm$ 7.72**
3.0	10	316.4 $\pm$ 24.20**	8.87 $\pm$ 0.862*	1.948 $\pm$ 0.1614	0.950 $\pm$ 0.0827	77.3 $\pm$ 8.06**

The ovariectomized rats were continuously administered E₂ by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), $P < 0.05$ and $P < 0.01$, respectively.

Table 4. Effect of E₂ replacement on serum and tissue GOT activity in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Serum GOT (IU/l)	Tissue GOT (IU/g tissue)		
			Liver	Kidney	Heart
0	10	90.9 $\pm$ 13.15	243.8 $\pm$ 21.20	139.6 $\pm$ 13.39	258.4 $\pm$ 27.21
0.3	10	109.9 $\pm$ 54.58	269.2 $\pm$ 23.78*	131.8 $\pm$ 20.16	242.2 $\pm$ 22.85
1.0	10	433.8 $\pm$ 457.70*	298.4 $\pm$ 31.75**	122.0 $\pm$ 13.79**	235.4 $\pm$ 17.31*
3.0	10	292.9 $\pm$ 353.68	280.6 $\pm$ 45.56*	107.6 $\pm$ 9.92**	235.0 $\pm$ 19.40*

The ovariectomized rats were continuously administered E₂ by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), $P < 0.05$ and $P < 0.01$, respectively.

Table 5. Effect of E₂ replacement on serum and tissue mGOT activity in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Serum mGOT (IU/l)	Tissue mGOT (IU/g tissue)			
			Liver	Kidney	Heart	Femoral muscle
0	10	20.7 $\pm$ 4.64	99.2 $\pm$ 12.26	57.6 $\pm$ 7.82	64.6 $\pm$ 0.20	16.3 $\pm$ 8.11
0.3	10	28.3 $\pm$ 17.55	114.8 $\pm$ 13.00*	56.0 $\pm$ 5.96	60.4 $\pm$ 9.51	17.8 $\pm$ 4.94
1.0	10	161.3 $\pm$ 149.70*	130.4 $\pm$ 12.61**	47.8 $\pm$ 9.31*	56.0 $\pm$ 6.99*	14.1 $\pm$ 10.84
3.0	10	63.9 $\pm$ 63.66	122.6 $\pm$ 10.71**	43.2 $\pm$ 7.67**	58.8 $\pm$ 12.37	20.2 $\pm$ 9.31

The ovariectomized rats were continuously administered E₂ by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), P<0.05 and P<0.01, respectively.

Table 6. Effect of E₂ replacement on serum and tissue GPT activity in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Serum GPT (IU/l)	Tissue GPT (IU/g tissue)			
			Liver	Kidney	Heart	Femoral muscle
0	10	24.0 $\pm$ 3.50	86.88 $\pm$ 10.250	33.10 $\pm$ 2.623	23.74 $\pm$ 2.784	25.44 $\pm$ 6.220
0.3	10	44.3 $\pm$ 26.08*	88.82 $\pm$ 11.999	31.30 $\pm$ 5.486	21.40 $\pm$ 2.140*	30.62 $\pm$ 12.816
1.0	10	177.8 $\pm$ 179.59*	71.90 $\pm$ 8.687**	29.66 $\pm$ 3.723*	21.18 $\pm$ 2.030*	27.32 $\pm$ 8.624
3.0	10	154.0 $\pm$ 207.29	59.48 $\pm$ 9.333**	26.40 $\pm$ 2.933**	21.04 $\pm$ 2.576*	31.94 $\pm$ 12.938

The ovariectomized rats were continuously administered E₂ by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), P<0.05 and P<0.01, respectively.

Table 7. Effect of E_2 replacement on serum and liver proteins in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Serum total protein (g/dl)	Serum albumin (g/dl)	Liver weight (g)	Liver protein (mg/g tissue)	Liver protein (mg/liver)
0	10	5.77 ± 0.298	3.23 ± 0.183	7.83 ± 0.797	218.72 ± 9.068	1713.71 ± 197.784
0.3	10	$6.41 \pm 0.621^*$	$3.82 \pm 0.377^{**}$	7.94 ± 0.657	222.20 ± 14.556	1769.55 ± 232.968
1.0	10	$7.16 \pm 0.135^{**}$	$4.32 \pm 0.092^{**}$	8.44 ± 0.593	209.92 ± 10.287	1770.54 ± 136.039
3.0	10	$7.31 \pm 0.296^{**}$	$4.38 \pm 0.175^{**}$	$8.87 \pm 0.862^*$	213.18 ± 9.819	1894.96 ± 247.409

The ovariectomized rats were continuously administered E_2 by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), $P < 0.05$ and $P < 0.01$, respectively.

