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# POTENTIAL BILE ACID PRECURSORS IN PLASMA—POSSIBLE INDICATORS OF BIOSYNTHETIC PATHWAYS TO CHOLIC AND CHENODEOXYCHOLIC ACIDS IN MAN

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**Summary**—The plasma concentrations of 3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-4-cholestenoic acid have been compared with that of 7α-hydroxy-4-cholesten-3-one in healthy subjects and in patients with an expected decrease or increase of the bile acid production. In controls and patients with liver disease, the level of 7α-hydroxy-3-oxo-4-cholestenoic acid was positively correlated to that of 3β,7α-dihydroxy-5-cholestenoic acid and not to that of 7α-hydroxy-4-cholesten-3-one. In patients with stimulated bile acid formation the levels of the acids were not correlated to each other but there was a significant positive correlation between the levels of 7α-hydroxy-3-oxo-4-cholestenoic acid and 7α-hydroxy-4-cholesten-3-one. These findings indicate that the precursor of 7α-hydroxy-3-oxo-4-cholestenoic acid differs depending on the activity of cholesterol 7α-hydroxylase. Since the activity of this enzyme is reflected by the level of 7α-hydroxy-4-cholesten-3-one in plasma the findings are compatible with a formation of 7α-hydroxy-3-oxo-4-cholestenoic acid from 3β,7α-dihydroxy-5-cholestenoic acid when the rate of bile acid formation is normal or reduced and from 7α-hydroxy-4-cholesten-3-one under conditions of increased bile acid synthesis. In support of this interpretation, 7α,26-dihydroxy-4-cholesten-3-one was identified at elevated levels in plasma from patients with ileal resection or treated with cholestyramine. The levels of 7α,12α-dihydroxy-4-cholesten-3-one were also higher than normal in these patients.

Based on these findings and previous knowledge, a model is proposed for the biosynthesis of bile acids in man. Under normal conditions, two major pathways, one "neutral" and one "acidic" or "26-oxygenated", lead to the formation of cholic acid and chenodeoxycholic acid, respectively. These pathways are separately regulated. When the activity of cholesterol 7α-hydroxylase is high, the "neutral" pathway is most important whereas the reverse is true when cholesterol 7α-hydroxylase activity is low. In cases with enhanced activity of cholesterol 7α-hydroxylase, the "neutral" pathway is connected to the "acidic" pathway via 7α,26-dihydroxy-4-cholesten-3-one, whereas a flow from the acidic pathway to cholic acid appears to be of minor importance.

## INTRODUCTION

The formation of bile acids plays an important role for cholesterol homeostasis in man. The bile acids are major end products of cholesterol metabolism and bile acids and their precursors may inhibit the production of cholesterol. Bile acids can be formed via several different routes as demonstrated by administration of isotopically labelled potential intermediates and by studies of enzyme kinetics *in vitro* [1]. Because of the multitude of enzymes involved and their usually broad substrate specificities, the quantitative significance of different pathways and their regulation *in vivo* have been difficult to establish. Although the nuclear transformations are usually considered to precede those of the side chain in the biosynthesis of cholic acid, alternative pathways can exist, particularly in the formation of chenodeoxycholic acid. Thus, 26-hydroxycholesterol is preferentially converted to chenodeoxycholic acid in humans [2, 3]. In any case, the introduction of the 7α-hydroxy group is considered to be the rate-limiting step in bile acid biosynthesis.

Recently we have reported the identification of three cholestenoic acids, 3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-4-cholestenoic acid, in human blood [4]. Further studies on their concentrations in plasma from patients with abnormal bile acid synthesis have shown that the concentration of 7α-hydroxy-3-oxo-4-cholestenoic acid is selectively increased in patients with enhanced bile acid production [5, 6]. In addition,

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the plasma level of 7α-hydroxy-4-cholesten-3-one, an established bile acid precursor, seemed to reflect the activity of cholesterol 7α-hydroxylase [7]. A similar positive correlation between plasma levels of 7α-hydroxycholesterol and an increased activity of cholesterol 7α-hydroxylase has previously been reported [8]. We have now studied the relationships between the levels of cholestenoic acids and 7α-hydroxy-4-cholesten-3-one in plasma in different patients. Based on the absolute and relative levels under physiological and pathological conditions and on existing knowledge of potential pathways, a model for bile acid biosynthesis in man is presented. Two additional bile acid precursors, 7α,12α-dihydroxy-4-cholesten-3-one and 7α,26-dihydroxy-4-cholesten-3-one, were also identified in blood and their levels in plasma under different conditions provided further support for the model.

## MATERIALS AND METHODS

### *Patients*

Blood was obtained from 20 healthy subjects (median age 37 yr) and from patients with abnormal bile acid production or metabolism. With a few exceptions, the controls and patients were those described more in detail in previous papers [5–7]: 11 patients (median age 68 yr) with large bile duct obstruction secondary to carcinoma or gallstones; 10 patients (median age 62 yr) with primary biliary cirrhosis; 8 patients (median age 56 yr) with moderate or severe forms of alcoholic liver cirrhosis; 6 patients (median age 37 yr) subjected to a complete colectomy due to polyposis coli; 11 patients (median age 40 yr) with Crohn's disease and subjected to a resection of terminal ileum (>60 cm) with or without colectomy, and 9 patients (median age 63 yr) with hypercholesterolemia and treated with 8 g cholestyramine (Questran®, Bristol) twice daily for 6–8 weeks. The blood was collected in tubes with or without heparin. Following centrifugation, plasma/serum was separated and stored at −20°C until analyzed.

### *Analytical procedures*

Chemicals, columns, reference compounds and the methods for analysis of cholestenoic acids and 7α-hydroxy-4-cholesten-3-one in plasma were described in previous papers [4, 7].

