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S Skrede, … , G Hopen, O Fausa


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A Novel Pathway for Biosynthesis of Cholestanol with 7α-Hydroxylated C27-Steroids as Intermediates, and Its Importance for the Accumulation of Cholestanol in Cerebrotendinous Xanthomatosis

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Abstract
A mixture of 7α-3H- and 4-14C-labeled cholesterol was administered intravenously to rats. Cholesterol with 20-30% lower ratio between 3H and 14C than in cholesterol could be isolated from different organs. In a healthy human control, cholestanol isolated from feces had a 3H/14C ratio which was 28% lower than in administered cholesterol. Cholesterol and coprostanol reisolated in these experiments had the same ratio between 3H and 14C as in the precursor. A previously unknown pathway for formation of cholestanol, involving 7α-hydroxylated intermediates, may explain these results. Under normal conditions, this pathway is responsible for at most 30% of the cholestanol synthesized from cholesterol.

Intravenous administration of the 7α-3H- and 4-14C-labeled cholesterol to a patient with cerebrotendinous xanthomatosis (CTX) resulted in formation of cholestanol which had 70-75% lower 3H/14C ratio. It is concluded that the novel pathway involving 7α-hydroxylated intermediates is accelerated in patients with CTX. This acceleration may contribute essentially to the accumulation of cholestanol, which is a predominant feature of this disease.

7α-Hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one might be intermediates in the novel pathway to cholestanol. After intravenous administration of 7β-3H-labeled 7α-hydroxycholesterol in a patient with CTX, significant amounts of 3H were incorporated into plasma and fecal cholestanol. Only small amounts of 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one are excreted into the intestine, and we therefore conclude that the 7α-dehydroxylation step mainly occurs in the liver. In CTX, the synthesis of cholestanol may be accelerated because the concentrations of 7α-hydroxylated bile acid intermediates in the liver are increased. A possible mechanism for the conversion of a minor fraction of 7α-hydroxycholesterol into cholestanol is suggested.

Introduction
Cerebrotendinous xanthomatosis (CTX) is a rare inherited disease. The most important symptoms are caused by increased biosynthesis of cholesterol and cholestanol, with depositions in brain, cerebellum, and tendons.

In CTX, the capacity to convert cholesterol to bile acids is impaired. The incomplete oxidation of the cholesterol side chain leads to the accumulation of abnormal tetra- and penta-hydroxylated bile alcohols. Feedback control of the cholesterol 7α-hydroxylase is decreased, and its activity is therefore increased several fold. By studies in vitro and in vivo, we have recently found evidence for the concept that the primary enzymic defect in CTX is a deficiency of a mitochondrial steroid 26-hydroxylase. Salén and co-workers have previously concluded that a microsomal 24β-hydroxylase active on 3α,7α,12α,25-tetrol is deficient in CTX.

It is difficult to understand the relation between the deficient cleavage of the cholesterol side chain and the increased biosynthesis of 5α-cholestan-3β-ol (cholestanol) in CTX. Cholestanol is derived from cholesterol, but apparently not from cholesterol precursors. It has been previously concluded that cholestanol and bile acids have no common intermediates in their biosynthesis. The biosynthesis of cholestanol from cholesterol is generally believed to proceed via 4-cholesten-3-one, but the capacity of this pathway is low and it has so far only been demonstrated in rat liver tissue. Recently, we showed that intermediates of bile acid biosynthesis are accumulated in liver tissue in CTX. Increased amounts of, e.g., 7α-hydroxy-4-cholesten-3-one are excreted in the bile. 7α-hydroxylated intermediates may be dehydroxylated in the gut, analogous to the formation of secondary bile acids, and 4-cholesten-3-one may thus be formed. However, the quantitative importance of a 7α-hydroxylation/dehydroxylation route for the formation of cholestanol in normal individuals is not known.

The present study was performed to evaluate the possibility that in man cholestanol may be formed from early 7α-hydroxylated bile acid intermediates, and to elucidate the role of this route in CTX.

