pend only on the basal plane area and thus on the number of stacked sheets, which we assume are double layers as in 2H molybdenite on the basis of the results discussed above:

$$\text{Surface area} = m^2/g = 327/n$$

where n is the number of stacked sheets or rags. For five to ten stacks this yields calculated surface areas in the range of about 33 to 66 m^2/g, in good agreement with the observed value but indicating that there is also a greater degree of stacking than indicated by the x-ray order length in the c-direction.

The asymmetric shape of the 100 envelope (as indicated in Fig. 4) is characteristic of random layer lattice structures (*11*), in which the layers are displaced randomly with respect to one another like a spread deck of cards. They are stacked almost normally, although the position of the 002 reflection is displaced slightly to lower angles; this displacement is presumably due to imperfect stacking, as has been described for graphite (*12*). When a mixed reflection such as the 103 reflection appears, its line width indicates that the two-layer molybdenite stacking sequence is maintained for at least two stacks as might be expected. We also observed that pressing the "poorly crystalline" MoS_2 in a laboratory press at approximately 1000 atm significantly increased the sharpness of the x-ray reflections, presumably improving the stacking of the rags. There is considerable non-Bragg scattering present at low angles (Fig. 4). This may be due to uncorrelated single layers and the pore structure that they generate by randomly folding and connecting with other sheets. All of the above observations are consistent with the stacking and folding of individual MoS_2 layers to form a highly disordered, poorly crystalline MoS_2.

This study demonstrates that the rag morphology in poorly crystalline MoS_2 arises from the two-dimensional macromolecular nature of the layered MoS_2 crystal structure. We believe that the anisotropic properties of MoS_2 rags will have important implications in catalysis and surface chemistry (*8*). The structure of amorphous MoS_2 and how this is related to the crystallization and growth of MoS_2 in the rag structure are still open questions.

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Defects of Bile Acid Synthesis in Zellweger's Syndrome

Abstract. *Abnormal mitochondrial structure and function have been documented in patients with Zellweger's syndrome (cerebrohepatorenal syndrome). In vitro studies have suggested that the formation of C_{24} bile acids (chenodeoxycholic acid and cholic acid) from C_{27} cholesterol requires mitochondrial oxidative cleavage of the terminal three carbons of the side chain. Therefore, three patients with Zellweger's syndrome were examined for the presence of mitochondrial defects in bile acid synthesis. All three excreted excessive amounts of 3α,7α-dihydroxy-5β-cholestan-26-oic acid, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid, and 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestan-26-oic acid (varanic acid), precursors of chenodeoxycholic acid and cholic acid that have undergone only partial side chain oxidation. These findings give added support to the role of mitochondrial oxidative side chain cleavage in the overall scheme of bile acid synthesis.*

Bile acids are synthesized from cholesterol in the liver and are important for the solubilization of cholesterol in bile and fat in the intestinal tract as well as being an excretory pathway for cholesterol. The synthesis of bile acids has been extensively studied in vitro, and it is thought that the initial steps of hydroxylation and rearrangement of the ring system of cholesterol take place in the endoplasmic reticulum and that side chain oxidation and cleavage take place in the mitochondria (*1*).

In 1964, Bowen *et al.* (*2*) described an autosomal recessive disease (Zellweger's syndrome) characterized by severe hypotonia, growth and mental retardation, renal cortical cysts, and liver dysfunction. Patients with this syndrome also have severe mitochondrial abnormalities (*3*). Thus, Zellweger's syndrome appears to be a unique "experiment of nature" in which the role of the mitochondria in bile acid synthesis could be assessed in vivo.

We studied three patients with typical features of this syndrome (*4*). Liver biopsies, obtained from two patients, showed abnormalities of the mitochondria consisting of disarrangement and twisting of the cristae. In addition, no peroxisomes could be identified. These electron microscopic findings are similar to those previously reported in Zellweger's syndrome (*3*).

Samples of urine from these three pa-

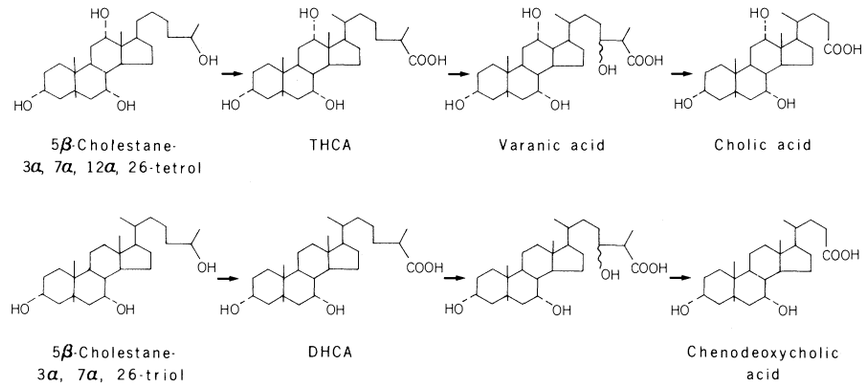

Fig. 1. Side chain oxidation in the synthesis of cholic acid from 5β-cholestane-3α,7α,12α,26-tetrol and chenodeoxycholic from 5β-cholestane-3α,7α,26-triol, respectively. Abbreviations: THCA, 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid; DHCA, 3α,7α-dihydroxy-5β-cholestan-26-oic acid; varanic acid, 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestan-26-oic acid.