*Analysis of oxygenated cholesterol derivatives in plasma.* Plasma or serum (1.5 ml) was added dropwise to 7 ml of ethanol in a tube placed in an ultrasonic bath. The mixture was ultrasonicated for 15 min, centrifuged and the supernatant was then transferred to another tube. To this tube was added 8 ml of water. The mixture was extracted on a washed (4) column (1 × 0.8 cm) of octadecylsilane-bonded silica (Preparative $C_{18}$; Waters Assoc. Inc., Milford, Mass). Following a wash with 10 ml of water and 5 ml of 60% aqueous methanol, sterols were eluted with two 10 ml-portions of 85% aqueous methanol which elutes only little cholesterol. To each portion was added 5 ml of water. The second portion was then reextracted on the same column (thoroughly washed and used in 60% aqueous methanol) followed by reextraction of the first portion. A gentle stream of nitrogen was then passed through the column for 1 min in order to remove excess water, and the sterols were eluted with 6 ml of methanol/chloroform, 1:1 (v/v). The solvent was evaporated under a stream of nitrogen and the residue was dissolved in 1 ml of hexane. To remove contaminating cholesterol, the sample was passed through a Sep-Pak silica cartridge (Waters Assoc. Inc., washed with 5 ml each of methanol, chloroform and hexane), followed by washing with 5 ml of hexane and 8 ml of hexane/isopropanol 99:1. The sample tube was rinsed with 6 ml of methanol/chloroform, 1:1 (v/v), which was then used to elute oxygenated sterols. The eluate was taken to dryness under a stream of nitrogen and the residue was dissolved in 0.5 ml of methanol. Acids were removed by passage of the solution through a column (4 × 0.4 cm) of triethylaminohydroxypropyl Sephadex LH-20 (TEAP-LH-20) in $HCO_3^-$-form, packed in methanol. Neutral steroids were recovered by an additional wash with 3 ml of methanol/chloroform, 1:1. To the combined eluate was added 128 ng of hexatriacontane (Fluka, Buchs, Switzerland) as internal standard prior to preparation of trimethylsilyl (TMS) ethers using a mixture of pyridine–hexamethyldisilazane–trimethylchlorosilane, 3:2:1 (by vol) and heating at 60°C for 30 min. For identification purposes, a few samples were converted to *O*-methyloxime-trimethylsilyl (MO-TMS) ether derivatives [4]. All solvents were degassed by ultrasonication prior to use.

The derivatized compounds were analyzed by gas chromatography–mass spectrometry (GC–MS) using a Finnigan 1020 instrument, housing a fused-silica column (30 m × 0.32 mm) coated with a 0.25 μm layer of SE-30 DB-1 (J&W Scientific, Rancho, Cordova, Calif.) and ending in the ion source. An on-column injection device was used. The oven temperature was about 50°C during injection and, after 6 min, was rapidly increased to 190°C. It was then programmed from this temperature to 285°C at a rate of 5°C × $min^{-1}$. The temperature of the ion source was 290°C and the electron energy was 40 eV. Repetitive scanning (20 scans × $min^{-1}$) over the $m/z$ range 50–800 was started after a suitable delay. The instrument was tuned so that intensities of fragment ions with $m/z$ values above 200–300 were enhanced relative to those of lighter fragments. Retention indices were calculated by comparison of retention times with those of normal $C_{30}$–$C_{38}$ hydrocarbons analyzed under the same conditions. The quantitation was based on the peak areas in selected ion chromatograms compared with those given by a known amount of the reference substance. When the authentic compound was not available, the amounts were estimated by converting selected ion current into

Table 1. Concentrations of potential bile acid precursors and bile acids in plasma/serum of healthy subjects and patients with abnormal bile acid production or metabolism

| Subjects[a] | Steroid[b] concentration (ng/ml)[c] | | | | | |
|---|---|---|---|---|---|---|
| | $C^4$-7α-ol-3-one | $C^5$-3β-ol-26-oate | $C^5$-3β,7α-ol-26-oate | $C^4$-7α-ol-3-one-26-oate | Unconjugated $C_{24}$ bile acids[d] | Conjugated $C_{24}$ bile acids[d,e] |
| Healthy | 12, 10–14 | 57, 39–64 | 30, 25–38 | 85, 67–93 | 146, 98–227 | ND[f] |
| Alcoholic liver cirrhosis | <1, <1–3 | 90, 33–177 | 76, 30–147 | 357, 118–515 | 598, 275–1260 | 12400, 6690–14900 |
| Primary biliary cirrhosis | <2, <1–10 | 67, 54–89 | 50, 34–85 | 170, 95–233 | 63, 47–393 | 6040, 3290–14300 |
| Extrahepatic cholestasis | <1, <1–2 | 46, 29–71 | 24, 13–37 | 84, 56–95 | <30, <30–38 | 12800, 4330–45000 |
| Colectomized | 24, 14–26 | 61, 49–90 | 27, 24–36 | 87, 75–105 | 113, 78–396 | 544, 418–634 |
| Cholestyramine-treated | 174, 105–366 | 62, 52–84 | 30, 26–36 | 189, 180–291 | 71, 55–111 | 264, 191–298 |
| Ileal resection | 370, 195–600 | 31, 24–42 | 14, 10–24 | 233, 210–320 | 685, 167–1960 | 611, 403–778 |

[a]Details on patients are given in Materials and Methods and in Refs 5 and 6.
[b]C, cholestane; superscript indicates position of double bonds; Greek letters denote configuration of hydroxyl groups.
[c]Concentration expressed as median, lower quartile–upper quartile.
[d]Includes chenodeoxycholic, cholic and deoxycholic acids.
[e]Expressed as ng unconjugated acids.
[f]ND, not determined.

total ion current and comparing the area of the total ion current peak with that given by reference compounds with analogous structures.

