Embryonic Lethality and Defective Neural Tube Closure in Mice Lacking Squalene Synthase*

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Squalene synthase (SS) catalyzes the reductive head-to-head condensation of two molecules of farnesyl diphosphate to form squalene, the first specific intermediate in the cholesterol biosynthetic pathway. We used gene targeting to knock out the mouse SS gene. The mice heterozygous for the mutation (SS+/−) were apparently normal. SS+/− mice showed 60% reduction in the hepatic mRNA levels of SS compared with SS+/+ mice. Consistently, the SS enzymatic activities were reduced by 50% in the liver and testis. Nevertheless, the hepatic cholesterol synthesis was not different between SS+/− and SS+/+ mice, and plasma lipoprotein profiles were not different irrespective of the presence of the low density lipoprotein receptor, indicating that SS is not a rate-limiting enzyme in the cholesterol biosynthetic pathway. The mice homozygous for the disrupted SS gene (SS−/−) were embryonic lethal around midgestation. E9.5–10.5 SS−/− embryos exhibited severe growth retardation and defective neural tube closure. The lethal phenotype was not rescued by supplementing the dams either with dietary squalene or cholesterol. We speculate that cholesterol is required for the development, particularly of the nervous system, and that the chorioallantoic circulatory system is not mature enough to supply the rapidly growing embryos with maternal cholesterol at this developmental stage.

Squalene synthase (SS)[1] (farnesyl-diphosphate:farnesyl-diphosphate farnesyltransferase, EC 2.5.1.21) catalyzes the reductive head-to-head condensation of two molecules of farnesyl diphosphate to form squalene, the first specific intermediate in the cholesterol biosynthetic pathway (1). This enzyme is an attractive target for cholesterol-lowering therapy, because the inhibition of this step theoretically may not perturb the nonsterol pathway, which is a potential problem in the use of the inhibitors of cholesterol synthesis (2). Indeed, several potent SS inhibitors, such as squalestatins or zaragozic acids (3), 1,1-bisphosphonates (4), and quinuclidine derivatives (5), successfully lower plasma cholesterol levels without adverse effects *in vivo*.

Accumulating evidence supports the notion that cholesterol metabolism plays an essential role in development, particularly of a like nervous system (see Ref. 6 for review). All of the naturally occurring inborn errors in cholesterol metabolism, such as Smith-Lemli-Opitz (SLO) syndrome (7, 8), desmosterolosis (9), and mevalonate kinase deficiency (10), are associated with severe developmental abnormalities. Moreover, mice lacking apoB (11, 12), microsomal triglyceride transfer protein (13), and megalin/gp330 (14) had anomalies in the nervous system. Recently, the Hedgehog family of proteins, which are crucial for the pattern formation in the vertebrate embryogenesis (see Ref. 15 for review), was shown to undergo post-translational modification by covalent attachment of a cholesterol molecule to the biologically active amino-terminal fragment of these peptides (16). Furthermore, Patched, a putative cognate receptor for the Hedgehog protein, shares homology with cholesterol-binding proteins (17, 18). Together, it is tempting to hypothesize that the derangement of cholesterol synthesis affects the development of the nervous system through distorting Hedgehog signaling.

To test the hypothesis that cholesterol itself is essential for normal embryogenesis and whether the reduction of SS activities is associated with cholesterol-lowering effects, we have generated SS knockout mice. The SS−/− mice were lethal between E9.5 and E12.5 and exhibited severe retardation of development. The SS+/− mice expressed only 50% SS activities in the liver and testes compared with SS+/+ mice, whereas their plasma lipoprotein profiles and responses to the dietary challenge were unaffected.

