Markedly increased tissue concentrations of 7-dehydrocholesterol combined with low levels of cholesterol are characteristic of the Smith-Lemli-Opitz syndrome

G. S. Tint,[1,*,†] Mary Seller,[§] Rhiannon Hughes-Benzie,[**] Ashok K. Batta,[†] Sarah Shefer,[†] David Genest,[††] Mira Irons,[§§] Ellen Elias,[§§] and Gerald Salen[*,†]

Research Service,[*] Veterans Affairs Medical Center, East Orange, NJ; Department of Medicine,[†] University of Medicine and Dentistry-New Jersey Medical School, Newark, NJ; Division of Medical and Molecular Genetics,[§] United Medical and Dental Schools of Guy's and St. Thomas Hospitals, London, England; Clinical Genetics,[**] Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada; Department of Pathology,[††] Brigham and Women's Hospital, Harvard Medical School, Boston, MA; and Section of Clinical Genetics,[§§] Department of Pediatrics, New England Medical Center, Tufts University School of Medicine, Boston, MA

Abstract The Smith-Lemli-Opitz syndrome is an autosomal recessive birth defect (frequency 1:20,000-1:40,000) that results in profound mental retardation, physical deformities, and failure to thrive. It is characterized biochemically by low plasma cholesterol and greatly elevated levels of two dehydrocholesterols, one of which is the cholesterol precursor 7-dehydrocholesterol. To determine whether the block in cholesterol biosynthesis affects tissue sterols, we assayed several organs from two affected individuals, a female who died at 27 hours and a 20-week male fetus. Cholesterol concentrations in abdominal wall, adrenal gland, and kidney from two or three unaffected fetuses, who served as controls, averaged 2.0, 1.5, and 1.4 mg/g wet weight, compared to 0.08, 0.44, and 0.14, respectively, for the homozygous fetus. Cerebral cortex cholesterol concentrations were 2.2 mg/g for two 20-22-week fetal controls but only 0.21 and 0.09 mg/g, respectively, for the homozygous child and fetus. Similarly, tissue cholesterol levels were abnormally low in the homozygous child being less than 1 mg/g in liver, adipose, thymus, muscle, and adrenal and 6.2 mg/dl in plasma. Dehydrocholesterols could not be detected by conventional means in any controls but were elevated enough in tissues from affected individuals to make total sterol concentrations nearly normal. These results suggest that a defect in 3β-hydroxysterol Δ^7-reductase leads to both a profound lack of cholesterol and its replacement by dehydrocholesterols. Such a combination may be lethal in the most severely affected individuals.—**Tint, G. S., M. Seller, R. Hughes-Benzie, A. K. Batta, S. Shefer, D. Genest, M. Irons, E. Elias, and G. Salen.** Markedly increased tissue concentrations of 7-dehydrocholesterol combined with low levels of cholesterol are characteristic of the Smith-Lemli-Opitz syndrome. *J. Lipid Res.* 1995. **36:** 89-95.

Supplementary key words cholesterol biosynthesis • plasma sterols • cholesta-5,8-dien-3β-ol • capillary gas chromatography • mass spectroscopy • birth defects • mental retardation

The Smith-Lemli-Opitz syndrome (1-3) is a not uncommon birth defect with an autosomal recessive mode of inheritance (4) and an estimated frequency of 1 in 20,000 to 1 in 40,000 (5, 6). It is frequently diagnosed by a distinctive dysmorphic facies (1-9) which includes microcephaly, ptosis of eyelids, prominent epicanthal folds, wide nasal bridge with anteverted nares, low-set retroverted ears, a short neck, micrognathia, and a highly arched and often cleft palate. Other signs include syndactyly, usually of the second and third toes, polydactyly, valgus foot deformities, cataracts, and hypotonia or hypertonia. Most organs, including heart, kidneys, brain, central and peripheral nervous system, skeleton, and digestive tract are adversely affected while, in males, genital malformations including cryptorchidism, hypospadias, and microphallus are common. In the most severely affected males there can be a complete failure to masculinize resulting in phenotypic females with a male (46,XY) karotype. There is often a severe failure to thrive requiring the placement of a gastrostomy tube, while spontaneous abortion and early death are common.