The statistical evaluation of data included calculation of Spearman's rank correlation coefficient and Kolmogorov–Smirnov two-sample test [9].

## RESULTS AND DISCUSSION

### *Potential bile acid precursors in plasma*

Little is known about the normal occurrence of bile acid precursors in blood. Although 7α- [8, 10–12] and 26-hydroxycholesterol [12, 13] have been determined, their origin has not been established. 7α-Hydroxycholesterol can be an autooxidation product of cholesterol which makes analyses of low levels in plasma very difficult [8, 10, 11], and sterol 26-hydroxylase can be found in many tissues [14].

The levels of 7α-hydroxycholesterol have been shown to be positively correlated to the activity of cholesterol 7α-hydroxylase in patients with enhanced bile acid synthesis [8]. Recently, we reported that the levels of 7α-hydroxy-4-cholesten-3-one were also increased in the same categories of patients [6]. The levels of this compound were subnormal in patients expected to have a reduced bile acid production. Thus, the levels in plasma of 7α-hydroxy-4-cholesten-3-one appeared to reflect both an increased and a decreased rate of bile acid formation and the corresponding activity of cholesterol 7α-hydroxylase. This has been confirmed in later studies (Axelson *et al.*, unpublished).

We have also shown that the cholesten-26-oic acid analogues of cholesterol, 7α-hydroxycholesterol and 7α-hydroxy-4-cholesten-3-one are present in plasma [4]. To evaluate a possible role in bile acid biosynthesis, their levels were compared with those of 7α-hydroxy-4-cholesten-3-one (reflecting cholesterol 7α-hydroxylase activity) in patients with reduced (alcoholic liver cirrhosis, primary biliary cirrhosis and extrahepatic cholestasis) and increased (cholestyramine treatment and ileal resection) bile acid production. The results are shown in Table 1 and Fig. 1.

The median concentrations of 7α-hydroxy-4-cholesten-3-one were low (<2 ng/ml) in patient groups with liver disease. In contrast, the median concentrations of the three cholestenoic acids were elevated in liver cirrhosis. Since the levels correlated well with those of conjugated bile acids in liver cirrhosis but not in cholestasis [5], the higher levels in cirrhosis are probably due to a reduced hepatic uptake/metabolism. In extrahepatic cholestasis, the levels of the cholestenoic acids were

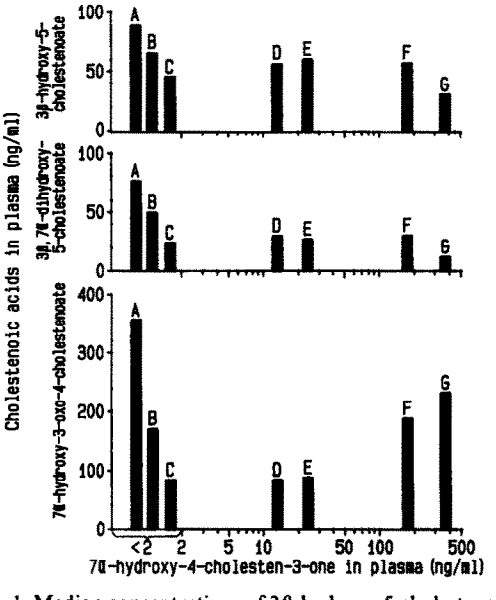


Fig. 1. Median concentrations of 3β-hydroxy-5-cholestenoic acid (top panel), 3β,7α-dihydroxy-5-cholestenoic acid (middle panel) and 7α-hydroxy-3-oxo-4-cholestenoic acid (bottom panel) in relation to those of 7α-hydroxy-4-cholesten-3-one in plasma from healthy subjects (D), and patients with alcoholic liver cirrhosis (A), primary biliary cirrhosis (B) extrahepatic cholestasis (C), colectomy (E), cholestyramine treatment (F), and ileal resection (G).

normal. The production of bile acids is expected to be low in this condition [15] which is consistent with the low levels of 7α-hydroxy-4-cholesten-3-one in plasma.

Treatment with cholestyramine or ileal resection, both known to increase bile acid production, results in high levels of 7α-hydroxy-4-cholesten-3-one [7]. This is accompanied by a selective increase of 7α-hydroxy-3-oxo-4-cholestenoic acid. The levels of the other $C_{27}$-acids remain normal or low (Table 1 and Fig. 1). A significant positive correlation ($r = 0.75$, $P < 0.0001$) between the levels of the neutral and acidic 3-oxo-4-ene steroids was observed in patients with expected high bile acid production, as illustrated in Fig. 2. Such a correlation was not seen in healthy controls or patients with liver disease. In these subjects, however, there was a strong correlation ($r = 0.77$, $P < 0.0001$) between the levels of 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-4-cholestenoic acid [5], not observed in patients with increased bile acid synthesis [6]. Colectomy did not result in any of the changes seen after ileal resection.

While correlations between levels in plasma do not prove metabolic relationships, the positive correlations between the levels of 7α-hydroxy-4-cholesten-3-one and 7α-hydroxy-3-oxo-4-cholestenoic acid in patients with an enhanced bile acid synthesis is compatible with a formation of the acid from the neutral compound via 7α,26-dihydroxy-4-cholesten-3-one. Support for this assumption was obtained following a screening for potential neutral intermediates in plasma.