Methods

Materials
Clinical. Two sisters (A.F., born 27 July 1941, and I.J., born 6 May 1935) with CTX were studied. Detailed records of clinical findings and laboratory data have been published previously (6, 7, 15). A.F. was only slightly mentally retarded when studied in June 1983, but had rather extensive xanthomatosis. Laboratory data (normal reference ranges are indicated in parentheses): serum total cholesterol, 10.2 mmol/L (3.5-7.5); nonesterified cholesterol, 23% of total cholesterol (18-33%); high density lipoprotein (HDL)-cholesterol, 2.2 mmol/L (0.9-1.9); triglycerides, 1.6 mmol/L (0.6-1.4); ASAT, 23 U/L (13-38); ALAT, 41 U/L (9-29); and alkaline phosphatases, 248 U/L (55-195). A needle liver biopsy specimen showed only slight steatosis by light microscopy.

When studied in November 1983, I.J. was more disabled than during hospitalization in 1981 (7), with mental retardation and neurological dysfunction. Laboratory data: serum total cholesterol, 6.8...
mmol/L; nonesterified cholesterol, 24% of total cholesterol; HDL-cholesterol, 0.6 mmol/L; triglycerides, 4.2 mmol/L; ASAT, 11 U/L; ALAT, 14 U/L; and alkaline phosphatases, 396 U/L. A needle liver biopsy specimen showed steatosis by light microscopy.

The ethical aspects of the present study were approved by the ethical board of the Norwegian Research Council for Science and the Humanities, Division for Medical Research. Informed consent was obtained from the patients and their family.

Three subjects without CTX served as controls for the analyses of faecal steroids (S.S., male, born 8 October 1932, L.A., female, born 25 September 1925, and P.L., male, born 18 December 1950). The last two subjects have been described previously (7). Samples from them were stored at −20°C for 2 yr before analysis, whereas unstored samples from S.S. were used. From both CTX patients, fresh samples and samples stored for 2 yr were analyzed.

**Labeled steroids.** 4-14C-cholesterol, with a specific radioactivity of 58.4 mCi/mmol, was obtained from The Radiochemical Centre (Amer sham, Great Britain).

7α-H chol esterol, with a specific radioactivity of ~18.7 mCi/mmol, was prepared as described previously (16). The radiochemical purity of 7α-4H-cholesterol was investigated by addition of 4-14C-cholesterol, dilution with unlabeled cholesterol, and conversion into the acetate of 7-oxocholesterol by the method described by Fieser (17).

The material obtained was purified by thin-layer chromatography (TLC), using benzene-ethylacetate 4:1 (vol/vol) as solvent, and was crystallized twice from methanol-water and acetone-water. The same preparation of 4-14C-labeled cholesterol (A) was used in all experiments, except in the experiment with the healthy subject S.S. The recrystallized material had lost >99% of the 4H (with both preparation A and B).

Thus, >99% of the 4H label is present in the 7α and/or 7β position. The mixture of 7α-4H- and 4-14C-cholesterol was further incubated with liver microsomes from a rat with a biliary fistula (18). The isolated and purified product, 5-chol esterol-3β,7α-diol (7α-hydroxycholesterol), had lost 81% of the 4H with preparation A and 92% with preparation B. Since this hydroxylation is known to occur with a stereospecific replacement of hydrogen (19), it may be concluded that ~81% of the label was present in the 7α position and ~19% in the 7β position in preparation A, whereas the corresponding fractions for preparation B were 92% (7α-H) and 8% (7β-H). Cholic acid was isolated from rabbits treated with the same mixture of 4-14C- and 7α-4H-cholesterol of preparation A and had lost ~84% of the 4H label (see below). The slightly lower retention of 4H in this case may be due to oxidation at C-7 during the enterohepatic circulation of cholic acid. The cholic acid isolated from the bile of A.F. collected 7 d after the administration of 4-14C- and 7α-4H-cholesterol also had a 4H/4C ratio corresponding to removal of ~75% of 7α-H but retention of all 7β-H present in precursor cholesterol. 4-14C-7α-hydroxy-4-cholesten-3-one (14) and 7β-H-7α-hydroxycholesterol (20) were synthesized as described previously. All compounds were purified by high performance liquid chromatography (HPLC) (see below) immediately before preparation of the mixture to be injected. To exclude the possibility of any cholestanol contamination of the labeled cholesterol preparation used, aliquots of 4-14C- and 7α-3H-cholesterol were added to unlabeled cholestanol and cholest erol and carried through the epoxidation procedures and analyzed by HPLC as described below (Fig. 1). All radioactivity had Rf as cholest erol before epoxidation, and as cholesterol epoxides after epoxidation. The cholestanol fraction had unchanged Rf (41 min) and was completely devoid of radioactivity (4H or 14C) both before and after epoxidation.