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tients were analyzed for bile acids as described previously with the use of gas-liquid chromatography–mass spectroscopy (*5*). Individual bile acids were identified by a combination of their gas chromatographic retention times and their characteristic chemical ionization and electron-impact mass fragments (*6*).

3α,7α - Dihydroxy - 5β-cholestan - 26 - oic acid (DHCA), 3α,7α,12α-trihydroxy-5β-cholestan-26-oic acid (THCA), and 3α,7α,12α,24ξ-tetrahydroxy-5β-cholestan-26-oic acid (varanic acid) were identified in the urine of all three patients (see Fig. 1 and Table 1). Figure 2 shows the electron-impact mass spectra of THCA isolated from patient 1 compared to the mass spectra of authentic THCA isolated from the bile of *Alligator mississippiensis*, a species in which THCA is the major primary bile acid (*7*). In contrast, no bile acid intermediates were found in samples collected from 12 control patients with noninherited forms of liver disease.

DHCA was not present in the urine of patient 2 until the patient was 12 months of age. Similarly, varanic acid was not present in the urine until patient 3 was 4 months old. The presence of progressive deficiencies in bile acid synthesis with time suggests that the metabolic defects are secondary to progressive alterations in mitochondrial function.

If the mitochondrial defects in bile acid synthesis were complete, no cholic acid or chenodeoxycholic acid production would be expected. Thus, the presence of cholic acid and chenodeoxycholic acid in these three patients suggests that the mitochondrial defects were incomplete (see Table 1). A second possibility exists; an alternate pathway, which requires only microsomal enzymes, has been suggested for oxidation and cleavage of the side chain of bile acid precursors (*8*). This pathway involves initial hydroxylation at C-25 with subsequent hydroxylation at position 24 and cleavage of C-25, C-26, and C-27 (*8*). This pathway does not involve DHCA, THCA, or varanic acid and may have been operating in these children.

The present report confirms that abnormalities in mitochondrial structure and function exist in Zellweger's syndrome and gives support to the hypothesis that mitochondria are involved in bile acid synthesis.

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Table 1. Bile acids present in patients with Zellweger's syndrome.

Patient	Age (months)	Total (μmole/ml)	Relative percentages of bile acids				
			Cholic acid	Chenodeoxycholic acid	THCA	DHCA	Varanic acid
1	4	1.2	39.2	26.3	9.2	1.3	24.0
2	9	0.6	36.3	22.7	18.3	0	22.7
	12	1.4	14.7	14.3	3.4	3.1	64.5
3	3	4.5	21.0	44.7	25.3	9.0	0
	4	10.1	31.0	28.6	4.4	2.4	33.6

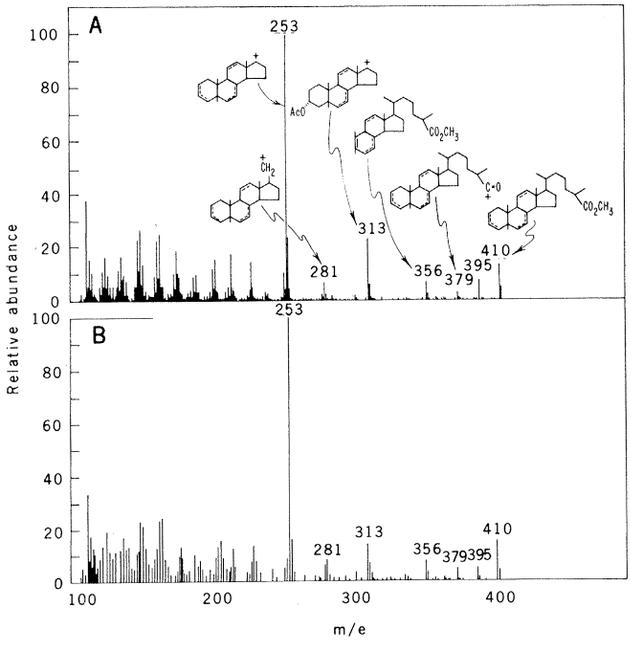

Fig. 2. Electron-impact mass spectra of (A) authentic THCA isolated from the bile of *Alligator mississippiensis* and of (B) THCA isolated from patient 1. The characteristic ion, mass to charge (*m/e*) 410 ($M^+ - 3CH_3CO_2$), for the triacetate derivative of methyl THCA was observed in each spectra.

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4. The features included severe hypotonia, difficulty in feeding, growth and psychomotor retardation, and seizures. All had mild hepatomegaly, high forehead, shallow supraorbital ridges, open fontanelles and sutures, and a broad nasal bridge. Laboratory evaluation showed high concentrations of serum iron which later returned to normal, mild liver function test abnormalities, and punctate epiphyseal calcifications noted on x-rays of the long bones. Concentrations of pipecolic acid in blood and urine were normal. Postmortem examination obtained on two of the patients showed renal cortical cysts, increased brain weight and hepatic fibrosis, and hemosiderosis.
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6. Gas-liquid chromatographic (GLC) separation of the methyl ester acetate derivatives of bile acids was carried out with the use of a glass column (inside diameter, 1.8 m by 1 mm) packed with 1 percent Poly S-179 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc.); column temperature ranged from 260° to 275°C. Mass spectra were obtained with isobutane reagent gas at a pressure of 0.6 torr and a source temperature of 150°C. Identification of specific bile acid compounds were confirmed by electron-impact mass spectrometry on a Perkin-Elmer mass spectrometer, model 270, at 70 eV; the same GLC conditions and authentic samples were used as reference standards.
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