Effects of Clofibrate on the Metabolism of Progesterone and Oestradiol in Rat Liver Microsomal Fraction

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1. The substrate conversion of [4-¹⁴C]progesterone and [4-¹⁴C]oestradiol during incubation with the liver microsomal fraction from both control and clofibrate-treated rats amounted to about 10–15 and 20–25% respectively. 2. The metabolites of progesterone formed by preparations from control rats were hydroxylated in the 16 α -position (14%), the 6 β -position (12%) and the 2 α -position (7%). Of the products formed from oestradiol 12% were recovered as a 16 α -hydroxylated derivative whereas 5% had a 6 β - and 2% a 6 α -hydroxyl group. 3. Clofibrate affected the microsomal metabolism of both progesterone and oestradiol. It induced 7 α -hydroxylation of both compounds, metabolic conversions not found in control rats. The 6 β -hydroxylation of progesterone and the 6 α -hydroxylation of oestradiol were enhanced by a factor of 2 and 3.5 respectively. The 2 α -hydroxylation, and the 20 α - and 20 β -hydroxy steroid reduction of progesterone were significantly decreased as were the 16 α - and the 6 β -hydroxylation of oestradiol.

Chlorophenoxyisobutyrate (clofibrate) is a hypolipidaemic agent whose mechanisms of action are not fully known. Clofibrate treatment increases liver weight (Best & Duncan, 1964), induces proliferation of smooth-surfaced endoplasmic reticulum in rat liver cells (Hess *et al.*, 1965) and increases cytochrome P-450 content in rat liver microsomal fraction (Salvador *et al.*, 1970). Clofibrate also stimulates the liver hydroxylation of testosterone (Salvador *et al.*, 1970). These effects of clofibrate may all be explained by the induction of drug-metabolizing hepatic microsomal enzymes.

Clofibrate alters the metabolism of androstendione in rat liver (Einarsson *et al.*, 1973). As clofibrate is a widely used drug it seemed important to study its effects on the hepatic metabolism of other steroid hormones as well. The present paper reports on the metabolism of progesterone and oestradiol in liver microsomal fractions from control and clofibrate-treated rats.

Experimental

Materials

Reference compounds. Several of the reference steroids used in this investigation were generous gifts from Dr. J. Babcock (progesterone, 20 α - and 20 β -hydroxypregn-4-en-3-one and 6 β - and 16 α -hydroxypregn-4-ene-3,20-dione). 3 β -Hydroxy-5 α -pregnan-20-one was purchased from Ikapharm (Ramat-Gan, P.O. Box 31, Israel).

Oestradiol, oestrone and oestriol were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

NaBH₄ reduction of 3,7 α -dihydroxyoestra-1,3,5(10)-trien-17-one (kindly given by Dr. P. Crabbé) gave oestra-1,3,5(10)-triene-3,7 α ,17 β -triol. LiAlH₄ reduction of 3,17 β -dihydroxyoestra-1,3,5(10)-trien-6-one 3,17-diacetate (kindly given by Professor W. Klyne) resulted in the formation of oestra-1,3,5(10)-triene-3,6 α ,17 β -triol and oestra-1,3,5(10)-triene-3,6 β ,17 β -triol, which were separated as silyl ethers on SE-30.

Oestra-1,3,5(10)-triene-3,16 β ,17 β -triol and oestra-1,3,5(10)-triene-3,16 α ,17 β -triol were donated by Professor W. J. A. VandenHeuvel and Professor Klyne respectively.

Clofibrate (Atromidin) was obtained from Scanmeda, Göteborg, Sweden.

Radioactive steroids. [4-¹⁴C]Progesterone (specific radioactivity, 2.0 μ Ci/mg) and [4-¹⁴C]oestradiol (specific radioactivity, 4.0 μ Ci/mg) were purchased from The Radiochemical Centre (Amersham, Bucks., U.K.).

Methods

Animals and preparation of homogenates. Two groups of six male white rats of the Sprague-Dawley strain weighing about 200 g had free access to a crushed commercial pellet diet for 3–4 weeks. The food given to one of the groups was supplemented with 0.3% (w/w) clofibrate. The drug was added to the diet dissolved in ethanol, which was allowed to evaporate at room temperature. The animals were killed by a blow on the neck and the liver was excised quickly, chilled and weighed. Liver homogenates (20%, w/v) were prepared in a modified Bucher

medium (Bergström & Gloor, 1955), pH 7.4, with a Potter-Elvehjem homogenizer equipped with a loosely fitting pestle. The homogenate was centrifuged at $20000g_{av}$ for 15 min. The supernatant fluid was centrifuged at $105000g_{av}$ for 1 h and the microsomal fraction obtained was suspended in Bucher medium in a volume corresponding to that of the $20000g_{av}$ supernatant fluid. The protein concentration of the microsomal fraction was determined by the method of Lowry *et al.* (1951) with albumin as standard.

