review

Abnormal cholesterol biosynthesis in the Smith-Lemli-Opitz syndrome

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Abstract The Smith-Lemli-Opitz syndrome is caused by an inherited defect in 7-dehydrocholesterol-Δ^7-reductase, the enzyme that catalyzes the last reaction in cholesterol biosynthesis, the conversion of 7-dehydrocholesterol to cholesterol. As a result, deficient cholesterol is produced and the precursor 7-dehydrocholesterol and derivatives (8-dehydrocholesterol and 19-nor-5,7,9(10)-cholestatrien-3β-ol) accumulate. Tissues (especially brain) deprived of cholesterol, or because of the deposited sterol precursors and derivatives, develop abnormally and function poorly. Replacement with dietary cholesterol may help correct the biochemical defects and improve symptoms.—**Salen, G., S. Shefer, A. K. Batta, G. S. Tint, G. Xu, A. Honda, M. Irons, and E. R. Elias.** Abnormal cholesterol biosynthesis in the Smith-Lemli-Opitz syndrome. *J. Lipid Res.* 1996. **37:** 1169–1180.

Supplementary key words 7-dehydrocholesterol • 7-dehydrocholesterol-Δ^7-reductase • lathosterol-5-dehydrogenase • 8-dehydrocholesterol

The Smith-Lemli-Opitz (RSH or SLO) syndrome is a fairly common, often lethal, birth defect that was first described clinically by David Smith, Luc Lemli, and John Opitz in 1964 (1, 2). Newborns with the Smith-Lemli-Opitz syndrome have a distinctive appearance with specific facial dysmorphism and suffer from multiple congenital anomalies including cleft palate, congenital heart disease, genitourinary abnormalities, and malformed limbs. They often manifest severe failure to thrive and virtually all are mentally retarded with significant central nervous system anomalies (3–17). The syndrome is inherited as an autosomal recessive disorder and obligate heterozygotes (parents) are clinically normal (13, 15). Recently, we have discovered a major abnormality in late cholesterol (5-cholesten-3β-ol) biosynthesis in homozygotes due to an inherited deficiency of the last enzyme in the pathway, 7-dehydrocholesterol-Δ^7-reductase, that catalyzes the conversion of the precursor, 7-dehydrocholesterol, to cholesterol. As a result, markedly reduced cholesterol concentrations with the accumulation of the precursor, 7-dehydrocholesterol (5,7-cholestadien-3β-ol) and derivatives, are found in plasma and tissues (18–28).

Based on the clinical phenotype, the suggested incidence of the Smith-Lemli-Opitz syndrome is 1:20,000 births with an estimated gene carrier frequency in the North American Caucasian population of 1 to 2% (2). However, Opitz has recently suggested that the true prevalence of the Smith-Lemli-Opitz syndrome may be much more common as a recent study from the Czech Republic reported the syndrome in 1 of 9,000 births (alive and stillborn) with 1 in 50 individuals carrying the defective gene (29). In comparison, the incidence of three other common inborn errors of metabolism noted in childhood, cystic fibrosis, phenylketonuria (PKU), and galactosemia vary from 1:800, 1:14,000, and 1:40,000 births, respectively (30). It also has been suggested that because cholesterol is necessary for structure in all cells, the more severe inherited defects in cholesterol biosynthesis may result in spontaneous termination of pregnancy so that the aborted fetuses are not counted in the epidemiologic statistics and the true incidence of the Smith-Lemli-Opitz syndrome may be underestimated.

Clinical

As cholesterol is an integral component of all cell membranes and serves as a substrate for the biosynthesis of steroid and sex hormones, and bile acids, it is not unexpected that the clinical expression should be varied and abnormalities would affect virtually every tissue. The most characteristic presentation includes a set of facial dysmorphic features with microcephaly, ptosis, cataracts, prominent epicanthal folds, anteverted nostrils, low set posteriorly rotated ears, cleft palate, and micrognathia. Some examples of these features are illustrated in **Fig. 1.** Also very common are limb defects with 2-3 syndactyly of the toes; postaxial polydactyly, and, much less frequently, oligodactyly; club foot deformities; and dislocated hips. Major organs are affected commonly and congenital heart disease (atrial and ventricular septal defects) impair cardiac function fre-

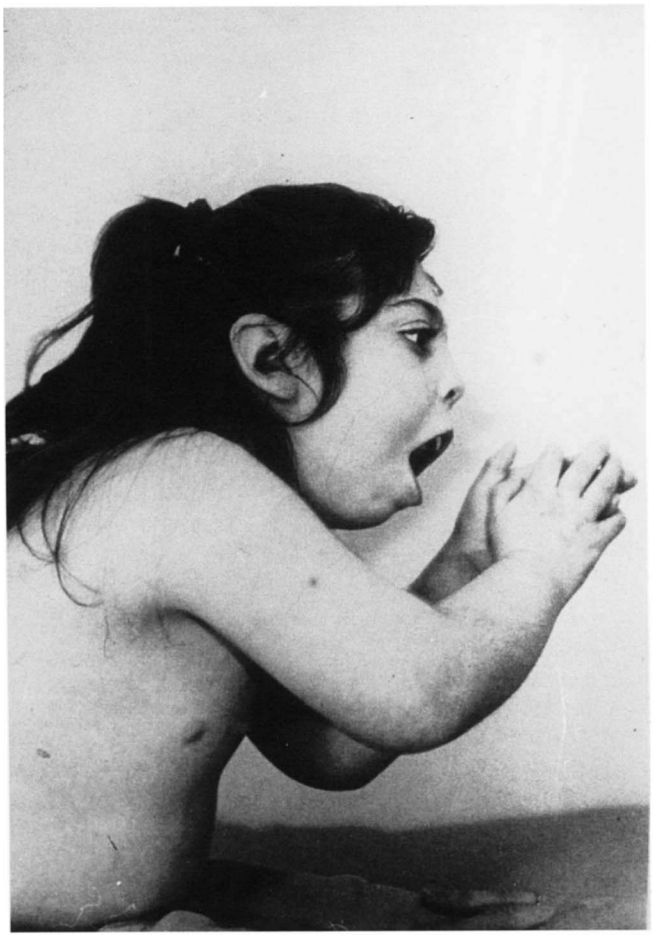

Fig. 1. Ten-year-old female Smith-Lemli-Opitz homozygote shown in profile. Microcephaly, anteverted nostrils, micrognathia, and low-set posteriorly rotated ears are prominently featured in the photograph. (Permission to use this photograph was obtained from the patient's parent.)

quently. Kidney cysts and structural malformations occur in the liver, lungs, urinary system, and adrenal glands. Hypotonia of the gastrointestinal tract is present and contributes to feeding problems in many homozygotes. Devastating developmental malformations of the brain and peripheral nervous system are frequent and help explain the disabling neurologic function and mental retardation. Central nervous system malformations include holoprosencephaly, irregular gyri, small cerebellum, loss of nerve cells with astrocyte proliferation, absence of cerebellar vermis, and hypomyelination. Hirschprung's disease with congenital agangliosis of the colon may be present. Abnormal striated muscle tone with hypotonia followed by muscle spacity is frequently noted. Failure to thrive is a key feature and many affected homozygotes require special diets and feeding through gastrostomy tubes. Males often manifest genital anomalies including cryptorchidism, hypospadias, and microphalus and in the most severe cases, karyotypic XY males show ambiguous or female external genitalia (3–18).