Abbreviations: Cholesterol, cholest-5-en-3β-ol; 7-dehydrocholesterol (7-DHC), cholesta-5,7-dien-3β-ol; coprostanol, 5β-cholestan-3β-ol; desmosterol, cholesta-5,24-dien-3β-ol; lathosterol, cholest-7-en-3β-ol; lanosterol, 4,4'14-trimethyl-5α-cholesta-8(9),24-dien-3β-ol; 24,25-dihydrolanosterol, 4,4'14-trimethyl-5α-cholest-8(9)-en-3β-ol; SLO, Smith-Lemli-Opitz syndrome.

[1]To whom correspondence should be addressed.

Perhaps the most devastating symptom in those children who do survive is mental retardation. While a few mild cases have been reported (5, 10, 11) it is most often quite profound. And, although not usually commented upon, many of these children are extremely difficult to control and are often violent and self destructive (12).

In 1987 Curry et al. (13) gathered data on a number of the most severely affected cases, 90% of whom did not survive for more than 1 year, and suggested that these might represent a separate phenotype: Smith-Lemli-Opitz type II. It is now suspected that these individuals represent the lower end of a continuum and may arise from a variant of the basic gene defect rather than being a completely new disease (14).

Although the Smith-Lemli-Opitz syndrome was first described in 1964, it could only be diagnosed from its clinical signs until 1993 when we identified the biochemical defect (12, 15–17). At that time, we reported that plasma cholesterol levels in homozygous children are abnormally low while concentrations of the cholesterol precursor, 7-dehydrocholesterol, are elevated several thousand-fold above normal. In addition to the plasma sterol abnormalities, we also detected extraordinarily high levels of 7-dehydrocholesterol as well as an isomeric dehydrocholesterol (isomeric dehydrocholesterol II) in erythrocytes, lens, bile, feces, and cultured fibroblasts from a number of homozygous children. These results suggested that the enzyme 3β-hydroxysteroid Δ^7-reductase, which saturates the C-7 double bond of the cholesterol precursors 7-dehydrocholesterol and cholesta-5,7,24-trien-3β-ol (**Fig. 1**), was defective (12, 15–17). Because reduction of the C-7 double bond is an obligatory step in the biosynthesis of cholesterol (18–21), a partial block of that reaction should result in reduced cholesterol production and the accumulation of 7-dehydrocholesterol.

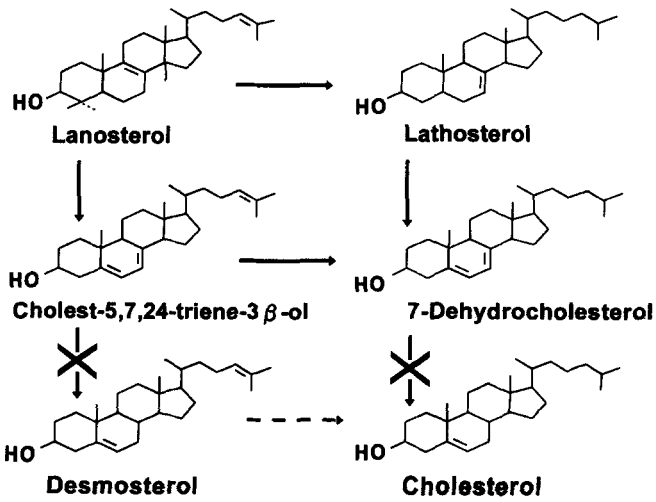

Fig. 1. The biosynthesis of cholesterol from lanosterol showing early (right-hand side) and late (left-hand side) reduction of the C-24 double bond. The symbol X represents the proposed block responsible for the Smith-Lemli-Opitz syndrome; a defect in the enzyme 3β-hydroxysterol Δ^7-reductase.

TABLE 1. Study subjects

Subject	Age (weeks)	Diagnosis[a]
C (Child)	1 day	SLO, died at 27 h
F (Fetus)	20	SLO, elective termination
Fetal controls		
F1	18	Fetal Turner syndrome
F2	19	Anencephalic
F3	19.5	Spina bifida
F4	22	Elective termination
F5	20	Elective termination

[a]SLO, Smith-Lemli-Opitz syndrome.

While we had noted the plasma sterol abnormalities in a number of children (12, 15–17), we had no firm evidence of the effect of the cholesterol biosynthetic defect on tissue sterol levels. In particular, because of the severe mental retardation encountered in the Smith-Lemli-Opitz syndrome, a knowledge of sterol composition of brain would seem to be especially important. We have recently analyzed a number of tissues from two Smith-Lemli-Opitz syndrome homozygotes, a newborn child and a fetus. We report here on their tissue sterol identification and concentration and compare them to sterols from several unaffected fetuses.