Figure 3 shows the GC–MS analysis of hydroxylated sterols in plasma from a patient with ileal resection. In addition to high levels of 7α-hydroxycholesterol (250 ng/ml) and 7α-hydroxy-4-cholesten-3-one (800 ng/ml), two 3-oxo-4-ene steroids, 7α,26-dihydroxy-4-cholesten-3-one (65 ng/ml) and 7α,12α-dihydroxy-4-cholesten-3-one (45 ng/ml) could be identified. Since the reference compounds were not available to us, the identification of these two compounds was based on retention indices (RI) and mass spectra [4]. The retention indices for the TMS (3555 and 3280, respectively) and MO-TMS (3510/3525 and 3215/3235, respectively) derivatives agreed with calculated RI-values (±10 units) [4], and the mass spectra were as expected for these compounds (Fig. 4). The fragmentation of compounds with a 7α-hydroxy-3-oxo-4-ene structure has previously been described. The ion of mass 103 is indicative of a 26-trimethylsiloxy group. Support for the identifications was also obtained when the steroids were treated with hydrochloric acid in methanol. This resulted in elimination of the 7α-hydroxy group from 7α,26-dihydroxy-4-cholesten-3-one to give a 3-oxo-4,6-diene structure [4]. The 12-hydroxylated compound appeared to be resistent to this treatment, as was also the case with authentic 7α,12α-dihydroxy-3-oxo-4-cholenoic acid.

The level of 26-hydroxycholesterol (RI 3460 for the TMS ether) in plasma from the patient with ileal resection was about 20 ng/ml. In addition, a compound was detected with properties expected of 7α,26-dihydroxycholesterol. Thus, the TMS ether

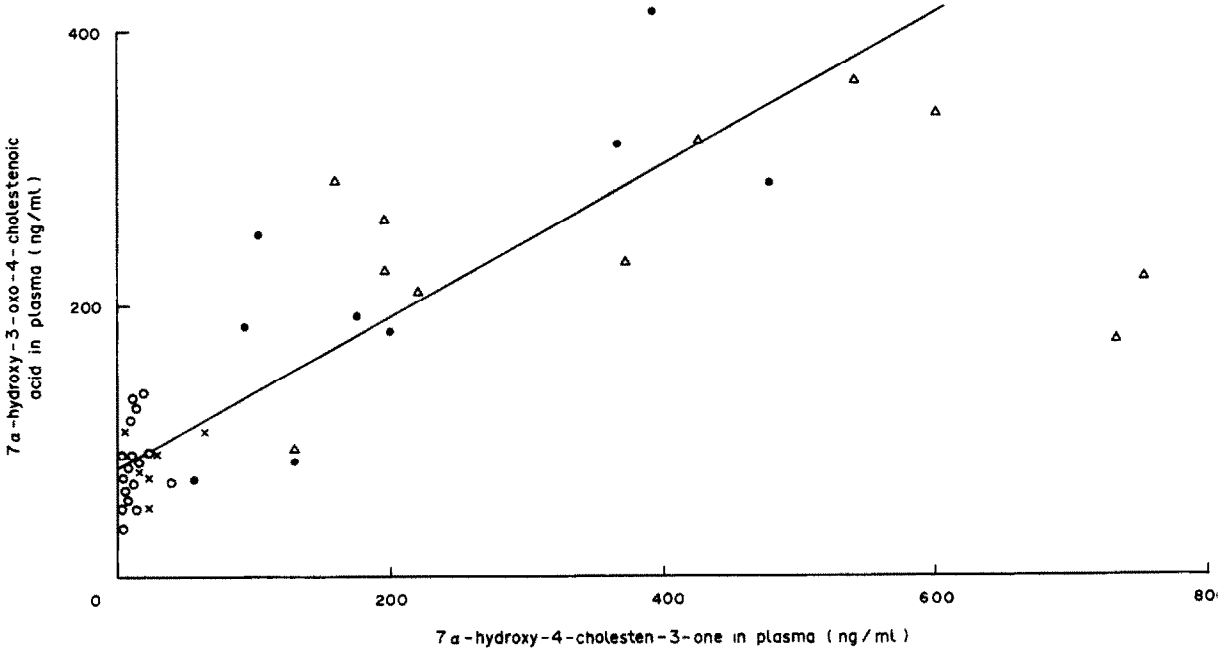


Fig. 2. The relationship between concentrations of 7α-hydroxy-4-cholesten-3-one and 7α-hydroxy-3-oxo-4-cholestenoic acid in plasma of control subjects (○), patients treated with cholestyramine (●), patients with colectomy (×) and patients with ileal resection (△). The drawn regression line does not include the values above 700 ng/ml of the neutral steroid.