In 1987, Curry et al. (11) and Bialer et al. (12) suggested that the Smith-Lemli-Opitz syndrome might be divided into two presentations with a more severe clinical phenotype that was designated Type II and manifested more serious facial dysmorphism, limb deformities, pseudohermaphodism in males, and life-threatening congenital anomalies involving the heart so that the majority of these subjects died before the age of 6 months. Before the discovery of the cholesterol biosynthetic defect in the Smith-Lemli-Opitz syndrome, debate often raged in the literature as to whether Smith-Lemli-Opitz syndrome Type I and Type II presentations represented two distinct disorders or a spectrum in severity of the same genetic defect. Opitz argued that Smith-Lemli-Opitz Type I and II syndromes occurred in the same sibship, which suggested a single syndrome of varying severity (2). With the discovery of the cholesterol biosynthetic defect, measurements of 7-dehydrocholesterol-Δ^7-reductase activity in liver and fibroblasts have confirmed that the last enzyme in cholesterol biosynthesis is abnormal in both Types I and II phenotypes (20, 21, 27), but that most patients with the clinically more serious Type II phenotype appear to show more severe plasma and tissue sterol abnormalities (31, 32).

Cholesterol biosynthesis

It has been amply authenticated that cholesterol can be synthesized from acetate (2-carbon fragments) in virtually every cell (**Fig. 2**). The reduction of hydroxymethylglutaryl CoA (a dicarboxylic acid) to mevalonic acid (a monocarboxylic acid) is considered the rate-controlling step in biosynthesis as virtually every molecule of mevalonic acid is committed to cholesterol with the exception of the diversion of small amounts of precursors to other isoprenoids. This first specific reaction in the pathway is catalyzed by the microsomal enzyme, HMG-CoA reductase. Importantly, it has been demonstrated that the formation of cholesterol correlates positively with the activity, mass, and mRNA abundance of HMG-CoA reductase. Enzyme activity undergoes diurnal cycling and is under sensitive negative feedback control by the hepatic cholesterol pool and the enterohepatic bile acid flux. Further, unused precursor (HMG-CoA) does not accumulate but any excess that is not converted to mevalonic acid may be eliminated as CO_2 and water through fatty acid oxidation. This difference in alternative utilization of unused precursors will become more important as we consider the possible deleterious effect of the precursor 7-dehydrocholesterol that accumulates because of the enzyme defect in late cholesterol biosynthesis that is responsible for the Smith-Lemli-Opitz syndrome.

In the pathway, six molecules of mevalonic acid are ultimately incorporated into cholesterol. Key reactions and intermediates in the pathway are listed in Fig. 2. Two isopentenyl pyrophosphates (5 carbons) are condensed to form geranyl pyrophosphate (10 carbons) and another enzymatic condensation adds a third isopentenyl pyrophosphate molecule to form farnesyl pyrophosphate (15 carbons). Two farnesyl pyrophosphates are converted enzymatically to give squalene, which in turn is cyclized into the first steroid, lanosterol (4,4′,14-trimethyl-8(9), 24(25)-cholestadien-3β-ol). At this junction, two pathways exist to transform lanosterol which contains 30 carbons to cholesterol with 27 carbons. According to the classical reaction sequence, the three methyl groups located at C-4,4′, and 14 are eliminated and the double bond at carbon 8(9) is transferred to carbon 5(6) so that desmosterol (5,24-cholestadien-3β-ol) becomes the last precursor and the saturation of the double bond at C-24 by a sterol-Δ^{24}-reductase becomes the final reaction (Fig. 2) (33). Alternatively, cholesterol can be produced when the double bond at C-24 in lanosterol is reduced early in the reaction sequence so that 24,25-dihydrolanosterol [4,4′,14-trimethyl-8(9)-cholesten-3β-ol] is formed (34) which in turn is converted to lathosterol (7-cholesten-3β-ol), 7-dehydrocholesterol (5,7-cholestadien-3β-ol), and finally cholesterol (35–40). Although illustrated separately, the pathways are interrelated as only a single sterol-Δ^{24}-reductase saturates the double bond at C-24, and both lathosterol 5-dehydrogenase and 7-dehydrocholesterol-Δ^{7}-reductase are necessary enzymes in the reactions that form cholesterol from either desmosterol or 7-dehydrocholesterol.

Biochemical defect

Until 1993–1994, there was no laboratory or biochemical test to diagnose the Smith-Lemli-Opitz syndrome. We then described a major defect in cholesterol biosynthesis in four children clinically affected with the Smith-Lemli-Opitz phenotype (18, 19). All were found to have unusually low levels of cholesterol in plasma and erythrocytes, that were well below the 5th percentile for age, associated with markedly elevated concentrations of the last cholesterol precursor, 7-dehydrocholesterol (Fig. 2). In addition, two additional sterols, whose structures have been conclusively identified, have been isolated from plasma of affected patients: 8-dehydrocholesterol, the 8-dehydro isomer (5,8-cholestadien-3β-ol) (41) and a 26-carbon derivative of 7-dehydrocholesterol with 3 double bonds located in ring B, now identified as 19-nor-5,7,9(10)-cholestatrien-3β-ol (42). **Figure 3** shows the gas chromatogram of the plasma sterols from an Smith-Lemli-Opitz homozygote. Normally, only a single major peak of cholesterol is present in the plasma as contrasted with diminished amounts of cholesterol and abundant quantities of 3 additional sterols. The structures of the sterols in the Smith-Lemli-Opitz syndrome homozygotes' plasma are shown in **Fig. 4.**

