Changes in serum sterols of rats treated with 7-dehydrocholesterol-Δ^7-reductase inhibitors: comparison to levels in humans with Smith-Lemli-Opitz syndrome

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Abstract The impaired conversion of 7-dehydrocholesterol to cholesterol, as a result of a permanent inhibition of the activity of 7-dehydrocholesterol-Δ^7-reductase, has been reported in the Smith-Lemli-Opitz (SLO) syndrome (1, 2). For the purpose of experimental teratology, an animal disease model consisting of the offspring of pregnant rats treated with AY 9944 or BM 15766, inhibitors of 7-dehydrocholesterol-Δ^7-reductase, was established. The present study compares the profiles of sterols in rat serum, obtained after transient treatment with inhibitors, with profiles of sterols obtained from patients with the permanent enzyme defect. AY 9944 (single dose of 50, 75, or 100 mg/kg) or BM 15766 (60, 75, or 90 mg/kg per day for 11 days) induces hypocholesterolemia and accumulation of 7-dehydrocholesterol and aberrant sterols in rat serum. The aberrant sterols in the treated rats are similar to those detected in human SLO patients by gas chromatography coupled to mass spectrometry (1, 3, 4) and were identified as 7- and 8-dehydrocholesterol, two trienols (I and II), and 19-nor-5,7,9(10)-cholestatrien-3β-ol. The time- and dose-dependences of the biochemical alterations are compared to the teratogenic abnormalities induced by inhibitors. The dietary cholesterol supplementation that suppresses embryo malformations induced by AY 9944 prevents severe hypocholesterolemia and decreases the aberrant sterol levels. As a function of time after intoxication, the 8-dehydrocholesterol to 7-dehydrocholesterol ratio increases, suggesting that 8-dehydrocholesterol is derived from the gradual conversion of the accumulated 7-dehydrocholesterol. The ratio of 8-dehydrocholesterol to 7-dehydrocholesterol is higher in human SLO than in the animal disease model. This may be explained by a permanent block in 7-dehydrocholesterol-Δ^7-reductase in SLO compared to a transient inhibition of this enzyme in the animal model.—**Wolf, C., F. Chevy, J. Pham, M. Kolf-Clauw, D. Citadelle, N. Mulliez, and C. Roux.** Changes in serum sterols of rats treated with 7-dehydrocholesterol-Δ^7-reductase inhibitors: comparison to levels in humans with Smith-Lemli-Opitz syndrome. *J. Lipid Res.* 1996. **37:** 1325–1333.

Supplementary key words cholesterol synthesis • animal disease model • aberrant sterols profiling • Smith-Lemli-Opitz syndrome

The Smith-Lemli-Opitz (SLO) syndrome is an autosomal recessive disorder detected on the basis of clinical criteria including failure to thrive, distinctive dysmorphic features, and severe mental retardation. The syndrome was originally described in 1964 (3) but only recently were homozygotes shown to have reduced cholesterol biosynthesis (1). The accumulation of the cholesterol precursor 7-dehydrocholesterol and of the related 8-dehydrocholesterol isomer in the serum, stools, and tissues of the patients (1, 4–6) has pointed to a deficiency in the enzyme 7-dehydrocholesterol-Δ^7-reductase thereby blocking the ultimate step in cholesterol synthesis. Reduced enzyme activity in liver tissue supported this assumption (7) and recently the single enzyme defect was established conclusively in cultured skin fibroblasts from SLO homozygotes (2). In addition, the degree of inhibition of the conversion of 7-lathosterol to cholesterol by deficient fibroblasts parallels the clinical severity.

The malformations observed in rats treated with one of two different inhibitors of 7-dehydrocholesterol-Δ^7-reductase are analogous to those observed in human SLO syndromes (8–10). AY 9944 and BM 15766 are chemically unrelated inhibitors but both induce similar defects in the offspring when fed to pregnant rats at the

Abbreviations: cholesterol, cholest-5-en-3β-1; 7-dehydrocholesterol (7-DHC), cholesta-5,7-dien-3β-ol; 8-dehydrocholesterol (8-DHC), cholesta-5,8-dien-3β-ol; nortrienol, 19-nor-5,7,9(10)- cholestatrien-3β-ol; epicoprostanol, 5β-cholestan-3α-ol; 7-lathosterol, cholest-7-en-3β-ol; 7-dehydrodesmosterol, cholesta-5,7,24-truen-3β-ol; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; GLC–MS, gas–liquid chromatography coupled to mass spectrometry; TMS, trimethylsilyl; RRT, relative retention time; SLO, Smith-Lemli-Opitz syndrome.

