The Metabolism of Steroids in the Fatty Liver Induced by
Orotic Acid Feeding

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1. The metabolism of 4-[4-14C]androstene-3,17-dione, 4-[4-14C]pregnene-3,20-dione,
5α-[4-14C]androstane-3α,17β-diol, [4-14C]cholesterol, 7α-hydroxy-4-[6β-3H]cholesten-
3-one, 5β-[7β-3H]cholestan-3α,7α-diol and [3H]lithocholic acid was studied in the
microsomal fraction of livers from control and orotic acid-treated male rats. 2. As a result
of the treatment the orotic acid-fed rats had fatty livers and subnormal concentrations of
cholesterol and triglycerides in serum. 3. The 6β- and 7α-hydroxylation of 4-androstene-
3,17-dione, the 2α-, 2β- and 18-hydroxylation of 5α-androstane-3α,17β-diol, and the
5α-reduction of 4-androstene-3,17-dione and 4-pregnen-3,20-dione were decreased by
40–50% in orotic acid-fed rats. Other oxidative and reductive reactions of the steroid
hormones were not significantly affected. 4. The 12α-hydroxylation of 7α-hydroxy-4-
cholesten-3-one was decreased by about 50%, whereas the 7α-hydroxylation of cholesterol
and the 26-hydroxylation of 5β-cholestan-3α,7α-diol were not significantly decreased.
The 6β-hydroxylation of lithocholic acid was stimulated by 40%. 5. The results are dis-
cussed in relation to present knowledge of the hepatic drug-metabolizing enzymes and to
the recent findings of an abnormal bile acid metabolism in liver disease.

The most common response of the injured liver is
an abnormal accumulation of fat in the parenchymal
cells. Indeed, various degrees of fatty infiltration have
been found in association with many disease states in
man, and there are many agents and means by which a
fatty liver can be induced in experimental animals.
Many agents seem to act via the same overall mecha-
nism, which makes it difficult to differentiate between
effects related to the specific agent and effects common
to the group of agents (Farber, 1967; Witting, 1972).
Ingestion of orotic acid in the rat is known to result in
an experimental model characterized by a fatty liver
and an almost complete inhibition of β-lipo-
protein formation in and/or secretion from the liver
(Standerfer & Handler, 1955; Handschumacher et al.,
1960; Windmueller, 1964; Novikoff et al., 1966). The
metabolic abnormalities associated with orotic acid
feeding are not severe and animals have been main-
tained on such a diet for up to 250 days without major
untoward effects (Creasey et al., 1961).

Orotic acid treatment of male rats is reported to
result in a decrease in the liver content of cytochrome
P-450 (Holtzman & Gillette, 1969), but it has not been
fully elucidated to what extent the various hydroxy-
lase activities in the liver microsomal fraction are

influenced (Rubin, 1965; Holtzman & Gillette, 1969;
Witting, 1972). We decided to find out whether the
diminished amount of cytochrome P-450 in the fatty
liver of orotic acid-treated rats would result in a
common decrease in the activity of and/or in an altered
substrate specificity of the microsomal hydroxylase
systems. In the present paper we have studied the
metabolism of some steroid hormones (androstene-
dione, progesterone and 5α-androstane-3α,17β-
diol), sterols (cholesterol, 7α-hydroxy-4-cholesten-
3-one and 5β-cholestan-3α,7α-diol) and one bile
acid (lithocholic acid) in liver microsomal fractions
from control and orotic acid-treated male rats.

Experimental

Materials

Radioactive steroids. 4-[4-14C]Androstene-3,17-
dione (specific radioactivity 1.7μCi/mg), 4-[4-14C]-
pregnen-3,20-dione (specific radioactivity 2μCi/mg)
and 4-[4-14C]cholesterol (specific radioactivity
145μCi/mg) were from Radiochemical Centre,
Amersham, Bucks., U.K. Before use, [4-14C]chole-
sterol was purified by chromatography on a column
(0.8cm × 6.0cm) of Al₂O₃, grade III (Woelm, Esch-
wege, Germany). 5α-[4-14C]Androstane-3α,17β-diol
(specific radioactivity 3μCi/mg) was synthesized as

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described by Berg & Gustafsson (1973). 7α-Hydroxy-4-[6β-3H]cholesten-3-one (specific radioactivity 6.7 μCi/mg) was prepared as described by Björkhem (1969). 5β-[7β-3H]Cholestan-3α,7α-diol (specific radioactivity 16.7 μCi/mg) was synthesized as described by Björkhem & Gustafsson (1973). [3H]-Lithocholic acid (specific radioactivity 10 μCi/mg) was from New England Nuclear Corp., Boston, MA, U.S.A. Before use, the radiochemical purity of the labelled compounds was tested.