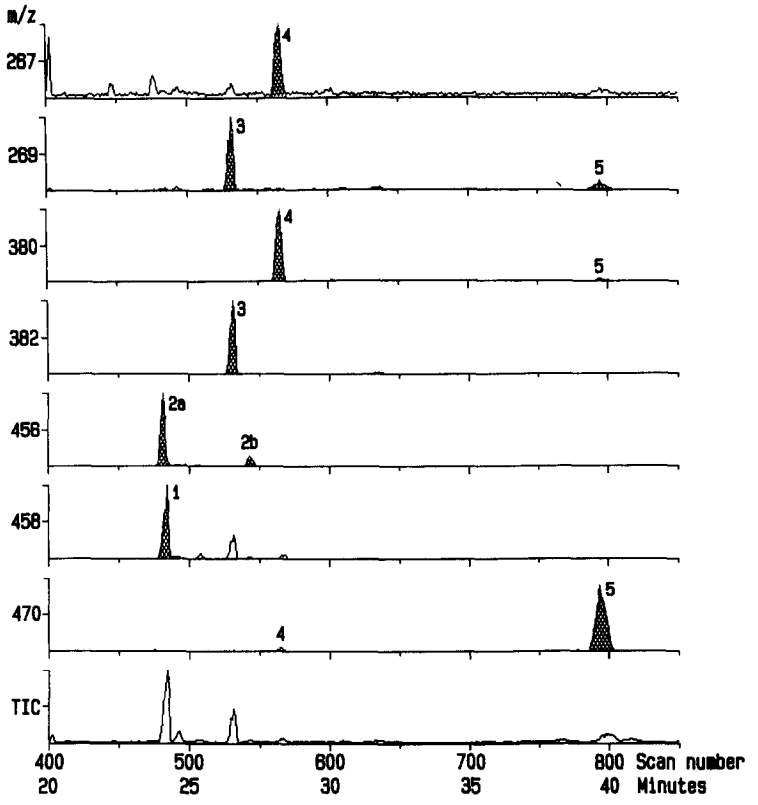


Fig. 3. Gas chromatographic–mass spectrometric analysis of neutral oxygenated sterols in a nonpolar fraction from plasma of a patient with ileal resection. Total ion current (TIC) and fragment ion current chromatograms characteristic of the trimethylsilyl ethers of cholesterol (1; *m/z* 458), 7α-hydroxy- (2a) and 7β-hydroxycholesterol (2b; *m/z* 456), 7α-hydroxy-4-cholesten-3-one (3; *m/z* 269, 382), 7α,12α-dihydroxy-4-cholsten-3-one (4; *m/z* 267, 380, 470) and 7α,26-dihydroxy-4-choleten-3-one (5; *m/z* 269, 380, 470) were constructed by the computer.

had an RI of 3440 and gave a very intense ion at *m/z* 544. This compound was only present in trace amounts (< 1 ng/ml), which did not permit a positive identification.

Analysis of plasma from two other patients with ileal resection also showed that the levels of 7α,26-dihydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one were about 1/10 and 1/20, respectively, of the level of 7α-hydroxy-4-cholesten-3-one (225 and 500 ng/ml in the 2 patients). When similar analyses were carried out on plasma from 6 healthy subjects, the concentrations of the 3-oxo-4-ene-$C_{27}$ steroids were much lower: 7α-hydroxy-4-cholesten-3-one about 10 ng/ml (range 3–14 ng/ml), 7α,26-dihydroxy-4-cholesten-3-one about 3 ng/ml (range 2–10 ng/ml), and 7α,12α-dihydroxy-4-cholesten-3-one at or below the detection limit (< 1 ng/ml). The levels of 7α-hydroxy-, 26-hydroxy- and 7α,26-dihydroxycholesterol were about 14 (range 7–15), 31 (range 13–41) and < 1 ng/ml, respectively, in the same subjects. The concentrations of most of these oxygenated neutral steroids in a patient with extrahepatic cholestasis were very similar to those found in healthy subjects, although the level of 7α-hydroxy-4-cholesten-3-one was lower (< 1 ng/ml) and that of 26-hydroxy-cholesterol, higher (about 90 ng/ml) than in the controls.

*Pathways of bile acid biosynthesis in man*

Bile acids may be formed via several different pathways as demonstrated by administration of isotopically labeled hypothetical intermediates and by studies of enzyme kinetics *in vitro*. While these methods demonstrate the potential of a compound to be a precursor, the quantitative importance of an intermediate/substrate *in vivo* is difficult to evaluate. This is due to the lack of information about the distribution of a given/added compound, its concentration in subcellular fractions, the presence of competing substrates, etc. Considering the broad specificities of several enzymes involved, knowledge about competing substrates is particularly important.

It is known that the concentrations of cholesterol precursors are increased in plasma of subjects with an increased rate of cholesterol synthesis [16]. It has also been shown that the levels of 7α-hydroxy-cholesterol and 7α-hydroxy-4-cholesten-3-one, known intermediates in bile acid biosynthesis [1], are increased in subjects with an increased rate of bile acid synthesis [7, 8]. In the present paper we have used an analogous reasoning in an attempt to

evaluate the role of cholestenoic acids in bile acid biosynthesis.

The measurements of plasma levels in health and disease revealed distinct relationships between the cholestenoic acids and other intermediates. These are illustrated in Figs 5 and 6. Arrows between compounds indicate possible reactions, and the width of the arrows an increased or decreased flow based on levels of the compounds in plasma. Statistically significant positive correlations are shown by filled arrows. On the basis of this data we propose a model for bile acid synthesis in humans in which the relative importance of different pathways is determined by the activity of cholesterol 7α-hydroxylase. The model is limited to humans and cannot be applied to animals. Preliminary data on the rat and rabbit indicate significant differences from the situation in man, as will be reported separately. According to the model, the formation of the two major primary bile acids under normal conditions (with a moderately inhibited cholesterol 7α-hydroxylase) proceeds mainly via two separate routes, cholic acid along a "neutral" pathway identical to that considered to be the major pathway, and chenodeoxycholic acid via an "acidic" or 26-oxygenated pathway (Fig. 5). This hypothesis is supported by the positive correlations between the three cholestenoic acids [4], and the absence of a correlation between 7α-hydroxy-4-cholesten-3-one and its corresponding acid. However, a small fraction of the acid is probably derived from the neutral compound, since 7α,26-dihydroxy-4-cholesten-3-one is normally present in blood.

