

### 32.1 Introduction

The primary bile acids (the taurine and glycine conjugates of chenodeoxycholic acid and cholic acid) are synthesised from cholesterol in the liver. The first and rate-limiting step in the synthetic pathway, cholesterol 7 $\alpha$ -hydroxylase, is inhibited by bile acids as they flow through the liver in the enterohepatic circulation. Bile acids also reduce hepatic sterol synthesis by feedback inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. Secretion of bile acids from the hepatocytes into the canaliculi drives water secretion so that a major component of bile flow is “bile acid dependent”. In the intestinal lumen, bile acids are responsible for assisting the digestion and absorption of lipids. Thus, in some inborn errors of bile acid synthesis, failure of bile flow (cholestasis) and malabsorption of fat and fat-soluble vitamins are prominent clinical features. Patients may present with prolonged neonatal jaundice, steatorrhoea, rickets and haemorrhage due to vitamin K deficiency. In some disorders of bile acid synthesis, however, symptoms of bile acid deficiency do not occur. Rather, symptoms appear to be caused mainly by accumulation of intermediates proximal to the site of the block and conversion of these intermediates to a product which is deposited in various tissues of the body. The deposition of cholestanol and cholesterol can lead to the formation of cataracts, to mental retardation in the first decade and neurological regression with dementia and motor dysfunction in later life. The lipid deposition also produces tendon xanthomata and premature atherosclerosis.

Treatment with bile acids is effective whether symptoms are due to bile acid deficiency or to precursor accumulation. In the latter case, the effect is mediated through inhibition of cholesterol 7 $\alpha$ -hydroxylase and HMG-CoA reductase. Because bile acid therapy leads to a dramatic improvement in liver function and malabsorption in some patients and to an improvement in intelligence quotient (IQ) in others, it is important to make a diagnosis as early as possible. The simplest way to screen a symptomatic individual for all inborn errors of bile acid synthesis is to analyse urine by liquid secondary ion-mass spectrometry (LSI-MS). Other methods are available for

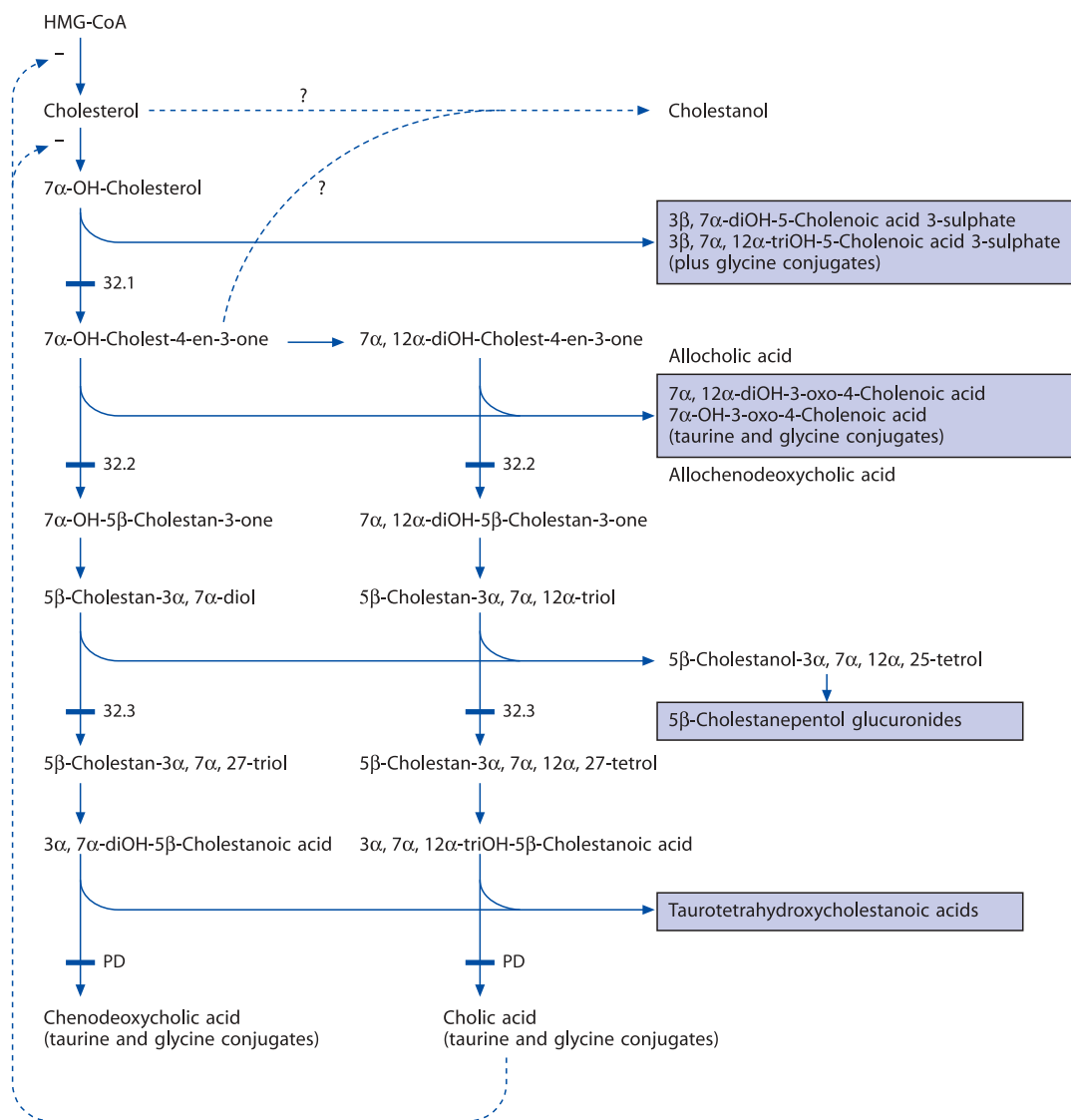
some of the individual disorders. Routine neonatal screening has not yet been described.