Reference compounds. Several of the reference compounds used in this investigation to identify metabolites of 4-androstene-3,17-dione, 4-pregnen-3,20-dione and 5α-androstane-3α,17β-diol were generous gifts from colleagues: Dr. K. Babcock, Upjohn Co., Kalamazoo, MI, U.S.A. (4-androstene-3,17-dione, 4,5-pregnen-3,20-dione, 3α- and 3β-hydroxy-5α-androstan-17-one, 7α-hydroxy-4-androstene-3,17-dione, 20α- and 20β-hydroxy-4-pregnen-3-one and 6β- and 16α-hydroxy-4-pregnen-3,20-dione); Dr. W. Klyne, Medical Research Council Steroid Reference Collection, London NW3 7ST, U.K. (6β- and 16α-hydroxy-4-androstene-3,17-dione); Dr. M. Wolff, San Francisco, CA, U.S.A. (5α-androstane-2β,3α,17β-triol); Dr. P. N. Rao, San Antonio, TX, U.S.A. (2α-hydroxy-4-pregnen-3,20-dione). 5α-Androstanol-3α( and 3β),17β-diol and 17β-hydroxy-5α-androstan-3-one were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). 3α-Hydroxy-5α-pregnene-20-one and 3β-hydroxy-5α-pregnene-20-one were from Ikhahparm (Ramat-Gan, P.O. Box 31, Israel).

Casein was manufactured by E. Merck, Darmstadt, Germany. Orotic acid (monosodium salt), NADP+, NADPH, dl-isocitric acid (trisodium salt) and isocitrate dehydrogenase (type IV) were from Sigma. Vitamins (vitamin diet fortification mixture) and salts (Hawk Oser salt mixture no. 3) were from Nutritional Biochemicals Corp., Cleveland, OH, U.S.A.

Methods

Animals and preparation of homogenates. Adult male Sprague-Dawley rats weighing about 150 g were used. They were divided into two groups each of 12 animals. The first group received a semisynthetic diet containing (w/w): 70% glucose; 18% casein; 5% corn oil; 2% vitamins; 5% salts. The second group was fed on the same semisynthetic diet supplemented with 1% orotic acid. This food and water were given ad libitum for 10 days. After starvation overnight, six rats from each group were anaesthetized with diethyl ether. Blood drawn from the abdominal aorta was analysed for plasma cholesterol and triglycerides. EDTA (0.1 M, 25 μl/ml of blood) was added as anticoagulant. The remaining six rats in each group were killed by a blow on the neck. The livers were excised, immediately chilled on ice, blotted, weighed and minced into small pieces. A 20% (w/v) homogenate was prepared in a modified Bucher medium (Bergström & Gloor, 1955) (0.1 M, pH 7.4), by using a Potter-Elvehjem homogenizer equipped with a loosely fitting pestle. A portion of this homogenate was refixed at 80°C for 1 h with 5 vol. of chloroform/methanol (1:1, v/v), and the lipid extract was used for analysis of cholesterol and triglycerides. The remaining part of the homogenate was centrifuged for 15 min at 4°C and 20000 g and the supernatant was spun at 4°C and 100000 g for 70 min. The microsomal pellet was resuspended with Bucher medium to the same volume as before ultracentrifugation and gently rehomogenized.

The cholesterol concentrations of plasma, liver extracts and of the 20000 g supernatant fluid were measured by the method of Hanel & Dam (1955). Plasma and liver triglycerides were determined as described by Laurell (1966). The protein concentrations of the 20000 g supernatant fraction and of the microsomal fraction were assayed by a modification of Lowry's technique (Lowry et al., 1951), with albumin as standard.