Incubations with $[4-^{14}C]$ progesterone. $[4-^{14}C]$ Progesterone ($250\mu g$ dissolved in $50\mu l$ of acetone) was added to a mixture of $0.5 ml$ of microsomal fraction and $2.5 ml$ of Bucher medium fortified with $3\mu mol$ of NADPH. The incubation was carried out for 10 min at $37^\circ C$ and subsequently terminated by the addition of $20 vol.$ of chloroform-methanol ($2:1, v/v$). The precipitate was filtered off and $0.2 vol.$ of NaCl (0.9% , w/v) was added. The chloroform phase was evaporated. The residue, dissolved in chloroform-methanol ($2:1, v/v$), was subjected to t.l.c. (pre-coated silica-gel plates; $250\mu m$; Merck, Darmstadt, Germany) with benzene-ethyl acetate ($3:1, v/v$) as solvent. The t.l.c. were subjected to radioautography with an exposure time of about 10 days. The radioactive zones on the t.l.c. (determined exactly from the X-ray film) were scraped off, eluted with methanol and measured for radioactivity.

Incubations with $[4-^{14}C]$ oestradiol. A mixture of $500\mu g$ of $[4-^{14}C]$ oestradiol dissolved in $50\mu l$ of acetone, $3 ml$ of the microsomal fraction and $1 ml$ of Bucher medium fortified with $4\mu mol$ of NADPH was incubated for 20 min at $37^\circ C$. The incubation was terminated by the addition of $15 vol.$ of chloroform-methanol ($2:1, v/v$). The precipitate was filtered off and the solvent evaporated. The residue, dissolved in chloroform-methanol ($2:1, v/v$), was subjected to t.l.c. with cyclohexane-ethyl acetate-ethanol ($10:9:1$, by vol.) as solvent. Further analysis was performed as described above for $[4-^{14}C]$ progesterone.

Gas-liquid chromatography-mass spectrometry (g.l.c.-m.s.) analysis of metabolites of $[4-^{14}C]$ progesterone and $[4-^{14}C]$ oestradiol. The methanol extracts of the radioactive zones pooled from six incubations were evaporated to dryness and all material left after radioactivity measurement was trimethylsilylated and analysed by g.l.c.-m.s. (LKB 9000 instrument) on a 1.5% SE-30 column.

Mass spectra were recorded on magnetic tape by using the incremental mode of operation and were then treated in an IBM 1800 computer (Reimendahl & Sjövall, 1972). Conventional g.l.c. for determination of relative retention times and of quantitative ratios between steroid metabolites in t.l.c. zones that contained more than one compound were carried out on a Pye gas chromatograph (series 104)

with 1.5% SE-30 and 3% OV-17 as stationary phases. A compound was considered identified if it had the same mass spectrum and g.l.c. behaviour as the reference compound.

Radioactivity measurements. Radioactivity was assayed in a Packard scintillation spectrometer, model 3003. The scintillation fluid contained $3 g$ of 2,5-diphenyloxazole (PPO) and $150 mg$ of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (dimethyl-POPOP) per litre of toluene. All samples were counted for radioactivity for at least 10 min.

Statistical analysis. Student's *t* test was used.

Results

Liver weight and microsomal protein

The liver weights of the clofibrate-treated rats ($13.7 \pm 1.5 g$, mean $\pm$ s.d.) were about 15% higher than those ($11.9 \pm 0.9 g$) of the control rats ($P < 0.05$). During treatment with clofibrate the protein concentration of the microsomal fraction increased from 3.01 ± 0.30 to $3.95 \pm 0.24 mg/ml$ ($P < 0.001$).

Incubation of $[4-^{14}C]$ progesterone

The conversion of substrate was about $10-15\%$ in both control and clofibrate-treated rats. On t.l.c. of extracts of the incubated material the labelled products separated into nine zones (Plate 1).