EXPERIMENTAL PROCEDURES

Generation of SS Knockout Mice—A fragment (528 base pairs) of mouse SS cDNA was amplified by using primers that were designed based on reported sequences of mouse SS cDNA (sense primer, 5′-GTCGCAAGGATGGGAGTTCGT-3′, and antisense primer, 5′-GTG-GCAGTACTTGTCCCAGT-3′) (19). This polymerase chain reaction product was used as a probe to clone a genomic DNA from the 129/Sv mouse genomic library as described previously (20). A replacement-type targeting vector was constructed; the short arm containing a 1.2-kb *Pst*I/*Bgl*II fragment in intron 3 and the long arm containing a 8.5-kb *Eco*RV fragment spanning exons 6–7 were inserted into the *Xho*I and *Not*I sites, respectively, of the vector pPolIIshort-neobpA-HSVTK as described previously (21). Thus, exons 4–5, which contained a putative catalytic site (22), were replaced by a neomycin-resistant cassette.

After linearization by digestion with *Sal*I, the vector was electroporated into JH1 embryonic stem cells. Targeted clones, which had been selected in the presence of G418 and 1-(2-deoxy, 2-fluoro-β-D-arabinofuranosyl)-5-iodouracil, were identified by polymerase chain reaction

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[1] The abbreviations used are: SS, squalene synthase; SLO, Smith-Lemli-Opitz; apo, apolipoprotein; kb, kilobase(s); LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HPLC, high performance liquid chromatography; TC, total cholesterol; TG, triglycerides; LDLR, low density lipoprotein receptor; E, embryonic day.
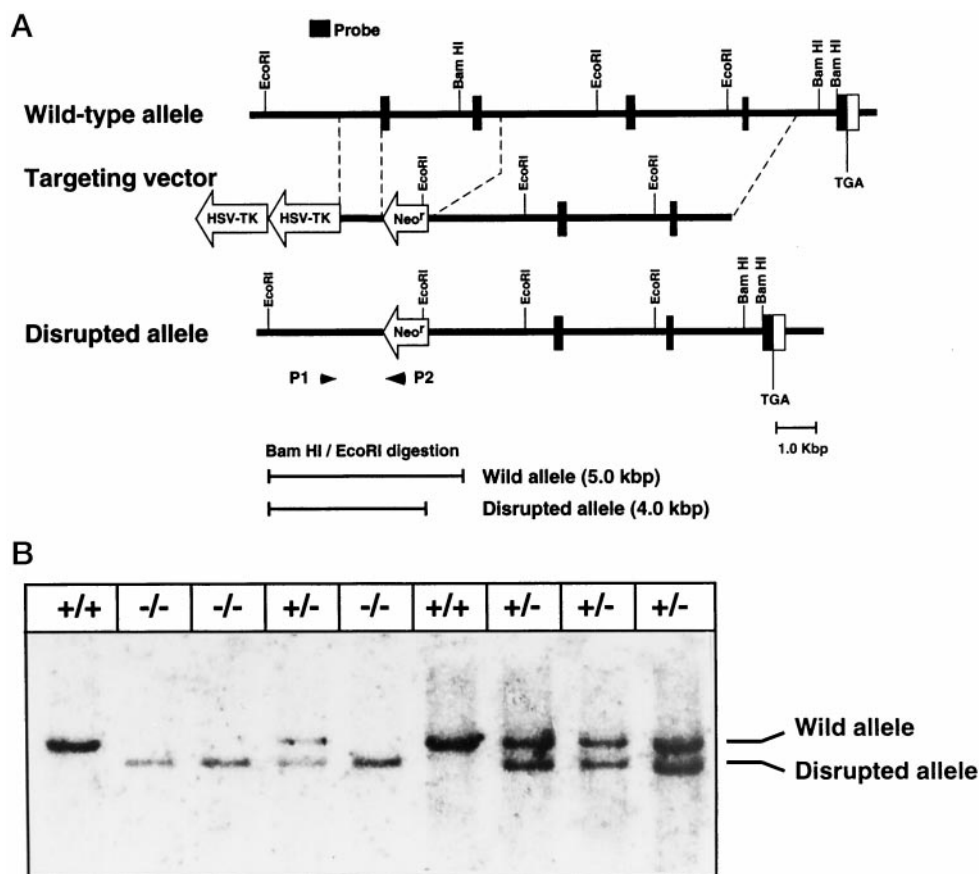
FIG. 1. **Targeted disruption of the SS gene.** *A,* a map of the SS locus, together with the sequence replacement gene-targeting vector and the targeted SS allele. Exons (*closed boxes*) and introns (*thick lines*) are indicated. The gene targeting event replaced exon 4–5 coding sequences and intron 4 with a cassette containing a neomycin resistance gene (*Neo*[r]) driven by the RNA polymerase II promoter. Two copies of herpes simplex virus thymidine kinase (*HSV-TK*) expression cassettes were used as a negative selection marker. Analysis of gene-targeting events and mouse genotyping was performed by Southern blot analysis of *Bam*HI/*Eco*RI-digested genomic DNA using a 0.5-kb probe (*closed bar*) in intron 3. *P1,* primer 1; *P2,* primer 2; *TGA,* stop codon; *Kbp,* kilobase pairs. *B,* Southern blot of *Bam*HI/*Eco*RI fragment in the targeted allele is 4.0 kb *versus* 5.0 kb in the wild-type allele. DNA was isolated from whole E10.5 embryos.