Incubations. 4-[4-[4-14C]Androstene-3,17-dione (300 μg), dissolved in 50 μl of acetone, was added to a mixture of 0.3 ml of microsomal fraction and 2.7 ml of Bucher medium supplemented with 3 μmol of NADPH. 4-[4-[4-14C]Pregnenolone-3,20-dione (250 μg), dissolved in 50 μl of acetone, was mixed with 0.5 ml of microsomal fraction and 2.5 ml of Bucher medium supplemented with 3 μmol of NADPH. 5α-[4-[4-14C]-Androstanol-3α,17β-diol (200 μg), in 50 μl of acetone, was added to 2.5 ml of the microsomal fraction, 1.5 ml of Bucher medium, 0.03 μmol of MnCl2, 3 μmol of NADP+, 12.5 μmol of isocitrate and 10 μl of isocitrate dehydrogenase solution (36 units/ml). Incubations were carried out for 10 min at 37°C and were terminated by the addition of 20 vol. of chloroform/methanol (2:1, v/v). The conditions under which the incubations were performed have been shown to give reactions that are linear with respect to enzyme concentration and time. The precipitate was filtered off, and 0.2 vol. of 0.9% NaCl was added. The chloroform phase was collected, and the solvent was evaporated. The residue was further analysed by t.l.c. (pre-coated silica-gel plates; 250 μm thick; Merck, Darmstadt, Germany). The extracts from the incubations with 4-[4-[4-14C]Androstene-3,17-dione were developed in chloroform/ethyl acetate (4:1, v/v) and the extracts from the incubations with 4-[4-[4-14C]-Pregnenolone-3,20-dione in benzene/ethyl acetate (3:1, v/v). The organic material recovered after incubations with 5α-[4-[4-14C]-Androstanol-3α,17β-diol was developed in the solvent system ethyl acetate/cyclohexane (3:2, v/v). The t.l.c. plates were subjected to radioautography with an exposure time of about 10 days. The radioactivity zones on the t.l.c. plates traced from the X-ray films were scraped off, eluted with methanol and measured for radioactivity.
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[4-14C]Cholesterol (13 µg), dissolved in 50 µl of acetone, was incubated for 20 min at 37°C with 3 ml of the 20000g supernatant fraction. The incubation was terminated by the addition of 20 vol. of chloroform/methanol (2:1, v/v). Analysis of the chloroform/methanol extract was performed as described previously (Danielsson et al., 1967). 7α-Hydroxy-4-[6β-3H]cholesten-3-one (50 µg), dissolved in 50 µl of acetone, was added to a mixture of 2 ml of microsomal fraction and 1 ml of Bucher medium supplemented with 3 µmol of NADPH. The incubation was carried out for 12 min at 37°C. The incubation was terminated as described above and the organic extract was analysed as described by Björkhem & Einarsson (1970).

[3H]Lithocholic acid (50 µg), dissolved in 50 µl of aq. 95% (v/v) ethanol, was added to 1 ml of microsomal fraction and 2 ml of Bucher medium supplemented with 3 µmol of NADPH. The incubation was carried out for 20 min at 37°C and was terminated with an equal volume of 95% ethanol. Further analysis was performed as reported previously (Einarsson & Gustafsson, 1974).

Gas–liquid chromatography–mass spectrometry analysis of metabolites of 4-[4-14C]androstene-3,17-dione, 4-[4-14C]pregnen-3,20-dione and 5α-[4-14C]-androstan-3α,17β-diol. The methanol extracts of radioactive zones pooled from the t.l.c. plates were evaporated to dryness, and all material left after radioactivity measurements was trimethylsilylated and analysed by g.l.c.–mass spectrometry (LKB 9000 instrument) with the use of a 1.5% SE-30 column (Shackleton & Gustafsson, 1971). Mass spectra were recorded on magnetic tape with the incremental mode of operation and were then processed in an IBM 1800 computer (Reimendal & Sjövall, 1972). A compound was considered identified only if it had the same mass spectrum and g.l.c. behaviour as the reference compound.

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

Recovery. The recovery of metabolites from the entire procedure of extraction and elution from the t.l.c. plate was determined to be 85–90%.

Statistical analysis. Student's t test was used.

Results

Body weight, liver weight, cholesterol, triglycerides and protein

Compared with the controls, the orotic acid-fed rats were less heavy, but their liver weights had increased by about 30% (Table 1). Orotic acid feeding also resulted in an increase in the liver triglycerides (5-fold) and cholesterol (2-fold). The two groups of animals did not differ with regard to the cholesterol concentration in the 20000g supernatant fluid or the amount of protein in the microsomal fraction, but the amount of protein in the 20000g supernatant was slightly decreased in the orotic acid-fed animals.

