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METABOLISM OF ^{3}H-ESTRONE SULFATE PERFUSED *IN VIVO* THROUGH A RHESUS MONKEY LIVER

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SUMMARY

The liver of a rhesus monkey was perfused *in vivo* with [^{3}H]-estrone sulfate (E_1SO_4). The injection was made via the portal vein and blood was collected from the hepatic vein, using a heart catheter. Analysis for free and conjugated metabolites was done. The sulfate fraction represented $\frac{2}{3}$ of the recovered activity, indicating a slow rate of desulfurylation. Conversion of estrone sulfate to free estrogens was indicated. 2-hydroxy-estrogens were found in substantial amounts in the conjugate fractions. Double conjugates of estriol and 2-hydroxy-estriol in the form of sulfoglucosiduronates were indicated. Evidence for retention of E_1SO_4 by the monkey liver was presented.

INTRODUCTION

THE METABOLISM of estrogen sulfates, naturally occurring esters, has received renewed interest[1, 2]. Estrone sulfate (E_1SO_4) was considered to be a very potent compound in effecting the production of estradiol[3]. The liver is one of the most important organs for metabolism of steroids. However, no *in vivo* liver perfusion of primates with estrogen conjugates has been reported. Most studies concerning estrogen conjugates mention E_1SO_4 only as an intermediary compound or report its metabolism *in vitro* or whole organs[4–9]. Therefore, a detailed analysis of the metabolism of E_1SO_4 itself in the liver *in vivo* was undertaken in a Rhesus monkey. The technique was the same as described by us in human experiments[10, 11].

MATERIALS AND METHODS

1. *Substrate*

[6,7^{3}H]-Estrone sulfate with a specific activity of 0·11 μCi/μg was purchased from New England Nuclear Corp. (Boston, Mass.) and was used immediately after delivery. The purity was more than 98%, as checked in system A (Table 1).

2. *Experimental*

The experimental procedure is basically the same as used in human experiments[10]. A polyvinyl catheter was placed in one of the liver veins via the heart. Entry was made from the right jugular vein. After opening the abdomen, [6,7^{3}H]-estrone sulfate (260×10^6 d.p.m.), dissolved in 0·5 ml of ethanol plus 1·5 ml of

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List of abbreviations used: Estrone (E_1) = 3-hydroxy-1,3,5(10)-estratrien-17-one; estradiol-17β (E_2) = 1,3,5(10)-estratriene-3,17β-diol; estriol (E_3) = 1,3,5(10)-estratriene-3,16α,17β-triol; 2-hydroxyestrone (2-OH-E_1) = 2,3-dihydroxy-1,3,5(10)-estratrien-17-one; 2-hydroxyestradiol-17β (2-OH-E_2) = 1,3,5(10)-estratriene-2,3,17β-triol; 2-hydroxyestriol (2-OH-E_3) = 1,3,5(10)-estratriene-2,3,16α, 17β-tetrol.

Table 1. Solvent systems used for thin layer chromatography (t.l.c.) and paper chromatography (p.c.) of estrogens

System	
	t.l.c. for Conjugates:
A	Choroform: Methanol: Ammonia 10:10:0·2 (by vol)
	t.l.c. for Steroids:
B	Benzene: Ethanol 85:15 (v/v)
C	Acetone: Isopropyl Ether 25:75 (v/v)
D	Methylene Chloride: Methanol 95:5 (v/v)
	t.l.c. for Acetates:
E	Benzene–Ethanol 99:1 (v/v)
F	Benzene: Chloroform 75:25 (v/v)
G	Benzene: Methylene Chloride 80:20 (v/v)
	p.c.
H	Methylcyclohexane: Propylene glycol

saline solution, was injected into the portal vein. Perfusion was carried out over a one-minute period, and 20 ml of blood was withdrawn from the hepatic vein at 1, 3, 5, 10, 15 and 20 min. (Fig. 1). As found in experiments with rhesus monkeys (unpublished), radioactivity injected into the right heart could not be found on the average, earlier than 3 min after injection. The recovered radioactivity is very low and remains on a constant level. Therefore, the earlier stage of the perfusion is highly specific for the liver metabolism, showing a slight decrease in specificity as the time period progressed.