METHODS

Clinical

All tissues (**Table 1**) were obtained at autopsy and appropriate parental and institutional consents were obtained. The child (C) (Children's Hospital of Eastern Ontario, Ottawa, Canada) with the Smith-Lemli-Opitz syndrome died 27 h after birth. This girl displayed features and malformations typical for the more severe type II phenotype including micrognathia, flat nose and anteverted nares, low set ears, syndactyly, valgus foot deformities, polydactyly, bilateral hydronephrosis, unilobular lung, and multiple severe cardiovascular abnormalities. Blocks of tissue (2–3 g) were excised during the post mortem examination, frozen immediately, and maintained at −20°C until they were analyzed. The affected fetus (F) (Guy's Hospital, London) had been aborted at 20 weeks following an ultrasound examination that revealed reduced liquor volume, abnormal fetal feet, and no detectable kidney or bladder. This fetus had multiple abnormalities which were not inconsistent with the Smith-Lemli-Opitz syndrome (22). Samples from F had been fixed in formalin for 2 months before being analyzed. Control tissue was obtained from five terminated pregnancies, F1–F5 (Table 1). Tissues from F1–F3 were ob-

tained at Guy's Hospital while F4–F5 were collected from the New England Medical Center, Boston, MA. The material from the first three fetal controls had been preserved in formalin for 0.5–24 months. Tissues from F4 and F5 were available both as frozen and as formalin-fixed (1–2 months).

Analytical

Tissue sterols were determined as previously reported (12, 23). Fixed material was first rinsed in distilled water. The tissues were then chopped into small pieces and the lipids were extracted by refluxing in 1 N NaOH for 2 h. Then, appropriate amounts of 5α-cholestane and coprostanol (Steraloids, Wilton, NH) were added as internal standards, the sterols were converted to their trimethylsilyl ethers and analyzed by capillary gas chromatography on 25 m × 0.25 mm ID nonpolar CP-Sil 5CB (polysiloxane) and polar CP-Wax 57CB (PEG Carbowax) columns (Chrompack, Raritan, NJ) using flame ionization detectors. Chromatographic conditions were 100°C for 2 min then an increase of 35°C/min to 265°C (CP-Sil 5CB) or an isothermal analysis at 225°C (CP-Sil 57CB) with He as the carrier gas at 1 ml/min. For liquid samples, 50 μg of coprostanol was added to 0.1–0.2 ml of plasma or bile and the mixture was subjected to saponification in 1 N NaOH. Identification was made by matching the retention times relative to 5α-cholestane of the natural compounds with those of authentic 7-dehydrocholesterol (Aldrich, Chemical, Milwaukee, WI) and cholesterol, desmosterol and lathosterol (Steraloids, Wilton, NH).

Kovats retention indices of tissue sterols were calculated by comparing the retention times relative to 5α-cholestane of the dehydrocholesterols to those of the normal C_{30}–C_{36} hydrocarbons. The nomenclature for the aberrant sterols is the same as that used previously (12). Retention times relative to 5α-cholestane after injecting onto the CP-Sil 5CB and CP-Sil 57CB columns for dehydrocholesterol II, compound III and 7-dehydrocholesterol were 1.40, 1.42, 1.46 and 1.87, 2.03, 2.21, respectively, while the absolute retention times of 5α-cholestane were 14.72 min and 6.70 min, respectively. A comparison of the areas of the sterol peaks with that of the internal standards yielded the mass.

Structural confirmation was obtained by gas chromatography–mass spectroscopy using a Hewlett-Packard model 5988.

Because desmosterol is poorly resolved from 7-dehydrocholesterol by gas chromatography, it was necessary to separate brain sterols by argentation thin-layer chromatography (24). Silica-gel HL plates (Analtech, Newark, DE) were impregnated by dipping in a solution of 5% $AgNO_3$ in methanol. After drying at 100°C, 50–100 μg of brain sterol extract was plated and developed in chloroform–acetone 85:15 (vol/vol) at 5°C in the dark. Relative mobilities (R_f) of 7-dehydrocholesterol, dehydrocholesterol II, cholesterol, and desmosterol were 0.29, 0.54, 0.72, and 0.69, respectively.

Tissues from the homozygous fetus as well as the first three controls were available only as formalin-fixed material. To assess the effect of fixation on tissue sterols we carried out a parallel analysis of frozen and formalin-fixed (1–2 months) brain and liver from fetal controls F4 and F5.