The concentrations of serum cholesterol and triglycerides in the control rats averaged 81±6 (mean±s.E.M.) and 53±7 mg/100 ml respectively. The corresponding values for the orotic acid-fed rats were significantly lower (P<0.02) and amounted to 61±4 (cholesterol) and 20±6 (triglyceride) mg/100 ml.

Incubations with 4-[4-14C]androstene-3,17-dione

The conversion of substrate averaged about 11% in the control rats. On the basis of their chromatographic and mass spectrographic characteristics (Einarsson et al., 1974) the various metabolites were identified as 6β-, 7α- and 16α-hydroxy-4-androstene-3,17-dione, 17β-hydroxy-4-androsten-3-one, 3α- and 3β-hydroxy-5α-androstan-17-one and 5α-androstan-3,17-dione. Individual values were not determined for the 3α- and 3β-hydroxy steroid reduction products as the corresponding derivatives were not separated in the t.l.c.

<table>
<thead>
<tr>
<th>Table 1. Body weight, liver weight and concentrations of lipids and protein in the liver of control and orotic acid-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>The values listed are the means±s.E.M. of experiments with six male rats. N.S., No significance (P&gt;0.05).</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
</tr>
<tr>
<td>Liver weight (g/100 g of rat)</td>
</tr>
<tr>
<td>Liver triglycerides (mg/g)</td>
</tr>
<tr>
<td>Liver cholesterol (mg/g)</td>
</tr>
<tr>
<td>Cholesterol concentration of the 20000g supernatant fraction (mg/ml)</td>
</tr>
<tr>
<td>Protein concentration of the 20000g supernatant fraction (mg/ml)</td>
</tr>
<tr>
<td>Protein concentration of the microsomal fraction (mg/ml)</td>
</tr>
</tbody>
</table>

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system used. The identified metabolites constituted about 90% of the total conversion products formed.

Compared with the controls, the orotic acid-fed rats showed a 50% decrease in the 6β- and 7α-hydroxylation reactions as well as in 3-hydroxy steroid reduction and 5α-reduction as evidenced by the combined formation of 5α-androstane-3,17-dione and 3α- and 3β-hydroxy-5α-androstan-17-one (Table 2). A similar tendency was also observed for the 16α-hydroxylation reaction, whereas the 17β-hydroxy steroid reduction was unaffected by orotic acid treatment.

Incubations with 4-[4-14C]pregnene-3,20-dione

The total conversion of this material when incubated with the microsomal liver fraction was 13–14% in the control rats. About 75% of the radioactivity not associated with progesterone was recovered in the various metabolites (Einarsson et al., 1973) shown in Table 3. The parent compound had been subjected to 2α-, 6β- and 16α-hydroxylation, 3β- and 20β-hydroxy steroid reduction, and 5α-reduction. The 20-hydroxy-4-pregnen-3-one fraction was most probably made up of both the 20α- and the 20β-isomers. The 5α-reduction (measured as the combined amount of 5α-pregnan-3,20-dione, 3β-hydroxy-5α-pregnane-20-one and 2α,3β-dihydroxy-5α-pregnane-20-one) was the major reaction, contributing in the controls to more than 50% of the metabolites formed. The effect of orotic acid feeding was reflected in a 50% decrease in 5α-reduction, whereas other reactions occurred at approximately the same rate in both groups of animals (Table 3).

Incubations with 5α-[4-14C]androstane-3α,17β-diol

Approx. 12% of the incubated material in the controls was converted into various polar products (Table 4), which were all identified as described by Berg & Gustafsson (1973). The major one in both groups was 5α-androstane-2α,3α,17β-triol. Other metabolites were 2β-, 7α-, 7β- and 18-hydroxylated derivatives of the parent compound. After treatment with orotic acid, the activities of the 2α-, 2β- and 18-hydroxylases decreased about 40%. The 7α- and 7β-hydroxylations were not significantly decreased (Table 4).