In disorders which affect cholesterol synthesis (e.g, mevalonic aciduria, 7-dehydrocholesterol reductase deficiency [Smith-Lemli-Opitz syndrome]) there may be markedly reduced bile acid synthesis – these disorders are beyond the scope of this chapter. As indicated in section 3, the synthesis of bile acids involves conversion of C<sub>27</sub> bile acids (cholestanoic acids) to their C<sub>24</sub> analogues (cholanic acids) and this occurs by a process of  $\beta$ -oxidation in the peroxisomes. Thus defective bile acid synthesis occurs in disorders of peroxisomal  $\beta$ -oxidation and in disorders of peroxisome biogenesis. These disorders affect pathways other than the bile acid synthesis pathway and are discussed in Chap. 23.

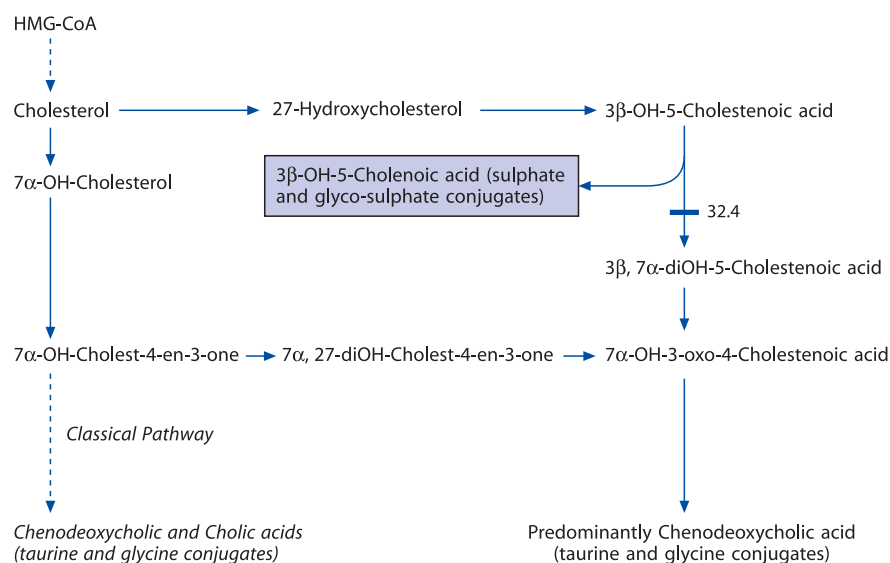
## 32.2 Nomenclature

Disorder	Affected component	Tissue distribution	Chromosomal localisation	MIM
32.1	3 $\beta$ -Hydroxy- $\Delta^5$ -C <sub>27</sub> -steroid dehydrogenase (3 $\beta$ -HSDH) deficiency	Fibroblasts	16p11.2–12	231100
32.2	$\Delta^4$ -3-Oxosteroid 5 $\beta$ -reductase deficiency (5 $\beta$ -reductase deficiency)	Liver	7q31	235555
32.3	Sterol 27-hydroxylase deficiency (cerebrotendinous xanthomatosis, CTX)	Fibroblasts	2q33-qter	213700
32.4	Oxysterol 7 $\alpha$ -hydroxylase (CYP7b) deficiency	Fibroblasts	8q21.3	231100

## 32.3 Metabolic Pathway



**Fig. 32.1.** The classical ('neutral') pathway for the synthesis of bile acids from cholesterol, where the modification of the steroid nucleus occurs prior to side-chain modification. Also illustrated are the inborn errors of bile acid synthesis and the resulting abnormal metabolites. 32.1, 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid dehydrogenase (3 $\beta$ -HSDH) deficiency; 32.2,  $\Delta^4$ -3-oxosteroid 5 $\beta$ -reductase deficiency; 32.3, sterol 27-hydroxylase deficiency (cerebrotendinous xanthomatosis, CTX); PD, peroxisomal disorders (defects of peroxisome biogenesis and peroxisomal  $\beta$ -oxidation). The abnormal metabolites that are readily detected by analysis of urine by LSI-MS are shown in boxes. Cholic acid can also be synthesised from 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol; this is the so-called microsomal or 25-hydroxylase pathway of cholic acid synthesis, which provides an alternative route for side-chain modification other than peroxisomal  $\beta$ -oxidation