using the following primers: primer 1, 5′-ATACAGGGGAGTGTGC-CTTTCTTGTG-3′ and primer 2, 5′-GATTGGGAAGACAATAGCAG-GCATGC-3′ (Fig. 1). Homologous recombination was verified by Southern blot analysis after *Bam*HI/*Eco*RI double digestion using a 0.5-kilobase pair *Pst*I/*Xho*I fragment as a probe (Fig. 1). Targeted embryonic stem clones were injected into the C57BL/6 blastocysts yielding five lines of chimeric mice, which transmitted the disrupted allele through the germ line. All experiments reported here were performed with 129/Sv-C57BL6 hybrid descendants (F1 and subsequent generations) of these animals.

Breeding Experiments—SS+/− mice were cross-bred to the LDL receptor knockout mice (*LDLR*−/−) (21) to produce mice that were heterozygous for the disrupted alleles of both SS and the LDL receptor loci. An intercross of these animals was performed.

Diets—Three diets were used: (i) a normal chow (MF, Oriental Yeast), (ii) a 1.25% cholesterol diet, which contains 1.25% (w/w) cholesterol, 5% (w/w) cocoa butter, and 0.5% (w/w) cholic acid (23), and (iii) a 2% squalene diet, which contains 2% (w/w) squalene (WAKO) (24). Timed mating was performed and dietary supplementation was initiated 2 weeks before the mating.

SS Activity Assay—Liver and testes were homogenized in a buffer containing 15 mM nicotinamide, 2 mM MgCl$_2$, and 100 mM potassium phosphate, pH 7.4, and centrifuged at $10,000 \times g$ for 20 min at 4 °C. The supernatants were centrifuged at $105,000 \times g$ for 1 h at 4 °C, and the resultant pellets, a microsome fraction, were washed, resuspended in the same buffer, and stored in aliquots at −80 °C. SS activities were measured according to a modified method of Cohen *et al.* (25). In brief, the microsome fractions (~20 μg) were incubated in 50 μl of a buffer containing 20 μM [1-³H] farnesyl pyrophosphate (25 μCi/μmol), 1 mM NADPH, 5 mM MgCl$_2$, 6 mM glutathione, and 100 mM potassium phosphate, pH 7.4, at 37 °C for 15 min. Reaction was terminated by the addition of 150 μl of chloroform/methanol (1:2, v/v) containing 0.2% unlabeled squalene. After 50 μl of chloroform and 50 μl of 3 M NaOH

were added, the reaction mixtures were vortexed and centrifuged. The infranatant organic phase was used for the determination of the radioactivities in the squalene produced.