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TABLE 1. Chromatogram of trimethylsilylether derivatives of sterols in the serum of rats treated with the cholesterol synthesis inhibitor AY 9944

Peak	Relative Retention Time	Characteristic Ion Mass Used to Quanitate the Compiund
Epicoprostanol*a*	0.8	370
Cholesterol	1 (reference RT = 11'30")	329,368
8-Dehydrocholesterol*b*	1.06	325,351
Trienol I	1.1	364
Isodehydrocholesterol III	1.13	351
7-Dehydrocholesterol	1.17	325,351
Nortrienol*c*	1.36	350
Trienol II	1.6	364

Identical patterns are recorded in children homozygotes for SLO. The separation of the sterol trimethylsilylether is achieved by GC on a capillary polar column (Supelcowax 10) (30 m, ID 0.32 mm, film 0.25 μm). Derivatives are detected by MS in the electron impact mode with repetitive scanning of the positive ions in the mass range 320–375 (2 sec).

[a]5β-Cholestan-3α-ol added as internal standard.

[b]Cholesta-5,8-dien-3β-ol (4).

[c]19-Nor-5,7,9(10)-cholestatrien-3β-ol (5).

appropriate time (11). A characteristic malformation of the rat fetus is holoprosencephaly, a malformation of the anterior part of the brain, the frequency of which rises above 95% when the maternal cholesterol level is below 30 mg/dl during early pregnancy (12). Holoprosencephaly in rats is associated with pituitary agenesis when AY 9944 is given on gestation day 3. When AY 9944 is given after day 10, it induces sexual malformations in the male offspring comparable with those noted in male patients with the SLO syndrome.

BM 15766 intoxication in rats was found to reproduce the deficient synthesis of cholesterol and bile acids seen in homozygotes with SLO (13). The latter study, where BM 15766 inhibits the distal step of the pathway, gave expected overexpression of the proximal key enzyme of cholesterol synthesis, HMG-CoA reductase. It has also been suggested that the abnormal level of aberrant metabolites may be modulated with a cholesterol-rich diet.

In the present study, the detailed profiles of the sterols accumulating in the serum after acute intoxication with AY 9944 have been studied with the aim of challenging the animal disease model with the recent biochemical findings in human patients (1, 4, 5).

In addition, we raise the question as to whether the acute intoxication of rats can serve as a model for the permanent enzyme block observed in human SLO. The animal specificities, rat versus human, may be one source of difference between the sterol profiles. Alternatively, different adaptation as a function of time of cholesterol metabolism after either a transient block versus a permanent deficit may be another source of difference.

With the aim of better understanding the involvement in fetal teratogenicity of the cholesterol deficiency and of the accumulation of aberrant sterols, the effects of a dietary cholesterol supplementation on the serum cholesterol and on the aberrant sterols levels were examined. Cholesterol supplementation in the diet has a demonstrated suppressive activity on the teratogenicity induced by AY 9944 (9, 14). Finally, to compare the animal model with the human disease, the variation in sterols as a function of time in intoxicated rats and in children homozygous for SLO were examined.

MATERIALS AND METHODS

Chemicals

BM 15766 (4-(2-[1-(4-chlorocinnamyl)piperazin-4-yl]ethyl)-benzoic acid) and AY 9944 (*trans*-1,4-bis(2-chlorobenzylaminomethyl)cyclohexane dihydrochloride) were generous gifts from Boehringer Mannheim Gmbh (Mannheim, Germany) and Ayerst Laboratories (New York, NY). Cholesterol, 7-dehydrocholesterol, epicoprostanol, 7-lathosterol, dihydrolanosterol, and lanosterol were obtained from Steraloids Inc. (Wilton, NH) and Sigma (St. Louis, MO).

Treatment of animals

Wistar rats weighing 200–250 g (Charles River Co., Cléon, France) were fed on a 103 diet (Union de l'Alimentation rationnelle, Villemoisson, France) containing 3100 kcal/kg with 4% lipid (75% from vegetable origin). Females were mated with males of the same strain. The day spermatozoa were present in the vagina was designated as gestation day 0. Rats were housed under a 12-h light/dark cycle and blood samplings were made after 4 h fasting. AY 9944 was administered by

gavage as an aqueous solution (15 mg/ml) at a single dose (50, 75, 100 mg/kg). BM 15766 was given from gestation day 1 to 11 as a suspension in methylcellulose (60, 75, 90 mg/kg per day). When indicated, cholesterol supplementation (500 mg/kg per day) was administered in olive oil.