The distribution of the radioactivity on the chromatographic plate, the relative retention times of the steroid during g.l.c. and the characteristic peaks on the mass spectra are shown in Table 1. No steroids were detected in zones 1 and 6. Unmetabolized progesterone was recovered in zone 8. The metabolites identified were as follows: 16α -hydroxypregn-4-ene-3,20-dione and 7α -hydroxypregn-4-ene-3,20-dione (zone 2), $2\alpha,3\alpha$ -dihydroxy- 5α -pregnan-20-one and $2\beta,3\alpha$ -dihydroxy- 5α -pregnan-20-one (zone 3), 6β -hydroxypregn-4-ene-3,20-dione (zone 4), 20α -hydroxypregn-4-en-3-one and 20β -hydroxypregn-4-en-3-one (zone 5), 3β -hydroxy- 5α -pregnan-20-one (zone 7) and 5α -pregnane-3,20-dione (zone 9). The ratio between the steroids within the various zones was determined by g.l.c.

In the control rats the hydroxylated derivatives 16α - and 6β -hydroxypregn-4-ene-3,20-dione and $2\alpha,3\alpha$ -dihydroxy- 5α -pregnan-20-one constituted about 14, 12 and 7% respectively of the metabolites formed. Only trace amounts of $2\beta,3\alpha$ -dihydroxy- 5α -pregnan-20-one were detected. The amounts of the reduced derivatives 20α -hydroxypregn-4-en-3-one, 20β -hydroxypregn-4-en-3-one, 3β -hydroxy- 5α -pregnan-20-one and 5α -pregnane-3,20-dione corresponded to 6, 10, 11 and 21% respectively of the total metabolites formed.

Clofibrate treatment influenced the metabolism of progesterone in several ways (Table 2). 6β -Hydroxylation was enhanced about twice and 7α -hydroxyl-

EXPLANATION OF PLATE I

T.l.c. of labelled material recovered after incubation of [4-¹⁴C]progesterone with the microsomal fraction of liver homogenates from control and clofibrate-treated rats fortified with NADPH

The solvent system used was benzene-ethyl acetate (3:1, v/v). The following compounds were identified in the radioactive zones: zone 2, 16 α -hydroxypregn-4-ene-3,20-dione and 7 α -hydroxypregn-4-ene-3,20-dione (this metabolite was only found in clofibrate-treated rats); zone 3, 2 α ,3 α -dihydroxy-5 α -pregnan-20-one; zone 4, 6 β -hydroxypregn-4-ene-3,20-dione; zone 5, 20 α - and 20 β -hydroxypregn-4-en-3-one; zone 7, 3 β -hydroxy-5 α -pregnan-20-one; zone 8, progesterone; zone 9, 5 α -pregnane-3,20-dione.

EXPLANATION OF PLATE 2

T.l.c. of labelled material recovered after incubation of [4-¹⁴C]oestradiol with the microsomal fraction of liver homogenates from control and clofibrate-treated rats fortified with NADPH

The solvent system used was cyclohexane-ethyl acetate-ethanol (10:9:1, by vol.). The following compounds were identified in the radioactive zones: zone 3, oestriol; zone 4, oestra-1,3,5(10)-triene-3,7 α ,17 β -triol, oestra-1,3,5(10)-triene-3,6 α ,17 β -triol and oestra-1,3,5(10)-triene-3,6 β ,17 β -triol; zone 7, oestradiol; zone 8, oestrone.

Table 1. Gas-liquid-chromatographic and mass-spectrometric characteristics of isolated metabolites of [4-¹⁴C]progesterone t_R = relative retention time (5 α -cholestane = 1.00); M^+ = molecular ion.