**Chemicals.** 5β-cholestan-3α-ol (epicoprostanol) was purchased from Steraloids, Inc., Wilton, NH. All other chemicals and solvents used in the present study were standard commercial high purity materials.

**Procedures.**

**Administration of labeled steroids and collection of samples.** The mixture of 4-14C-cholesterol (22.3 µCi) and 7α-3H-cholesterol (60 µCi) given to A.F. was dissolved in 12 ml of ethanol/water containing 1% Tween 80 and was passed through a 0.22-µm filter Millex (Millipore Corp., Bedford, MA). These procedures were performed at the Norwegian Institute of Energy Technology, Kjeller, Norway. The 7β-3H-7α-hydroxycholesterol (32.4 µCi) and 4-14C-7α-hydroxy-4-cholesten-3-one (0.84 µCi) given to I.J. were dissolved in 0.5 ml of ethanol together with 1% Tween 80 in 4.5 ml of water and sterile-filtered as above. The solutions were slowly injected intravenously. In CTX patient A.F., blood samples were drawn after 15 min (contralateral arm) and after 16, 88, and 160 h. In both CTX patients, urine and feces were collected quantitatively for 8 d after the injection of the labeled precursor. In A.F., bile was collected by duodenal incubation 160 h after the administration of the labeled cholesterol, as described previously (7). Duodenal contents were collected on ice in 10-min periods. The sampling period was 60 min after the intravenous injection of cholecystokinin (1 clinical unit/kg body weight). The total volume obtained during this period was 89 ml (in the 10- to 20-min period the volume was 32 ml). Control subject S.S. received the mixture of 4-14C-cholesterol (19.5 µCi) and 7α-H-cholesterol (60.2 µCi) in olive oil orally to ensure sufficient radioactivity in coprostanol for calculation of the 4H/14C ratio. Feces was collected quantitatively for 8 d.

**Isolation and analyses of sterols and bile acids.** Cholesterol, cholestanol, and 5β-cholestan-3β-ol (coprostanol) were isolated from feces, bile, and blood plasma by the following procedures: the samples were weighed, and hydrolysis and lipid extractions were performed as described previously (14) by refluxing at 80°C for 1 h with KOH/ethanol (5:100 wt/vol), followed by extraction twice with n-hexane. The hexane extracts of feces were initially subjected to TLC on plates coated with silica gel H using benzene/ethyl acetate (5:1, vol/vol) as solvent. The combined sterol zone (Rr 0.26–0.44) was scraped off, epicoprostanol was added as internal standard, and eluted on a glass silica plate with chloroform/methanol (2:1, vol/vol). Epicoprostanol was added directly to hexane extracts of plasma and bile. The hexane extracts were blown to dryness, and the sterols were separated by HPLC. A Supelcosil LC-18 column (250 × 4.6 mm, 5-µm particle size, obtained from Supelco, Inc., Bellefonte, PA) was used with methanol/water (95:5, vol/vol) as the mobile phase and a flow rate of 1 ml/min. The chromatograph was equipped with a constant flow pump (ConstaMetric III, Laboratory Data Control, Milton Roy Co., St. Petersburg, FL) and a differential refractometer (R-401, Waters Associates, Milford, MA). The retention times (minutes) were as follows: cholesterol-5,6a-epoxide 18; cholesta-4,6-dien-3-one 27; epicoprostanol 27; 4-cholesten-3-one 31; cholesterol 36; coprostanol 36; cholesterol 42; coprostanol 42; 24-methyl-5β-cholestan-3β-ol 42; and 24-ethyl-5β-cholestan-3β-ol 48.

