Defective conversion of 7-dehydrocholesterol to cholesterol in cultured skin fibroblasts from Smith-Lemli-Opitz syndrome homozygotes

Akira Honda, G. S. Tint,[1] Gerald Salen, Ashok K. Batta, Thomas S. Chen, and Sarah Shefer

Departments of Medicine and Pathology, Veterans Affairs Medical Center, East Orange, NJ 07018, and Departments of Medicine and Pathology, the University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ 07103

Abstract The Smith-Lemli-Opitz syndrome is a common birth defect syndrome characterized biochemically by low plasma cholesterol levels and high concentrations of the cholesterol precursor 7-dehydrocholesterol. The present study was undertaken to prove that the enzyme defect is at the step in which 7-dehydrocholesterol is converted into cholesterol and to establish a new biochemical method for the diagnosis of this disease. We assayed the latter part of the cholesterol biosynthetic pathway by incubating [^{3}H]lathosterol (the immediate precursor of 7-dehydrocholesterol) with cultured skin fibroblasts from 15 homozygous patients, 14 obligate heterozygous parents, and 8 controls, and measuring its conversion to 7-dehydrocholesterol and cholesterol. The formation of cholesterol from lathosterol in parents was not significantly different from that in controls. In contrast, cells from patients made very little cholesterol ($P < 0.0001$, patients vs. parents or vs. controls) but readily converted lathosterol to 7-dehydrocholesterol. The defect was especially profound in a subgroup of 8 of the most severely clinically affected patients, as virtually no label was detected in the cholesterol fraction. These results provide compelling evidence that *1)* this disease is caused by a primary defect in 7-dehydrocholesterol Δ^7-reductase, an essential enzyme in the biosynthesis of cholesterol; *2)* the most clinically severe form of the syndrome may be associated with the most inhibited enzyme; and *3)* the enzyme lathosterol 5-desaturase that converts lathosterol to 7-dehydrocholesterol is fully intact. The present method using fibroblast and amniocyte cultures establishes it as a useful procedure for the biochemical diagnosis of this syndrome.—**Honda, A., G. S. Tint, G. Salen, A. K. Batta, T. S. Chen, and S. Shefer.** Defective conversion of 7-dehydrocholesterol to cholesterol in cultured skin fibroblasts from Smith-Lemli-Opitz syndrome homozygotes. *J. Lipid Res.* 1995. **36:** 1595–1601.

Supplementary key words amniocytes · 7-dehydrocholesterol Δ^7-reductase · lathosterol 5-desaturase

The Smith-Lemli-Opitz syndrome (1) is probably the third most common recessive disorder after cystic fibrosis and phenylketonuria in North American Caucasian populations (2). The prevalence is estimated to be 1 in 20,000 births with a carrier frequency of 1–2%. Patients are characterized clinically by profound mental retardation, failure to thrive, and multiple organ abnormalities including microcephaly, unusual facial appearance, limb anomalies, genital disorders, endocrine malfunction, cataracts, and heart and kidney malformations (1, 3–6).

In 1987, Curry et al. (4) described a more severe phenotype of the Smith-Lemli-Opitz syndrome characterized by a high frequency of males (46,XY karyotype) with ambiguous genitalia, a greater number of more severe congenital abnormalities, together with frequent fetal or neonatal death, which they called Smith-Lemli-Opitz syndrome-type II. However, as the biochemical abnormalities of neither form of the syndrome had been identified and the syndrome had been diagnosed only from its clinical presentation, it remained unclear whether these two types shared the same metabolic defect.

Recently we reported (7–9) the combination of abnormally low plasma cholesterol levels and high concentrations of the cholesterol precursor 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) and its isomer 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) (which we had previously called dehydrocholesterol II (8)) in homozygous patients, and hypothesized that the observation demon-

Abbreviations: 7-dehydrocholesterol, cholesta-5,7-dien-3βol; 8-dehydrocholesterol, cholesta-5,8-dien-3β-ol; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; lathosterol, cholest-7-en-3β-ol; TLC, thin-layer chromatography.