T.l.c. zone	Radioactivity recovered (%)		t_R	Characteristic mass-spectrometric peaks (m/e)	Compound isolated	Cf. with reference
	Control rats	Clofibrate-treated rats (on SE-30)				
1	0.69	0.56	—	—	—	—
2	1.32	1.95	1.30	402 (M^+), 186, 172 (base peak), 159, 157, 109, 96	16 α -Hydroxypregn-4-ene-3,20-dione	Lisboa & Gustafsson (1968)
			1.12	402 (M^+), 312 ($M^+ - 90$), 279 ($M^+ - 123$), 224 ($M^+ - 178$)	7 α -Hydroxypregn-4-ene-3,20-dione	Einarsson & Gustafsson (1973)
3	0.66	0.46	1.02	478 (M^+), 463 ($M^+ - 15$), 299 ($M^+ - [90 + 89]$, base peak), 143, 142, 129	2 α , 3 α -Dihydroxy-5 α -pregnan-20-one	Einarsson & Gustafsson (1973)
			0.86	478 (M^+), 463 ($M^+ - 15$), 299 ($M^+ - [90 + 89]$, base peak), 143, 142, 129	2 β , 3 α -Dihydroxy-5 α -pregnan-20-one	Eriksson & Gustafsson (1970)
4	1.21	3.57	1.07	402 (M^+), 387 ($M^+ - 15$), 346 ($M^+ - 56$, base peak)	6 β -Hydroxypregn-4-ene-3,20-dione	Einarsson & Gustafsson (1973)
5	1.59	0.76	1.16	388 (M^+), 117 (base peak)	20 β -Hydroxypregn-4-en-3-one	Lisboa & Gustafsson (1969)
			1.24	388 (M^+), 117 (base peak)	20 α -Hydroxypregn-4-en-3-one	
6	1.19	1.15	—	—	—	—
7	1.04	0.88	0.84	390 (M^+), 375 ($M - 15$, base peak)	3 β -Hydroxy-5 α -pregnan-20-one	Eriksson <i>et al.</i> (1968)
8	89.88	87.24	0.90	314 (M^+ , base peak)	Progesterone	
9	2.05	3.00	0.80	316 (M^+ , base peak)	5 α -Pregnane-3,20-dione	

Table 2. Metabolism of progesterone in liver microsomal fractions from control and clofibrate-treated rats

The conversions are calculated from the amounts of radioactivity in the different zones after t.l.c. and from peak-area determinations after g.l.c. The values listed are the means $\pm$ s.d. of experiments with six male rats. N.S. = no significance.

Compound	nmol of metabolite formed/10min per mg of protein		Significance of differences
	Control rats	Clofibrate-treated rats	
16 α -Hydroxypregn-4-ene-3,20-dione	5.02 $\pm$ 0.64	4.78 $\pm$ 0.36	N.S.
7 α -Hydroxypregn-4-ene-3,20-dione	—	1.36 $\pm$ 0.10	
2 α , 3 α -Dihydroxy-5 α -pregnan-20-one	5.61 $\pm$ 1.32	3.32 $\pm$ 0.24	$P < 0.005$
6 β -Hydroxypregn-4-ene-3,20-dione	6.43 $\pm$ 0.98	14.29 $\pm$ 3.08	$P < 0.001$
20 β -Hydroxypregn-4-en-3-one	5.32 $\pm$ 0.47	0.85 $\pm$ 0.11	$P < 0.001$
20 α -Hydroxypregn-4-en-3-one	3.15 $\pm$ 0.28	2.21 $\pm$ 0.27	$P < 0.001$
3 β -Hydroxy-5 α -pregnan-20-one	5.67 $\pm$ 2.64	3.55 $\pm$ 0.39	$P < 0.10$
5 α -Pregnane-3,20-dione	11.19 $\pm$ 5.15	12.02 $\pm$ 3.39	N.S.
Total 5 α -reduced metabolites	22.47 $\pm$ 8.90	18.89 $\pm$ 3.61	N.S.

ation (not measurable in the control rats) became a significant metabolic pathway. The 2 α -hydroxylation, and 20 α - and 20 β -hydroxysteroid reductions decreased, whereas 16 α -hydroxylation, 5 α -reduction

(measured as the sum of synthesized 5 α -pregnane-3,20-dione, 3 β -hydroxy-5 α -pregnan-20-one and 2 α , 3 α -dihydroxy-5 α -pregnan-20-one) and 3 β -hydroxysteroid reduction were unaffected by treatment.

Incubations of [4-¹⁴C]oestradiol

The conversion of substrate was about 20–25% in both control and clofibrate-treated animals. Labelled products were recovered in eight zones on the chromatographic plate (Plate 2).