Chemical analysis

Lipids were extracted from serum with chloroform–methanol 2/1 (vol/vol) in the presence of butylated hydroxytoluene as antioxidant. Saponification of sterol esters was carried out in 0.5 N methanolic KOH

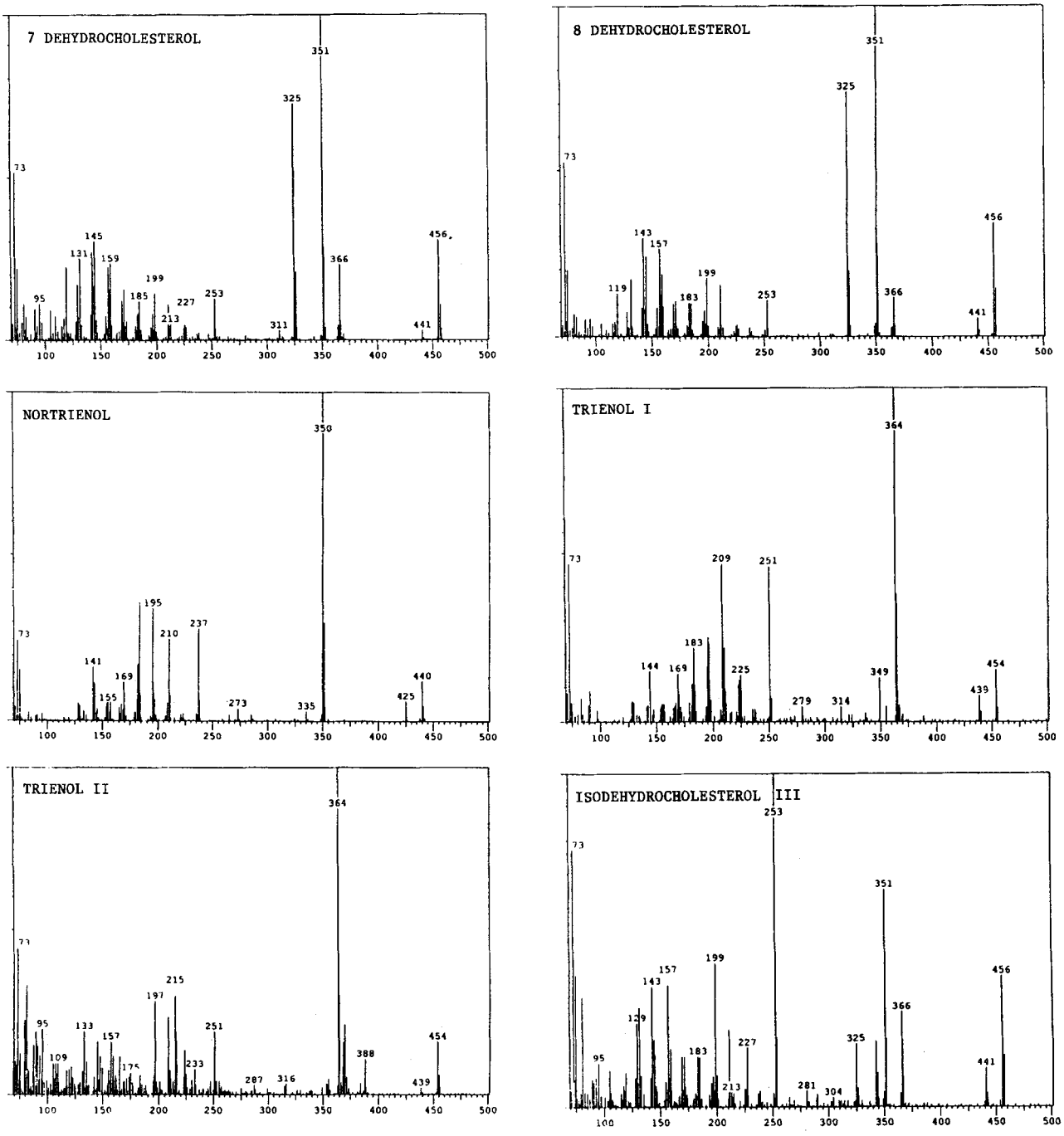

Fig. 1. Mass spectra of the aberrant sterols: 7-dehydrocholesterol, 8-dehydrocholesterol, isodehydrocholesterol III, trienol I and II, and nortrienol (19-nor-5,7,9(10)-cholestatrien-3β-ol). Mass spectra are produced after ionization in the electron impact mode (70 eV; source temperature 220°C) and the positive ions are scanned in the mass range 70–500 amu (2 sec).