**Fig. 32.2.** The alternative ('acidic') pathway for the synthesis of bile acids, which starts with conversion of cholesterol to 27-hydroxycholesterol and results predominantly in the production of chenodeoxycholic acid. Also shown is the route of the classical pathway via 7 $\alpha$ -hydroxycholesterol. The alternative pathway illustrates that side-chain modification can proceed modification of the steroid nucleus, although the exact sequence and relative contributions of these different pathways is unclear, with most of the synthetic enzymes being shared by both pathways. A deficiency of oxysterol 7 $\alpha$ -hydroxylase (32.4), which is unique to the alternative pathway, is shown, and the abnormal metabolites that are readily detected in urine by LSI-MS are highlighted

## 32.4 Signs and Symptoms

**Table 32.1.**  $3\beta$ -Hydroxy- $\Delta^5$ - $C_{27}$ -steroid dehydrogenase deficiency

System	Symptoms/marker	Neonatal	Infancy	Childhood
Characteristic clinical findings	Jaundice	+	±	±
	Steatorrhoea	+	+	+
	Rickets	+	+	+
	Vit. K-reponsive bleeding	±	±	±
	Pruritus		±	±
Routine laboratory	Conjugated bilirubin (P)	↑	n-↑	n-↑
	ASAT/ALAT (P)	↑	↑	↑
	$\gamma$ -GT (P)	n	n	n
	Alkaline phosphatase (P)	↑	↑	↑
	Albumin (P)	n	n	n
	Calcium (P)	↓-n	↓-n	↓-n
	Cholesterol (P)	↓-n	↓-n	↓-n
	Prothrombin ratio	n-↑	n-↑	n-↑
	Periportal inflammation	+	+	+
Liver biopsy	Giant cells	+	+	
	Cholestasis	+	±	±
	Bridging fibrosis		+	+
	Cirrhosis		±	±
	Sulfated $3\beta,7\alpha$ -dihydroxy- and $3\beta,7\alpha,12\alpha$ -trihydroxy-5-cholenoic acids (P, U) (m/z 469, 485, 526, 542 on LSIMS)	+	+	+
Special laboratory	Vitamin A (P)	↓-n	↓-n	↓-n
	25-Hydroxy-vitamin D (P)	↓	↓	↓
	Vitamin E (P)	↓	↓	↓
	Chenodeoxycholic acid (P)	↓	↓	↓
	Negative ion LSI-MS (U) (m/z 469, 485, 526, 542)	+	+	+

**Table 32.2.**  $\Delta^4$ -3-Oxosteroid 5 $\beta$ -reductase deficiency<sup>a</sup>

System	Symptoms/markers	Neonatal	Infancy
Characteristic clinical findings	Jaundice	+	±
	Hepatosplenomegaly	±	±
	Ascites, edema	±	±
Routine laboratory	Conjugated bilirubin (P)	↑	n-↑
	ASAT/ALAT (P)	↑	↑
	$\gamma$ -GT (P)	n-↑	n-↑
	Alkaline phosphatase (P)	n-↑	n-↑
	Albumin (P)	↓-n	↓-n
	Cholesterol (P)	↓-n	↓-n
	Prothrombin ratio	↑	n-↑
	Ferritin (S)	n-↑	n-↑
Liver biopsy	Periportal and lobular inflammation	±	±
	Giant cells	+	+
	Cholestasis	+	+
	Pseudoacinar transformation	+	±
	Small caniculi, few microvilli	+	±
Special laboratory	7 $\alpha$ -Hydroxy-3-oxo- and 7 $\alpha$ ,12 $\alpha$ -dihydroxy-3-oxo-4-cholenoic acids (U) (m/z 444, 460, 494, 510 on LSI-MS)	+	+
	Tyrosine, methionine (P)	↑	n-↑
	Allocholic and allochenodeoxycholic acid (P)	+	+
	Reduced content of immunoreactive 5 $\beta$ -reductase in liver biopsy/truncated protein	+	+

<sup>a</sup> There is no test currently available that can prove that a patient has reduced activity  $\Delta^4$ -3-oxosteroid 5 $\beta$ -reductase as a result of a defect in the gene coding for this enzyme and it is known that reduced activity of the enzyme (resulting in excretion of 3-oxo- $\Delta^4$  bile acids) can occur as a non-specific consequence of severe liver damage in infancy/childhood. Thus, at present the features of 5 $\beta$ -reductase deficiency cannot be accurately defined. Nor can its relationship to other recessive disorders causing severe liver damage (e.g. neonatal haemochromatosis).