3. *Extraction and analysis*

The analysis followed a general procedure which has been described in detail [9–14]. The characterization of double conjugates is described below and follows techniques described in detail elsewhere[15]. After protein precipitation with ethanol: acetone (1:1), the organic solvents were evaporated. Unconjugated estrogens were extracted with chloroform and methylene chloride from the water

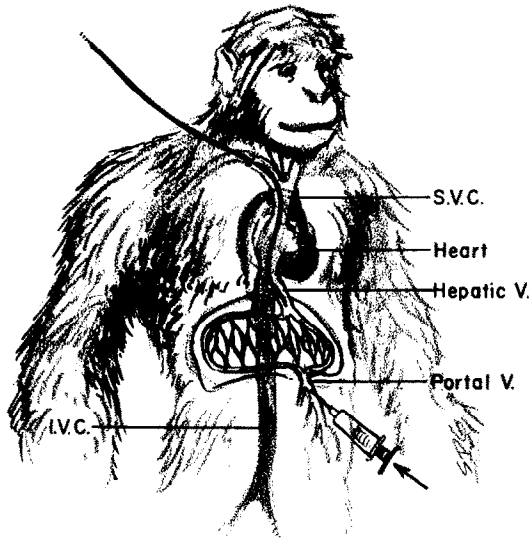

Fig. 1. Method for perfusion of the monkey liver.

residue. In the aqueous residue remaining, conjugated estrogens were extracted thereafter with ethylacetate immediately after saturation with ammonium sulfate and acidification to pH 1. The conjugates were separated by thin layer chromatography (t.l.c.) in system A (Table 1)[11, 13]. The sulfoglucuronides and the glucuronides remained near the starting line, while the sulfates migrated to the upper half of the plate. The sulfate fraction was hydrolyzed with Mylase P (Nutritional Biochemicals) and extracted. The glucuronide plus sulfoglucuronide fraction was extracted after β-glucuronidase (Sigma II) hydrolysis for glucuronides. The remaining aqueous residue containing partly hydrolyzed sulfoglucuronides of estrogens was re-incubated with Mylase P and extracted with chloroform and methylene chloride. This sequential hydrolysis with glucuronidase and Mylase P served to release the sulfoglucuronides[9, 13, 14]. After separation of freed steroids and their conjugates, enzyme hydrolysis was performed. Then 1, 3 and 5 min samples and 10, 15 and 20 min samples were combined.

The free steroids were mixed with 50 μg carrier estrogens and separated on t.l.c. and PC in different systems (Table 1).

Purification resulted from successive t.l.c. in systems B, C and D, followed by acetylation and further purification on paper in system H, as well as on t.l.c. in systems E, F and G. For identification, a recently described technique was used [16]. During the last 3 t.l.c. a standard of each estrogen acetate was separately spotted on the plate for determination of recovery after elution from the plate. The amount was determined by *in situ* spectrodensitometry. The ratio of recovered radioactivity of the purified estrogen acetate and the corresponding standard served for calculation of specific activity (d.p.m./μg). The ratio did not change more than 5% for all compounds during the last 2 t.l.c. This was indication for purity of the compounds[16].

Radioactivity was assayed in a Packard Tri-Carb Scintillation Spectrometer, Model 3375, which allowed quench correction by external standardization. Recovery from the plate was determined after eluting all zones and blank areas of the plate and measuring the total radioactivity.

RESULTS AND DISCUSSION

After perfusion of the monkey liver *in vivo* with [6,7^{3}H]-estrone sulfate, free and conjugated compounds were found in all samples obtained from the hepatic vein. Most of the radioactivity was found in the collections up to 3 min. (Figs. 2–5). This was followed by a fall and finally 10–15 min after perfusion a second increase of ^{3}H-activity was detected. This may indicate a retention of E_1SO_4 by the liver. The speed of metabolism was not calculated, because data for blood flow through the monkey liver were not available. The recovery of injected radioactivity was 1·5%. This is in the expected range, since only an aliquot (20 ml) of the blood passing the liver was collected at the time intervals.