Measurement of Hepatic Cholesterol Synthesis—Cholesterol synthesis in the liver was estimated by a modified method of Eisele *et al.* (26). In brief, 6 SS+/+ and 6 SS+/− mice (9–10-week-old females) were given food and water *ad libitum* and injected intraperitoneally with [2-¹⁴C]acetate (37 kBq/kg body weight). After 30 min, animals were euthanized, and two pieces of the liver (0.3 g/each) were removed. [1,2-³H]cholesterol (~10,000 dpm) was added as an internal standard, and tissue samples were saponified in 2 ml of 15% KOH, 90% ethanol at 70 °C overnight. After the addition of 2 ml of water, nonsaponified lipids were extracted with *n*-hexane, evaporated, and dissolved in ethanol/acetone (1:1, v/v). Sterols were precipitated with digitonin. After washing the precipitates with 50% acetone, the radioactivities were determined. The results were expressed as ¹⁴C dpm/g wet weight of liver/30 min.

Northern Blot Analyses—Poly(A)⁺ RNA was isolated and pooled from the livers of six animals. 1.2 μg were subjected to 1% agarose electrophoresis in the presence of formalin. The fractionated RNA was transferred to Hybond N (Amersham Pharmacia Biotech). The filters were hybridized to ³²P-labeled cDNA probes: the LDL receptor, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and cholesterol 7α-hydroxylase.

Plasma Lipoprotein Analyses—After a 12-h fast, blood was collected in tubes containing EDTA. Plasma levels of total cholesterol (TC) and triglycerides (TG) were determined enzymatically using kits (Determiner TC555 and Determiner TG555, Kyowa Medex). Lipoproteins were fractionated by high performance liquid chromatography (HPLC) as described (23), and the cholesterol contents in each lipoprotein fraction were determined.

Morphological Studies—Embryonic tissues were examined by standard histological techniques.

TABLE I
Genotypes of offspring from intercrosses of SS+/− mice

Genotypes were confirmed by Southern blot analysis.

Age	SS+/+	SS+/−	SS−/−	Resorbed[a]	Total
E9.5	12	18	2	9	41
E10.5	19	42	8	14	83
E9.5–10.5[b]	4	14	3	12	33
E12.5	6	7	0	4	17
E13.5	12	25	0	21	58
Postnatal	50	99	0	NA[c]	149

[a] Significant number of embryos exhibited appearance of resorption. Genotyping of these embryos were ambiguous because of small DNA contents.

[b] E9.5 and E10.5 embryos were analyzed together.

[c] NA, not applicable.

Statistics—Data are represented as mean ± S.D. The Student's *t* test was used to compare the mean values between two groups.

RESULTS

SS Knockout Mice—Two genomic clones were isolated from a library with a cDNA probe. The restriction mapping revealed that one genomic clone (Fig. 1*A*) consisted of 8 exons spanning more than 26 kb. A replacement-type vector, which allowed the deletion of exon 4–5, was constructed and used to generate heterozygous SS knockout mice (*SS+/−*). *SS+/−* mice, which were viable and fertile, were intercrossed to obtain homozygous SS knockout mice (*SS−/−*). No viable *SS−/−* mice were identified among 149 weaned offspring, although 99 were heterozygotes (Table I). To determine the developmental stage where the embryos were lethal, the embryos were genotyped at four different embryonic stages: 9.5, 10.5, 12.5, and 13.5 days postcoitum (Table I, Fig. 1*B*). At E9.5–10.5, *SS−/−* was identified at the frequency significantly lower than the expected mendelian frequency (χ^2 = 8.35, $p < 0.05$). All *SS−/−* embryos were significantly smaller than *SS+/+* embryos with comparable developmental age. *SS−/−* embryos exhibit a wide variety of morphology. Among 13 *SS−/−* embryos, only 1 at E9.5 showed a nearly normal shape with a small forebrain (Fig. 2*C*), 6 had a shape that is similar to E8.5 embryos, early somite-stage embryos, with an open head fold (Fig. 2*D*). Their microscopic examination showed necrotic neuronal cells with condensation of nuclei, *i.e.* apoptotic cells (Fig. 2, *F* and *G*). Four were much smaller and ambiguous and did not have somites (Fig. 2*B*). At E12.5–13.5, however, no *SS−/−* were identified (Table I).