In plasma from control subjects, these 3 abnormal sterols were either absent or detected in only trace amounts (43). Since the initial reports (19, 20), about 100 biochemically documented Smith-Lemli-Opitz subjects have been reported in the literature from Baltimore, MD (22), Munster, Germany (25), Paris, France (44), and East Orange, NJ (31). It should also be noted that, rarely, a subject with Smith-Lemli-Opitz syndrome shows a milder phenotype (45) with normal plasma cholesterol levels and only slightly elevated 7-dehydrocholesterol concentrations. The diagnosis in these individuals with borderline abnormal sterol concentrations

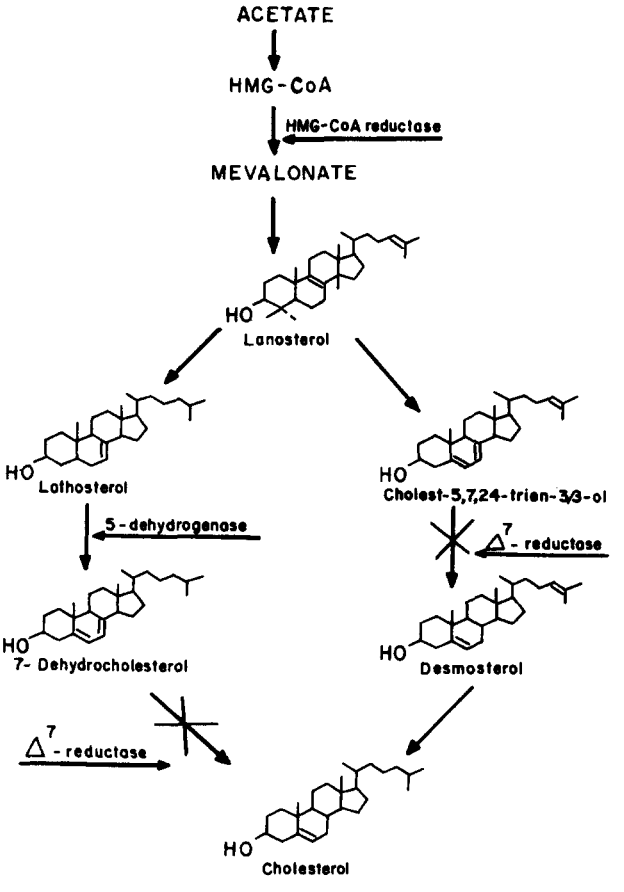

Fig. 2. Flow diagram for the formation of cholesterol. Key intermediates are listed. The formation of mevalonate catalyzed by HMG-CoA reductase is considered the rate-limiting step for the entire pathway. Lanosterol, the first sterol in the pathway is transformed to cholesterol by two mechanisms, via desmosterol or 7-dehydrocholesterol. As illustrated, both pathways share the Δ^{7}-reductase enzyme. The accumulation of the precursor, 7-dehydrocholesterol, points to this reaction as the inherited abnormality in the Smith-Lemli-Opitz syndrome.

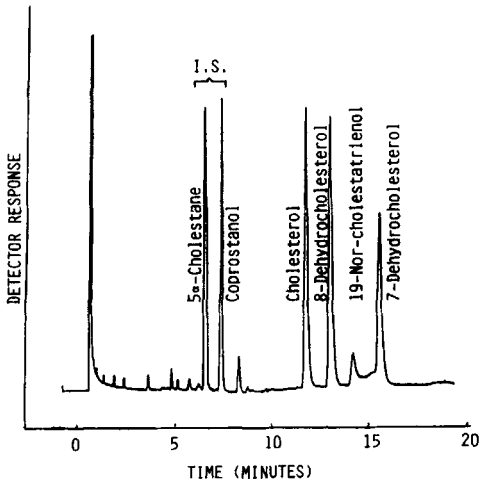

Fig. 3. Capillary gas chromatogram of plasma sterols from Smith-Lemli-Opitz homozygote. In addition to reduced amounts of cholesterol, three additional sterols, 7-dehydrocholesterol, 8-dehydrocholesterol, and 19-nor-5,7,9(10)-cholestatrien-3β-ol, were conclusively identified. 5α-Cholestane and coprostanol (5β-cholestan-3β-ol) were added as internal standards (I.S.).

can be established conclusively by measuring 7-dehydrocholesterol-Δ^7-reductase activity in fibroblasts that is several-fold higher in controls than in homozygotes. Also, it is important to emphasize that the elucidation of the biochemical abnormalities associated with the Smith-Lemli-Opitz syndrome has identified some subjects who appear remarkably similar, but have normal cholesterol biosynthesis and do not accumulate the precursor, 7-dehydrocholesterol. Thus, the discovery of the biochemical defect has refined the specific diagnosis of the syndrome to those subjects with abnormal late cholesterol biosynthesis. Other inherited inborn errors not involving cholesterol biosynthesis may be responsible for clinical syndromes with some features similar to the Smith-Lemli-Opitz syndrome.

Plasma sterol concentrations

We have measured plasma sterol concentrations by capillary gas–liquid chromatography (Fig. 3) in 50 clinically affected subjects (**Table 1**) (age 1 day to 35 years). Mean ± SD values (mg/dl) for cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol were 49 ± 41 mg/dl, 16 ± 10 mg/dl, and 12 ± 5 mg/dl, respectively. The cholesterol concentrations in 47 out of 50 subjects were less than 110 mg/dl as contrasted with values of more than 120 mg/dl in 95% of children 2–3 years old. Mean 7-dehydrocholesterol concentrations for healthy children and adults have ranged from 0.005 to 0.01 mg/dl while the 8-dehydro isomer, 8-dehydrocholesterol, usually cannot be detected (41). In addition to 7- and 8-dehydrocholesterols, a third sterol with 26 carbons and 3 double bonds has been conclusively identified as 19-nor-5,7,9(10)-cholestatrien-3β-ol (42) (Figs. 3 and 4). Although 7-dehydrocholesterol is well known as the final precursor of cholesterol, the significance of 8-dehydrocholesterol and 19- nor-5,7,9(10)-cholestatrien-3β-ol in the pathway is unknown. It has been suggested that 8-dehydrocholesterol may accumulate because it is chemically more stable than 7-dehydrocholesterol. The reaction to form 8-dehydrocholesterol from 7-dehydrocholesterol is enzymatically controlled by a microsomal sterol 7(8)-isomerase that catalyzes the reaction but either is not distributed or active in every tissue. As a result, the formation of 8-dehydrocholesterol is limited.