To obtain cholestanol and coprostanol fractions free from unsaturated sterols, an additional purification by HPLC was performed after quantitative conversion of cholesterol and phytosterols to epoxides. Aliquots of the hexane extract (blown to dryness with N2) were incubated with 10 or 20 mg of n-chloroperbenzoic acid in 1 ml of chloroform at 45°C for 30 min. Identification (and in some cases also quantitation) of cholestanol, coprostanol, 24-methyl-5β-cholestan-3β-ol, and 24-ethyl-5β-cholestan-3β-ol was performed by gas chromatography/mass spectrometry (GC/MS) as described previously (14). 7α-hydroxy-4-cholesten-3-one was quantitated by isotope dilution mass spectrometry as described previously (14).

**Hydrolysis of bile acids.** The water phase of the bile remaining after the alkaline hydrolysis and the extraction with hexane was adjusted to pH 8 with HCl (5 mol/L), incubated at 80°C for 10 min, and centrifuged. Bile acids were isolated by means of SepPak cartridges, essentially as described by Sethell (21). The bile acid conjugates were hydrolyzed by choloylglycine hydrolase essentially as described by Ross et al. (22), except that incubations were performed at 37°C overnight. Extraction of bile acids with ethyl-acetate was performed as described by Ross et al. (22). The bile acids were separated by HPLC as described for sterols (see above) except that the mobile phase was methanol/water (70:30, vol/vol). The water was adjusted to pH 3.0 with phosphoric acid. The retention times (minutes) were: cholic acid 18; chenodeoxycholic acid 36; deoxycholic acid 44%; and lithocholic acid 90.

**Measurement of radioactivity.** A Packard Tri-Carb liquid scintillation spectrometer, model 3355 (Packard Instrument Co., Inc., Downers Grove, IL) was used. Simultaneous estimation of 4H and 14C activity was usually performed in 1-ml fractions (methanol/water 95:5, vol/
Results

We have previously shown that cholestanol may be formed from 7α-hydroxy-4-cholesten-3-one by intestinal 7α-dehydroxylation (14), but we concluded that this route probably was of minor quantitative importance. Table I shows results of studies evaluating the relative contribution of 7α-hydroxylated intermediates in the biosynthesis of cholestanol, by estimating the loss of 7α-3H from the precursor 7α-3H cholesterol. Rats and a normal human subject were studied. Table I shows that cholesterol was recovered from different rat organs and from rat and human feces with the same 3H/4C ratio as in ingested cholesterol. This ratio was the same in coprostanol as in its precursor cholesterol. In cholestanol, however, the 3H/4C ratio was 20–30% lower than in cholesterol, both in the rat and in the normal human subject. (Cholestanol was purified and identified as described below.) The extent of the decrease of the 3H/4C ratio suggests that at most ~4 of cholesterol is normally formed by a pathway involving 7α-hydroxylated intermediates.

Table II. 3H/4C Ratios and Specific Activities of Cholesterol and Cholestanol Isolated from a Patient with CTX (A.F.) Given 4-14C- and 7α-3H-Labeled Cholesterol

<table>
<thead>
<tr>
<th>Source</th>
<th>3H/4C ratio</th>
<th>Specific activity of 14C (cpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3H/4C</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Plasma</td>
<td>1.54</td>
<td>0.44 (0.010)</td>
</tr>
<tr>
<td>Bile</td>
<td>1.54</td>
<td>0.35 (0.008)</td>
</tr>
<tr>
<td>Feces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 June 1983</td>
<td>0.48</td>
<td>(0.008)</td>
</tr>
<tr>
<td>20 June 1983</td>
<td>0.45</td>
<td>(0.013)</td>
</tr>
</tbody>
</table>

The mixture of 3H- and 14C-labeled cholesterol (with a 3H/4C ratio of 1.54) was given intravenously to A.F. on 13 June 1983 (refer to Methods). Time (days) for sampling after precursor administration: plasma, 8; bile, 7; feces, 6–7. Amount of cholestanol (milligram) isolated: plasma, 0.7; bile, 1.5; feces, 0.8. Arithmetical means for 15–30 repeated countings/calculations are shown (in parentheses, SEM). (The amounts of cholestanol in bile and feces are shown in Table IV.)