The detection limit for 7-dehydrocholesterol using the CP-Sil 5CB column in a Hewlett-Packard model 5890 gas chromatograph and our routine aliquot, injection volume, and split-ratio is the equivalent of about 0.0005 mg/g while the lower limit when the CP-Wax 57CB column (Hewlett-Packard model 5840) was used was 0.005 mg/g. The ultimate practical sensitivity for the gas chromatographic method using splitless injection is approximately 5 ng/g.

RESULTS

Using our standard gas chromatographic analysis, we were able to detect 7-dehydrocholesterol, dehydrocholesterol II, and compound III (12, 15–17) in substantial quantities in all tissues from both of the homozygotes but in no tissues from any of the controls (Tables 2, 3). Sterol concentrations, with the exception of brain, from both the affected child and the affected fetus as well as sterol levels in similar tissues from six control fetuses are shown in **Table 2.** Also listed in Table 2 are plasma and biliary neutral sterols from the homozygous child. The results for brain sterols are given separately in **Table 3.**

Retention indices (Kovats) for the two major aberrant sterols, dehydrocholesterol II and 7-dehydrocholesterol, were found in our system to be 3140 and 3180, respectively. The mass spectrum of compound III, *m/z* 440 (5%), 425 (5%), 350 (80%), 207 (100%), suggests that it is probably not a 3β-hydroxy-cholestadiene (i.e., a dehydrocholesterol).

In the homozygous child (Table 2), cholesterol accounted for only 16–20% of total tissue sterols (mean ± SD, 20 ± 2%, n = 7) with most of the remainder being the dehydrocholesterols (7-dehydrocholesterol plus dehydrocholesterol II) so that 7-dehydrocholesterol or dehydrocholesterol II was the major sterol in every tissue. As in the newborn homozygote, cholesterol concentrations in the tissues of the affected fetus (Table 2) were extremely low but, because of the greatly increased content of dehydrocholesterols, total sterol concentrations were often comparable to the controls.

In brain (Table 3), the disparity between control and homozygous sterols was even greater. For the two affected individuals, cholesterol constituted, at most, 4% of total brain sterols while 7-dehydrocholesterol was found to be the major sterol. However, as we had noted above, be-

TABLE 2. Sterol concentrations and cholesterol as % of total sterols in tissues from child (C) and fetus (F) with the Smith-Lemli-Opitz Syndrome and in five fetal controls (F1–F5)

Tissue	Cholesterol (%)[a]	7-DHC	DHC II	III	Total
		mg/g wet weight			
Affected child (C)					
Muscle	0.46 (20)	0.92	0.68	0.19	2.25
Adrenal	0.74 (20)	1.13	1.33	0.19	3.39
Kidney	1.18 (21)	2.38	1.74	0.33	5.63
Liver	0.92 (22)	1.15	1.77	0.34	4.18
Adipose	0.90 (20)	1.53	1.84	0.30	4.57
Lungs	1.21 (21)	2.51	1.63	0.36	5.71
Thymus	0.53 (16)	1.40	1.32	0.15	3.40
Plasma (mg/dl)	6.25 (20)	12.55	12.70	0.39	31.89
Bile (mg/dl)	3.49 (16)	19.14	10.39	1.20	34.22
Affected fetus (F):					
Muscle	0.08 (19)	0.17	0.16	nd	0.42
Adrenal	0.44 (25)	0.89	0.40	nd	1.73
Kidney	0.14 (30)	0.15	0.09	0.08	0.46
Testis	0.29 (12)	1.33	0.87	nd	2.49
Fetal controls (F1–F3):					
Abdominal wall	2.04 ± 0.4 (n = 3)	nd	nd	nd	2.04[b]
Fetal controls (F4; F5):					
Muscle	0.75; 1.08	nd	nd	nd	0.92[b]
Adrenal	1.48; 1.54	nd	nd	nd	1.51[b]
Kidney	1.27; 1.59	nd	nd	nd	1.43[b]
Liver	1.26; 1.22	nd	nd	nd	1.24[b]

Abbreviations: 7-DHC, 7-dehydrocholesterol; DHC II, dehydrocholesterol II; III, compound III; nd, not detected by usual gas chromatography assay.

[a] In parentheses, cholesterol as % of total sterols.

[b] Mean of two or three values.

cause the concentration of aberrant sterols was more than sufficient to make up for the deficiency in cholesterol, total sterol content did not appear to be abnormally low. It should be noted that the concentrations of lathosterol in homozygous and control fetal brains were comparable but that the cholesterol precursor desmosterol was not detectable at all in the brain from the affected fetus. In contrast, desmosterol was found at a concentration of 0.17 and 0.18 mg/g, respectively, in the two fetal controls.