Table 8. Effect of E_2 replacement on kidney and heart proteins in ovariectomized rats.

Doses ($\mu\text{g}/\text{body}/\text{day}$)	No. of animals	Kidney weight (g)	Kidney protein (mg/g tissue)	Heart weight (g)	Heart protein (mg/g tissue)
0	10	1.842 ± 0.1349	152.52 ± 8.938	1.012 ± 0.0744	62.810 ± 7.1455
0.3	10	1.932 ± 0.2463	148.47 ± 12.399	1.001 ± 0.0895	59.765 ± 4.2778
1.0	10	1.846 ± 0.1409	150.68 ± 8.893	0.981 ± 0.0799	61.685 ± 4.9162
3.0	10	1.948 ± 0.1614	$138.52 \pm 6.263^{**}$	0.950 ± 0.0827	60.695 ± 5.2445

The ovariectomized rats were continuously administered E_2 by subcutaneous implantation of mini osmotic pumps for 28 days.

Each value represents group mean $\pm$ standard deviation.

* and ** Significantly different from control (0 $\mu\text{g}/\text{body}/\text{day}$), $P < 0.05$ and $P < 0.01$, respectively.

Estrogen replacement on liver function

Histopathology :

Results of histopathology are shown in Table 9.

In comparison with the control group there were no histopathological changes in the liver at all doses which were suggestive of the elevated serum transaminase levels. The doses 1.0 and 3.0 $\mu\text{g}/\text{body}/\text{day}$ of E_2 caused a lower incidence of microgranulomas in the liver (0–10%) compared with the control group (60%). Vacuolation (fatty change) of hepatocytes was observed at a lower incidence and a lower severity in all E_2 treatment groups (20–40%, very slight) than in the control group (80%, very slight to moderate). In adrenals, the incidence of dilation of sinusoids was slightly increased by E_2 treatment. Atrophy of zona reticularis cells was observed dose-relatedly. There were no treatment-related changes in the kidney, heart and femoral muscle.

Table 9. Effect of E_2 replacement on histology of liver and adrenal in ovariectomized rats.

Organs	Findings	Grade	Doses ($\mu\text{g}/\text{body}/\text{day}$)			
			0 (n)	0.3 (10)	1.0 (10)	3.0 (10)
Liver	Microgranuloma	—	4	6	10	9
		±	3	1	0	0
		+	2	2	0	1
		++	1	1	0	0
	Vacuolation of hepatocyte	—	2	7	8	6
		±	2	3	2	4
		+	5	0	0	0
		++	1	0	0	0
Adrenal	Atrophy of zona reticularis cell	—	4	2	3	0
		±	6	3	4	4
		+	0	5	0	1
		++	0	0	3	5
	Sinus dilation	—	4	1	4	1
		±	1	3	1	1
		+	3	2	0	3
		++	2	4	5	5

The ovariectomized rats were continuously administered E_2 by subcutaneous implantation of mini osmotic pumps for 28 days.

Figures represent the number of animals which showed findings.

— : Normal ± : Very slight + : Slight ++ : Moderate

Ultrastructural histology :

There were no ultrastructural changes in all E₂ treatment groups which were suggestive of liver dysfunction. In the control group dysplasia of rough endoplasmic reticulum (rER) was observed in both centri- and peri-lobular hepatocytes compared with the non-ovariectomized and age-matched female rats (Photo. 1, 2), while all E₂ treatment groups showed normal development of rER (Photo. 3). The inclusion of fat droplets was remarkable in the perilobular hepatocytes of the control group (Photo. 2).

Dilatation and a decrease of microvilli of bile canaliculi, both findings which are known to be as the ultrastructural indication of ethinyl estradiol-induced cholestasis (Schaffner, 1966), were not observed in the E₂ treatment groups.

DISCUSSION

Bilateral ovariectomy significantly decreased serum transaminase activity, and estrogen replacement increased the activity up to or above the non-ovariectomized level without histological changes suggestive of liver dysfunction which were detectable by light microscope or by electron microscope. The elevation of the activity peaked not at the highest but at the intermediate dose (1.0 μ g/body/day). Serum E₂ concentrations were determined by RIA method in the same experimental

Photo. 1. Perilobular hepatocyte from a non-ovariectomized and age-matched female rat, showing normal development of rER. $\times 5300$.