**Table 32.3.** Sterol 27-Hydroxylase deficiency (Cerebrotendinous xanthomatosis, CTX)

System	Symptoms/marker	Neonatal	Infancy	Childhood	Adolescence	Adult
Characteristic clinical findings	Developmental delay/↓IQ			±	±	±
	Regression/dementia				±	±
	Spastic paresis/pyramidal signs			±	±	±
	Ataxia				±	±
	Expressive dysphasia				±	±
	Tendon xanthomatoma				±	±
	Ischaemic heart disease, angina/ myocardial infarction					±
	Cataracts			±	±	±
Routine laboratory	Spinal cord myelopathy					±
	Cholesterol (P)				n-↑	n-↑
Special laboratory	Cholestane pentol glucuronides (U) (m/z 627 in LSI-MS)	+	+	+	+	+
	25-Hydroxy-vitamin D (P)				↓-n	↓-n
	Abnormal EEG ± evoked potentials			±	±	±
	Cholestanol (P)	↑	↑	↑	↑	↑
	27-Hydroxylase (FB)	↓	↓	↓	↓	↓
GI	Diarrhoea		±	±		
Respiratory	Failure					±
CNS	Peripheral neuropathy					±
	Parkinsonian symptoms, hypokinesia/tremor				±	
	Demyelination and lipid deposition on MRI				±	
	Convulsions			±	±	±
	Hypo-/hyperthyroidism					±
Endocrine	Adrenal insufficiency					±
	Pituitary/hypothalamic dysfunction					±
Musculoskeletal	Fractures (osteoporosis)					±
	Foot deformity (pes cavus)					±
Hepatobiliary	Pigment granules in liver biopsy					±
	Gall stones/cholecystectomy					±

**Table 32.4.** Oxysterol 7 $\alpha$ -hydroxylase deficiency

System	Symptoms/marker	Infancy <sup>a</sup>
Characteristic clinical findings	Jaundice	+
	Hepatosplenomegaly	+
	Vit. K-responsive bleeding	+
Routine laboratory	Total/direct bilirubin (S)	↑
	ALAT/ASAT (S)	↑
	$\gamma$ -GT (S)	n
	Alkaline phosphatase (S)	↑
	Prothrombin time	↑
	Cholesterol (S)	n
Liver biopsy	Cholestasis	+
	Portal inflammation/lobular disarray	+
	Bridging fibrosis	+
	Giant cells	+
	Bile duct proliferation	+
	3 $\beta$ -Hydroxy-5-cholenoic acids (U)	+
Special laboratory	(m/z 453, 510 by LSI-MS)	
	Vitamin E (S)	n
	Retinol (S)	↓

<sup>a</sup> Taken from the case report of a single patient.

## 32.5 Reference Values

### ■ Determination of Urinary Cholanoid (Bile Acid and Bile Alcohol) Profile by LSI-MS

The mass spectrometer scans negative ions over the range m/z 350–700 and draws a spectrum with the largest peak as 100% intensity. In the following table, – indicates that the peak is not detectable above the background,  $\pm$  indicates undetectable to 20% of the largest peak,  $\uparrow$  indicates 20–100% intensity of largest peak.



Ion	Identity	Normal	Cholestasis
444	7 $\alpha$ -Hydroxy-3-oxo-4-cholenoic acid (Gly)	–	±
448	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Gly)	±	±/↑
453	3 $\beta$ -Hydroxy-5-cholenoic acid (SO <sub>4</sub> )	–	±
460	7 $\alpha$ ,12 $\alpha$ -Dihydroxy-3-oxo-4-cholenoic acid (Gly)	–	±
464	Trihydroxy-cholanoic acids (e.g. cholic acid) (Gly)	±	±/↑
469	3 $\alpha$ ,7 $\alpha$ -Dihydroxy-5-cholenoic acid (SO <sub>4</sub> ) also steroid sulphate <sup>a</sup>	– ±	– –
485	3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -Trihydroxy-5-cholenoic acid (SO <sub>4</sub> )	–	–
494	7 $\alpha$ -Hydroxy-3-oxo-4-cholenoic acid (Tau)	–	±
498	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Tau)	±	±/↑
510	7 $\alpha$ ,12 $\alpha$ -Dihydroxy-3-oxo-4-cholenoic acid (Tau), 3 $\alpha$ -Hydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )	±	±
514	Trihydroxy-cholanoic acids (e.g. cholic acid) (Tau)	±	±/↑
526	3 $\alpha$ ,7 $\alpha$ -Dihydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )	–	±
528	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Gly, SO <sub>4</sub> )	±	±/↑
530	Tetrahydroxy-cholanoic acids (Tau)	±	±/↑
542	3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -Tetrahydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )	–	–
572	Tetrahydroxycholestanoic acids (Tau)	–	–
613	27-Nor-cholestanepentol (SO <sub>4</sub> ) (Gluc)	±	±
627	Cholestanepentols (Gluc)	±	±

Gly, glycine conjugate; Tau, taurine conjugate; SO<sub>4</sub>, sulphate; Gluc, glucuronic

<sup>a</sup> An ion of mass/charge ratio 469 can occur in urine samples from patients who do not have 3 $\beta$ -HSDH deficiency; the other ions which are characteristic of 3 $\beta$ -HSDH (485, 526, 542) are not present and GC-MS fails to show increased excretion of 3 $\alpha$ ,7 $\alpha$ -dihydroxy-5-cholenoic acid.

## ■ Urinary Cholanoid Excretions Determined by GC-MS

Cholanoid	µmol/mmol Creat	% Total bile acid excretion
3 $\beta$ ,7 $\alpha$ -diOH-5-cholenoic acid <sup>a</sup>	<0.1	<2%
3 $\beta$ ,7 $\alpha$ ,12 $\alpha$ -triOH-5-cholenoic acid <sup>a</sup>	<0.1	<2%
7 $\alpha$ -OH-3-oxo-4-cholenoic acid <sup>b</sup>	trace <sup>d</sup>	<sup>d</sup>
7 $\alpha$ ,12 $\alpha$ -triOH-3-oxo-4-cholenoic acid <sup>b</sup>	trace <sup>d</sup>	<sup>d</sup>
Cholestanepentols <sup>c</sup>	<1.0	

<sup>a</sup> Following mild solvolysis and enzymatic hydrolysis of glycine conjugates.