Both 7- and 8-dehydrocholesterols are esterified like cholesterol with long chain fatty acids. About 65% of 7-dehydrocholesterol and 83% of 8-dehydrocholesterol were transported as esters compared with 75% of esterified cholesterol in LDL (19). Clearly, the presence of the additional double bond in ring B of the precursor was not recognized and both ACAT (acyl-CoA:cholesterol acyltransferase) and LCAT (lecithin:cholesterol acyltransferase) actively esterify 7-dehydrocholesterol and its 8-dehydro isomer about equal to cholesterol (G. Salen, unpublished observation).

It is also noteworthy that the proportion of cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol in tissues reflected their percentage in plasma. This distribution strongly suggested that although the enzyme defect is expressed in all tissues, most cholesterol is synthesized in the liver and that tissue sterols are derived from plasma lipoproteins that are probably formed in the liver. The major exception might be in the brain where de novo cholesterol biosynthesis in the fetus and neonate is very active so that brain sterol composition is quite abnormal in homozygotes. Moreover, because the blood–brain barrier prevents sterols

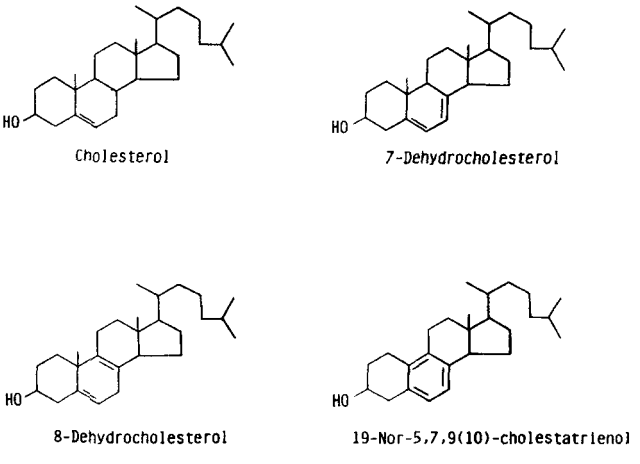

Fig. 4. Structures of the sterols identified in the plasma and tissues of Smith-Lemli-Opitz homozygotes.

TABLE 1. Plasma cholesterol, 7-dehydrocholesterol (7-DHC), 8-dehydrocholesterol (8-DHC) and total sterols in 50 subjects with the Smith-Lemli-Opitz syndrome

Sterols	All Subjects n=50	Type 1 n=36	Type 11 n=14	Controls n-14
		mg/dl (mean ± SD [range])		
Cholesterol	49 ± 41 [3.7–190]	65 ± 38[a] [18–190]	9.4 ± 6.5 [3.7–29]	143 ± 20 [109–176]
7-DHC	16 ± 10 [0.15–61]	14 ± 7[b] [0.15–30]	20 ± 14 [6–61]	nd
8-DHC[c]	12 ± 5 [1–26]	11 ± 4[b] [1–19]	15 ± 7 [7–26]	nd
Total sterols[c]	79 ± 37 [22–200]	91 ± 34[a] [32–200]	48 ± 25 [22–104];	143 ± 20 [143 ± 20]
Age (years)	7.2 ± 9.9 [1 d–35 y]	9.9 ± 11 [1 d–35 y]	0.26 ± 0.66 [1 d–2.5 y]	2 ± 2

Abbreviations: d, day; y, year; nd, not detected.
[a] $P < 0.001$ versus Type II.
[b] $P < 0.05$ versus Type II.
[c] Includes 19-nor-5,7,9(10)-cholestatrien-3β-ol (42).

in the plasma from gaining access to the brain sterol pool, brain sterol composition is little influenced by circulating plasma sterols that might be enriched by absorbed dietary cholesterol. This difference between brain and other tissues was most notable in a 20-week Type II female fetus who died soon after birth (**Table 2**) (32). Seven nonbrain tissues removed at post mortem, including striated muscle, adrenal gland, kidney, liver, thymus, lung, and adipose tissue, contained 20% cholesterol and 72% a mixture of 7-dehydro- and 8-dehydrocholesterols. In contrast, brain sterols from this subject were composed of only 4.1% cholesterol with about 92% a mixture of 7-dehydro- and 8-dehydrocholesterols. Thus, the brain is more vulnerable to the inherited cholesterol biosynthetic defect and plasma sterol measurements may not completely reflect the true deprivation of cholesterol and the accumulation of 7- and 8-dehydrocholesterol precursors and derivatives in the central nervous system.

As the same inherited enzyme defect is responsible for the Type I and clinically more severe Type II phenotypes, it might be expected that the biochemical abnormalities should be more exaggerated in the plasma of the Type II homozygotes (31). This was observed in 14 homozygotes classified Type II as compared with 36 Type I homozygotes (Table 1). Although the Type II homozygotes were younger as a group than those designated Type I, plasma sterols showed much lower cholesterol concentrations and more elevated 7-dehydro- and 8-dehydrocholesterol levels in Type II subjects. Further, when age was equalized by limiting the examination to the 11 Type I subjects out of 36 who were younger than 1.5 years, the differences between Type I and II remained (data not shown). Thus, in spite of objections that the Type II clinical description is artificial, when supported by measurements of plasma sterols it denotes a more serious enzymatic defect with more pronounced cholesterol deficiency and higher 7- and 8-dehydrocholesterol levels.

Furthermore, an additional practical point can be made about very low plasma cholesterol levels seen in subjects with the Smith-Lemli-Opitz syndrome: children

TABLE 2. Tissue sterol composition in 20 week fetus

Tissues	Total	Cholesterol	7DHC	8DHC
	mg/g		%	
Striated muscle, Liver, Kidney, Adipose tissue, Lung, Thymus, Adrenal gland	4.2 ± 1.1	20 ± 2	37 ± 5	35 ± 5
Brain	5.1	4.1	61	31

7DHC, 7-dehydrocholesterol; 8DHC, 8-dehydrocholesterol.

TABLE 3. Amniotic Fluid Sterol Composition

Amniotic Fluid	Cholesterol	7-DHC	8-DHC
		mg/dl	
SLO homozygote[a]	1.38	0.57	0.26
Controls[a] (n = 20)	1.56 ± 0.66	trace[b]	trace[b]

[a]Obtained at 16 weeks.
[b]< 0.001 mg/dl.

with plasma cholesterol levels below 7 mg/dl probably will not survive. This result was suggested earlier by Bialer et al. in 1987 (12) based on their analysis of the spectrum of clinical abnormalities in these children. Thus, the clinical designation Type II remains a useful tool to describe the most severely affected children with the lowest cholesterol and highest 7-dehydrocholesterol levels and the poorest prognosis. This designation will become even more meaningful when the actual gene mutations responsible for the abnormal enzyme protein can be correlated with measurements of 7-dehydrocholesterol-Δ^7-reductase activity.