Table I. 3H/4C Ratios of Cholesterol and
Cholestanol Isolated from Rats and a Healthy Human Subject
Given 7α-3H and 4-14C-Cholesterol Orally

<table>
<thead>
<tr>
<th>Organ or material</th>
<th>Animal or subject</th>
<th>3H/4C</th>
<th>Cholesterol</th>
<th>Cholestanol</th>
<th>Coprostanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small intestinal</td>
<td>Rat A</td>
<td>1.21</td>
<td>0.91</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>and cecal wall</td>
<td>Rat B</td>
<td>1.40</td>
<td>1.06</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>Rat B</td>
<td>1.38</td>
<td>1.09</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Feces</td>
<td>Rat B</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.38</td>
</tr>
<tr>
<td>Human subject</td>
<td>S.S.</td>
<td>1.74*</td>
<td>1.25</td>
<td>1.74</td>
<td></td>
</tr>
</tbody>
</table>

The total doses given to two rats in the chow during 10 d were as follows: 4-14C-cholesterol: rat A, 3.2 μCi; rat B, 15.3 μCi; 7α-3H-cholesterol: rat A, 6.9 μCi; rat B, 38.0 μCi. Normal human subject S.S. received a single oral dose of 4-14C-cholesterol (19.5 μCi) and 7α-3H-cholesterol (60.2 μCi) (refer also to Methods). The 3H/4C ratios (cpm/cpm) in ingested cholesterol were rat A, 1.21; rat B, 1.40; and S.S., 1.74.

* Measured in cholesterol epoxide separated from coprostanol by HPLC.

The results shown in Table II indicate that in CTX a major part of the cholesterol is formed via 7α-hydroxylated intermediates. In the normal state, on the other hand (Table I), such a pathway can at the most only account for 20–30%
I. epicoprostanol and Traces of this fraction of administration were also present in the mammalian organism. Since plant sterols could only be synthesized in the mammalian organism and were not present in the bile of A', it could be concluded that the addition of 3H-cholesterol to the bile of A' was not due to a dietary factor. A mixture of 7α-4C-cholesterol and 4α-cholesterol was administered to patient A' with CTX and to patient A with CTX (refer to Table II). The ratio of the specific activity of the administered 3H-cholesterol present in the bile of A' and A was about the same as that in the bile of the control subjects L.A., L.I. and C.I. which were given only 4α-cholesterol. Table II shows that the specific activity of the administered 3H-cholesterol was much higher in the bile of A' than in the bile of A. The relative contribution of the 7α-dehydroxylation to the 3H-activity in the bile of A' was 1.64. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The relative contribution of the 7α-dehydroxylation to the 3H-activity was 11.5 in the bile of A'. The ratio of the specific activity of the administered 3H-cholesterol present in the bile of A' and A was about the same as that in the bile of the control subjects L.A. and L.I. who were given only 4α-cholesterol. Table II shows that the specific activity of the administered 3H-cholesterol was much higher in the bile of A' than in the bile of A. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol present in the bile of A' was about 1.64. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg. The specific activity of the administered 3H-cholesterol was 770 dpm/mg.
above normal by a factor of \(~10\) in both our patients with CTX. In A.F., bile was collected, and its relative concentration of cholestanol was 6.5% of total sterols. This is abnormally high, as previously found in patients with CTX (10). In the bile of A.F., the concentration of 7α-hydroxy-4-cholesten-3-one was \(4 \mu g/ml\), which was only \(~6\)% of the concentration of cholestanol.