TABLE 3. Sterol concentrations in brain of child and fetus with the Smith-Lemli-Opitz syndrome and in two control fetuses

	SLO		Fetal Controls	
Sterol	Child	Fetus	F4	F5
		mg/g wet weight		
Cholesterol	0.21	0.09	2.16	2.20
7-DHC	3.12	2.10	nd	nd
DHC II	1.58	1.00	nd	nd
Compound III	0.20	0.25	nd	nd
Desmosterol	0.004	nd	0.17	0.18
Lathosterol	0.01	0.04	0.02	0.02
Total	5.13	3.48	2.35	2.40

Abbreviations: 7-DHC, 7-dehydrocholesterol; DHC II, dehydrocholesterol II; nd, not detected by usual gas chromatography assay.

Plasma and biliary sterols in the affected child (Table 2) also reflect the biosynthetic defect. Cholesterol constituted only 20% and 10%, respectively, of total neutral sterols in these fluids with the remainder being dehydrocholesterols. The major biliary bile acids were found to be chenodeoxycholic acid (0.90 mg/ml), cholic acid (0.27 mg/ml), and deoxycholic acid (0.13 mg/ml).

We found no deleterious effect of formalin fixation on tissue sterols. The concentrations of cholesterol in formalin-fixed sections of liver and brain from controls F4 and F5 were found to be 1.23 and 2.52 mg/g and 1.12 and 1.64 mg/g, respectively, compared to concentrations in the corresponding frozen tissues (Tables 2 and 3) of 1.26 and 2.16 mg/g and 1.22 and 2.20 mg/g, respectively.

DISCUSSION

Total tissue sterol concentrations in the homozygous child (Table 2) are comparable to levels we have reported in an 18-year-old male control and in an 18-year-old male with sitosterolemia (23). In these two subjects, as in the homozygous child, liver was rather more cholesterol-rich (4.6 and 4.3 mg/g, respectively) than was muscle at about 1.5 mg cholesterol/g. Similarly, total brain sterols in the newborn homozygote, fetal homozygote, and our fetal

controls (Table 3) are not too different from reported concentrations of 6.95 ± 0.86 and 7.46 ± 0.81 mg/g for small and average gestational age term infants, respectively (25). Forebrain cholesterol has been determined to range between 5.5 and 8.5 mg/g in newborns and to be between 3.4 and 4.9 mg/g (26) in 20 week fetuses.

The cholesterol biosynthetic pathway following the formation of lanosterol is branched (18). That is, the side chain C-24 double bond can be saturated immediately (right hand side of Fig. 1) or as the final step in the process (left hand side of Fig. 1). After early C-24,25 saturation, cholesterol precursors include 24,25-dihydrolanosterol, lathosterol, and 7-dehydrocholesterol. In contrast, in the other arm, cholesterol biosynthesis proceeds through a number of intermediates all having an unsaturated side chain, such as cholesta-5,7,24-trien-3β-ol and desmosterol. It is generally agreed (19–21), however, that the enzymes that reduce the C-24 double bond of either lanosterol or desmosterol are, in reality, the same enzyme. Similarly, there is probably only a single 3β-hydroxy-Δ^7-reductase capable of saturating the C-7 double bond of both cholesta-5,7,24-trien-3β-ol and 7-dehydrocholesterol. Thus, if the C-7 reductase were to be defective one would expect reduced cholesterol biosynthesis coupled with increased levels of the precursor proximal to the reaction (Fig. 1). It is for this reason that we have postulated a partial block of this particular reaction as the biochemical cause of the Smith-Lemli-Opitz syndrome (12, 15–17).

In further support of our hypothesis concerning the defect in cholesterol biosynthesis in the Smith-Lemli-Opitz syndrome is our observation (Table 3) that desmosterol was virtually undetectable in the brain of the affected fetus, but concentrations of lathosterol were similar in both homozygotes and controls. It has been suggested that Δ^{24}-reductase is rate-limiting during cholesterol synthesis by the early fetal brain but that the effect disappears once myelination begins (27, 28). Thus, desmosterol is reported to account for up to 6% of total brain sterols at 34 weeks gestation but is virtually undetectable at birth (28). In contrast, because C-7 reductase is defective in the Smith-Lemli-Opitz homozygotes one would predict that they would be able to form desmosterol very slowly but could make lathosterol, an immediate precursor of 7-dehydrocholesterol, quite readily (Fig. 1). Our finding of extraordinarily low cholesterol concentrations in brain may well explain many of the neurological manifestations of the syndrome because it is known that 7-dehydrocholesterol is rather poorly incorporated into myelin (29). It should be noted, however, that because Smith-Lemli-Opitz homozygotes are all microcephalic with reduced brain size (1–3), the mass of brain sterols in all cases is probably abnormally low, even though total sterol concentration is nearly normal.