[1]To whom correspondence should be addressed.

strated a block in cholesterol biosynthesis at the step in which 7-dehydrocholesterol is transformed to cholesterol.

Cholesterol in mammals is synthesized from acetate with the final two steps being the conversion of lathosterol to 7-dehydrocholesterol (10) and 7-dehydrocholesterol to cholesterol (11) (**Fig. 1**). To assay the activities of these two transformations we incubated radiolabeled lathosterol with cultured skin fibroblasts from both type I and II phenotypes, from their parents, and from controls, purified 7-dehydrocholesterol and cholesterol by TLC, and measured the incorporation of label into each compound.

The objectives of this study were to *1)* prove the hypothesized enzyme defect; *2)* quantify the reduction in cholesterol biosynthesis; and *3)* establish a new biochemical method for the definitive diagnosis of the syndrome.

MATERIALS AND METHODS

Patients

Patients 2, 4, 9, 18, 29, and 36, three females and three males, age 2–25 years, (group I) were the least clinically affected. The subjects assigned to group II were done so prospectively on the basis of syndrome severity. Patients 5, 14, 30, 32, RD, FVR, and SL were a group of generally younger children with clinical presentations consistent with the most profoundly affected type II phenotype (4). SV was aborted at 14 weeks because of early fetal death and both amniocytes and fetal fibroblasts were cultured and analyzed. 7-Dehydrocholesterol was found to be markedly elevated in her amniotic fluid (**Table 1**). Patients 5, 14, and 32 were males (46,XY karyotype) with ambiguous genitalia while 30, RD, FVR, and SL were females with multiple congenital major organ abnormalities. Subjects 14, 30, 32, RD, and SL had died by 4 weeks, 13 weeks, 9 weeks, 5 months, and 6 months, respectively, while FVR died at age 7 years. Subject 24 (2½ years old) was a male (46,XY karyotype) with ambiguous genitalia, but was assigned to group I because his plasma cholesterol level was 2–3 times that of the other patients in group II (Table 1). Subjects 2 and 4 (8) and RD (12) have been described previously. Patient 29 who has a normal plasma cholesterol concentration is a 25-year-old man and one of a pair of siblings originally described by Johnson (13). At 4½ years he manifested typical features of the syndrome but had normal genitalia and no major organ abnormalities. He possesses some language, is able to walk, and, when younger, was able to attend special classes at school. His sister is also affected. She is 31 years old and her plasma cholesterol concentration was 132 mg/dl.

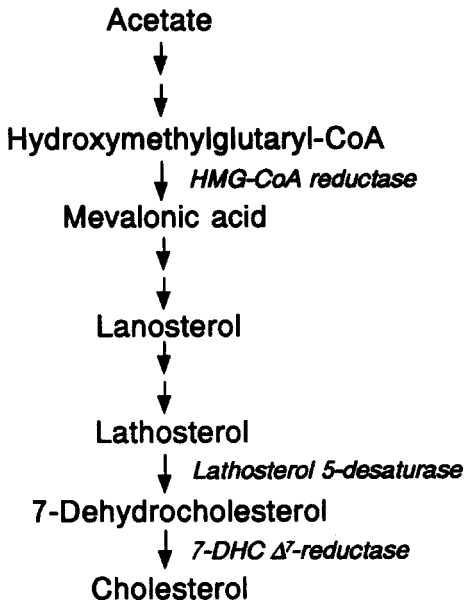

Fig. 1. Cholesterol biosynthesis from acetate. The rate-controlling enzyme is HMG-CoA reductase; the reactions studied in this report are lathosterol 5-desaturase and 7-dehydrocholesterol (7-DHC) Δ^7-reductase.