The free fraction represented 7·0% of the recovered activity. The largest amount of the radioactivity was found in the first 5 min (Table 2). Estriol was the main metabolite followed by estradiol (E_2) and estrone (E_1). In the second period of collection (10–20 min) 2-OH-E_2 dominated. The small amount of radioactive metabolites in the free fraction and the larger amount of radioactive metabolites in the sulfate fraction indicate metabolism without removal of the sulfate group.

The conjugate fraction represented 93% of the recovered radioactivity and the

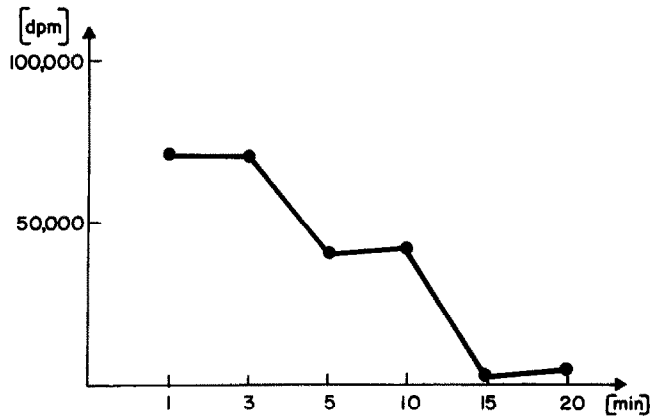

Fig. 2. Recovered radioactivity in the unconjugated fraction at different times from plasma of the hepatic vein.

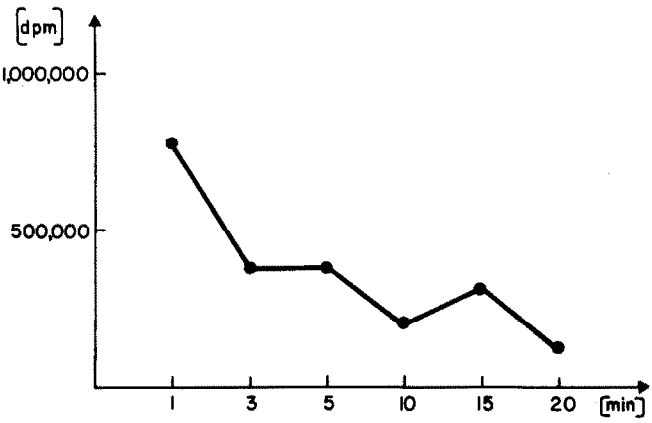

Fig. 3. Recovered radioactivity in the sulfate fraction at different times from plasma of the hepatic vein.

Table 2. Radioactivity in different estrogen fractions* (d.p.m. × 10^3)

	Free fraction		Sulfates		Glucuronides		Sulfoglucuronides	
	1, 3, 5† min	10, 15, 20† min	1, 3, 5 min	10, 15, 20 min	1, 3, 5 min	10, 15, 20 min	1, 3, 5 min	10, 15, 20 min
E_1	12·4	X	756·2	59·0	17·4	28·2	X	X
E_2	13·6	5·9	28·0	87·0	X	X	X	X
E_3	49·9	6·3	X	12·4	X	82·2	5·4	X
2-OH-E_1	11·4	X	56·0	80·0	X	38·8	X	X
2-OH-E_2	9·8	22·2	74·6	55·9	X	28·9	X	X
2-OH-E_3	8·2	X	X	6·2	14·8	19·2	16·5	17·6
Epi-E_3	X	X	X	X	8·3	X	X	X

X Experimental value was below the limits of detection.
*Corrected for recovery after hydrolysis and extraction steps have been completed.
†Collections of 1, 3, 5 and 10, 15, 20 min respectively were pooled.