With regard to the pathogenesis of the clinical phenotype and developmental malformations, it is not clear whether low cholesterol or elevated 7-dehydrocholesterol concentrations or their combination is responsible. Certainly, the plasma sterol concentrations cannot predict brain sterol levels because, as noted in Table 2, cholesterol concentrations were much lower in brain than other tissues because the blood–brain barrier prevented equilibrium.

Fecal and biliary sterols and bile acids

Fecal and biliary neutral sterols and bile acids reflect the underlying defect as expressed in hepatic cholesterol biosynthesis. Both 7-dehydrocholesterol and 8-dehydrocholesterol are excreted in the bile and appear in feces associated with little cholesterol (19). As a consequence of markedly reduced cholesterol biosynthesis, and depletion of hepatic cholesterol, bile acid synthesis is reduced and virtually no bile acids were detected in the feces of one child with the Type II phenotype (19). When this child was fed cholesterol for several weeks, both primary bile acids appeared in the bile and feces which indicated that deficient cholesterol substrate pool was responsible for absent bile acid synthesis (46). Natowicz and Evans (47) have reported unnatural bile acids excreted in the urine of some Smith-Lemli-Opitz homozygotes, but the identity of these urinary bile acids remains unknown.

Amniotic fluid sterols

Examination of amniotic fluid sterols from more than 20 normal pregnancies revealed only cholesterol with trace amounts of 7-dehydrocholesterol that are barely detected (**Table 3**). In contrast, amniotic fluid obtained at 16 weeks gestation from a pregnant woman carrying a Smith-Lemli-Opitz fetus contained at least 1000 times more 7-dehydrocholesterol (48). In addition, the cholesterol concentration in the amniotic fluid sample tended to be lower than controls, but the difference was not statistically significant. Most importantly, the assay of amniotic fluid sterols appears to be a more sensitive indicator of this inborn error than ultrasonography, especially in detecting the less severely affected Type I phenotypes. Thus, Smith-Lemli-Opitz homozygotes can now be detected prenatally by the demonstration of elevated amounts of 7-dehydrocholesterol in amniotic fluid after 16 weeks (23, 49, 50). Interestingly, it may be possible to establish the diagnosis earlier by examining the sterols produced by chorionic villi. (personal communication, Dr. H. Mandel, Haifa, Israel).

Cholesterol biosynthesis defect in liver

The demonstration of reduced plasma cholesterol with markedly elevated 7-dehydrocholesterol concentrations suggested that the last enzyme in the cholesterol biosynthesis pathway, 7-dehydrocholesterol-Δ^7-reductase, was inherited abnormally in the Smith-Lemli-Opitz syndrome (19). To test this hypothesis, we incubated the

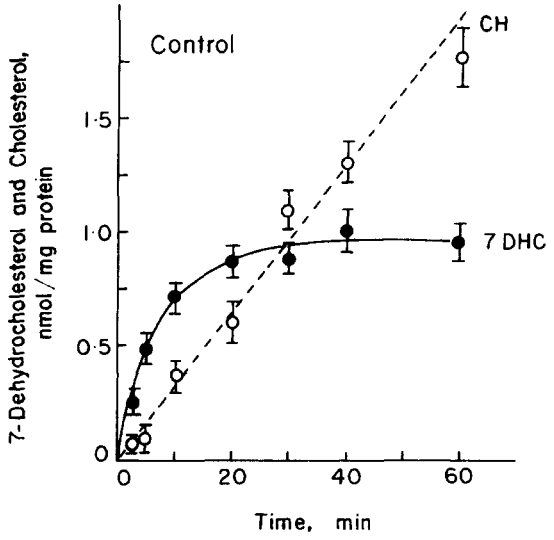

Fig. 5. Conversion of [3α-^{3}H]lathosterol to 7-dehydrocholesterol (7DHC) and cholesterol (CH) by control microsomes. Each point represents the mean ± SD for seven subjects. 7-Dehydrocholesterol was produced rapidly after the reaction was started, and its mass increased linearly over 5 min. Cholesterol was not formed abundantly until after 10 min and then was synthesized linearly from newly formed 7-dehydrocholesterol over the next 50 min. A precursor–product relationship was suggested between 7-dehydrocholesterol and cholesterol (from reference 20 with copyright permission from the Journal of Clinical Investigation and the American Society of Clinical Investigation)

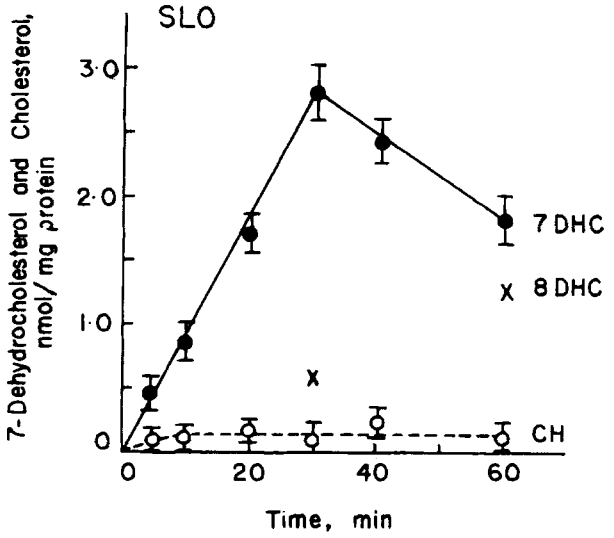

Fig. 6. Conversion of [3α-^{3}H]lathosterol to 7-dehydrocholesterol (7DHC) and cholesterol (CH) by Smith-Lemli-Opitz liver microsomes. Mean ± SD are presented for four subjects. 7-Dehydrocholesterol was produced abundantly over 30 min while virtually no cholesterol was formed. A small quantity of 8-dehydrocholesterol (8DHC) first appeared at 30 min with increased amounts at 60 min (from reference 20 with copyright permission from the Journal of Clinical Investigation and the American Society of Clinical Investigation).