TABLE 1. Plasma concentrations of 7-dehydrocholesterol, 8-dehydrocholesterol, and cholesterol in patients and parents

Subject	Plasma Concentration		
	7-DHC[a]	8-DHC[b]	Cholesterol
	mg/dl		
Patients (Group I)			
2	26	19	28
4	23	15	101
9	17	8.0	74
18	13	12	44
24	24	26	29
29	0.2	1.0	154
36	1.3	3.8	98
Mean ± SEM	15 ± 4[c]	12 ± 3[c]	75 ± 17[c]
Patients (Group II)			
5	23	20	15
14	61	26	7.0
30	24	12	4.0
32	12	7.2	4.7
SV (amniotic fluid)	0.2[d]	0.3[d]	1.2[d]
Mean ± SEM	30 ± 11[c]	16 ± 4[c]	7.7 ± 2.5[c,e]
Parents			
Mean ± SEM (n = 11)	ND[f]	ND[f]	182 ± 13

[a]7-Dehydrocholesterol.
[b]8-Dehydrocholesterol.
[c]$P < 0.0005$, significantly different from parents.
[d]Data from amniotic fluid (excluded from the calculation of mean ± SEM). 7-Dehydrocholesterol and 8-dehydrocholesterol not detectable in controls (27).
[e]$P < 0.05$, significantly different from group I patients.
[f]Not detectable.

Fibroblast culture

Skin biopsies were obtained from patients and from their parents and the fibroblasts were grown and maintained as monolayers in Dulbecco's modified Eagle's medium (Life Technologies, Inc., New York) supplemented with 17% fetal bovine serum. Controls were patients from other metabolic studies without abnormalities in cholesterol metabolism. All cells were used before the 15th passage.

Labeled steroids

[4-^{14}C]cholesterol, [5-^{3}H]mevalonolactone, and [^{3}H]sodium borohydride were obtained from DuPont NEN Research Products (Boston, MA). [3α-^{3}H]lathosterol was synthesized by chromium trioxide oxidation of lathosterol followed by reduction with [^{3}H]sodium borohydride. The pure compound (purity > 98%) was obtained by preparative TLC.

Lathosterol conversion assay

On day 1, 75-cm^2 tissue culture flasks were seeded with 1×10^6 cells/flask. On day 7 when the cells were nearly confluent, the original growth medium was replaced with 4 ml of fresh medium containing 10% fetal bovine serum. [^{3}H]lathosterol (1.3 μM, 1.3×10^5 cpm) was then added in 50 μl dimethyl sulfoxide and incubated at 37°C, after which the cells were harvested with trypsin and washed three times in isotonic salt solution. After the addition of 20,000 cpm of [^{14}C]cholesterol as an internal recovery standard, lipids were extracted from the cells with chloroform–methanol 2:1 (14). The extracts were applied to argentation TLC plates (15, 16), developed in chloroform–acetone 9:1, and the radioactivity from the 7-dehydrocholesterol band (R_f = 0.25) was determined by liquid scintillation counting. The cholesterol band (R_f = 0.55), which could have been contaminated with lathosterol (R_f = 0.60), was eluted with chloroform–methanol 9:1, applied to a normal-phase silica gel plate (20 cm × 20 cm × 0.25 mm, Analtech Inc., Newark, DE), and developed in n-hexane–diethyl ether 1:5. The complete elimination of lathosterol (R_f = 0.62) from the cholesterol band (R_f = 0.70) was achieved by the second chromatographic step. ^{3}H- and ^{14}C-label in the cholesterol was measured by dual-label liquid scintillation counting with appropriate corrections for quench. Assays in all subjects were carried out in duplicate or triplicate.

Incorporation of mevalonolactone into sterols

[^{3}H]mevalonolactone (3.8 μM, 1.9×10^6 cpm) was added to confluent cells obtained from two patients and one parent and incubated at 37°C for 24 h. After the addition of [^{14}C]cholesterol and 50 μg of cold lathosterol as internal standards, lathosterol, 7-dehydrocholesterol, and cholesterol were extracted and purified as described above. The amount of the recovered cold lathosterol was measured by gas chromatography–mass spectrometry (17, 18).

Plasma and cell sterols

Plasma cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol concentrations were determined by capillary-column gas chromatography as described previously (8). The sterol concentrations in fibroblasts were measured by gas chromatography–mass spectrometry (17, 18).