<sup>b</sup> Following enzymatic hydrolysis of glycine and taurine conjugates.

<sup>c</sup> Following hydrolysis with glucuronidase.

<sup>d</sup> Amount of urinary bile acids in healthy neonates, including 3-oxo- $\Delta^4$  bile acids, has been shown to be elevated in the first month of life – 7 $\alpha$ ,12 $\alpha$ -diOH-3-oxo-cholenoic acid <13.0 µmol/mmol creat (<30% total bile acid excretion) and 7 $\alpha$ -OH-3-oxo-cholenoic acid <0.4 µmol/mmol creat (<1% total bile acid excretion). See ref. [14].

### ■ Plasma Cholanoid Concentrations

The data below refers to results obtained by GC-MS following hydrolysis of glycine and taurine conjugates with cholyglycine hydrolase. Normal plasma bile acid concentrations are higher in the postprandial period (0.5–3 h following a fat-containing meal) than in the fasting state. They are also higher in the neonatal period than later in infancy. For the purposes of diagnosis of inborn errors these differences are not of great importance and have not been included in the reference data.

Plasma cholanoid	Concentration (μmol/l)	
	Normal	Cholestatic
Chenodeoxycholic acid	0.22–12.4	25–359
Cholic acid	0.05–4.55	7–317
Other cholanoids of diagnostic significance <sup>a</sup>	<0.25	<0.25

<sup>a</sup> 3β,7α-Dihydroxy-5-cholenoic, 3β,7α,12α-trihydroxy-5-cholenoic, 7α-hydroxy-3-oxo-4-cholenoic, 7α,12α-diOH-3-oxo-4-cholenoic, allocholic, allochenodeoxycholic and 3α,7α,12α-trihydroxy-5β-cholestanoic acid (THCA).

### ■ Plasma Cholesterol Concentrations

The values below refer to total plasma cholesterol concentration determined by GC-MS analysis following hydrolysis of cholesterol esters.

Age	<15 y	>15 y	Cholestatic
Cholesterol (P) (μmol/l)	1–9	4–18	4–50

## 32.6 Pathological Values

### ■ Urinary Cholanoid (Bile Acid and Alcohol) Profile by LSI-MS

Ion	Identity	32.1 3 $\beta$ -HSDH	32.2 5 $\beta$ - Reductase <sup>a</sup>	32.3 Sterol 27- hydroxylase (CTX)	32.4 Oxysterol 7 $\alpha$ -hydroxy- lase	Peroxisomal disorders
444	7 $\alpha$ -Hydroxy-3-oxo-4-cholenoic acid (Gly)		↑			
448	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Gly)		-/±			±
453	3 $\beta$ -Hydroxy-5-cholenoic acid (SO <sub>4</sub> )				↑	
460	7 $\alpha$ ,12 $\alpha$ -Dihydroxy-3-oxo-4-cholenoic acid (Gly)		↑			
464	Trihydroxy-cholanoic acids (e.g. cholic acid) (Gly)		-/±			±
469	3 $\beta$ ,7 $\alpha$ -Dihydroxy-5-cholenoic acid (SO <sub>4</sub> ) also steroid sulphate?	↑				
485	3 $\beta$ ,7 $\alpha$ ,12 $\alpha$ -Trihydroxy-5-cholenoic acid (SO <sub>4</sub> )	↑				
494	7 $\alpha$ -Hydroxy-3-oxo-4-cholenoic acid (Tau)		↑			
498	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Tau)		-/±			±
510	7 $\alpha$ ,12 $\alpha$ -Dihydroxy-3-oxo-4-cholenoic acid (Tau) or 3 $\beta$ -Hydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )		↑		↑	
514	Trihydroxy-cholanoic acids (e.g. cholic acid) (Tau)		-/±			±
526	3 $\beta$ ,7 $\alpha$ -Dihydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )	↑				
528	Dihydroxy-cholanoic acids (e.g. chenodeoxycholic acid) (Gly, SO <sub>4</sub> )					±
530	Tetrahydroxy-cholanoic acids (Tau)					±
542	3 $\beta$ ,7 $\alpha$ ,12 $\alpha$ -Trihydroxy-5-cholenoic acid (Gly, SO <sub>4</sub> )	↑				
572	Tetrahydroxycholestanoic acids (Tau)					±/↑ <sup>b</sup>
613	27-Nor-cholestanepentol(s) (Gluc)			↑		
627	Cholestanepentols (Gluc)			↑ <sup>c</sup>		

<sup>a</sup> In patients considered to have a genetic deficiency of 5 $\beta$ -reductase, the LSI-MS spectrum shows peaks due to 3-oxo- $\Delta^4$  bile acids that are 4–5 times larger than those due to the corresponding saturated bile acids ie 444 → 448, 460 → 464, 494 → 498, 514 → 510. The saturated bile acids may not be detectable above the background. By contrast a LSI-MS spectrum which shows 3-oxo- $\Delta^4$  peaks of similar size to the corresponding saturated bile acid i.e. 444 approx.=448 etc. indicates severe hepatocyte damage due to something other than genetic 5 $\beta$ -reductase deficiency. In these patients the excretion of 3-oxo- $\Delta^4$  bile acids will disappear when the hepatocyte function improves.