radioactive precursors, [3α-^{3}H]lathosterol, and [1,2-^{3}H]7-dehydrocholesterol, separately with liver microsomes from four Smith-Lemli-Opitz homozygotes and seven control subjects (normal liver was obtained from the University of Minnesota Liver procurement program (LTPDS, NIH Contract No 1-DK-62274) when no suitable transplant recipient could be identified. When [3α-^{3}H]lathosterol was incubated with control hepatic microsomes and optimum co-factors including 100,000 *g* hepatocyte cytosolic fraction, labeled 7-dehydrocholesterol appeared almost immediately in the reaction mixture (**Fig. 5**), and was produced linearly over the first 5 min and then this precursor was converted into radioactive cholesterol which was formed efficiently (20). The mass of labeled cholesterol increased abundantly for the next 50 min and intersected the curve for the mass of 7-dehydrocholesterol at its maximum which is consistent with a precursor–product relationship. In comparison, when [3α-^{3}H]lathosterol was incubated with liver microsomes from four Smith-Lemli-Opitz homozygotes and optimum cofactors, radioactive 7-dehydrocholesterol was formed immediately and continued to be produced linearly for the next 30 min (**Fig. 6**). However, virtually no cholesterol was synthesized in the reaction mixture. Interestingly, after 30 min, the quantity of 7-dehydrocholesterol declined in the incubation mixture and a new sterol, 8-dehydrocholesterol, appeared and its concentrations increased several-fold during the next 30 min coincident with a further decrease in the mass of 7-dehydrocholesterol in the reaction mixture (20, 21).

In separate experiments, [1,2-^{3}H]7-dehydrocholesterol was incubated with microsomes from seven controls and four Smith-Lemli-Opitz subjects, and the rates of cholesterol formation were compared (**Fig. 7**). Cholesterol was produced efficiently and the mass increased linearly over the entire 90-min incubation in control microsomes while virtually no cholesterol was synthesized from this precursor by the Smith-Lemli-Opitz homozygotes' microsomes (20, 21).

The specific activities or reaction rates for both enzymes, lathosterol-5-dehydrogenase and 7-dehydrocholesterol-Δ^7-reductase, can be calculated from the slopes of the linear part of the product formation curves (**Table 4**). For lathosterol-5-dehydrogenase, which catalyzes the formation of 7-dehydrocholesterol, specific activities were similar in the control and Smith-Lemli-Opitz homozygotes' microsomes. This finding not only indicated normal function for this enzyme in the Smith-Lemli-Opitz livers, but also is evidence that the homozygotes' liver microsomes were as viable as controls. In contrast, the activity of 7-dehydrocholesterol-Δ^7-reductase was 9-fold higher in control microsomes than in microsomes from Smith-Lemli-Opitz homozygotes: 365 ± 4 compared to 40 ± 4 pmol/mg protein per min (20). Further, a substantial amount of 7-dehydrocholesterol that was not converted into cholesterol in the Smith-Lemli-Opitz homozygotes' microsomes was transformed

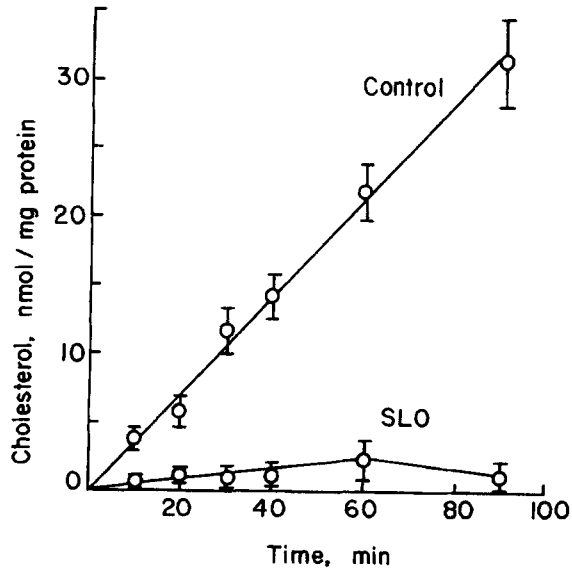

Fig. 7. Conversion of [1,2-^{3}H]7-dehydrocholesterol to cholesterol by control (n = 7) and Smith-Lemli-Opitz (n = 4) liver microsomes. Mean ± SD are plotted. Cholesterol was produced linearly over 90 min in the control incubations while little cholesterol was formed from 7-dehydrocholesterol by the Smith-Lemli-Opitz microsomes (from reference 20 with copyright permission from the Journal of Clinical Investigation and the American Society of Clinical Investigation).

TABLE 4. Liver Microsomal Enzyme Activities

Enzyme	Controls n=7	SLO n=4
	pmol/mg protein/min	
Lathosterol 5-dehydrogenase	120 ± 8	100 ± 7
7-Dehydrocholesterol-Δ^7-reductase	365 ± 4	40 ± 4[a]

[a] $P < 0.0001$ versus controls.

into 8-dehydrocholesterol that appeared in the reaction mixtures about 0.5 h after the reaction was started (Fig. 6). This fact indicated that sterol 7(8)-isomerase required about 30 min to activate and might only be important when the conversion of 7-dehydrocholesterol to cholesterol was blocked.

Thus, the accumulation of 7-dehydrocholesterol and reduced cholesterol levels in the plasma and tissues of Smith-Lemli-Opitz homozygotes can be attributed to the severe deficiency of microsomal 7-dehydrocholesterol-Δ^7-reductase activity (20, 21). Further, because of the enzyme deficiency, when 7-dehydrocholesterol accumulates in liver microsomes of homozygotes and cannot be transformed to cholesterol, a portion was converted to 8-dehydrocholesterol through the action of sterol 7(8)-isomerase. The 8-dehydrocholesterol that is formed is chemically more stable than 7-dehydrocholesterol, but is probably not a precursor of cholesterol.