Reduction in mRNA Levels and Activities of SS in Heterozygotes of SS Knockout Mice—The SS mRNA levels in the *SS+/−* mice were reduced by 60% in the liver (Fig. 3*A*). Consistently, their SS activities were significantly reduced by 50% both in the liver and testes (Fig. 3*B*). In the liver, the mRNA levels of the LDL receptor appeared to be increased by 20% in the *SS+/−* mice (Fig. 3*A*). On the other hand, the mRNA of cholesterol 7α-hydroxylase appeared to be decreased by 20%. There was no significant change in the mRNA levels of HMG-CoA reductase between the *SS+/+* and *SS+/−* mice.

Cholesterol Synthesis in the Liver—If SS is the rate-limiting step for TC synthesis, cholesterol synthesis is expected to be reduced in the liver. However, the amounts of [^{14}C]acetate incorporated into cholesterol in the liver were not different between the wild-type and *SS+/−* mice (10,173 ± 6444 *versus* 13,992 ± 10,276 dpm/g liver/30 min, $p > 0.05$, $n = 6$).

Plasma Lipoprotein Analyses—To determine whether the reduction in the SS activities in the liver is associated with the changes in plasma lipoprotein profile, we determined the plasma lipid levels. Neither plasma TC nor TG levels were different between the wild-type and *SS+/−* mice, which were
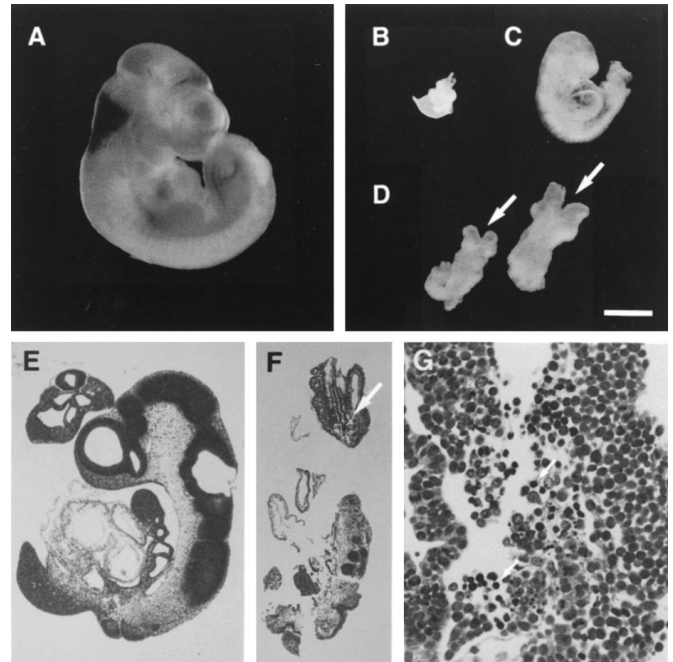

FIG. 2. **Phenotype of SS−/− embryos.** *A,* an E9.5 *SS+/+* embryo with normal developmental morphology; *B,* an E9.5 *SS−/−* embryo that lacked somite structure; *C,* an E9.5 *SS−/−* embryo that was much smaller than wild-type embryos but apparently almost normal except for an undeveloped forebrain; *D,* E10.5 *SS−/−* embryos that had open heads (indicated by *white arrows*). *Bar,* 1 mm. *E,* a sagittal section of an E9.5 *SS+/+* embryo as a normal control; *F,* a sagittal section of an E10.5 *SS−/−* embryo that is shown in *D*. The unclosed head is indicated by an *arrow*. *G,* microscopic examination of a part of the head that is shown in *F*. Some of the cells with condensed nuclei are indicated by *white arrows*. Hematoxylin eosin staining was used.