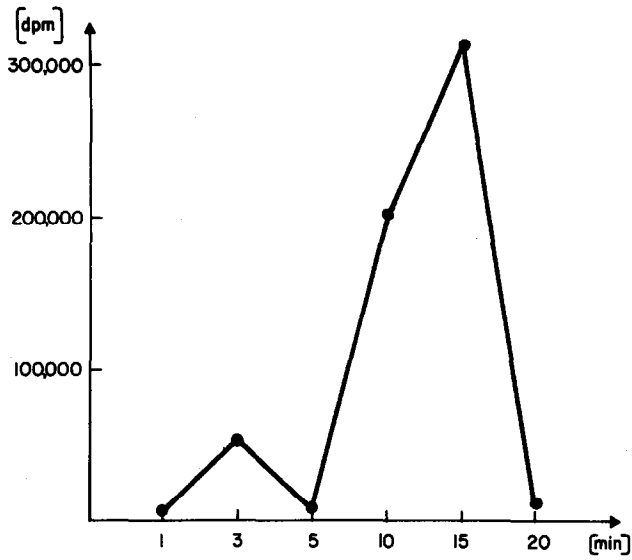

Fig. 4. Recovered radioactivity in the glucuronide fraction at different times from plasma of the hepatic vein.

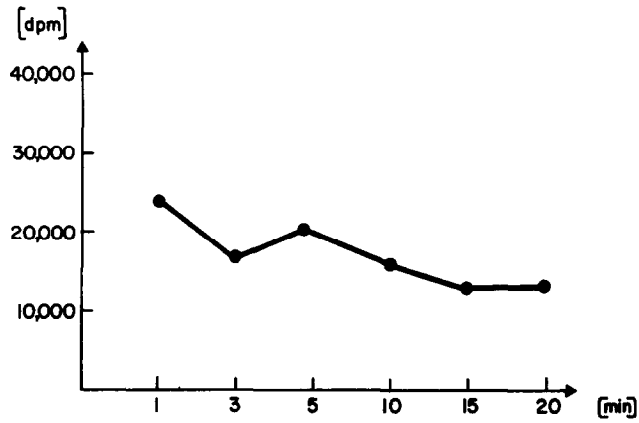

Fig. 5. Recovered radioactivity in the sulfoglucuronide fraction at different times from plasma of the hepatic vein.

sulfates were the largest. The ratio of other conjugates/sulfates in pool I (1, 3, 5 min samples) was 0·22; in pool II (10, 15, 20 min samples) it was 0·5, indicating a slowly increasing conversion of sulfates in the monkey liver.

The sulfoglucuronides have not previously been isolated from the liver under *in vivo* conditions. 2-OH-E_3 and E_3 were found in the sulfoglucosiduronate fraction. Sulfoglucuronides were also found after incubation of rat liver microsomes with estrone[15]. The sulfoglucuronides were 3% of the conjugate fraction and 2·7% of the recovered radioactivity.

The glucuronide fraction represented 21% of the conjugate fraction Comparing pools I and II, the latter showed more radioactivity. In contrast to the first pool, where mainly 2-OH-E_3 was detected, the second pool showed a variety of metabolites with E_1 as a major compound. The 2-OH-estrogens were also present, as shown by other investigators[17, 18]. The glucuronides represented 18% of the recovered radioactivity.

The sulfates represented the largest proportion, with 72% in the conjugate fraction and 57% of the total recovered radioactivity. While the sulfate fraction decreased during the 20 min period, the glucuronides increased. This indicates a preferred conversion to glucuronides as one of the main metabolic pathways for E_1SO_4 in the liver. The largest variety of different estrogen metabolites was found in the 10–20 min pool. The main metabolite in the first pool was E_1, while in the second pool it was E_2. This is in agreement with other work[3]. 2-OH-E_1 was found in about the same amount as E_1 in the second portion. More 2-hydroxysteroids were found in the sulfate fraction than in the glucuronide fraction. E_3 was present in low amounts.

It can be concluded that the conversion of E_1SO_4 to free, glucuronides or double conjugates in the monkey liver is slow. The conversion of estrone sulfate to free estrogens was demonstrated *in vivo* in the liver. These results indicate the ability of the liver for conversion of E_1SO_4 to biologically active estradiol-17β. The conversion of E_1SO_4 to E_2SO_4 or estriol was rapid and is in agreement with other reports[3]. The formation of sulfoglucuronides of E_3 and 2-OH-E_3 in a liver perfusion *in vivo* was demonstrated. The sulfoglucuronide of estriol has previously been isolated from maternal blood[9], from bile[19], and after incubation of rat liver microsomes with estrone[15]. 2-Hydroxyestrogens were found also in the conjugated fractions. Evidence was presented for retention of E_1SO_4 by the liver during the time period studied.

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