TABLE 2. Comparison of the concentration (mg/dl) of cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol in rats treated with the cholesterol synthesis inhibitor AY 9944 and in humans with the Smith-Lemli-Opitz syndrome

Treatment	Cholesterol		7-Dehydrocholesterol (GC-MS)[c]	8-Dehydrocholesterol (GC-MS)[c]
	(Enzyme)[a]	(GC-MS)[b]		
	mg/dl			
No treatment	51	41	<0.05	<0.05
Rats: AY 9944, 75 mg/kg (day 3)	33	21	10.7	0.5
Rats: AY 9944, 75 mg/kg (day 5)	26	13	14	1.4
Rats: AY 9944, 75 mg/kg (day 9)	15	8	9	1.9
Rats: AY 9944, 75 mg/kg (day 18)	51	31	4	0.8
Rats: AY 9944, 75 mg/kg + DCS[d] (day 3)	48	42	3.6	0.2
Rats: AY 9944, 75 mg/kg + DCS (day 5)	60	37	4.3	0.4
Rats: AY 9944, 75 mg/kg + DCS (day 9)	49	28	4.4	0.4
Rats: AY 9944, 75 mg/kg + DCS (day 18)	58	34	0.1	<0.05
Children with SLO before DCS (n = 18)	54	30	11	11

[a]Cholesterol was assayed by the cholesterol-oxidase routine test.
[b]Cholesterol was assayed by gas chromatography–mass spectrometry.
[c]7-Dehydrocholesterol and 8-dehydrocholesterol were assayed by GC–MS after calibration for ion-fragment at *m/z* 325 with a weight standard of 7-dehydrocholesterol (the response factor of 8-dehydrocholesterol is assumed to be identical with 7-dehydrocholesterol)
[d]Rats received daily by gavage a dietary cholesterol supplementation (DCS) of 500 mg/kg per day.

(15 min at 56°C) and the released fatty acids were converted to methyl esters with BF_3 methanol 20% (Merck, Darmstadt, Germany) (15 min at 56°C). The methyl ester fatty acids do not interfere with the elution of sterols under the following conditions for gas chromatography. Sterols extracted in hexane were derivatized to trimethylsilylether with BSTFA-TMCS 10% (Chrompack, Middleburg, Netherlands) (15 min at 56°C).

Sterol trimethylsilyl ethers were chromatographed on a bonded polar polyethylene glycol capillary column (internal diameter 0.3 mm; length 30 m; film thickness 0.25 μm) (Supelcowax 10, Bellefonte, PA). Two microliters of TMS sterol ethers in *n*-heptan (100 μl) was injected in the splitless mode (Hewlett-Packard Automatic Sampler 6890 thermostated at 6°C). The split flow was opened after 1.5 min transfer and the oven temperature was raised to 220°C. Inlet temperature was 250°C; the carrier gas (helium) pressured at 55 kPa gave the linear velocity of 31 cm/sec at the initial temperature of 60°C. TMS sterol ethers were eluted along a linear temperature ramp between 220°C and 280°C at 3°C/min.

Sterol derivatives were detected by coupled gas chromatography–mass spectrometry (GC–MS) running in the electron impact mode (70 eV) with detection of the positive ion (Nermag R10-10 C, Rueil, France). Identification of sterols was obtained by comparison of their mass spectra to NIST library or to the published spectra (4, 5, 14) or to commercially available standards. Sterols were quantified after normalization with an internal standard (epicoprostanol) and calibration was done with weight standards of cholesterol and 7-dehydrocholesterol. The 7-dehydrocholesterol standard, after treatment identical to serum (saponification and derivatization), did not contain the 8-dehydrocholesterol isomer but GC–MS revealed contaminating traces of trienols (I and II) and of a nortrienol. A recent publication (5) established that the nortrienol (19-nor-5, 7, 9(10)-cholestatrien-3β-ol) is not an artefact formed during the preparation of 7-dehydrocholesterol but a contaminant representing 4.5% of the commercial standard. There is a possibility that under the present experimental conditions the conversion by photolysis or by thermal isomerization of 7-dehydrocholesterol to previtamin D and to vitamin D has been eliminated (ion-fragments at *m/z* 136 and 118 are not traceable in the chromatograms) (15).