**Discussion**

Pathways for the biosynthesis of cholestanol in the normal state. It is well established that cholesterol may serve as precursor of cholestanol in man (9, 11). Cholestanol is readily formed from 4-cholesten-3-one (26), which in all probability is an intermediate in the route from cholesterol (11, 12). This precursor is converted into cholestanol in liver tissue by a Δ4-3-oxo-steroid 5α-reductase (27) and a 3β-hydroxysteroid dehydrogenase (28) (route A in Fig. 2). The mechanism for formation of 4-cholesten-3-one in mammalian tissues is not clear, however, and this step is evidently rate limiting. A very slow NAD*-dependent conversion of cholesterol to 4-cholesten-3-one has been demonstrated in rat liver microsomes (12), but not convincingly in human tissues.

In a previous study (14) we discussed the possibility that 7α-hydroxylated bile acid intermediates might be precursors of 4-cholesten-3-one and cholestanol. It was shown that 7α-hydroxy-4-cholesten-3-one, which is a key intermediate in bile acid biosynthesis, could be converted into cholestanol after oral administration to rabbits. The conversion occurred via 7α-dehydroxylation and intermediate formation of cholesta-4,6-dien-3-one and 4-cholesten-3-one. We concluded from radiotracer experiments and quantitation of 7α-hydroxy-4-cholesten-3-one in human bile that formation of cholestanol with intestinal 7α-hydroxy-4-cholesten-3-one as an intermediate only can represent a minor pathway (14).

**Table IV. Amounts of Sterols in Plasma, Bile, and Feces from Two Patients with CTX and in Control Subjects**

<table>
<thead>
<tr>
<th>Source</th>
<th>Subject</th>
<th>Date of sampling</th>
<th>Cholesterol</th>
<th>Coprostanol</th>
<th>Cholestanol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mmol/L</td>
<td>total amount (mg)</td>
<td>total amount (mg)</td>
</tr>
<tr>
<td>CTX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>A.F.</td>
<td>21 June 1983</td>
<td>10.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>L.J.</td>
<td>10 Nov. 1983</td>
<td>6.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bile</td>
<td>A.F.</td>
<td>20 June 1983</td>
<td>2.8</td>
<td>83‡</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17 June 1983</td>
<td>871</td>
<td>Trace</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 June 1983</td>
<td>913</td>
<td>Trace</td>
<td>—</td>
</tr>
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<td></td>
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<td>20 June 1983</td>
<td>—</td>
<td>Trace</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>L.J.</td>
<td>21 May 1981</td>
<td>Trace</td>
<td>—</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 May 1981</td>
<td>34</td>
<td>113</td>
<td>Trace</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11 Nov. 1983</td>
<td>151</td>
<td>671</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Nov. 1983</td>
<td>—</td>
<td>—</td>
<td>428§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13 Nov. 1983</td>
<td>—</td>
<td>—</td>
<td>295§</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 Nov. 1983</td>
<td>—</td>
<td>—</td>
<td>496§</td>
</tr>
<tr>
<td>Controls</td>
<td>Feces§</td>
<td>L.Aa.</td>
<td>272</td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P.I.</td>
<td>698</td>
<td>Trace</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>S.S.</td>
<td>57</td>
<td>428</td>
<td>5.8</td>
</tr>
</tbody>
</table>

—, not examined. * Level in normal controls, 0.001-0.019 mmol/L (15). ‡ In 89 ml collected from duodenum after cholecystokinin. § mg/24 h. ‡‡ Cholesterol plus coprostanol.
The above experiments did not exclude that other 7α-hydroxylated bile acid intermediates might be precursors of cholestanol, or that 7α-dehydroxylation might occur in other organs than the intestine. In the present work we used a more general approach by studying the loss of 3H from 7α-3H-labeled cholesterol during its conversion into cholestanol in rats and in one human control subject. There was a significant (20–30%) loss of tritium during the conversion both in the rats and in the human subject.