The predicted effect of such a defect, that is, reduced amounts of cholesterol and elevated levels of 7-dehydrocholesterol, which we had noted previously in the plasma of these children is evident in every tissue (Tables 2 and 3). It is apparent, however, that the remainder of the cholesterol biosynthetic pathway is fully intact because total tissue sterols, that is the sum of cholesterol and the dehydrocholesterols, are often within the normal range. Thus, sufficient quantities of 7-dehydrocholesterol and the dehydrocholesterol II are synthesized to make up for the deficit. However, because of the clinical (1–11, 13) and histological (29–31) abnormalities of the central and peripheral nervous systems (mental retardation, hypotonia, hypertonia, poor enervation of the digestive tract, etc.) it is unlikely that the dehydrocholesterols are adequate functional replacements for cholesterol (30). Also, it is probably defective local tissue synthesis of cholesterol and not merely an abnormally reduced circulating plasma cholesterol level (12, 15–17, Table 2) that is responsible for the malformations seen in the Smith-Lemli-Opitz syndrome. Homozygotes with the codominant genetic disorder hypobetalipoproteinemia, a disease caused by truncations of apolipoprotein B, can have plasma total cholesterol concentrations as low as 13–18 mg/dl (32). Yet, these individuals have a normal appearance and are not usually developmentally impaired. They are affected primarily with fat malabsorption and with neuropathies, pigmentary retinopathies and a red blood cell acanthocytosis directly attributed to deficiencies of the fat-soluble vitamins E, A, and K. Unlike patients with the Smith-Lemli-Opitz syndrome there is no accumulation of aberrant sterols and their cholesterol synthesis is elevated 2-fold above normal (33). In contrast to these latter individuals, are fetal, embryonic, or pregnant rats treated with AY 9944, a pharmacological inhibitor of sterol C-7 reductase. In these animals, cholesterol biosynthesis is suppressed, 7-dehydrocholesterol accumulates in most tissues, and a number of malformations develop that are analogous to the functional and physical deformities noted in Smith-Lemli-Opitz homozygotes (12, 30, 31, 34, 35).

Biliary bile acids in the homozygous newborn were also abnormal because the concentration of chenodeoxycholic acid (0.90 mg/ml) was three times greater than that of cholic acid (0.27 mg/dl). In contrast, studies in normal fetal bile (36) and in meconium (37, 38) suggest that this ratio should normally be somewhat less than 1.0. The finding of deoxycholic acid is not unusual in newborns and fetuses, but the source of this secondary bile acid is obscure.

The identity and origin of dehydrocholesterol II is still not entirely clear but its mass spectrum suggests that it is one of the trace dehydrocholesterols first identified by Axelson (39) in normal adult plasma. Following his chromatographic analysis, the measured retention index of 3140 for dehydrocholesterol II, compared to an index of 3180 for 7-dehydrocholesterol, suggests that dehydrocholesterol II is most likely cholest-5,8-diene-3β-ol. In contrast, the mass spectrum of the trimethylsilyl ether derivative of compound III in-

dicates that it is probably not a diunsaturated derivative of cholesterol (dehydrocholesterol). We often find it as a minor (about 5%) impurity in commercial 7-dehydrocholesterol.

Lowry and Young (5), after examining birth records in British Columbia for the period 1964–1971, concluded that the frequency of the syndrome was between 1:20,000 and 1:40,000. However, because of our discovery of the biochemical cause of the syndrome, we feel that the above estimate must be carefully reevaluated. Thus, only individuals with elevated plasma or tissue dehydrocholesterol levels in addition to the major clinical signs should be diagnosed as having the Smith-Lemli-Opitz syndrome. It has been our experience that children can exhibit some of the clinical features of the syndrome without demonstrating the biochemical defect so that there must be other biochemical diseases that give rise to clinical signs reminiscent of the Smith-Lemli-Opitz syndrome.

We also suggest that the very low tissue cholesterol levels in the newborn who was able to survive for only one day might be indicative of a cholesterol biosynthetic defect of such magnitude as to be incompatible with life.