In liver disease, with elevated levels of bile acids in blood, the activity of cholesterol 7α-hydroxylase is low [15, 17] resulting in decreased formation/levels of the neutral intermediates. However, the levels of the 7α-hydroxylated cholestenoic acids are not decreased and the model predicts an intact or moderately decreased production of chenodeoxycholic acid, while the production of cholic acid is markedly reduced (Fig. 6). This prediction agrees well with results of studies on bile acid production in cirrhotic patients [17]. A constant formation/level of 3β,7α-dihydroxy-5-cholestenoic acid could be due to an accumulation in the liver cells of the precursors (26-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid) or to the presence of a different 7α-hydroxylase active on 26-oxygenated intermediates and not down-regulated in the same way as cholesterol 7α-hydroxylase. The fact that the level

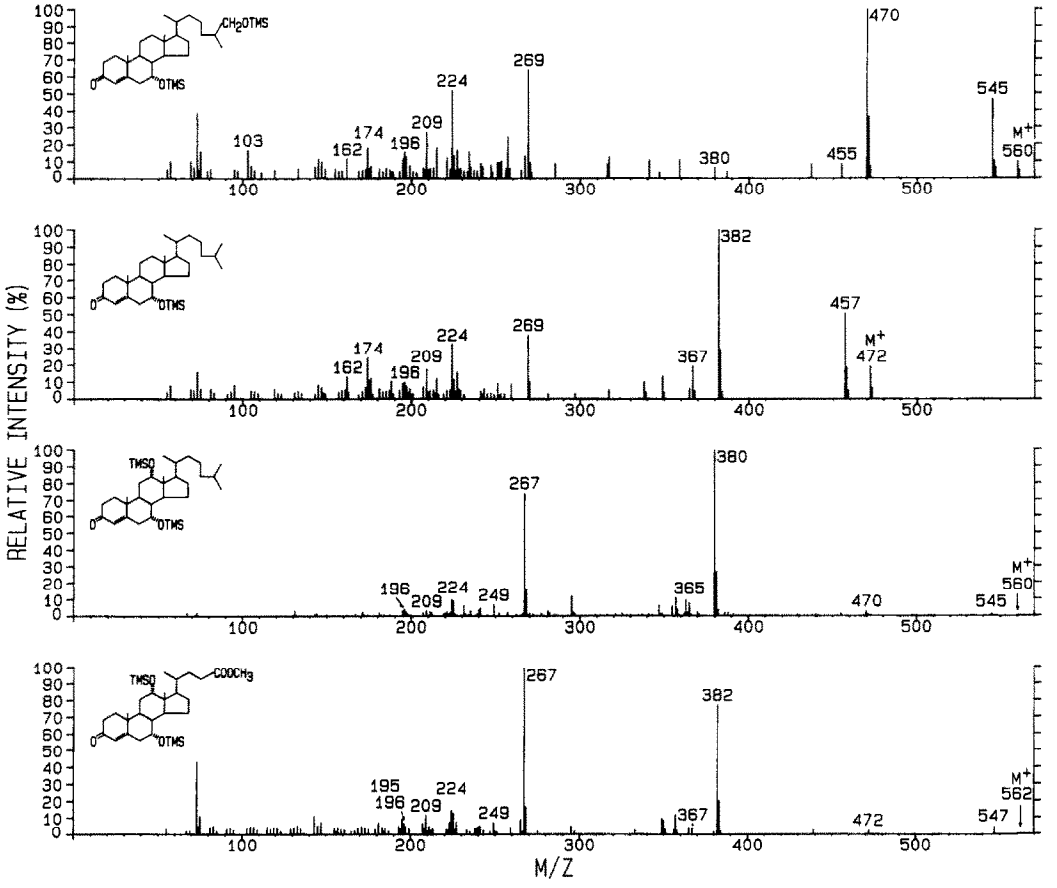


Fig. 4. Mass spectra of the trimethylsilyl ethers of the two steroids in plasma identified as 7α,26-dihydroxy-4-cholesten-3-one and 7α,12α-dihydroxy-4-cholesten-3-one. Mass spectra of reference steroids with analogous structures are shown for comparison.