Statistics

Data are reported here as the mean ± SEM. The statistical significance of differences between the results in the different groups was evaluated with the Student's two-tailed *t* test or Wilcoxon signed-ranks two-tailed test and significance was accepted at the level of $P < 0.05$.

RESULTS

Table 1 lists the plasma sterols measured in the patients and their parents. Abnormally low cholesterol levels and high concentrations of 7-dehydrocholesterol and 8-dehydrocholesterol were found in all patients except for patient 29. Although the concentrations of cholesterol were especially reduced in the most severely affected group II patients ($P < 0.05$, compared to group I), 7-dehydrocholesterol and 8-dehydrocholesterol levels did not differ significantly between the two groups. 7-Dehydrocholesterol and 8-dehydrocholesterol were not detectable in parents' plasma when our conventional gas chromatographic methods (8, 18) were used.

The concentrations of 7-dehydrocholesterol were markedly elevated in fibroblasts from both groups of patients. The ratios of 7-dehydrocholesterol to cholesterol were 2.9 ± 0.5% (n = 6), 2.6 ± 0.5% (n = 6), 0.14 ± 0.01% (n = 14), and 0.06 ± 0.01% (n = 6) in group I patients, group II patients, parents, and controls, respectively. In contrast, only trace amounts of 8-dehydrocholesterol were found in patients' fibroblasts and no 8-dehydrocholesterol was detected in fibroblasts from parents and controls.

Figure 2 illustrates the effects of incubation time on the relative amount of [^{3}H]lathosterol taken up by the cells (approximately 4×10^6 cells/flask) and then converted to 7-dehydrocholesterol and cholesterol in a typical patient (RD), a parent, and a control. Because incubations longer than 24 h did not further increase the uptake of [^{3}H]lathosterol, this time interval was chosen for the standard assay. The conversion of lathosterol to 7-dehydrocholesterol and cholesterol was calculated as the percentage of the radioactivity found in each sterol

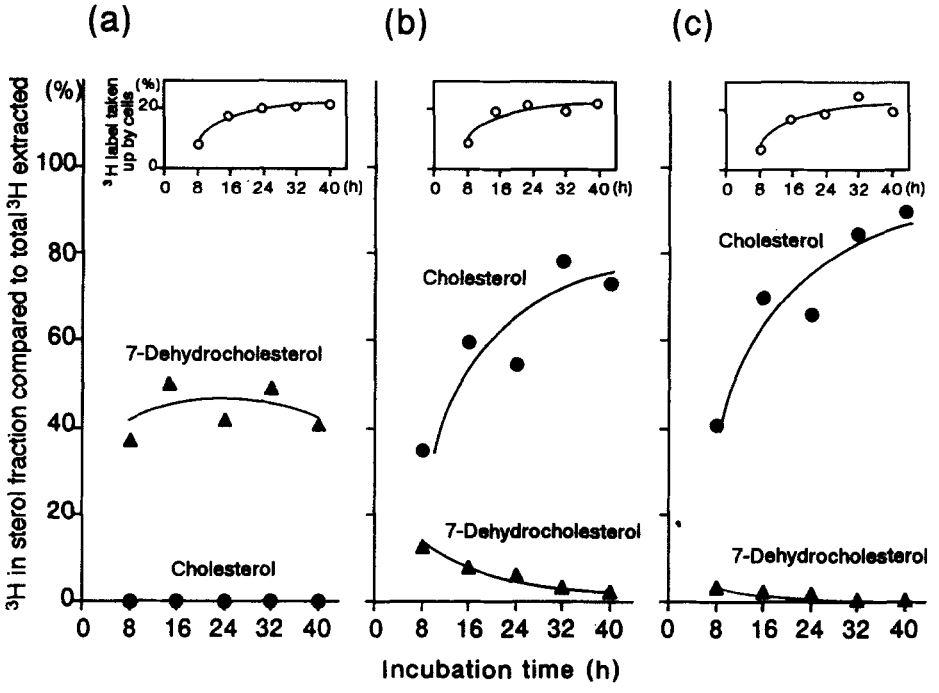

Fig. 2. ^{3}H label recovered in 7-dehydrocholesterol (▲) and cholesterol (●) as a function of time of incubation. The inset plots depict the time course of [^{3}H]lathosterol uptake by cells compared to total [^{3}H]lathosterol added; (a), patient (RD); (b), parent; (c), control.