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Photo. 2. Perilobular hepatocyte from an ovariectomized rat continuously administered the vehicle for 28 days, showing poor development of rER and remarkable inclusion of fat droplets compared with the non-ovariectomized and age-matched female rat (Photo. 1). $\times 5300$.

Photo. 3. Perilobular hepatocyte from an ovariectomized rat continuously administered 1.0 $\mu\text{g}/\text{body}/\text{day}$ of E_2 for 28 days, showing normal development of rER. $\times 5300$.

condition as the present study, and 0.3, 1.0 and 3.0 $\mu\text{g/body/day}$ maintained E_2 levels of approximately 20, 70 and 200 pg/ml , respectively. In 4-day estrous cycled rats, the E_2 concentration peaked at 80 pg/ml in proestrus and other days ranged from 20 to 50 pg/ml (Butcher et al., 1974). Therefore 0.3 and 1.0 $\mu\text{g/body/day}$ could be considered as physiological dosage levels, while 3.0 $\mu\text{g/body/day}$ is somewhat higher than a physiological level.

Elevation of serum transaminase levels has been long taken as an indication of liver dysfunction (Wadsworth et al., 1977), because the enzymes have been thought not to be leaked from intact hepatocytes (Kawaguchi, 1966). In order to determine whether the elevation of serum transaminase activity due to the replaced physiological dosage levels of E_2 is attributable to possibly accelerated synthesis of the enzymes in original organs, we estimated the enzyme activity in homogenized tissues of the liver, kidney, heart and skeletal muscle. Both GOT and mGOT activity was increased in the liver by E_2 treatment, while those in the kidney and heart were decreased. In the liver the peak of elevation was not in the highest dose but in the intermediate dose and correlated with serum GOT and mGOT levels. The elevated serum GOT and mGOT could be attributable to the accelerated synthesis of the enzymes in the liver, due to the increased weight of the liver and consequently the enhanced activity of total liver enzymes compared with the kidney and heart. On the other hand, the elevation of serum GPT was not accompanied by increased tissue activity in the original organs. In addition to the accelerated synthesis of transaminases, a possible change of cell membrane permeability in the organs could be considered as one of mechanisms responsible for the increase in serum transaminases, since steroid hormones are well known to have effects on it (Seeman, 1972). Further studies are needed to clarify the effect of physiological doses of natural estrogen on the permeability.

Since transaminases are the enzymes contributing to amino acid metabolism, the accelerated synthesis of GOT in the liver might be related to a change of albumin synthesis in the liver. Bilateral ovariectomy decreased serum albumin and E_2 replacement increased it above the non-ovariectomized level. A reduction of serum albumin has been also taken as an indication of liver dysfunction because the synthesis of albumin is depressed in cases of liver disease such as cirrhosis (Hirayama et al., 1970). Estrogens, in particular higher doses of synthetic estrogens, have been reported to reduce serum albumin or total proteins as result of liver dysfunction (Musa et al., 1967 ; Shinkawa et al., 1985). Contrary to these reports, in the present study serum albumin was increased by E_2 treatment, and BUN, which is known to be altered by anabolic/catabolic balance, was decreased. Although E_2 induced no significant changes in liver protein concentrations, the increase in liver weight consequently caused the greater accumulation of total liver proteins compared with the control group. Results of the ultrastructural investigation of the liver suggest the presence of an accelerated synthesis of protein, i.e., bilateral ovariectomy inhibited development of rER in hepatocytes and physiological dosage levels of E_2

returned the rER development to normal.

Cortisone is also known to increase the rates of albumin synthesis in liver (Rothschild et al., 1961). Since adrenal cortex is hypertrophic in rats during estrus and with the administration of estrogens (Anderson and Capen, 1978), in the present study we examined weight and histology of adrenals and found that E₂ increased adrenal weights and induced histological changes in the adrenal cortex. Although our data could not be used to determine whether the accelerated albumin synthesis by the E₂ treatment originated from possibly enhanced glucocorticoid synthesis, some reports which have indicated that the hepatic activity of GPT but not GOT is elevated by cortisone treatment (Rosen et al., 1959) and that hydrocortisone decreases serum GPT levels (Nakata et al., 1963) suggest that estrogens and glucocorticoids have different effects on liver function.

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