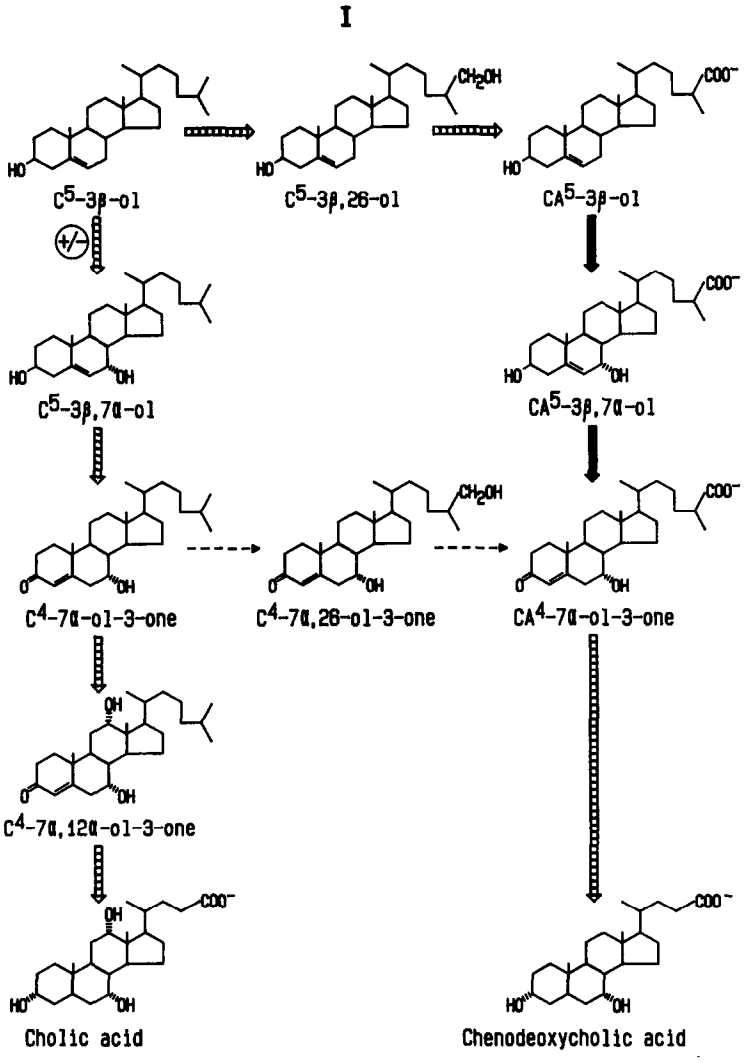


Fig. 5. A proposed model for the biosynthesis of cholic and chenodeoxycholic acids in (I) healthy subjects with a moderate inhibition of cholesterol 7α-hydroxylase (indicated by ±). The model is based on the identification, absolute levels and correlations between levels of these bile acid precursors in plasma. Arrows indicate probable reactions and their width the rate of flow (levels of compounds). Filled arrows indicate statistically significant positive correlations between levels.

of 3β-hydroxy-5-cholestenoic acid in plasma is not increased in extrahepatic cholestasis appears to support the second explanation.

When the activity of cholesterol 7α-hydroxylase is increased, the neutral intermediates increase markedly in blood. According to the model, the major part of 7α-hydroxy-3-oxo-4-cholestenoic acid is now derived from the corresponding neutral compound (Fig. 6). This is supported by the elevated levels of 7α,26-dihydroxy-4-cholesten-3-one in blood, and the positive correlation between the levels of 7α-hydroxy-4-cholesten-3-one and the corresponding acid under these conditions. In these patients there is no correlation between levels of 3β,7α-dihydroxy-5-cholestenoic acid and 7α-hydroxy-3-oxo-4-cholestenoic acid [6]. The model predicts an increased production of both cholic and chenodeoxycholic acid, which is in agreement with kinetic studies *in vivo* [18]. However, the positive correlation between the levels of 3β-hydroxy-5-cholestenoic acid and its 7α-hydroxylated form ($r = 0.82$, $P < 0.0001$) is still seen in these patients [5], indicating that the latter is formed from the acid and not from 7α-hydroxycholesterol. Although 7α-hydroxycholesterol was determined in only a few cases in our study, comparisons with the results of Björkhem *et al.*[8] show that the concentration of 3β,7α-dihydroxy-5-cholestenoic acid cannot be correlated to that of 7α-hydroxycholesterol in patients with increased bile acid synthesis.

The levels of the 3β-hydroxy-5-ene-$C_{27}$ acids in blood were significantly lower in patients with ileal resection than in patients treated with cholestyramine (Table 1 and Fig. 1) [6]. This interesting difference could be related to the availability of the precursor cholesterol. The patients with ileal resection had

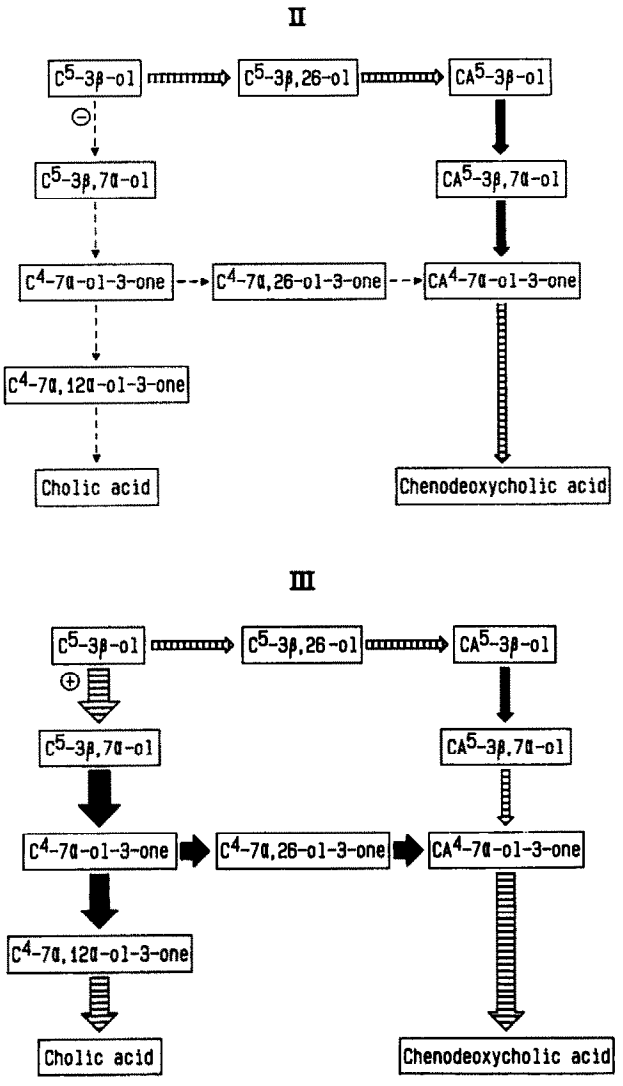


Fig. 6. The model for the biosynthesis of cholic and chenodeoxycholic acids in patients with a decreased activity of cholesterol 7α-hydroxylase (II, due to liver diseases) and in patients with an increased activity of cholesterol 7α-hydroxylase (III, due to a reduced intestinal reabsorption of bile acids). Arrows indicate probable reactions and their width the rate of flow (levels of compounds). Filled arrows indicate statistically significant positive correlations between levels. For abbreviations, see Fig. 5.

low levels of cholesterol in blood, while the patients given cholestyramine were treated for hypercholesterolemia. Hypercholesterolemic patients treated with cholestyramine have been reported to have normal or elevated levels of 26-hydroxycholesterol in plasma [11]. Patients with ileal resection have an increased synthesis of cholesterol [19] and the newly synthesized cholesterol may be extensively 7α-hydroxylated, possibly affecting the availability of substrate for 26-hydroxylation. The production/level of 3β,7α-dihydroxy-5-cholestenoic acid appeared to be relatively independent of the activity of cholesterol 7α-hydroxylase (Fig. 1). Thus, the formation of this acid may depend on access to or source of the precursor cholesterol in a pathway starting with 26-hydroxylation. Other factors regulating this pathway may obviously exist.