Incubations with [4-14C]cholesterol

The main products formed under the present conditions have previously been identified as 5-cholesten-3β,7α-diol, 7α-hydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one (Danielsson & Einarsson, 1966). The pattern of metabolites formed in the present experiments was the same in both groups of animals. The conversion of cholesterol into 7α-hydroxylated products was about 0.3% (0.079 ± 0.008 nmol/mg of protein) in the control rats and almost the same in the orotic acid-fed animals (Table 5).

Incubations with 7α-hydroxy-4-[6β-3H]cholesten-3-one

The main metabolites formed from 7α-hydroxy-4-cholesten-3-one in the presence of the microsomal liver fraction were 7α,12α-dihydroxy-4-cholesten-3-one, 7α-hydroxy-5α-cholestan-3-one and 5α-cholestan-3β,7α-diol (Danielsson & Einarsson, 1966; Björkhem & Einarsson, 1970). In the control rats,

Table 2. Metabolism of 4-[4-14C]androstene-3,17-dione in the hepatic microsomal fraction of control and orotic acid-treated rats

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Identified metabolites</th>
<th>Control</th>
<th>Orotic acid-treated</th>
<th>Significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>6β-Hydroxylation</td>
<td>6β-Hydroxy-4-androstene-3,17-dione</td>
<td>21.2 ± 2.3</td>
<td>10.9 ± 0.5</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>7α-Hydroxylation</td>
<td>7α-Hydroxy-4-androstene-3,17-dione</td>
<td>4.95 ± 0.81</td>
<td>2.76 ± 0.11</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>16α-Hydroxylation</td>
<td>16α-Hydroxy-4-androstene-3,17-dione</td>
<td>21.4 ± 4.7</td>
<td>13.2 ± 1.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>17β-Hydroxy steroid reduction</td>
<td>17β-Hydroxy-4-androsten-3-one</td>
<td>19.7 ± 1.7</td>
<td>20.9 ± 1.2</td>
<td>N.S.</td>
</tr>
<tr>
<td>3α-+3β-Hydroxy steroid reduction</td>
<td>3α- and 3β-Hydroxy-5α-androstan-17-one</td>
<td>15.5 ± 1.0</td>
<td>7.35 ± 0.26</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>5α-Reduction</td>
<td>5α-Androstone-3,17-dione, and 3α- and 3β-hydroxy-5α-androstan-17-one</td>
<td>78.4 ± 6.3</td>
<td>40.6 ± 3.4</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

The conversions are calculated from the amounts of radioactivity in the different zones after t.i.c. and from peak-area determinations after g.l.c. The values listed are the means ± S.E.M. of experiments with six male rats. N.S., No significance (P > 0.05).
Table 3. Metabolism of 4-[4-14C]pregnene-3,20-dione in the hepatic microsomal fraction of control and orotic acid-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±S.E.M. of experiments with six male rats. N.S., No significance (P>0.05).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Identified metabolites</th>
<th>Control</th>
<th>Orotic acid-treated</th>
<th>Significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>2α-Hydroxylation</td>
<td>2α-Hydroxy-4-pregnen-3,20-dione and 2α,3α-dihydroxy-5α-pregnan-20-one</td>
<td>7.26±1.50</td>
<td>4.78±0.38</td>
<td>N.S.</td>
</tr>
<tr>
<td>6β-Hydroxylation</td>
<td>6β-Hydroxy-4-pregnen-3,20-dione</td>
<td>8.46±0.85</td>
<td>6.22±0.35</td>
<td>N.S.</td>
</tr>
<tr>
<td>16α-Hydroxylation</td>
<td>16α-Hydroxy-4-pregnen-3,20-dione</td>
<td>5.18±0.64</td>
<td>4.60±0.33</td>
<td>N.S.</td>
</tr>
<tr>
<td>20α- + 20β-Hydroxy steroid reduction</td>
<td>20α- and 20β-Hydroxy-4-pregnen-3-one</td>
<td>3.96±0.28</td>
<td>3.56±0.18</td>
<td>N.S.</td>
</tr>
<tr>
<td>3β-Hydroxy steroid reduction</td>
<td>3β-Hydroxy-5α-pregnan-20-one</td>
<td>5.00±0.30</td>
<td>6.18±0.66</td>
<td>N.S.</td>
</tr>
<tr>
<td>5α-Reduction</td>
<td>5α-Pregnan-3,20-dione, 3β-hydroxy-5α-pregnan-20-one and 2α,3α-dihydroxy-5α-pregnan-20-one</td>
<td>44.0±2.6</td>
<td>20.9±1.3</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Metabolism of 5α-[4-14C]androstane-3α,17β-diol in the hepatic microsomal fraction of control and orotic acid-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±S.E.M. of experiments with six male rats. N.S., No significance (P>0.05).