This work was supported in part by grants from the Department of Veterans Affairs Research Service, by grants DK-18707 and HL-17818 from the National Institutes of Health, and by a grant from the Herman Goldman Foundation.

Manuscript received 4 April 1994 and in revised form 8 July 1994.

REFERENCES

1. Smith, D. W., L. Lemli, and J. M. Opitz. 1964. A newly recognized syndrome of multiple congenital anomalies. *J. Pediatr.* **64:** 210–217.
2. Gorlin, R. J., and M. Cohen, editors. 1990. Syndromes of the Head and Neck. 3rd edition. Oxford University Press, New York. 890–895.
3. Prober, B. 1990. Smith-Lemli-Opitz syndrome. *In* Birth Defects Encyclopedia. Blackwell Scientific Publications. Cambridge, MA. 1570–1572.
4. Dellaire, L. 1969. Syndrome of retardation with urogenital and skeletal anomalies (Smith-Lemli-Opitz syndrome): clinical features and mode of inheritance. *J. Med. Genet.* **6:** 113–120.
5. Lowry, R. B., and S-L. Young. 1980. Borderline intelligence in the Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* **5:** 413–425.
6. Chasalow, F. L. 1985. Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents. *Steroids.* **46:** 827–843.
7. Bialer, M. G., V. H. Penchaszadeh, E. Kahn, R. Libes, G. Krigeman, and M. L. Lesser. 1987. Female external genitalia and Müllerian duct derivatives in a 46,XY infant with the Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* **28:** 723–731.
8. Cherstvoy, E. D., G. I. Lazjuk, T. I. Ostrovskaya, I. A. Shved, G. I. Kravtzova, I. W. Lurie, and A. I. Gerasimovich. 1984. The Smith-Lemli-Opitz syndrome. A detailed pathological study as a clue to etiological heterogeneity. *Virchows Arch. [Pathol. Anat.]* **404:** 413–425.
9. Donnai, D., I. D. Young, W. G. Owen, S. A. Clark, P. F. W. Miller, and W. F. Knox. 1986. The lethal multiple congenital anomaly syndrome of polydactyly, sex reversal, renal hypoplasia, and unilobular lungs. *J. Med. Genet.* **23:** 64–71.
10. Hoefnaegel, D., D. Wurster, J. Pomeroy, and R. Benz. 1969. The Smith-Lemli-Opitz syndrome in an adult male. *J. Ment. Defec. Res.* **13:** 249–257.
11. Deaton, J. G., and L. O. Mendoza. 1973. Smith-Lemli-Opitz syndrome in a 23-year-old man. *Arch. Intern. Med.* **132:** 422–423.
12. Tint, G. S., M. Irons, E. R. Elias, A. K. Batta, R. Frieden, T. S. Chen, and G. Salen. 1994. Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N. Engl. J. Med.* **330:** 107–113.
13. Curry, C. J., J. C. Carey, J. S. Holland, D. Chopra, R. Fineman, M. Golabi, S. Sherman, R. A. Pagon, J. Allanson, S. Shulman, et al. 1987. Smith-Lemli-Opitz syndrome-type II: multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. *Am. J. Med. Genet.* **26:** 45–57.
14. Opitz, J. M., and F. de la Cruz. 1994. Cholesterol metabolism in the RSH/Smith-Lemli-Opitz syndrome: summary of an NICHD conference. *Am. J. Med. Genet.* **50:** 326–338.
15. Irons, M., E. R. Elias, G. Salen, G. S. Tint, and A. K. Batta. 1993. Defective cholesterol synthesis in the Smith-Lemli-Opitz syndrome. *Lancet.* **341:** 1414.
16. Tint, G. S. 1993. Cholesterol defect in Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* **47:** 574–575.
17. Irons, M., E. R. Elias, G. S. Tint, G. Salen, R. Frieden, T. M. Buie, and M. Ampola. 1994. Abnormal cholesterol metabolism in the Smith-Lemli-Opitz syndrome: report of clinical and biochemical findings in four patients and treatment in one patient. *Am. J. Med. Genet.* **50:** 347–352.
18. Clayton, R. B. 1965. Biosynthesis of sterols, steroids and terpenoids. Part I. Biogenesis of cholesterol and the fundamental steps in terpenoid biosynthesis. *Q. Rev. Chem. Soc.* **19:** 168–200.
19. Steinberg, D., and J. Avigan. 1960. Studies of cholesterol biosynthesis II. The role of desmosterol in the biosynthesis of cholesterol. *J. Biol. Chem.* **11:** 3127–3129.
20. Fumigalli, R., R. Niermiro, and R. Paoletti. 1965. Investigation of the biogenetic reaction sequence of cholesterol in rat tissues, through inhibition with AY-9944. *J. Am. Oil Chem. Soc.* **42:** 1018–1023.
21. Koroly, M. J., and M. E. Dempsey. 1981. Synthesis of $\Delta^{5,22}$-cholestadien-3β-ol by a liver enzyme. *Lipids.* **16:** 755–758.
22. Seller, M. J., J. Russell, and G. S. Tint. 1994. An unusual case of Smith-Lemli-Opitz syndrome "Type II." *Am. J. Med. Genet.* In press.
23. Salen, G., I. Horak, M. Rothkopf, J. L. Cohen, J. Speck, G. S. Tint, V. Shore, B. Dayal, T. Chen, and S. Shefer. 1985. Lethal atherosclerosis associated with abnormal plasma and tissue composition in sitosterolemia with xanthomatosis. *J. Lipid Res.* **26:** 1126–1133.
24. Tint, G. S., and G. Salen. 1974. Transformation of 5α-cholsten-7-en-3β-ol to cholesterol and cholestanol in cerebrotendinous xanthomatosis. *J. Lipid Res.* **15:** 256–262.
25. Chase, H. P., N. N. Welch, C. S. Dabiere, N. S. Vasan, and L. J. Butterfield. 1972. Alterations in human brain biochemistry following intrauterine growth retardation. *Pediatrics.* **50:** 403–411.
26. Dobbing, J., and J. Sands. 1973. Quantitative growth and development of human brain. *Arch. Dis. Child.* **48:** 757–767.