The simplest explanation of this finding is that there is a pathway to cholestanol involving 7α-hydroxylated intermediates which is responsible for 20–30% of the biosynthesis of cholestanol (route B in Fig. 2). Although less likely, the possibility cannot be completely excluded that the reduced 3H/14C ratio to some extent may be caused by an isotope effect in the conversion of 7α-3H-cholesterol into 7α-3H-4-cholesten-3-one via the previously known, direct pathway (12). Attempts to study this possibility in vitro have failed owing to the very low conversion (12). The results of the experiments with the control subject, however, do not favor the possibility of such an isotope effect. Thus the coprostanol formed from the mixture of 7α-3H- and 4,6-dien-3-one labeled cholesterol had the same 3H/14C ratio as the precursor. Since the biosynthesis of coprostanol also involves 4-cholesten-3-one as an intermediate (29), an isotope effect in the conversion of cholesterol into 4-cholesten-3-one should have been reflected by a lowered 3H/14C ratio in coprostanol.

_Acceleration of the novel pathway for formation of cholestanol from bile acid intermediates in patients withCTX._ In the patient with CTX who received 7α-3H- and 4,6-dien-3-one labeled cholesterol, the ratios between 3H and 14C in cholestanol isolated from plasma, bile, and feces were reduced as compared with corresponding ratios in a healthy control. Our findings indicate that the novel pathway discussed above (Fig. 2 B) accounts for ~75% of the total biosynthesis of cholestanol in this patient. The remaining 25% may be formed by the direct route which would not lead to removal of 7α-3H. The novel pathway for biosynthesis of cholestanol is thus considerably accelerated in this patient as compared with the normal state. Our evidence for the presence of the novel pathway for biosynthesis of cholestanol in the normal state might not be accepted because of the possibility of an isotope effect in the conversion of 7α-3H cholesterol into cholestanol in vivo. It is still evident from the additional decrease of the 3H/14C ratio in CTX, however, that at least 50% of all the cholestanol synthesized in this patient must have been formed by the pathway involving 7α-hydroxylated intermediates. If there is an isotope effect, it must be the same in the patient with CTX as in the normal subject. Our results thus provide complete evidence that the novel pathway is responsible for between 50 and 75% of the total synthesis of cholestanol in the patient with CTX.

_Direct evidence that 7α-hydroxycholesterol is a precursor in the novel pathway to cholestanol._ A possible mechanism for conversion. In the present study we have shown that 3H from 7β-3H-labeled 7α-hydroxycholesterol was incorporated to a significant extent into plasma and fecal cholestanol in one of our CTX patients. It is thus established that 7α-hydroxycholesterol may serve as an intermediate in a novel route for the biosynthesis of cholestanol. However, we found little or no incorporation of 14C from 14C labeled 7α-hydroxy-4-cholesten-3-one. The latter finding is in accordance with previous investigations (7, 10, 30). Less 14C labeled precursor than desirable was available for our previous study (7) and the present work. (Informations on the amounts of labeled 7α-hydroxy-4-cholesten-3-one used in Salen's study were not given [10, 30].) It is also evident that a labeled precursor administered intravenously may be metabolized in a manner different from when it is formed within the cell. We therefore believe that our study does not exclude the possibility that 7α-hydroxy-4-cholesten-3-one is a cholestanol precursor of biological significance. We have previously shown that 7α-hydroxy-4-cholesten-3-one may be dehydroxylated in the gut and thereby serve as a cholestanol precursor. The concentrations of 7α-hydroxy-4-cholesten-3-one (14) and 7α-hydroxycholesterol (31) in the bile from control subjects and from patients with CTX, however, were very low. In view of this it seems more likely that the dehydroxylation step occurs in the liver than in the intestine. A quite large fraction of cholestanol newly synthesized from 7β-3H-7α-hydroxycholesterol was found in the plasma of our patient. This finding may also point to the liver as the site of synthesis of plasma cholestanol, which is probably incorporated into nascent lipoproteins. Studies are in progress to investigate whether administration of cholesta-4,6-dien-3-one may lead to its incorporation into lipoproteins and conversion to cholestanol in organs in which cholestanol deposits are found in CTX (e.g., brain).