compared to the total ^{3}H extracted from the cells (19). The plots of the incorporation of ^{3}H into 7-dehydrocholesterol and cholesterol versus incubation time from the parent (Fig. 2b) and the control (Fig. 2c) cell cultures were similar. However, the plot from the patient (Fig. 2a) was very different. In the case of parent and control, increasing amounts of ^{3}H were incorporated into cholesterol while the amount of label in 7-dehydrocholesterol decreased rapidly. In contrast, in cells from the patient, the quantity of label in 7-dehydrocholesterol was virtually constant while little, if any, ^{3}H could be found in the cholesterol fraction. In patients ^{3}H was detected in 8-dehydrocholesterol. However, the amounts were small and did not exceed 10% of those in 7-dehydrocholesterol.

Table 2 summarizes the results. Approximately 20% of the added radiolabeled lathosterol was taken up by the washed fibroblasts from all subjects. Although the transformation of [^{3}H]lathosterol to cholesterol in fibroblasts from parents was not different from that in controls, the amount of ^{3}H label found in the 7-dehydrocholesterol fraction was significantly elevated ($P < 0.01$). When we measured the ^{3}H label remaining in 7-dehydrocholesterol for incubations carried out with cells from the earliest passages (5 ± 1 passages) and compared those to the amount of label found in the 7-dehydrocholesterol fraction in cells from later passages (8 ± 1 passages), the origin of the above difference was found. Significantly more ^{3}H was found in the 7-dehydrocholesterol fraction (12 ± 3% vs. 4.7 ± 0.8%, $P < 0.05$) and less ^{3}H was detected in cholesterol (53 ± 4% vs. 65 ± 2%, $P < 0.05$) in the younger cells (Wilcoxon signed-ranks test). However, due to the wide variation it was not possible to discriminate easily between fibroblasts from controls and from parents.

In contrast, cells from patients made very little cholesterol but readily converted lathosterol to 7-dehydrocholesterol. The defect was especially profound in the clinically more severe group II patients as virtually no label could be detected in the cholesterol fraction. We also performed the same conversion assay by using amniocytes from SV with a result very similar to that obtained with the fibroblasts from her fetus. In the amniocytes 34% of the label was recovered in the 7-dehydrocholesterol fraction while no ^{3}H was detected in cholesterol, compared to 21% and 0%, respectively, in fetal fibroblasts.

Table 3 describes the results obtained when labeled mevalonolactone was incubated with cultured fibroblasts from two affected children and one of the parents. The labeling pattern paralleled that noted in the lathosterol conversion assay. In cells from the patients mevalonolactone was readily incorporated into lathosterol and 7-dehydrocholesterol but not into cho-

TABLE 2. Conversion of [^{3}H]lathosterol to 7-dehydrocholesterol and cholesterol in cultured fibroblasts and amniocytes from patients, parents, and controls

Subject	^{3}H in Sterol Fraction Compared to Total ^{3}H Extracted	
	7-Dehydrocholesterol	Cholesterol
	%	
Fibroblasts		
Patients (Group I)		
2	53	1.5
4	53	1.6
9	49	1.9
18	48	0.1
24	44	0.6
29	53	1.1
36	53	1.0
Mean ± SEM	50 ± 1[a]	1.1 ± 0.2[a]
Patients (Group II)		
5	48	0.2
14	51	0.0
30	50	0.0
32	56	0.0
RD	42	0.0
SV	21	0.0
FVR	48	0.0
SL	48	0.0
Mean ± SEM	45 ± 4[a]	0.03 ± 0.03[a,b]
Parents		
Mean ± SEM (n = 14)	7.9 ± 1.2[c]	59 ± 2
Controls		
Mean ± SEM (n = 8)	3.2 ± 0.7	64 ± 3
Amniocytes		
Patient (SV)	34	0.0
Controls		
Mean ± SEM (n = 3)	3.0 ± 1.3	39 ± 19