<sup>b</sup> In patients with peroxisome biogenesis defect over the age of 18 mo the LSI-MS analysis may give a negative result.

<sup>c</sup> Also observed in a patient postulated to have an inborn error of the 25-hydroxylase pathway for bile acid side-chain synthesis (along with a large m/z 611 – cholestanetetrol glucuronides), who presented with neonatal jaundice and hepatomegaly (see ref. [11]).

## ■ Further Analysis of Urinary Cholanoid Profile by GC-MS

GC-MS analysis is used to confirm the identities of ions in the LSI-MS urine spectrum and show that the excretion of abnormal cholanooids is >20 times normal. In the case of 5 $\beta$ -reductase deficiency GC-MS analysis should show that 3-oxo- $\Delta^4$  bile acids account for >70% of the total urinary bile acid excretion. In the case of sterol 27-hydroxylase deficiency (CTX), GC-MS analysis should indicate that the major cholestane pentols in the urine are 3,7,12,22,25 and 3,7,12,23,25-pentols. (One patient has been described who had familial cholestatic liver disease associated with greatly increased urinary excretion of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,24 S,25-pentol [see previous table]). Liquid secondary ion-tandem mass spectrometry (LSI-MS/MS) is an alternative method to GC-MS and can rapidly confirm the identity of a number of diagnostic ions that are found in the LSI-MS spectrum of urine. These include sulphated and taurine-conjugated abnormal metabolites such as those observed in 3 $\beta$ -HSDH deficiency (32.1), 5 $\beta$ -reductase deficiency (32.2), oxysterol 7 $\alpha$ -hydroxylase deficiency (32.4) and peroxisomal disorders [13].

Plasma cholanooids ( $\mu\text{mol/l}$ )	32.1 3 $\beta$ -HSDH	32.2 5 $\beta$ -Reductase	32.3 Sterol 27-hydroxylase (CTX)	32.4 Oxysterol 7 $\alpha$ -hydroxylase	Peroxisomal disorders
Chenodeoxycholic acid	↓ (<0.1)	↑/↓ (0–25)	↓	n	n/↑
Cholic acid	n/↓ (0–4.5)	n/↓	n/↓	n	n/↓
3 $\beta$ ,7 $\alpha$ -Dihydroxy-5-cholenoic <sup>a</sup>	↑↑ (1–80)				
3 $\beta$ ,7 $\alpha$ ,12 $\alpha$ -Trihydroxy-5-cholenoic <sup>a</sup>	↑ (0.05–30)				
3 $\beta$ ,7 $\alpha$ -Dihydroxy-5-cholestenoic	↑↑ (3–40)				
7 $\alpha$ -Hydroxy-3-oxo-4-cholenoic		↑ (0.8–10.0) <sup>b</sup>			
7 $\alpha$ ,12 $\alpha$ -Dihydroxy-3-oxo-4-cholenoic		↑ (0.3–2.0) <sup>b</sup>			
Allochenodeoxycholic acid		↑ (0.5–10.0) <sup>c</sup>			
Allocholic acid		↑ (0.5–8.0) <sup>c</sup>			
3 $\beta$ -Hydroxy-5-cholenoic acid				↑↑ (87)	
3 $\beta$ -Hydroxy-5-cholestenoic acid				↑↑ (24)	
3 $\alpha$ ,7 $\alpha$ -Dihydroxycholestanoic acid					↑↑ (0.5–12)
3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -Trihydroxycholestanoic acid					↑↑ (0.8–30)
5 $\beta$ -Cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ ,25-tetrol			↑↑		
Other compounds detected in cholanooid profile			d		e
Cholestanol			↑ (19–400)		

<sup>a</sup> These compounds are present almost entirely as sulphates in the plasma of patients with 3 $\beta$ -HSDH deficiency and will not be detected unless plasma is subjected to a mild solvolysis procedure.

<sup>b</sup> 3-Oxo- $\Delta^4$ -bile acids constitute >10% of total plasma bile acids.

<sup>c</sup> Allo-bile acids constitute >20% of total.

<sup>d</sup> 7 $\alpha$ -Hydroxycholesterol, 7 $\alpha$ -hydroxy-cholest-4-en-3-one, 7 $\alpha$ ,12 $\alpha$ -dihydroxy-cholest-4-en-3-one, 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ ,12 $\alpha$ -triol.

<sup>e</sup> C<sub>29</sub>-dicarboxylic acid and tetrahydroxycholestanoic acids in disorders of peroxisome biogenesis. Varanic acid in disorders of D-bifunctional protein and thiolase deficiencies.

## 32.7 Loading Tests

Not applicable.