fed a normal chow (Table II). There was no significant difference in the amounts of VLDL, LDL, or HDL cholesterol as measured by HPLC (Table III). To evaluate the effects of the suppression of SS activities in the liver on the plasma lipoprotein profile in the setting of LDL deficiency, we further crossbred these animals with LDL receptor knockout mice to generate *SS+/−;LDLR−/−* mice. Neither plasma TC nor TG levels were different between the *SS+/+;LDLR−/−* and *SS+/−;LDLR−/−* mice (Table II).

Attempt to Rescue the Homozygotes by Feeding with Squalene or Cholesterol—If the embryonic lethality of the homozygotes results from the cholesterol deficiency, it is theoretically possible that dietary supplementation of squalene or cholesterol would reverse the phenotype. To test this hypothesis, we fed the pregnant *SS+/−* mice with the diets supplemented with either 2% squalene or 1.25% cholesterol. No viable *SS−/−* mice were identified among 21 offspring for squalene rescue (4

A

Squalene synthase LDL receptor

W H   W H

β -actin

Fold Increase   0.4   1.2

HMG-CoA   Cholesterol
reductase   7 α -hydroxylase

W H   W H

β -actin

Fold Increase   0.9   0.8

B

FIG. 3. **SS expression and SS activities in *SS+/−* mice.** *A,* Northern blot for SS, LDL receptor, HMG-CoA reductase, cholesterol 7α-hydroxylase, and β-actin as a loading control using liver poly(A$^+$) RNA. *W,* wild-type; *H,* SS+/−. *B,* SS activities in the liver from *SS+/+* and *SS+/−* mice. SS activities in the microsome prepared from the liver ($n = 6$) and testis ($n = 3$) were measured as described under "Experimental Procedures."
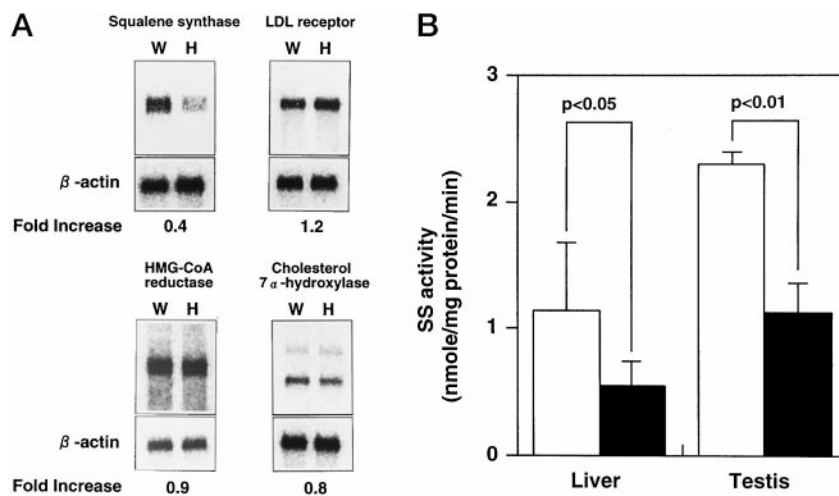
TABLE II
Plasma lipid levels

Mice aged 10–11 weeks were maintained on normal chow. After a 12-h fast, blood was collected for determination of TC and TG. Data are expressed as mean ± S.D. No significant difference was noted between *SS+/+* and *SS+/−* for either plasma TC or TG levels, irrespective of the presence of the *LDLR* mutation.