Fibroblasts

To extend the investigation of the biochemical defect to other tissues, lathosterol 5-dehydrogenase and 7-dehydrocholesterol-Δ^7-reductase activities were measured in fibroblasts from control subjects and Smith-Lemli-Opitz homozygotes. When [1,2-^{3}H]7-dehydrocholesterol was incubated with fibroblasts from controls and Smith-Lemli-Opitz homozygotes that were grown to confluency in media supplemented with 10% fetal calf serum that contained cholesterol (4 mg/dl), 7-dehydrocholesterol-Δ^7-reductase activity was 3.6 times lower in homozygotes and 2.2 times less in obligate heterozygotes' fibroblasts than controls' cells. Stimulating cholesterol biosynthesis by exposing the confluent fibroblast cultures to lipoprotein-deficient serum with added lovastatin increased 7-dehydrocholesterol-Δ^7-reductase activity 2-fold in controls' fibroblasts, but did not raise the markedly inhibited enzyme activities in both homozygotes' and heterozygotes' fibroblasts. As a result, in the controls' fibroblasts where cholesterol biosynthesis was stimulated, 7-dehydrocholesterol-Δ^7-reductase activity was now 7.2 times and 4.4 times higher than in homozygotes' and heterozygotes' cells, respectively. This suggested that in homozygotes and heterozygotes, not only is 7-dehydrocholesterol-Δ^7-reductase activity reduced, but enzyme activity is expressed maximally and cannot be up-regulated further when the cells are exposed to cholesterol-deficient media as seen with controls' cells. In contrast, both HMG-CoA reductase and lathosterol 5-dehydrogenase, two enzymes that precede 7-dehydrocholesterol-Δ^7-reductase in the biosynthetic pathway, were equally active in the fibroblasts from controls, Smith-Lemli-Opitz homozygotes and heterozygotes. These large differences in 7-dehydrocholesterol-Δ^7-reductase activities among controls, Smith-Lemli-Opitz homozygotes and heterozygotes can be used to confirm the diagnosis in homozygotes especially with atypical clinical and biochemical presentations. More importantly, the intermediate levels of 7-dehydrocholesterol-Δ^7-reductase activity that are significantly lower than controls but higher than homozygotes establish the enzyme deficiency in heterozygotes who are carriers of a single defective gene for the Smith-Lemli-Opitz syndrome (S. Shefer and G. Salen, unpublished data).

In other studies, Honda et al. (27, 28) reported reduced formation of cholesterol from [^{3}H]lathosterol with the accumulation of the precursor, 7-dehydrocholesterol, in fibroblasts from 15 Smith-Lemli-Opitz homozygotes. In contrast, fibroblasts from 8 controls and 14 obligate heterozygotes with an intermediate deficiency of 7-dehydrocholesterol-Δ^7-reductase activity converted [^{3}H]lathosterol to cholesterol efficiently with only slightly more 7-dehydrocholesterol in the heterozygotes' than controls' cells (27, 28). Thus, despite low levels of 7-dehydrocholesterol-Δ^7-reductase, sufficient enzyme activity remains in heterozygotes' fibroblasts to convert the 7-dehydrocholesterol to cholesterol so that the precursor does not accumulate. We suggest, there-

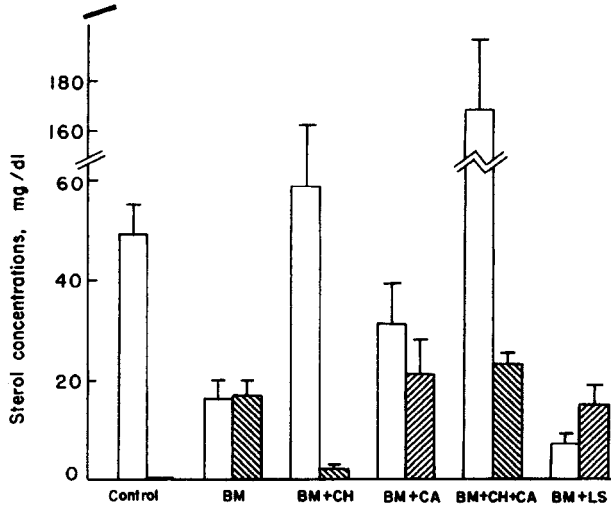

Fig. 8. Effect of different treatments on plasma cholesterol (□) and 7-dehydrocholesterol (striped column) concentrations (mean ± SD). Control; BM, BM 15.766 (30 mg/kg/day) fed for 2 wks; BM + CH, BM + 2% cholesterol, fed for 2 wks; BM + CA, BM + 1% cholic acid, fed for 2 wks; BM + 2% cholesterol + 1% cholic acid, fed for 2 wks; BM + LS, BM + lovastatin 0.04%, fed for 2 wks.

TABLE 5. Comparison of rat hepatic HMG-CoA reductase and lathosterol-5-dehydrogenase activities

	HMG-CoA reductase;t	Lathosterol-5-dehydrogenase
	pm/mg protein/min	
Control	53 ± 9	273 ± 35
	(n = 6)	(n = 7)
BM[a]	92 ± 34[b]	434 ± 96[b]
	(n = 9)	(n = 4)
BM + CH[a]	24 ± 9[c]	59 ± 21[c]
	(n = 5)	(n = 4)
BM + CA[a]	47 ± 16[d]	422 ± 99[b,e]
	(n = 5)	(n = 3)

The Bonferroni correction was used to estimate statistical significance when multiple comparisons were performed.

[a]BM 15.766 (30 mg/kg per day) was fed for 2 weeks; during the second week of treatment, 2% cholesterol (CH) or 1% cholic acid (CA) was fed.

[b]$P < 0.05$ as compared with the control value.

[c]$P < 0.001$ as compared with BM alone.

[d]$P < 0.01$ as compared with BM alone.

[e]$P < 0.001$ as compared with CH + BM.

fore, that combining the measurements of 7-dehydrocholesterol-Δ^7-reductase activity with the mass of 7-dehydrocholesterol in fibroblasts can distinguish homozygotes (low activity with high concentration of 7-dehydrocholesterol) from heterozygotes (intermediate low activity with only trace amounts of 7-dehydrocholesterol) from controls (normal enzyme activity with trace amounts of 7-dehydrocholesterol) in the cells.