## 32.8 Diagnostic Flow Chart

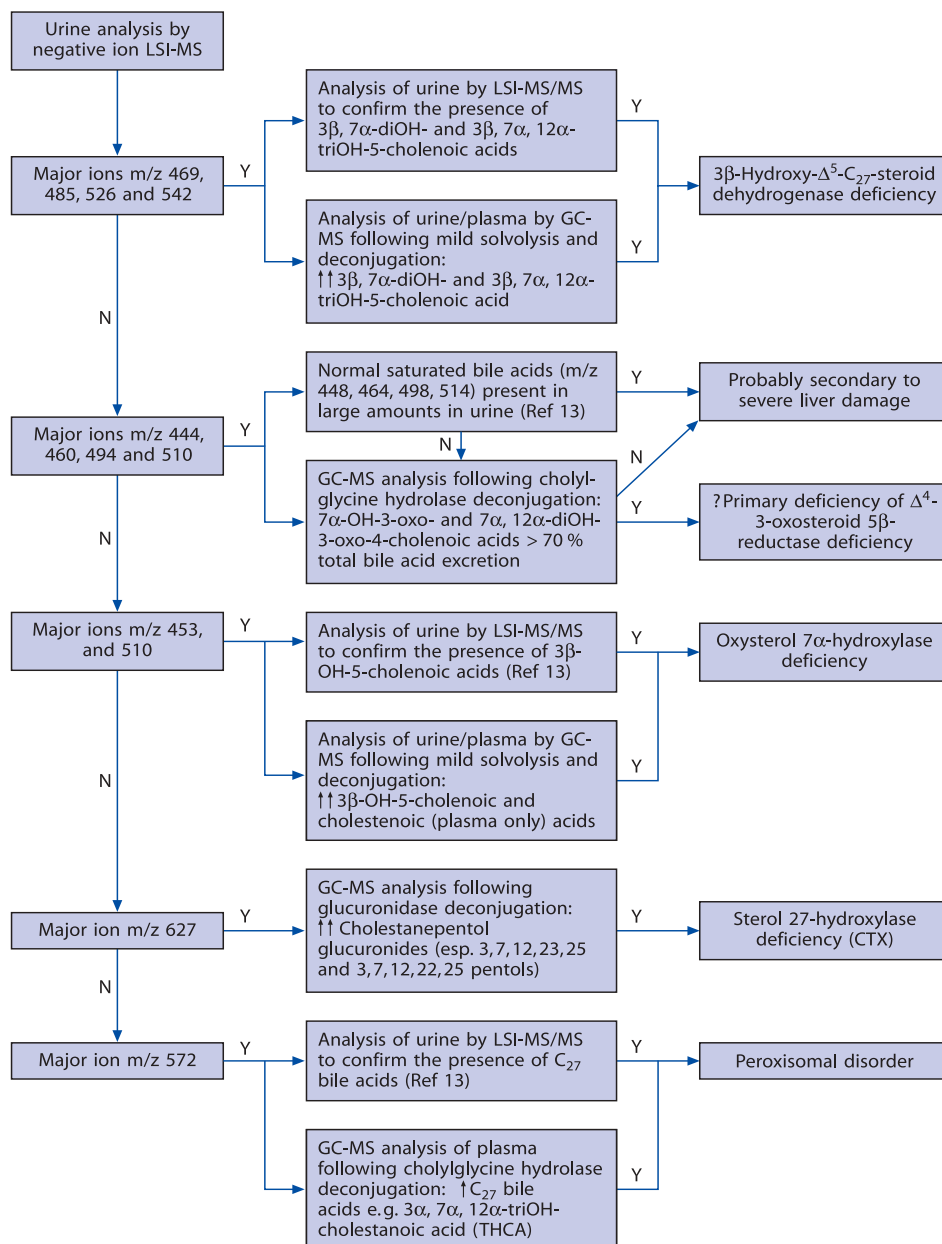


Fig. 32.3

## 32.9 Specimen Collection

Test	Conditions	Material	Handling	Pitfalls
Urine cholanoïd profile by LSI-MS	No bile acid therapy	Urine $\geq 0.5$ ml	Ambient temp. 12 h, 4 °C for 48 h, -20 °C for >6 months	Drugs and radiographic contrast media may produce large peaks on the LSI-MS spectrum
		Cholanoids from urine adsorbed on C <sub>18</sub> cartridge (volume of urine and creatinine recorded)	Ambient temp. 48 h	
Further analysis of urinary cholanoïds by GC-MS	No bile acid therapy	Urine $\geq 2.0$ ml (can be sent on C <sub>18</sub> cartridge as above)	As above	
Plasma bile acids	No bile acid therapy	Plasma/serum 0.5–2.0 ml	Ambient temp. 12 h, 4 °C for 48 h, -20 °C for >6 months	
Plasma cholestanol	No bile acid therapy	Plasma/serum 0.2–1.0 ml	As above	

## 32.10 Prenatal Diagnosis

32.1  $3\beta$ -Hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid dehydrogenase deficiency: Prenatal diagnosis has not been recorded but the enzyme can be assayed in cultured skin fibroblasts and could probably therefore be assayed in chorionic villus cells or amniocytes.

32.2  $\Delta^4$ -3-oxosteroid  $5\beta$ -reductase: Mutation screening of the  $5\beta$ -reductase gene is likely to be available soon.

32.3 Sterol 27-hydroxylase deficiency (CTX): This enzyme deficiency is also detectable in cultured fibroblasts. In addition DNA-based methods could be used for the detection of common mutations.

