The plasma bile acid profiles of three children with the inherited metabolic disorder, infantile Refsum's disease, were found to contain 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid. This intermediate in the synthesis of cholic acid was identified by combined gas-liquid chromatography–mass spectrometry and accounted for approximately 25% of the total bile acids which were present at elevated concentrations in plasma. Infantile Refsum's disease appears to share several biochemical features with the cerebro-hepato-renal syndrome (Zellweger's disease), including abnormal bile acid metabolism.

The bile acids, cholic acid and chenodeoxycholic acid, are synthesized exclusively in the liver from cholesterol and represent catabolic products of cholesterol metabolism (Salen and Shefer, 1983). In the last decade, abnormalities in bile acid biosynthesis have been detected in several inherited diseases. These include Zellweger's disease or the cerebro-hepato-renal syndrome (Hanson et al., 1979; Monnens et al., 1980; Mathis et al., 1980), cerebrotendinous xanthomatosis (Salen and Shefer, 1983; Oftebro et al., 1980) and neonatal cholestasis associated with biliary atresia (Hanson et al., 1975).

In all these diseases, various intermediates in the synthesis of cholic and chenodeoxycholic acids accumulate and can be detected in serum, bile or urine. In Zellweger's disease, altered bile acid metabolism leads to the overproduction of 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA), 3α,7α-dihydroxy-5β-cholestan-26-oic acid (DHCA) and 3α,7α,12α,24α-tetrahydroxy-5β-cholestan-26-oic acid (varanic acid) as shown in Figure 1 (Hanson et al., 1979; Monnens et al., 1980; Mathis et al., 1980). It has been postulated that these intermediates accumulate as a result of abnormal mitochondrial structure and function (Hanson et al., 1979; Mathis et al., 1980) or because of the possible absence of peroxisomes in the liver of patients with this syndrome (Hanson et al., 1979; Borst, 1983).

In addition to abnormalities in bile acid metabolism, the Zellweger syndrome is characterised biochemically by elevated levels of pipecolic acid in serum and urine (Mathis et al., 1980; Trijbels et al., 1979) and the accumulation of very long chain fatty acids, especially 5β-Cholestane 3α,7α,12α,26-tetrol

3α,7α,12α,26-Tetrol

5β-Cholesterol 3α,7α,12α,26-Tetrol

THCA

Varanic Acid

Cholic Acid

5β-Cholesterol 3α,7α,12α,26-Triol

DHCA

Chenodeoxycholic Acid

Figure 1 Oxidation of the sterol side chain in the synthesis of the bile acids, cholic acid and chenodeoxycholic acid. The abbreviations used are THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; DHCA, 3α,7α-dihydroxy-5β-cholestan-26-oic acid; varanic acid, 3α,7α,12α,24α-tetrahydroxy-5β-cholestan-26-oic acid

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hexacosanoic acid (Brown et al., 1982). These compounds have recently been found in excess in patients with the infantile form of Refsum's disease (Poulos et al., 1984). This inherited disorder is primarily identified by low levels of phytanic acid oxidase (Poulos et al., 1984). However, because of the similarities in the patterns of abnormal biochemical metabolites which are produced in these diseases, we have examined the plasma of three patients with infantile Refsum's disease for the presence of intermediates in bile acid biosynthesis.

**RESULTS**

The gas–liquid chromatograms of bile acids extracted from plasma of a patient with infantile Refsum's disease and a patient with Zellweger's disease were compared to the chromatogram obtained from the plasma of a healthy control subject (Figure 2). Major peaks in each plasma sample corresponded to chenodeoxycholic acid and the internal standard (7-ketodeoxycholic acid). However, in plasma samples

**METHODS**

**Collection of plasma**

Blood plasma was obtained from three patients with the infantile form of Refsum's disease. The diagnosis was based on the clinical features of this disease and also the biochemical abnormalities of elevated plasma phytanic acid and reduced phytanic acid oxidase activity in cultured skin fibroblasts (Poulos et al., 1984). A plasma sample was also obtained from one patient with Zellweger's disease (courtesy of Professor D. M. Danks, Melbourne University). This particular diagnosis was based on the clinical history, postmortem findings, and on the detection of increased plasma pipecolic acid levels.

**Plasma bile acid analysis**

Plasma samples (0.7–2.0 ml), plus 10 nmol of 7-ketodeoxycholic acid as internal standard, were analysed for bile acids by gas–liquid chromatography as described by Whiting and Watts (1980). Briefly, this method involves the extraction of bile acids from plasma or serum using Amberlite XAD-7 resin, chemical hydrolysis of the bile acid conjugates with sodium hydroxide, and conversion of the free bile acids to the methyl ester trifluoroacetate derivatives. Gas–liquid chromatography was carried out using a silanized glass column (1.2 m x 2 mm internal diameter) packed with 5% SP-2401 on Supelcoport 100–120 mesh (Supelco Inc., Bellafonte, PA, USA) with nitrogen (25 ml min⁻¹) as carrier gas at a temperature of 245°C. Under these conditions, the retention time for the internal standard, 7-ketodeoxycholic acid, was around 19 min. Peak height ratios of bile acid to internal standard were determined by comparison with known amounts of standards analysed simultaneously. THCA was kindly supplied by Professor D. N. Kirk, Curator of the Steroid Reference Collection, Westfield College, London.

Identification of specific bile acids was accomplished by comparison of gas–liquid chromatographic retention times with standards and by electron-impact mass spectrometry, using a Hewlett-Packard model 5992B mass spectrometer (Hewlett-Packard, Avondale, PA, USA). A BP-1 bonded phase silica capillary column (12 m x 0.3 mm internal diameter) equipped with an on-column injector (SGE Scientific Pty. Ltd., North Melbourne, Australia) was used to separate bile acids prior to the recording of mass spectra. Electron energy was 70 eV.

![Figure 2](image-url)  
**Figure 2** Gas–liquid chromatograms comparing bile acids isolated from the plasma of a patient with infantile Refsum's disease (tracing A) and a patient with Zellweger’s disease (tracing B) with a control sample (tracing C). Retention times of bile acid standards relative to the internal standard 7-ketodeoxycholic acid (7KDCOA) were deoxycholic acid (DCA) 0.39; chenodeoxycholic acid (CDCA) 0.49; ursodeoxycholic acid (UDCA) 0.54; cholic acid (CA) 0.72; 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA) 1.13