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Identified metabolites</th>
<th>Control</th>
<th>Orotic acid-treated</th>
<th>Significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>2α-Hydroxylation</td>
<td>5α-Androstane-2α,3α,17β-triol</td>
<td>6.76±0.38</td>
<td>3.94±0.61</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>2β-Hydroxylation</td>
<td>5α-Androstane-2β,3α,17β-triol</td>
<td>2.11±0.19</td>
<td>1.31±0.17</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>7α-Hydroxylation</td>
<td>5α-Androstane-3α,7α,17β-triol</td>
<td>2.50±0.37</td>
<td>1.95±0.31</td>
<td>N.S.</td>
</tr>
<tr>
<td>7β-Hydroxylation</td>
<td>5α-Androstane-3α,7β,17β-triol</td>
<td>0.77±0.08</td>
<td>0.62±0.11</td>
<td>N.S.</td>
</tr>
<tr>
<td>18-Hydroxylation</td>
<td>5α-Androstane-3α,17β,18-triol and 5α-androstane-3β,17β,18-triol</td>
<td>2.30±0.30</td>
<td>1.28±0.18</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

6–7% (1.78±0.12 nmol/mg of protein) of the 7α-hydroxy-4-cholesten-3-one was converted into 7α,12α-dihydroxy-4-cholesten-3-one and 8–9% of the incubated radioactivity was recovered as 7α-hydroxy-5α-cholestan-3-one and 5α-cholestone-3β,7α-diol. The 12α-hydroxylation and the 5α-reduction were both decreased by about 50% in the orotic acid-fed rats (Table 5).

Incubations with 5β-[7β-3H]cholestone-3α,7α-diol

The main metabolite formed from lithocholic acid upon incubation with the microsomal fraction of rat liver homogenates is 3α,6β-dihydroxy-5β-cholanoic acid (Einarsson & Johansson, 1969; Einarsson & Gustafsson, 1974). The extent of 6β-hydroxylation in the present study was about 11% (6.1±0.7 nmol/mg of protein) in the control rats and about 15% in the
The oxidative metabolism of bile acids is important in the conversion of cholesterol into bile acids. This conversion is influenced by the presence of orotic acid in the diet. In a study by Windmueller et al. (1969), the conversion of cholesterol into bile acids was found to be decreased by orotic acid treatment. This finding was confirmed by another study (Danielsson et al., 1974), which showed a decrease in the conversion of cholesterol to bile acids in rats fed orotic acid.

### Table 5. Metabolism of [4-14C]cholesterol, 7α-hydroxy-4-[6β-3H]cholesten-3-one, 5β-[7β-3H]cholestane-3α,7α-diol and [3H]lithocholic acid in control and orotic acid-treated rats

The conversions are calculated from the amounts of radioactivity in different zones after t.l.c. N.S., No significance (P>0.05).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction</th>
<th>Control</th>
<th>Orotic acid-treated</th>
<th>Significance of differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>7α-Hydroxylation</td>
<td>0.079±0.008</td>
<td>0.066±0.009</td>
<td>N.S.</td>
</tr>
<tr>
<td>7α-Hydroxy-4-cholesten-3-one</td>
<td>12α-Hydroxylation</td>
<td>1.78±0.12</td>
<td>0.86±0.07</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5α-Reduction</td>
<td>2.27±0.24</td>
<td>1.26±0.16</td>
<td>P&lt;0.02</td>
</tr>
<tr>
<td>5β-Cholestane-3α,7α-diol</td>
<td>26-Hydroxylation</td>
<td>2.3±0.2</td>
<td>1.8±0.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>6β-Hydroxylation</td>
<td>6.1±0.7</td>
<td>8.5±0.4</td>
<td>P&lt;0.05</td>
</tr>
</tbody>
</table>

Animals fed with orotic acid and this difference was significant (P<0.05) (Table 5).