RESULTS

The chromatograms reveal quantitative differences in the sterol profiles between humans with SLO and rats receiving a distal inhibitor of cholesterol synthesis, but no qualitative difference is noted. The similarity in the profiles suggests that the inhibitor target is the enzyme deficient in the SLO syndrome, 7-dehydrocholesterol-Δ^7-reductase. The chromatogram and fragmentograms of the aberrant sterols are presented in **Table 1** and **Fig. 1.** The polar column used, compared favourably with an apolar column of 95% dimethylsiloxane–5% diphenyl polysiloxane for the resolution of trienols and nor-

trienol. Table 1 displays the *m/z* values of the fragment-ions used to monitor the sterols by GC–MS when the mass range (320–375 amu) is scanned for routine detection. Two different fragment-ions at *m/z* 325 and 351 are used for identification of dehydrocholesterols. The rationale in selecting these abundant and specific ions is found in fragmentograms of Fig. 1. Cholestanol, 7-lathosterol, and plant sterols (campesterol and sitosterol) are also detectable in trace amounts.

The fragmentograms of 7-dehydrocholesterol and 8-dehydrocholesterol are indistinguishable as noted previously (4, 14). In the electron impact mode, the cleavages releasing the abundant ions at *m/z* 351 and 325 are M^+–90 (silanol)–15 (methyl) and M^+–90–15–26 (C_2H_2 from the B ring). The ion at *m/z* 325 indicates that both double-bonds are in the same ring (B) of dehydrocholesterol (4). The structure of 8-dehydrocholesterol was previously assigned to cholesta-5,8-dien-3β-ol on the basis of UV absorbance and NMR (4). 7- And 8-dehydrocholesterols were found previously to be moderately increased in amount when the cholesterol synthesis rate was high (16). The dehydrocholesterol isomer quoted as isodehydrocholesterol III elutes before 7-dehydrocholesterol on profiles of serum from SLO patients or from treated rats. This peak is also detected in the commercial standard of 7-dehydrocholesterol. It was also detected on the chromatogram on a polar CP-Sil 57 capillary column (1). The double-bond position of isodehydrocholesterol III is not established but the low abundance of the fragment at *m/z* 325, which is expected after a neutral loss of C_2H_2 from vicinal double bonds (4) (Fig. 1), favors a heteroannular diene structure. The mass spectrum shows abundant ion-fragments at *m/z* 253 (M^+–90–113 (saturated iso-octyl lateral chain)) and 199 (M^+–144 (ring A)–113). As isodehydrocholesterol III increases as compared to 7-dehydrocholesterol (data not shown) when the temperature and time for the derivation of sterols increase from 40°C (10 min) to 60°C (15 min), an artefactual migration of the double-bonds in the ring structure of 7-dehydrocholesterol is suggested. This migration is comparable to that observed under acidic conditions (17). The stability of 7-dehydrocholesterol toward heat and alkali has been established (5) but the activity of the silylating reagents could be envisaged.

Table 1 shows the presence of two trienols (mol wt = 454). The presence of a sterol with three double-bonds was also noted in the fecal sterols of patients with SLO syndrome (18). The peak was assigned to 7-dehydrodesmosterol (cholesta-5,7,24-trien-3β-ol) in agreement with a block in Δ^7 reductase acting on both of the final branched pathways of cholesterol synthesis. On a non-polar column, the 7-dehydrodesmosterol peak eluting just before cholesterol has also been noted (19). The fragmentogram of trienol I shows the cleavage of a saturated iso-octyl side-chain of mass 113 which releases an ion-fragment at *m/z* 251 = M^+–90–113 (Fig. 1). The compound does not have the unsaturated side-chain expected for cholesta-5,7,24-trien-3β-ol (7-dehydrodesmosterol) meaning that all three double-bonds are located in the ring structure. Trienol I is absent in control serum but trienol II is present in trace amounts in pathological sera and in a commercially available standard of 7-dehydrocholesterol. The mass spectrum of trienol II suggests also a saturated side-chain as judged from the fragment *m/z* 251 = M^+–90–113.