No steroids were identified in material eluted from zones 1 and 2 (Table 3). The silyl ether of the steroid in zone 3 yielded a molecular ion at *m/e* 504, which is compatible with a hydroxylated derivative of oestradiol. Peaks were also present at *m/e* 414 ($M^+ - 90$), 324 ($M^+ - 2 \times 90$), 386, 360, 345, 311, 297 and 270, i.e. a cracking pattern characteristic of the silyl ether of an oestra-1,3,5(10)-triene-3,16,17-triol (Adlercreutz & Luukkainen, 1968). The silyl ether of the metabolite in zone 3 had the same retention time as that of the corresponding derivative oestra-1,3,5(10)-triene-3,16 α ,17 β -triol, i.e. oestriol (t_R 1.20 and 1.23 on columns coated with SE-30 and OV-17 respectively). The retention times were different from those of the silyl ethers of oestra-1,3,5(10)-triene-3,16 β ,17 β -triol (t_R 1.30, both columns), oestra-1,3,5(10)-triene-3,16 α ,17 α -triol (t_R 1.15 and 1.16 on SE-30 and OV-17 respectively) and oestra-1,3,5(10)-triene-3,16 β ,17 α -triol (t_R 1.03, SE-30, cf. Eriksson *et al.*, 1972). Based on these findings the steroid recovered in zone 3 was tentatively identified as oestra-1,3,5(10)-triene-3,16 α ,17 β -triol.

Zone 4 contained several hydroxylated metabolites of oestradiol. One of the compounds was present only in extracts from clofibrate-treated rats. The mass spectrum of the silyl ether of this compound showed peaks at *m/e* 504 (molecular ion), 414 ($M^+ - 90$, base peak), 324 ($M^+ - 2 \times 90$), 309 [$M^+ - (2 \times 90 + 15)$], 298, 283 and 230. This compound was identified as oestra-1,3,5(10)-triene-3,7 α ,17 β -triol by comparison with the mass spectrum and gas-liquid chromatographic behaviour of the silyl ether of the appropriate reference substance.

In addition to the 7 α -hydroxylated metabolite of oestradiol zone 4 contained two other metabolites formed in both treated and untreated rats. The silyl ethers of these compounds gave mass spectra identical with those of oestra-1,3,5(10)-triene-3,6 β ,17 β -triol and oestra-1,3,5(10)-triene-3,6 α ,17 β -triol respectively. The close similarities in g.l.c. behaviour between the investigated and the reference compounds further supported the identifications. The ratios between the 6 β -, 6 α - and 7 α -hydroxylated metabolites of oestradiol eluted from zone 4 were determined by g.l.c.

No steroids were detected in zones 5 and 6. Zone 7 contained oestradiol and zone 8 oestrone. In the control animals the various metabolites accounted for the following percentages of the total amount of

Table 3. Gas-liquid-chromatographic and mass-spectrometric characteristics of isolated metabolites of [4-¹⁴C]oestradiol

t_R = relative retention time (5 α -cholestane = 1.00); M^+ = molecular ion.

T.l.c. zone	Radioactivity recovered (%)		t_R		Characteristic mass-spectrometric peaks (<i>m/e</i>)	Compound isolated	Cf. with reference
	Control rats	Clofibrate-treated rats	On SE-30	On OV-17			
1	5.88	5.28	—	—	—	—	
2	0.90	0.72	—	—	—	—	
3	1.96	1.28	1.20	1.23	504 (M^+ , base peak), 414 ($M^+ - 90$), 386, 360, 345, 324 ($M^+ - 2 \times 90$), 311, 297, 270	Oestriol	Adlercreutz & Luukkainen (1968)
4	1.17	3.36	0.72	0.67	504 (M^+), 414 ($M^+ - 90$, base peak), 324 ($M^+ - 2 \times 90$), 309, 298, 283, 230	Oestra-1,3,5(10)-triene-3,7 α ,17 β -triol	
			0.88	0.86	504 (M^+), 414 ($M^+ - 90$, base peak), 324 ($M^+ - 2 \times 90$), 309, 295, 283, 242, 229, 216	Oestra-1,3,5(10)-triene-3,6 β ,17 β -triol	Adlercreutz & Luukkainen (1968)
			0.96	0.95	504 (M^+), 414 ($M^+ - 90$, base peak), 324, ($M^+ - 2 \times 90$), 309, 295, 283, 229, 216	Oestra-1,3,5(10)-triene-3,6 α ,17 β -triol	Adlercreutz & Luukkainen (1968)
5	1.04	1.93	—	—	—	—	
6	1.37	2.32	—	—	—	—	
7	80.84	76.43	0.67	—	416 (M^+ , base peak), 285, 244, 232, 218, 129	Oestradiol	
8	4.36	5.64	0.54	—	342 (M^+ , base peak), 314 ($M^+ - 28$), 286 ($M^+ - 56$), 257 ($M^+ - 85$), 244, 231, 218	Oestrone	

Table 4. *Metabolism of [4-¹⁴C]oestradiol in liver microsomal fractions from control and clofibrate-treated rats*

The conversions are calculated from the amounts of radioactivity in the different zones after t.l.c. and from peak-area determinations after g.l.c. The values listed are the means $\pm$ s.d. of experiments with six male rats. N.S. = no significance.