LDLR locus	*SS* locus	n	TC	TG
+/+	+/+	7	96.4 ± 18.6	132.3 ± 54.5
+/+	+/−	18	93.8 ± 25.0	119.1 ± 38.7
−/−	+/+	11	306.4 ± 21.4	167.5 ± 30.9
−/−	+/−	7	294.4 ± 24.2	119.1 ± 38.7

TABLE III
HPLC lipoprotein profiles

Five animals aged 8–10 weeks were maintained on normal chow. After a 12-h fast, blood was collected in tubes containing EDTA. Plasma was subjected to HPLC lipoprotein analyses. Total cholesterol contents in three lipoprotein fractions (VLDL, LDL, and HDL) were calculated and expressed as mean ± S.D. No significant differences were noted between *SS+/+* and *SS+/−* for each lipoprotein.

	VLDL	LDL	HDL
SS+/+	6.3 ± 4.4	5.5 ± 2.4	61.8 ± 14.6
SS+/−	4.7 ± 2.0	7.7 ± 3.4	65.9 ± 7.5

+/+ *versus* 17 +/− *versus* 0 −/−, $\chi^2 = 6.9$, $p < 0.05$,) and 40 offspring for cholesterol rescue (10 +/+ *versus* 30 +/− *versus* 0 −/−, $\chi^2 = 6.7$, $p < 0.05$).

DISCUSSION

In the present study, we demonstrate that SS is essential for the normal development of the embryo. 50% reduction in the SS activities in the liver did not result in alterations in the plasma lipoprotein profiles. Moreover, the lethal phenotypes of *SS−/−* embryos were not reversed by oral administration of either cholesterol or squalene to the dams.

During the early development of animals, particularly during fetal stage, there is a marked demand for new sterol. Cholesterol is required for new membrane synthesis, for maintenance of existing membranes, and for the synthesis of hormone and bile acids. As in the adult, this cholesterol is supplied by either *de novo* synthesis within the fetal compartment or by transfer from the maternal compartment to the fetus through uptake of cholesterol carried in lipoproteins. Previous studies have shown that sterol synthesis is markedly increased in the developing fetus including a preimplantation embryo (27–29). In addition, transport of maternal LDL and HDL to the fetal

yolk sac is drastically increased (30). Evidence suggests that several lipoprotein receptor systems mediate the transfer of maternal lipoprotein: the LDL receptor, megalin/gp330, VLDL receptor, and low density lipoprotein receptor-related protein for the uptake of lipoproteins containing either apoB-100 or apoE (reviewed in Refs. 6 and 31), and scavenger receptor B-1 for the binding of apoA-I containing lipoproteins (32). Because mice lacking the LDL receptor (21) or VLDL receptor (33) are fertile, there may be some redundancy in the receptor function. On the other hand, mice lacking low density lipoprotein receptor-related protein (34) or megalin/gp330 (14) are embryonic lethal, suggesting the pivotal role of these receptor in embryogenesis.

In this concern, it is noteworthy that *SS−/−* embryos develop to the stage around E9.5. Results indicate that cholesterol required for growth is exclusively supplied from the dams in *SS−/−* mice. Indeed, it has not been reported that pharmacological doses of HMG-CoA reductase inhibitors or SS inhibitors cause preimplantation lethality (35). It is known that nonpolar squalene, an immediate product of squalene synthase, is transported in association with VLDL in the plasma (36) and subsequently by a squalene and sterol carrier protein (37). Therefore, embryos may utilize squalene that is diffused from extra embryonic tissues for cholesterol synthesis. Furthermore, in the rodents, the nutritional supply of the embryo during the immediate postimplantation period is dependent on the resorptive and synthetic capacity of the yolk sac (38). Presumably, cholesterol transported across this maternal fetal barrier is utilized for development.