Studies attempting to evaluate the relative importance of different pathways have usually indicated that the pathway starting with 26-hydroxylation is of minor importance [1–3]. This result may have been affected by the experimental conditions, since the studies have been performed on patients with partial or complete bile fistulas, leading to an enhanced bile acid synthesis. Our model predicts that a 26-oxygenated pathway would be of minor importance in such patients, while it could be the major pathway to chenodeoxycholic acid in normal subjects and particularly in patients with liver disease. The model is also compatible with the formation of cholic and chenodeoxycholic acids from the same cholesterol pool in patients with bile fistulas [20] and from different pools when the activity of cholesterol 7α-hydroxylase is low.

The pathway starting with 26-hydroxylation has been believed to involve 26-hydroxylated sterols [3] or $C_{24}$ bile acids [21] and not the corresponding $C_{27}$ acids. However, the levels of cholestenoic acids are comparable to those of the corresponding neutral intermediates while the concentration of 7α,26-dihydroxycholesterol (if present) is very low. This could suggest that the latter is not an important intermediate but does not exclude it from being so. Evidence against 3β-hydroxy-5-cholestenoic acid being an intermediate has been obtained in the rabbit [22], but species differences prevent an extrapolation to humans. Even if the $C_{27}$ acids turn out to be side products of the corresponding 26-hydroxysterols, the basic concepts of our model are still valid.

Our results support the view that 7α-hydroxy-4-cholesten-3-one is the key bifurcation compound to cholic and chenodeoxycholic acid in patients with enhanced bile acid synthesis. This is in good agreement with results obtained by administration of labelled 7α-hydroxy-4-cholesten-3-one to bile fistula patients [3]. It is not clear to what extent 5β-reduction may occur prior to 26-hydroxylation, but our data seem to suggest that 26-hydroxylation of the 3-oxo-4-ene compound is a major pathway. 7α,12α-Dihydroxy-4-cholesten-3-one is an established precursor of cholic acid in man, whereas 7α-hydroxy-3-oxo-4-cholestenoic acid has never been tested as a precursor for chenodeoxycholic acid. However, one of the precursors of this $C_{27}$ acid, 7α,26-dihydroxy-4-cholesten-3-one, favoured chenodeoxycholic over cholic acid by a factor of 7 when tested *in vivo* [3]. Indirect evidence that this compound was converted to 7α-hydroxy-3-oxo-4-cholestenoic acid prior to reduction of the A-ring was obtained following administration of the reduced compound, 5β-cholestane-3α,7α,26-triol, which yielded chenodeoxycholic and cholic acid in approximately equal amounts [3].

Since we did not find 5β-saturated intermediates in plasma, we can only rely on previous data for establishing the further fate of the 3-oxo-4-ene compounds. Thus, the existence of minor pathways between the two major ones following 5β-reduction cannot be excluded. Conversion of 7α,26-dihydroxy-4-cholesten-3-one to cholic acid has been demonstrated following administration of the labeled compound to patients but the results indicated that this is of minor importance [3]. Other pathways may also exist e.g. those involving 25-hydroxylation [1, 23]. This is a major pathway to cholic acid in patients with cerebrotendinous xanthomatosis [23] but we have not found such intermediates in the blood from the patients studied.

The proposed model is in good agreement with many previous studies on bile acid formation in man. It explains how the production of cholic acid can be selectively decreased when the activity of cholesterol 7α-hydroxylase is low, and how the production of both cholic and chenodeoxycholic acid increases with enhanced 7α-hydroxylase activity. It may be argued that levels of steroids and bile acids in plasma are determined by so many factors that relationships to metabolic pathways cannot be inferred from these levels. However, the model is compatible with the concentrations of the proposed intermediates in plasma, the absence of intermediates in alternative pathways and previous results of studies of the metabolism of radiolabelled intermediates in man [3].

Cholesterol and bile acid biosynthesis are regulated in a closely linked fashion [1, 24]. Oxysterols, e.g. 25- and 26-hydroxycholesterol [12, 25–27], and nonpolar bile acids [28–30] inhibit 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. The feed-back inhibition of both HMG-CoA reductase and cholesterol 7α-hydroxylase seems to be inversely proportional to the polarity of the bile acid [30]. Inhibitory effects of 26-oxygenated steroids or their metabolite(s) on the activity of HMG-CoA reductase can be suspected since patients with cerebrotendinous xanthomatosis who lack these compounds have an increased production of cholesterol [31]. Thus, the nonpolar 26-oxygenated sterols and $C_{27}$ bile acids in plasma are possible regulatory links between cholesterol and bile acid synthesis, and their qualitative and quantitative composition under various conditions could be important. The persistent formation of these compounds (and their metabolite(s)) also in cases with reduced bile acid synthesis is interesting. Although 3β-hydroxy-5-cholestenoic acid is a less potent inhibitor of HMG-CoA reductase than 26-hydroxycholesterol in Chinese hamster ovary cells [26], the elevated levels in patients with liver cirrhosis might inhibit peripheral cholesterol synthesis in these patients.

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