### Discussion

The present findings that the ingestion of a semi-synthetic diet containing 1% orotic acid results in an accumulation of triglycerides and cholesterol in the liver and a lower concentration of both lipids in serum are in agreement with previous reports (Standerfer & Handler, 1955; Handschumacher et al., 1960; Windmueller, 1964; Novikoff et al., 1966). Perfusion studies in rats have demonstrated that the lower concentrations of serum lipids are due to inability of the liver of orotic acid-fed rats to secrete and/or produce more than a fraction of the low-density lipoproteins excreted by normal livers (Windmueller & Levy, 1967). This defect does not seem to reflect an impaired synthesis of the protein constituents of the lipoproteins, since both low-density-lipoprotein and very-low-density-lipoprotein antigens have been isolated from the liver cells of orotic acid-treated animals (Pottenger & Getz, 1971).

As evidenced by the present study orotic acid feeding influenced the male rat liver metabolism of steroids in different and somewhat unpredictable ways. It led to a decreased activity of several hydroxylases involved in the hepatic metabolism of 4-androstene-3,17-dione (6β- and 7α-) and 5α-androstane-3α,17β-diol (2α-, 2β- and 18-) and of the 12α-hydroxylase involved in cholic acid synthesis. On the other hand it did not significantly affect the hydroxylations of 4-pregnen-3,20-dione, the 16α-hydroxylation of 4-androstene-3,17-dione and the 7α- and 7β-hydroxylation of 5α-androstane-3α,17β-diol. Neither were the 7α- and 26-hydroxylases, involved in bile acid biosynthesis, influenced by orotic acid treatment. The oxidative metabolism of steroid hormones in the liver is catalysed by a microsomal enzyme system, which also participates in the inactivation of drugs. Lu et al. (1972, 1973) have resolved this enzyme system and isolated a CO-binding haemoprotein (cytochrome P-450), and NADPH-dependent reductase and phosphatidylcholine as indispensable components. Using the reconstituted hydroxylation system from liver microsomal fraction, they also demonstrated that enzyme systems prepared from rats pretreated with a drug (phenobarbital) or a chemical agent (pregnenolone-16α-carbonitrile) showed different substrate specificities and further that these specificities reside in the cytochrome fraction. Among the bile acid hydroxylases, the 6β-hydroxylase seems to be similar to the drug-metabolizing enzymes. Although the hydroxylases involved in the conversion of cholesterol into bile acids seem to be more specific, they are all dependent on the activity of the cytochrome P-450 fraction (Björkhem & Daniellson, 1974). Since orotic acid feeding appears to result in a decrease in cytochrome P-450 in the male rat liver (Holtzman & Gillette 1969), it is possible that part of the enzymic changes recorded in the present study may be due to a diminished amount of the cytochrome. However, several hydroxylases were not affected by orotic acid treatment and the 6β-hydroxylase active on lithocholic acid was stimulated by about 40%, which indicates that not only had the bulk of cytochrome P-450 been diminished, but the properties of the cytochrome had also been altered. 5α-Reduction of 4-androstene-3,17-dione, 4-pregnen-3,20-dione and 7α-hydroxy-4-cholesten-3-one was decreased by about 50% in the orotic acid-fed rats. These enzymic changes cannot be ascribed to a smaller amount of cytochrome P-450, but may suggest a more general effect on the liver system.

The finding that the 12α-hydroxylase was more affected by liver damage than the 7α-hydroxylase (i.e., the enzyme conducting the rate-limiting step in the conversion of cholesterol into bile acids) and the 26-hydroxylase may have a bearing on the following observations in human subjects with portal liver cirrhosis. In patients with mild to moderate liver damage the total bile acid formation remains within...
normal limits. However, probably because of a defective 12α-hydroxylase, the formation of cholic acid is subnormal, whereas the synthesis of chenodeoxycholic acid tends to be correspondingly increased (Einarsson et al., 1975). With increasing degree of liver cirrhosis, the formation of cholic acid decreases gradually (Vlahcevic et al., 1972; McCormic et al., 1973).

As the liver concentration of cytochrome P-450 is decreased by about 50% in patients with severe hepatitis and cirrhosis (Schoene et al., 1972) and further, the demethylation rate of aminopyrine and p-nitroanisole is similarly decreased, the hepatic metabolism of steroid hormones might be expected to undergo changes in the damaged human liver.

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References
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