Animal models

It is now about 30 years since Roux and colleagues (51–53) first demonstrated that blocking cholesterol biosynthesis with AY 9944, a compound discovered by Dvornik et al. (54–56) that inhibits the enzyme, 7-dehydrocholesterol-Δ^7-reductase, was teratogenic. When fed to pregnant rats, growth retardation and cell necrosis, particularly in the central nervous system, developed in their offspring. When either AY 9944 or BM 15.766, which are both 7-dehydrocholesterol-Δ^7-reductase inhibitors (57, 58), was given during the first 4 days of gestation, characteristic, holoprosencephalic malformations from either cyclopia to pituitary agenesis occurred around day 9 (58). During this period, maternal cholesterol levels declined more than 50%, and the precursor, 7-dehydrocholesterol, increased substantially. The rat fetuses exposed to the 7-dehydrocholesterol-Δ^7-reductase inhibitors were also born with facial clefting and limb deformities. Further, when the 7-dehydrocholesterol-Δ^7-reductase inhibitors were introduced on gestational day 6, a different pattern of malformations with abnormal distension of the hind brain was observed on gestational day 13. These embryologic defects could be prevented when a high cholesterol diet was fed to the pregnant rats treated with the 7-dehydrocholesterol-Δ^7-reductase inhibitors (53). Apparently, the absorbed dietary cholesterol expanded the cholesterol pool to substitute for newly synthesized cholesterol and to shut down abnormal (blocked by AY 9944 or BM 15.766) de novo cholesterol biosynthesis so that 7-dehydrocholesterol was not produced in either the mother or fetus. It should be noted that a different inhibitor of cholesterol biosynthesis (triparanol) when administered to pregnant rats also produced similar congenital malformations. In particular Roux et al. (58) reported that triparanol, which inhibits the reduction of the double bond at C-24,25 of desmosterol, also caused holoprosencephalic deformities in rat fetuses. Further, the inhibition of other steps in cholesterol biosynthesis i.e., HMG-CoA reductase by mevanolic acid, during pregnancy can also be dangerous to the fetus (59). We obtained additional information about the formation of cholesterol and the regulation of key enzymes in the pathway i.e., HMG-CoA reductase and lathosterol 5-dehydrogenase, when the inhibitor of 7-dehydrocholesterol-Δ^7-reductase, BM 15.766 was fed to rats for 2 weeks and the countereffects of dietary cholesterol (2%), cholic acid (1%), lovastatin (0.04%) alone and in combination were evaluated (60, 61). With inhibitor (BM 15.766) treatment (**Fig. 8**), plasma cholesterol concentrations declined 67% and the precursor 7-dehydrocholesterol rose from trace to 17 mg/dl which about equaled the mass of cholesterol in the plasma. In response to the drop in cholesterol, hepatic HMG-CoA reductase activity was stimulated 74% (**Table 5**). Not only did the changes in plasma cholesterol and 7-dehydrocholesterol concentrations mimic those in Smith-Lemli-Opitz homozygotes, but the rise in the rate-limiting enzyme of the cholesterol biosynthetic pathway indicated that 7-dehydrocholesterol is not a feedback regulator of HMG-CoA reductase. The up-regulation of HMG-CoA reductase occurred in response to the decline in plasma and tissue cholesterol despite the substantial accumulation of structurally similar precursor, 7-dehydrocholesterol. In contrast, when inhibitor (BM 15.766)-treated rats were fed cholesterol, plasma concentrations increased 3.7 times, 7-dehydrocholesterol levels decreased 88%, and elevated HMG-CoA reductase activity declined 74%. In comparison, feeding cholic acid increased plasma cholesterol 30% without a drop in 7-dehydrocholesterol concentrations. The combination of cholic acid (1%) plus cholesterol (2%) enhanced plasma cholesterol levels 9.5 times, also without decreasing 7-dehydrocholesterol concentrations. Feeding lovastatin, a competitive inhibitor of HMG-CoA reductase, with BM 15.766 depressed cholesterol in plasma further without reducing 7-dehydrocholesterol. These results demonstrated conclusively that cholesterol is essential

to correct abnormal cholesterol biosynthesis when 7-dehydrocholesterol-Δ^7-reductase is inhibited. The cholesterol is needed to expand the pool and to inhibit HMG-CoA reductase, the rate-controlling enzyme in cholesterol biosynthesis, so fewer precursors enter the blocked pathway to end as 7-dehydrocholesterol. Cholic acid and lovastatin were not effective individually and neither lowered plasma 7-dehydrocholesterol concentrations. However, the combination of cholic acid with cholesterol produced plasma hypercholesterolemia, which might offer some benefit initially to raise the depleted cholesterol pool especially in the Type II homozygotes.

To explain why only cholesterol reduced 7-dehydrocholesterol levels, measurements of lathosterol 5-dehydrogenase, which catalyzes the formation of 7-dehydrocholesterol from lathosterol, were correlated with HMG-CoA reductase in livers from rats fed BM 15.766 to reproduce the biochemical abnormalities as seen in the Smith-Lemli-Opitz syndrome (Table 5). Cholesterol and cholic acid alone and in combination were fed as countertreatments. As expected, cholesterol inhibited HMG-CoA reductase activity and early cholesterol biosynthesis and also profoundly suppressed lathosterol 5-dehydrogenase activity and directly decreased the formation of 7-dehydrocholesterol from lathosterol. In contrast, cholic acid did not inhibit lathosterol 5-dehydrogenase and, as a result, 7-dehydrocholesterol continued to form when cholic acid was fed with BM 15.766. Thus, treatment strategies must include cholesterol to inhibit both HMG-CoA reductase and lathosterol 5-dehydrogenase. Cholic acid fed together with cholesterol may facilitate absorption to raise plasma concentrations but in our opinion should not be used alone in long term therapy because of its lack of effect on the formation of 7-dehydrocholesterol.

Treatment

Although it is not possible to extrapolate with certainty the treatment of abnormal cholesterol biosynthesis in rats treated with BM 15.766 to Smith-Lemli-Opitz homozygotes, some information is available on the effects of cholesterol feeding on plasma sterol levels. In 12 homozygous subjects (7 females and 5 males), Irons et al. (46, 62) reported that plasma cholesterol rose more than 140% while the proportion of 7-dehydrocholesterol relative to cholesterol declined when cholesterol was added to the diet. The greatest rise in cholesterol occurred in those subjects who started with the lowest plasma cholesterol levels. Moreover, in one subject who initially showed no bile acid formation, cholic acid and chenodeoxycholic acid appeared in the feces after cholesterol was fed, which suggested that reduced bile acid synthesis reflected the deficiency of the cholesterol substrate (19, 46). However, long term treatment of cholesterol will be necessary to determine whether beneficial changes in plasma cholesterol can be related to improved neurologic function. It is not clear whether dietary cholesterol can reach the brain through the blood–brain barrier. Moreover, it may not be possible to correct developmental anomalies caused by a deficiency of cholesterol or accumulation of 7-dehydrocholesterol during crucial periods of gestation. With respect to dietary treatment trials, comparison of fed cholesterol treatment with placebo treatment will have to be made to unequivocally prove the benefit of the cholesterol. Further, the value of cholic acid that produces plasma hypercholesterolemia without decreasing 7-dehydrocholesterol when fed with cholesterol to rats needs to be established.

Gene defect

Neither the gene for 7-dehydrocholesterol-Δ^7-reductase nor chromosome location has been identified. However, there are two reports of the association of the Smith-Lemli-Opitz syndrome with translocations that involve the long arm of chromosome 7 (11, 63). Importantly, Alley et al. (64) have created a yeast artificial chromosome (YAC) which spans the translocation breakpoint at 7q32.1 in a biochemically confirmed Smith-Lemli-Opitz homozygote with the Type II phenotype.

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