[a]$P < 0.0001$, significantly different from parents and controls.
[b]$P < 0.0005$, significantly different from group I patients.
[c]$P < 0.01$, significantly different from controls.

lesterol. In contrast, in the parent most of the label was found in the cholesterol band while little radioactivity could be detected as 7-dehydrocholesterol.

DISCUSSION

These results provide compelling evidence that patients with the Smith-Lemli-Opitz syndrome have a primary defect in the reduction of the C-7 double bond of 7-dehydrocholesterol catalyzed by the microsomal enzyme 7-dehydrocholesterol Δ^7-reductase (3β-hydroxysteroid Δ^7-reductase) (11). Although our experiments could not differentiate between markedly reduced cholesterol biosynthesis as a result of an enzyme with greatly reduced activity or because of low enzyme mass, the reduced conversion of lathosterol to cholesterol demonstrates conclusively that the Smith-Lemli-Opitz syndrome is caused by this specific defect in cholesterol biosynthesis. In contrast, because cells from patients readily converted lathosterol to 7-dehydrocholesterol, the enzyme lathosterol 5-desaturase is intact. This latter result also demonstrates that the cells from patients retained viability during the course of the assay. To determine the functionality of the pathway between mevalonic acid and 7-dehydrocholesterol, we also incubated [^{3}H]mevalonolactone with cultured fibroblasts and assayed its incorporation into sterols (Table 3). The observation that cells from patients made very little cholesterol but readily converted mevalonolactone to 7-dehydrocholesterol indicates that in the Smith-Lemli-Opitz syndrome 7-dehydrocholesterol Δ^7-reductase is probably the only defect in the cholesterol biosynthetic pathway. It should be noted that the high levels of ^{3}H found in the lathosterol fraction are consistent with both previous in vitro and in vivo studies (16, 19).

Plasma concentration of 8-dehydrocholesterol in patients was often equal to or higher than that of 7-dehydrocholesterol (Table 1). However, the concentration of 8-dehydrocholesterol in fibroblasts was considerably lower than that of 7-dehydrocholesterol. In the [^{3}H]lathosterol conversion assay, small amounts of ^{3}H were detected in 8-dehydrocholesterol. These results indicate that very little 8-dehydrocholesterol is synthesized by skin fibroblasts. Similarly, 8-dehydrocholesterol was detected in plasma, but only at low concentrations, in rats fed BM 15.766, an inhibitor of 7-dehydrocholesterol Δ^7-reductase (20).

Measurements of the conversion of lathosterol to cholesterol are also a useful and accurate method for making the diagnosis of the syndrome. The extraordi-

TABLE 3. Incorporation of [^{3}H]mevalonolactone into lathosterol, 7-dehydrocholesterol, and cholesterol in cultured fibroblasts[a]

Subject	Total ^{3}H Label Extracted from Cells	^{3}H in Sterol Fraction Compared to Total ^{3}H Extracted		
		Lathosterol	7-DHC[b]	Cholesterol
	cpm		%	
Patient 2	4.2×10^3	28	34	0.5
Patient 24	3.9×10^3	34	20	0.6
Parent	3.5×10^3	24	2.3	65

[a]Average of two assays.
[b]7-Dehydrocholesterol.

nary variability of clinical expression is one of the characteristics of this disease and has led some investigators to postulate the existence of Smith-Lemli-Opitz syndrome-type II (4) or different but closely related entities ("Lowry-Miller-Maclean syndrome" (21); "Gardner-Silengo-Wachtel syndrome" (22), etc.). However, it was unclear whether this variability indicated a biochemical heterogeneity or the individual responses of different patients to the same disease process. Our finding that fibroblasts from a group of more clinically affected patients (group II), most of whom had been diagnosed as the type II phenotype, exhibited the almost complete absence of 7-dehydrocholesterol Δ^7-reductase activity demonstrates that all Smith-Lemli-Opitz patients share the same metabolic abnormality. But there appears to be a correlation between the degree of clinical severity and the magnitude of the enzyme defect.