The presence of a nortrienol is a reliable marker of SLO as demonstrated recently (5). The occurrence of 19-nor-5,7,9(10)-cholestatrien-3β-ol is also a constant finding in rats receiving AY 9944 or BM 15766. The peak

TABLE 3. Cholesterol and aberrant sterols in the serum of eight children homozygotes for Smith-Lemli-Opitz

Subjects	Cholesterol (Enzyme)[a]	Cholesterol (GC–MS)[b]	7-Dehydrocholesterol (GC–MS)[b]	8-Dehydrocholesterol (GC–MS)b	Trienols[c]	Nortrienol[c]
	mg/dl		mg/dl		%	%
M.D.	nd	17	10	7	9.7	1.9
E.L.	76	62	15	12	2.0	1.7
C.G.	91	59	9	7	4.7	1.4
P.P.	55	26	15	12	7.4	3.8
L.M.	72	37	10	14	0.6	3.8
A.C.	21	8	8	10	1.7	2.7
V.F.	43	19	14	16	2.8	2.6
M.L.	22	10	7	9	2.9	4.3
Mean	54	30	11	11	4	2.8

[a]Cholesterol was assayed by a routine cholesterol-oxidase method; nd, not determined.
[b]Cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol were quantitated after calibration with weight standards for the fragment-ion at *m/z* 329 for cholesterol and *m/z* 325 for dehydrocholesterols. 7- And 8-dehydrocholesterol are assumed to have an identical response coefficient.
[c]Values are given as % areas under 364 and 350 amu for trienols and nortrienol, respectively, relative to the total ionization current.

is well resolved on the polar stationary phase of GLC and its mass spectrum is identical to that published previously (5). The molecular ion at *m/z* 440 of the trimethylsilylether derivative in the electron impact mode is confirmed in the chemical ionization mode with ammonia as the reagent gas by the quasi-molecular adduct at *m/z* 458 (=440 + NH_4^+).

As the enzyme block obtained by inhibitors AY 9944 or BM 15766 is transient, the serum sterol profiling in intoxicated rats could follow the interconversion of the sterols. In rats receiving a single dose of AY 9944, the pattern varies as a function of the dose (50, 75, or 100 mg/kg) and time. **Table 2** focuses on the levels of 7- and 8-dehydrocholesterol observed after intoxication with 75 mg/kg. The cholesterolemia decreases to a minimum, reached after 9 days (-80% of the original value). At day 9 the summed peak areas for the abnormal sterols (peaks under 325 amu (7- and 8-dehydrocholesterol) + peaks under 364 amu (trienols) + peak under 350 amu (nortrienol)) represent 48% of the area under 329 amu (cholesterol). Aberrant sterols are detectable 18 days after the initial treatment while cholesterol returned to 75% of its initial level. The cholesterol in treated rats is significantly lower when determined by GC–MS than when determined by the enzyme assay (Table 2). We assume that this results from the lack of specificity of the cholesterol-oxidase used in the routine test. In a preliminary study, unesterified 7-dehydrocholesterol added to serum responds in the enzyme assay with a relative sensitivity of 31% compared to unesterified cholesterol added to the serum (not shown).

The influence of the dose modifies the delay and the severity of the hypocholesterolemia (11). At a low dose of AY 9944 (50 mg/kg), the hypocholesterolemia peak is observed only after 3 days (-15%) whereas at high dose (100 mg/kg), AY 9944 induces delayed and severe hypochocholesterolemia that is only partially reversed after 18 days. Assay of AY 9944 by GLC has shown a sustained concentration in adrenal and liver tissues suggesting accumulation of the drug in tissues from which a gradual release induces a lasting hypocholesterolemic activity (C. Roux and E. Rey, personal communication).

BM 15766, a drug with a teratogenic potency comparable to AY 9944 (10), also produces hypocholesterolemia (13). When given for 11 days the cholesterol level decreases to a plateau at -71% to -75% for 60, 75, or 90 mg/kg per day. Hypocholesterolemia is associated with the appearance of aberrant sterols such as those described after AY 9944 intoxication. The low dose-dependence is noted for BM 15766. At 30 mg/kg per day for 14 days it induces a comparable hypocholesterolemia (-66%) in experiments described previously (13). Yet Sprague-Dawley rats have been shown

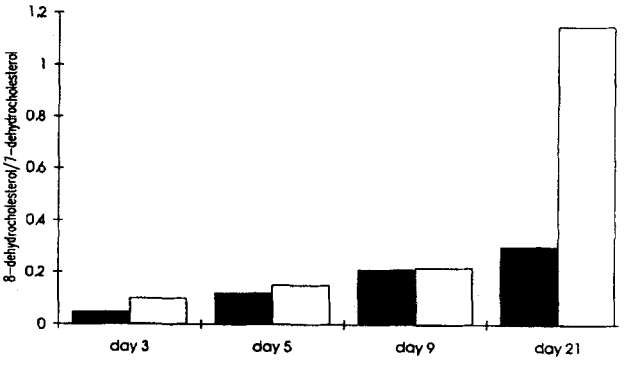

Fig. 2. Variation in the ratio of 8-dehydrocholesterol to 7-dehydrocholesterol in rat serum as a function of time after treatment with AY 9944 or BM 15766. The ratio is estimated from the peak areas under the ion current at *m/z* 325 at RRT 1.06 (8-dehydrocholesterol) and RRT 1.17 (7-dehydrocholesterol). Treatment consisted of a single dose of AY 9944 (75 mg/kg) on day 1 (solid bar) or daily administration of BM 15766 (75 mg/kg per day) for 11 days (open bar).