Compound	nmol of metabolite formed/10 min per mg of protein		Significance of differences
	Control rats	Clofibrate-treated rats	
Oestrinol	3.98 $\pm$ 0.99	1.94 $\pm$ 0.20	$P < 0.001$
Oestra-1,3,5(10)-triene-3,7 α ,17 β -triol	—	2.28 $\pm$ 0.30	
Oestra-1,3,5(10)-triene-3,6 β ,17 β -triol	1.76 $\pm$ 0.15	0.76 $\pm$ 0.10	$P < 0.001$
Oestra-1,3,5(10)-triene-3,6 α ,17 β -triol	0.59 $\pm$ 0.05	2.05 $\pm$ 0.27	$P < 0.001$
Oestrone	8.76 $\pm$ 1.03	8.56 $\pm$ 1.04	N.S.

metabolites formed: 16 α -hydroxylated oestradiol 12%, 6 β -hydroxylated oestradiol 5%, 6 α -hydroxylated oestradiol 2% and oestrone 26%.

The enzyme systems participating in the liver metabolism of oestradiol were affected in different ways by clofibrate treatment (Table 4). 6 α -Hydroxylation was stimulated more than three times. 7 α -Hydroxylation, which was not observed in control animals, became the most important hydroxylation reaction studied after clofibrate treatment, whereas 6 β - and 16 α -hydroxylation decreased by a factor of about two. 17 β -Hydroxysteroid oxidation, as reflected by the formation of oestrone, was not affected by clofibrate treatment.

Discussion

Several studies indicate that clofibrate belongs to the group of drugs that stimulates the activity of a variety of hepatic microsomal drug-metabolizing enzymes (Best & Duncan, 1964; Hess *et al.*, 1965; Salvador *et al.*, 1970). Clofibrate influences the metabolism of androstenedione in the rat liver by enhancing all hydroxylation reactions and the activity of 17 β -hydroxy steroid reductase. Other oxidoreductase activities were only slightly affected. In view of its hypolipidaemic effects it is interesting that clofibrate does not influence the 7 α -hydroxylation of cholesterol, i.e. the rate-limiting step in the conversion of this sterol into its main degradation products, the bile acids (Einarsson *et al.*, 1973).

The present study demonstrates that clofibrate causes changes of the pattern of products formed from two other steroid hormones. In the rat liver it induces 7 α -hydroxylation of both progesterone and oestradiol, which are metabolic pathways not encountered in the control animals. The former hormone is also affected to a higher extent by 6 β -hydroxylation, whereas the 2 α -hydroxylation and the 20 α - and the 20 β -hydroxy steroid reductions decrease. The 16 α - and the 6 β -hydroxylated derivatives are the major metabolites of oestradiol in the control rats,

whereas the 16 α -, 7 α - and 6 α -hydroxylated metabolites dominate after treatment with clofibrate.

Lu & Coon (1968), Lu *et al.* (1969, 1972) and Strobel *et al.* (1970) have been able to resolve and reconstitute the drug-metabolizing enzyme system of the liver microsomal fraction. Cytochrome *P*-450, an NADPH-dependent reductase and phosphatidylcholine were the three components isolated. Lu *et al.* (1973) used the reconstituted hydroxylation system to show that the cytochrome *P*-450 fractions from control and phenobarbital-treated rats have different substrate specificity. The fractions from both groups of animals were about equally active in the hydroxylation of testosterone at positions 6 β and 7 α . When compared with control rats this fraction from phenobarbital-treated rats was much more efficient in 16 α -hydroxylation of testosterone. It appears then that the cytochrome *P*-450 fractions contain material with different catalytic activity. The study of Lu *et al.* (1973) might explain our results. Thus it seems that the cytochrome *P*-450 fractions from control and clofibrate-treated rats also have different substrate specificity.

Previous reports (Salvador *et al.*, 1970; Einarsson *et al.*, 1973) and the present study have shown that clofibrate influences the metabolism of various steroid hormones in rat liver. As this drug is a commonly used hypolipidaemic agent it would be of great interest to study further its influence on the steroid metabolism in man.

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