Although the clinical diagnosis of Smith-Lemli-Opitz syndrome cannot always be made with certainty because of this variability of expression, the syndrome can usually be diagnosed quite easily from the biochemical abnormalities, reduced plasma cholesterol, and markedly elevated 7-dehydrocholesterol concentrations (Table 1) (7, 8). However, occasionally older subjects, such as patient 29, may exhibit normal plasma cholesterol levels with concentrations of 7-dehydrocholesterol that are considerably below that reported for most individuals with the syndrome. We recently assayed plasma sterols in 33 Smith-Lemli-Opitz patients (23) and found cholesterol concentrations in four patients to be more than 100 mg/dl and to be within the normal range in two of them. It is possible that diets rich in cholesterol, especially when used for many years, may raise plasma cholesterol levels and reduce plasma 7-dehydrocholesterol concentrations (20, 24), thus masking the biochemical indicators of the syndrome. In addition, although plasma levels of 7-dehydrocholesterol in healthy subjects are normally very low, marked elevations can be observed in patients with ileal resection or in those undergoing treatment with cholestyramine (25), that is, in patients who are expected to have a greatly increased rate of cholesterol biosynthesis (26). Therefore, because plasma 7-dehydrocholesterol in patient 29, while elevated 40- to 50-fold above controls, was within the range found in such patients (25) and because his plasma cholesterol concentration was normal, the diagnosis of the Smith-Lemli-Opitz syndrome if determined solely by plasma sterols might have been missed. However, the lathosterol fibroblast conversion assay easily detected the biochemical defect (Table 2).

In addition, it is important to note that because we obtained very similar results in both fibroblasts and amniocytes from the same fetus, the assay probably can also be used for a definitive prenatal diagnosis of the syndrome.

The excellent technical assistance of Bibiana Pcolinsky is greatly appreciated. This work was supported in part by grants from the Department of Veterans Affairs Research Service, by grants DK-18707, HL-17818 and HD-31932 from the National Institutes of Health, and by a grant from the Herman Goldman Foundation.

Manuscript received 12 January 1995 and in revised form 16 March 1995.

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. Opitz, J. M. 1994. RSH/SLO ("Smith-Lemli-Opitz") syndrome: historical, genetic, and developmental considerations. *Am. J. Med. Genet.* **50:** 344–346.
3. Chasalow, F. I., S. L. Blethen, and K. Taysi. 1985. Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents. *Steroids.* **46:** 827–843.
4. Curry, C. J. R., J. C. Carey, J. S. Holland, D. Chopra, R. Fineman, M. Golabi, S. Sherman, R. A. Pagon, J. Allanson, S. Shulman, M. Barr, V. McGravey, C. Dabiri, N. Schimke, E. Ives, and B. D. Hall. 1987. Smith-Lemli-Opitz syndrome-type II: multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. *Am. J. Med. Genet.* **26:** 45–57.
5. Gorlin, R. J., M. M. Cohen, Jr., and L. S. Levin. 1990. Syndromes of the Head and Neck. 3rd ed. Oxford University Press, New York, NY. 890–895.
6. Pober, B., C. J. R. Curry, and J. C. Rutledge. 1990. Smith-Lemli-Opitz syndrome. *In* Birth Defects Encyclopedia. M. L. Buyse, editor. Center for Birth Defects Information Services, in association with Blackwell Scientific, Dover, MA. 1570–1572.
7. Irons, M., E. R. Elias, G. Salen, G. S. Tint, and A. K. Batta. 1993. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. *Lancet.* **341:** 1414.
8. 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.
9. Batta, A. K., G. S. Tint, S. Shefer, D. Abuelo, and G. Salen. 1995. Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) in patients with Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **36:** 705–713.
10. Kawata, S., J. M. Trzaskos, and J. L. Gaylor. 1985. Microsomal enzymes of cholesterol biosynthesis from lanosterol. *J. Biol. Chem.* **260:** 6609–6617.
11. Kandutsch, A. A. 1962. Enzymatic reduction of the Δ^7 bond of 7-dehydrocholesterol. *J. Biol. Chem.* **237:** 358–362.
12. Wallace, M., R. T. Zori, T. Alley, E. Whidden, B. A. Gray, and C. A. Williams. 1994. Smith-Lemli-Opitz syndrome in a female with a de novo, balanced translocation involving 7q32: probable disruption of an SLOS gene. *Am. J. Med. Genet.* **50:** 368–374.