to be considerably more resistant to teratogenicity induced by AY 9944 and by triparanol than Wistar rats (20).

In rats receiving either BM 15766 or AY 9944, the ratio of 8-dehydrocholesterol to 7-dehydrocholesterol increases as a function of the time after the intoxication (**Fig. 2**). After treatment with BM 15766 has been discontinued (after day 11), the ratio increases sharply to a value over 1 comparable with the values reported for humans (**Table 3**) with the permanent Δ^7 reductase block of the SLO syndrome. The increment of this ratio suggests that the accumulated 7-dehydrocholesterol is slowly converted to 8-dehydrocholesterol as a function of time elapsed after intoxication.

As cholesterol supplementation after severe AY 9944 intoxication (75 mg/kg) (7) has been shown to prevent pituitary agenesis (the frequency decreases by -92%) and to reduce embryolethality in the offspring of pregnant rats, the effect of a cholesterol-rich diet on the metabolic alterations induced by AY 9944 was tested. Cholesterol dietary supplementation (500 mg/kg per day) constrains hypocholesterolemia to -32% compared to -80% in the absence of supplementation (Table 2). The ratio of aberrant sterols to cholesterol remains at a value much lower than that recorded without cholesterol supplementation, meaning that the dietary supplementation acts not only to restore cholesterolemia but also to retroinhibit the neosynthesis of aberrant sterols. An inhibition of 7-dehydrocholesterol synthesis (-55%) by dietary cholesterol via the negative feedback exerted on HMG-CoA reductase was documented previously for BM 15766-intoxicated rats supplemented with 2% cholesterol added to the diet (13).

The analytical thin-layer chromatography or reverse phase HPLC studies (20) showed the presence of a

dehydrocholesterol in AY 9944-treated rats. The present study using GC–MS describes the qualitative similarity in the serum profiles for aberrant sterols of rats intoxicated with the distal cholesterol synthesis inhibitors and of humans homozygous for the deficiency in 7-dehydrocholesterol-Δ^7-reductase. Quantitative weight data can be supplied for cholesterol and 7-dehydrocholesterol. 8-Dehydrocholesterol has been tentatively quantitated assuming the same response factor as 7-dehydrocholesterol (Table 3). Trienols I and II and nortrienol cannot be quantified in the absence of commercially available standards but the percentages relative to the total ionization current give an estimation of the aberrant sterols in SLO homozygotes. These aberrant sterols have not been observed in the absence of 7-dehydrocholesterol.

The comparison of the animal model with children homozygous for the SLO syndrome displays the influence of animal specificity and phenotypic adaptation to the permanent enzyme defect and to a cholesterol-rich diet received after birth. Table 3 summarizes the data for a series of eight homozygotes with SLO. All patients displayed a marked hypocholesterolemia that was detected by the routine cholesterol oxidase assay. The levels of hypocholesterolemia are lower when estimated by GC–MS. The difference between values obtained by GC–MS and by the enzyme assay lies in the lack of specificity of cholesterol oxidase which also converts aberrant 3β-OH sterols. The 8-dehydrocholesterol level is higher in the human SLO syndrome as compared to treated animals. In four patients, it is even higher than the 7-dehydrocholesterol level, the chemical marker of the SLO condition.

DISCUSSION

The observation that SLO syndrome is associated with a defect in 7-dehydrocholesterol-Δ^7-reductase (1, 2), has provided the basis for a prenatal diagnosis of this fetal condition (19). The possibility is envisioned of prenatal or postnatal therapy for SLO homozygotes by supplying a cholestesterol-rich diet. Such therapy may improve the myelination process in particular.