The detailed mechanism for conversion of 7α-hydroxycholesterol into cholestanol has not been investigated in the present work. Since a 7α-dehydroxylation is required, cholesta-4,6-dien-3-one would be a likely intermediate. Cholesta-4,6-dien-3-one has previously been shown to be an efficient precursor of 4-cholesten-3-one, and therefore also of cholestanol (14, 32). In view of the great amounts of 7α-hydroxycholesterol formed in the liver, conversion of only a low percentage into cholesta-4,6-dien-3-one would be required to explain the biosynthesis of cholestanol (refer below). In our laboratories, we have recently shown that 7α-hydroxycholesterol is partly converted to cholesta-4,6-dien-3-one via 7α-hydroxy-4-cholesten-3-one (Skrede, S., M. Buchmann, and I. Björkhem, unpublished observations) by rat liver microsomes in the presence of NAD (results not shown).

The possible mechanisms for formation of 4-cholesten-3-one from 7α-hydroxycholesterol and the different metabolic interrelations are summarized in Fig. 2.

_Basic metabolic defect in CTX and its relation to cholestanol biosynthesis._ We have previously presented evidence from in vitro (6) and in vivo (7) studies for the concept that the metabolic defect in CTX is a deficiency of a mitochondrial C27-steroid 26-hydroxylase, which belongs to the physiologically most important pathway for the side chain degradation (6, 7). Our conclusions are thus at variance with those of Salen and coworkers (8), who interpreted their results as due to a deficiency of a microsomal 24α-hydroxylase active on 5β-cholestan-3α,7α,12α,25-tetrol, which was apparently more slowly converted to cholic acid than normal (reduced to ¼). We think that their findings can be explained by an increased endogenous pool of 3α,7α,12α,25-tetrol, which is present in CTX (6). Our results also indicate that side-chain degradation initiated by 25-hydroxylation is normally a minor pathway (6,}

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7). In CTX, however, this route probably substitutes for normal 26-hydroxylation, but the capacity is too low for normal biosynthesis of bile acids. Accordingly, abnormal 25-hydroxylated tetrads and pentols accumulate and are excreted in bile (4) and urine\(^2\) (33), whereas 26-hydroxylated bile alcohols are not found\(^2\) (4, 33).

Because of reduced feedback control, the activity of the \(7\alpha\)-hydroxylation is increased (5). Bile acid intermediates, such as \(7\alpha\)-hydroxycholesterol and \(7\alpha\)-hydroxy-4-cholesten-3-one, are accumulated in the liver (7). The rate of \(7\alpha\)-dehydroxylation may be increased because of increased substrate concentrations. Increased flow through the \(7\alpha\)-hydroxylation combined with the deficient side-chain degradation leads to enormously increased formation of bile alcohols (4, 34). In our patients, the output of bile alcohols in feces and urine sometimes exceeded 1,000–1,500 mg/d.\(^2\) The present study shows that the fecal excretion of cholestanol was 20–50 mg/24 h, i.e., 1–3% of the sum of \(7\alpha\)-hydroxylated cholesterol metabolites excreted.\(^5\) This fraction is of the same order of magnitude as the relative amount of \(^3\)H found in cholestanol when compared with the total amount of \(^3\)H-labeled metabolites excreted after injection of \(7\beta\)-\(^3\)H-\(7\alpha\)-hydroxycholesterol in one of our patients (I.J.). It can further be roughly calculated from the decrease of the \(^3\)H/\(^1\)C ratio during conversion of cholesterol to cholestanol in the other patient (A.F.) that the novel pathway accounts for a maximum of 70–75% of all cholestanol formed, i.e., 30–40 mg/d in this CTX patient. The increased activity of the novel pathway and the deficiency of the 26-hydroxylation—which would be expected to affect also the metabolism of cholestanol—are probably the most important causes of the accumulation of cholestanol in CTX. Another expression of the quantitative importance of the pathway described in the present study is that treatment with cholestyramine, which increases \(7\alpha\)-hydroxylation of cholesterol, strongly increases the output of cholestanol in CTX (34). Our observations probably also explain why treatment with chenodeoxycholic acid, which decreases \(7\alpha\)-hydroxylation of cholesterol, leads to decreased biosynthesis of cholestanol in patients with CTX (34).

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References