27. Fumigalli, R., and R. Paoletti. 1963. The identification of desmosterol in the developing human and animal brain. *Life Sci.* **5:** 291–295.
28. Smith, M. E., R. Fumigalli, and R. Paoletti. 1967. The occurrence of desmosterol in myelin of developing rats. *Life Sci.* **6:** 1085–1091.
29. Fumigalli, R., M. E. Smith, G. Urna, and R. Paoletti. 1969. The effect of hypocholesteremic agents on myelinogenesis. *J. Neurochem.* **16:** 1329–1339.
30. Garcia, C. A., P. A. McGarry, M. Voirol, and C. Duncan. 1973. Neurological involvement in the Smith-Lemli-Opitz syndrome: clinical and neuropathological findings. *Dev. Med. Child. Neurol.* **15:** 48–55.
31. Fierro, M., A. J. Martinez, J. W. Harbison, and S. H. Hay. 1977. Smith-Lemli-Opitz syndrome: neuropathological and ophthalmological observations. *Dev. Med. Child. Neurol.* **19:** 57–62.
32. Linton, M. F., R. V. Farnese, Jr., and S. G. Young. 1993. Familial hypobetalipoproteinemia. *J. Lipid. Res.* **34:** 521–541.
33. Illingworth, D. R., A. S. Papu, and R. E. Gregg. 1989. Increased urinary mevalonic acid excretion in patients with abetalipoproteinemia and homozygous hypobetaproteinemia. *Arteriosclerosis.* **76:** 21–27.
34. Suzuki, K., and L. D. De Paul. 1971. Cellular degeneration in developing central nervous system of rats produced by hypocholesterolemic drug AY9944. *Lab. Invest.* **25:** 546–555.
35. Roux, C., and A. Aubry. 1966. Action tèratogène chez le rat d'un inhibiteur del la synthèse du cholestérol. *C. R. Soc. Biol.* **160:** 1353–1357.
36. Colombo, C., G. Zuliani, M. Ronchi, J. Breiderstein, and K. D. R. Setchell. 1987. Biliary bile acid composition of the human fetus in early gestation. *Pediatr. Res.* **21:** 197–200.
37. Back, P., and K. Walter. 1980. Developmental pattern of bile acid metabolism as revealed by bile acid analysis of meconium. *Gastroenterology.* **78:** 671–676.
38. Watkins, J. B., D. Ingall, P. Szczepanik, P. D. Klein, and R. Lester. 1973. Bile salt metabolism in the newborn. Measurement of pool size and synthesis by stable isotope technic. *N. Eng. J. Med.* **288:** 431–434.
39. Axelson, M. 1991. Occurrence of isomeric dehydrocholesterols in human plasma. *J. Lipid Res.* **32:** 1441–1448.