13. Johnson, V. P. 1975. Smith-Lemli-Opitz syndrome: review and report of two affected siblings. *Z. Kinderheilk.* **119:** 221–234.
14. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
15. Truswell, A. S., and W. D. Mitchell. 1965. Separation of cholesterol from its companions, cholestanol and Δ^7-cholestenol, by thin-layer chromatography. *J. Lipid Res.* **6:** 438–441.
16. Tint, G. S., and G. Salen. 1974. Transformation of 5α-cholest-7-en-3β-ol to cholesterol and cholestanol in cerebrotendinous xanthomatosis. *J. Lipid Res.* **15:** 256–262.
17. Kelley, R. I. 1994. Quantification of neutral sterols by gas chromatography/mass fragmentometry for diagnosis of inborn errors of sterol metabolism. *Am. J. Med. Genet.* **50:** 335.
18. Tint, G. S., M. Seller, R. Hughes-Benzie, A. K. Batta, S. Shefer, D. Genest, M. Irons, E. Elias, and G. Salen. 1995. Markedly increased tissue concentrations of 7-dehydrocholesterol combined with low levels of cholesterol are characteristic of the Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **36:** 89–95.
19. Tint, G. S., and G. Salen. 1982. Biosynthesis of cholesterol, lanosterol, and Δ^7-cholestenol, but not cholestanol, in cultured fibroblasts from normal individuals and patients with cerebrotendinous xanthomatosis. *J. Lipid Res.* **23:** 597–603.
20. Xu, G., G. Salen, S. Shefer, G. C. Ness, T. S. Chen, Z. Zhao, and G. S. Tint. 1995. Reproducing abnormal cholesterol biosynthesis as seen in the Smith-Lemli-Opitz syndrome by inhibiting the conversion of 7-dehydrocholesterol to cholesterol in rats. *J. Clin. Invest.* **95:** 76–81.
21. 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.
22. Greenberg, F., M. V. Gresik, R. J. Carpenter, S. W. Law, L. P. Hoffman, and D. H. Ledbetter. 1987. The Gardner-Silengo-Wachtel or genito-palato-cardiac syndrome: male pseudohermaphroditism with micrognathia, cleft palate and conotruncal cardiac defect. *Am. J. Med. Genet.* **26:** 59–64.
23. Tint, G. S., G. Salen, A. K. Batta, S. Shefer, M. Irons, E. R. Elias, D. N. Abuelo, V. P. Johnson, M. Lambert, R. Lutz, C. Schanen, C. A. Morris, G. Hoganson, and R. Hughes-Benzie. 1995. Severity and outcome correlate with plasma sterol levels in type I and type II variants of the Smith-Lemli-Opitz syndrome. *J. Pediatr.* In press.
24. 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.
25. Axelson, M. 1991. Occurrence of isomeric dehydrocholesterols in human plasma. *J. Lipid Res.* **32:** 1441–1448.
26. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
27. Abuelo, D. N., G. S. Tint, R. Kelley, A. K. Batta, S. Shefer, and G. Salen. 1995. Prenatal detection of the cholesterol biosynthetic defect in the Smith-Lemli-Opitz syndrome by the analysis of amniotic fluid sterols. *Am. J. Med. Genet.* **56:** 281–285.