The animal disease model developed for the purpose of experimental teratology caused by three different "distal" inhibitors of cholesterol synthesis should provide useful information for understanding the clinical situation. These compounds include two inhibitors of dehydrocholesterol-Δ^7-reductase (AY 9944 and BM 15766) and one inhibitor of desmosterol-Δ^{24}-reductase (triparanol) (20). These inhibitors have unrelated chemical structures, different physical properties (AY 9944 is water-soluble) and two different target enzymes, but share the same teratogenic potency. The latter characteristics argue against a specific role of the drug but support a common mechanism of action leading to a severe hypocholesterolemia in the fetus at a critical time of the gestation.

Studies into experimental teratology have delineated the effects on offspring of the administration of these distal inhibitors as a function of dose and timing during gestation. Maternal plasma cholesterol level correlated with the teratogenic effect (12) but is an indirect estimation of the metabolic deficiency taking place in the fetal tissues. A threshold value (30 mg/dl) at which the defect frequency rises abruptly in the offspring is observed. The fetal defects induced by "distal" inhibitors can be suppressed by dietary cholesterol (9, 14). Pituitary agenesis, a defect observed in rat holoprosencephaly after early AY 9944 administration, is suppressed when cholesterol is given simultaneously with the drug. Table 2 shows that with supplementation, hypocholesterolemia in the intoxicated pregnant rat remains above the teratogenic threshold value of 30 mg/dl (14).

The early administration of AY 9944 produces a high frequency of anterior brain abnormalities but when the drug is administered later (after day 10) it gives a different spectrum of teratogenicity altering the sexual characters in the male offspring (21). In human SLO the permanency of the Δ^7-reductase block results in both types of neurological and genital abnormalities.

"Proximal" inhibitors of cholesterol synthesis ("statins", competitive inhibitors of HMG-CoA reductase) do not share the same teratogenic activity yet they are able to induce hypocholesterolemia as do "distal" inhibitors. Their teratogenic potency is limited to skeletal abnormalities (malformations of the vertebrae and ribs) and is reversed by supplementation with mevalonic acid but not with cholesterol (22, 23). Accordingly, the deficiency of polyprenoids and dolichol derived from an intermediary step of cholesterol synthesis has been assumed to cause the defects induced by statins.

The different teratogenic potencies of "proximal" and "distal" inhibitors could also be explained not only by the cholesterol deficiency but also by the toxicity of the aberrant sterols that accumulate in the rat fetus. Direct toxicity or reinforced competitive inhibition of cholesterol by aberrant sterols on the developmental genes of the anterior part of the brain could be a possible consequence in embryos exposed to AY. Table 2 shows that the aberrant sterols are decreased by a high cholesterol diet as a consequence of negative feedback on HMG-CoA reductase. Consequently it becomes difficult to decipher whether moderate hypocholesterolemia or the low level of aberrant sterols is the reason for the suppression of teratogenicity by the high cholesterol diet.

Comparison of the biochemical data obtained from the animal model and from children with SLO syn-

drome reflects a moderate difference in species susceptibility. Significant genetic differences within the same animal species have already been reported, where Sprague-Dawley rats are much more resistant to teratogenicity induced by AY 9944 or by triparanol than Wistar rats (20). Unpublished data also show greater resistance of mice compared to rats (C. Roux, personal communication). We now suggest that the intoxicated Wistar rat offers a sensitive model of the human disease. The difference is mostly the high level of 8-dehydrocholesterol in human homozygotes for SLO as compared with intoxicated rats. However, we established that the difference is related to the difference between the permanent enzyme block in human patients and transient intoxication in rats. As a function of time, 8-dehydrocholesterol increases with a delay relative to 7-dehydrocholesterol after the administration of AY 9944. The gradual conversion of 7-dehydrocholesterol to 8-dehydrocholesterol is presumed to occur in the liver where the ratio of 8-dehydrocholesterol to 7-dehydrocholesterol is high (6). The ratio could also increase as a result of slow clearance of the 8-isomer compared with the 7-isomer which is converted to previtamin D (16) and to abnormal bile acids (24). In humans, measurement of the 8-dehydrocholesterol level could serve to follow the long-term effect of a diet. In one instance we followed the increase of the ratio of 8-dehydrocholesterol to 7-dehydrocholesterol under the influence of a 5-month high cholesterol diet (to be published).

Finally, we conclude that rats intoxicated with a distal inhibitor of the cholesterol synthesis pathway may be used as a reliable model for the Smith-Lemli-Opitz syndrome that results from a block of 7-dehydrocholesterol-Δ^7-reductase in humans. The respective role of cholesterol deficiency versus toxicity by one of the aberrant sterols in embryos remains to be established in an experimental plan where biochemical parameters are varied independently.

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