32.4 Oxysterol 7 $\alpha$ -hydroxylase: Gene analysis has been conducted on the sole patient described with this disorder.

### 32.11 Initial Treatment

Infants with severe cholestasis due to  $3\beta$ -HSDH deficiency may have hypocalcaemia due to malabsorption of vitamin D or severely deranged clotting due to malabsorption of vitamin K. Vitamins D and K should be given either parenterally or orally in a form that is absorbed despite intestinal bile salt deficiency (e.g.  $1\alpha$ -hydroxy-cholecalciferol or  $1,25$ -dihydroxy-cholecalciferol). Fresh frozen plasma and intravenous calcium supplement may occasionally be required.

### 32.12 Summary/Comments

Diagnosis of the 4 inborn errors of bile acid synthesis discussed in this chapter is important because three are treatable by oral bile acid supplementation (such treatment was not successful in the single reported case of oxysterol  $7\alpha$ -hydroxylase deficiency). Liquid secondary ion mass spectrometry (LSI-MS) is a simple and rapid method which can be used to screen urine samples for abnormal cholanooids (bile acids and bile alcohols). It should be applied to neonates with unexplained cholestatic liver disease, particularly if familial and associated with steatorrhoea and fat-soluble vitamin malabsorption, to infants and children with developmental delay whether or not this is associated with specific features suggestive of a peroxisomal disorder (e.g. hypotonia, seizures, dysmorphic features, ocular and auditory abnormalities and hepatic dysfunction) or CTX (e.g. juvenile cataracts).

### References

1. Björkhem I. Mechanism of bile acid biosynthesis in mammalian liver. In: Danielsson H & Sjövall J (eds) *Sterols and Bile Acids. New Comprehensive Biochemistry Volume 12*. 1985. Elsevier, Amsterdam, New York, Oxford, pp 231–278
2. Clayton PT. Inborn errors of bile acid metabolism. *J Inher Metab Dis* 1991 14: 478–496
3. Russell DW & Setchell KDR. Bile acid biosynthesis. *Biochemistry* 1992 31: 4737–4749
4. Verrips A, Hoefsloot L, Steenbergen G, Theelan J, Wevers R et al. Clinical and molecular characteristics of patients with cerebrotendinous xanthomatosis *Brain* (2000) 123: 908–919
5. Kuriyama M, Fujiyama J, Yoshidome H, Takenaga S, Matsumoro K, Kasama T, Fukada K, Kuramoto T, Hoshita T, Seyama Y et al. Cerebrotendinous xanthomatosis: clinical features of eight patients and a review of the literature. *J Neurol Sci* (1991) 102: 225–232
6. Berginer VM, Shany S, Alkalay D, Berginer J, Dekel S, Salen G, Tint GS & Gazit D. Osteoporosis and increased bone fractures in cerebrotendinous xanthomatosis. *Metabolism* 1993, 42: 69–74

7. Bjorkhem I, Boberg KM. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Basis of Inherited Disease*. McGraw-Hill, 1995: 2073–2101
8. Clayton PT. Delta 4-3-oxosteroid 5 beta-reductase deficiency and neonatal hemochromatosis [letter; comment]. *J Pediatr* 1994, 125: 845–846
9. Shneider BL, Setchell KD, Whittington PF, Neilson KA, Suchy FJ. Delta 4-3-oxosteroid 5 beta-reductase deficiency causing neonatal liver failure and hemochromatosis [see comments]. *J Pediatr* 1994; 124: 234–238
10. Leitersdorf E, Reshef A, Meiner V *et al*. Frameshift and splice-junction mutations in the sterol 27-hydroxylase gene cause cerebrotendinous xanthomatosis in Jews of Moroccan origin. *J. Clin. Invest.* 1993, 91, 2488–2496
11. Clayton PT, Casteels M, Mieli Vergani G, Lawson AM. Familial giant cell hepatitis with low bile acid concentrations and increased urinary excretion of specific bile alcohols: a new inborn error of bile acid synthesis? *Pediatr Res* 1995, 37: 424–431
12. Setchell KD, Schwarz M, O'Connell NC *et al*. Identification of a new inborn error in bile acid synthesis: mutation of the oxysterol 7 $\alpha$ -hydroxylase gene causes severe neonatal liver disease. *J Clin Invest* 1998, 102: 1690–1703
13. Lemonde HA, Johnson AW, Clayton PT. Identification of unusual bile acid metabolites by tandem mass spectrometry: use of low energy collision induced dissociation to produce informative spectra *Rapid Commun Mass Spectrom* 1999, 13(12):1159–64.
14. Kimura A, Mahara R, Inoue T, *et al*. Profile of urinary bile acids in infants and children: developmental pattern of excretion of unsaturated ketonic bile acids and 7 $\beta$ -hydroxylated bile acids. *Ped Res* (1999) 45(4): 603–609
15. Schwarz M, Wright A, Davies D, *et al*. The bile acid synthetic gene 3 $\beta$ -hydroxy- $\Delta^5$ -C<sub>27</sub>-steroid oxido-reductase is mutated in progressive intrahepatic cholestasis. *J Clin Invest* (2000) 106: 1175–1184