The embryonic lethality of the *SS−/−* mice may result from either cholesterol deficiency, toxic precursor buildup, or both. The cholesterol-supplying system in the early gestational period may no longer be effective beyond midgestation when a functional chorioallantoic circulatory system is established (38). It is conceivable that the placental system is too immature to fully compensate for the complete deficiency of the *de novo* cholesterol synthesis around this developmental stage. Belknap and Dietschy (27) have shown that the *de novo* cholesterol synthesis remains highly active during the whole midgestational period and that the contents of newly synthesized sterols are maximal at E17 in the rat fetus. Similar failure to catch up with the increasing demand may underlie the lethality of mice lacking apoB (11, 12, 39) or microsomal triglyceride transfer protein (13), because both mutants die at the same developmental stage. Alternatively, precursor buildup may have lethal effects on the embryos as postulated for SLO syndrome (7, 40). Inhibition of SS by compounds resulted in mas-

sive urinary excretion of farnesol-derived dicarboxylic acids (41), indicating the elevation of the plasma concentration of farnesol and its metabolites. Farnesol has recently been found to be a biologically active substance; it inhibits arterial vaso-constriction through blocking L-type Ca^{2+} channels in vascular smooth muscle cells (42, 43). Other studies have reported the existence of a farnesol-specific, orphan nuclear receptor in vertebrate cells, the farnesoid X-activated receptor, but its precise functions remain unknown (44). Therefore, it is possible that farnesol or its metabolites may have toxic effects on the embryos. This toxic precursor buildup hypothesis may be more plausible, because supplementation of the dams with dietary squalene failed to rescue the lethal phenotype.

As mentioned above, neural developmental anomalies are common in mice with the induced mutations in cholesterol transport systems (11–14), humans with genetic defects in the enzymes of the cholesterol biosynthetic pathway (7–9), and fetuses born to animals treated with the inhibitors of these enzymes (36, 40). These observations lead us to the speculation that $SS-/-$ mice develop anomalies in the nervous system. In this regard, it is noteworthy that $SS-/-$ had an open neural plate (Fig. 2*D*). Because the general growth of $SS-/-$ was severely impaired, the defective neural tube closure may simply reflect general growth retardation. Interestingly, the phenotypes of $SS-/-$ mice are different from those of infants with SLO syndrome, despite the fact that the responsible enzymes are involved in the proximal part of cholesterol synthesis (8). In the SLO syndrome, Δ^7-reductase deficiency may be partially compensated by Δ^{24}-reductase, an alternative enzyme involved in the terminal step of cholesterol biosynthesis, thereby leading to a milder phenotype (9). The differences may also arise from either species differences or build up of different precursors.

Recently, a link has emerged between cellular cholesterol metabolism and signaling molecules that are crucial for early development, the Hedgehog proteins (16–18). *Drosophila* Hedgehog and murine sonic Hedgehog proteins undergo posttranslational modification in which a cholesterol moiety is attached to its amino terminus (16). Patched, the putative receptor for Hedgehog protein (17), shares homology with the Nieman-Pick C1 protein (18), HMG-CoA reductase (45), and sterol regulatory element-binding protein cleavage-activating protein (46). This shared domain may function as a cholesterol-binding domain that is crucial for their functions. A certain part of the developmental anomalies of SLO syndrome may be ascribed to the defective function of the sonic Hedgehog signaling pathway; indeed, sonic Hedgehog knockout mice develop a holoprosencephalic phenotype similar to that in some SLO syndrome cases and in megalin-deficient mice (47). The phenotypes of $SS-/-$ are rather similar to Patched-deficient mice that have open and overgrown neural tubes (48). Further studies will be needed to elucidate the similarity in the phenotypes of these two mutants.

$SS+/-$ mice are apparently normal. Both mRNA levels and enzymatic activities for SS were reduced by 50% in the liver, but the overall hepatic cholesterol synthesis was not reduced, confirming that SS is not the rate-limiting enzyme in cholesterol biosynthesis (Fig. 3). The apparent changes in the LDL receptor and cholesterol 7α-hydroxylase mRNA levels may reflect hepatic cholesterol homeostasis. In this concern, it is noteworthy that zaragozic acid, an SS inhibitor, caused a compensatory increase in the hepatic mRNA levels of the LDL receptor, HMG-CoA reductase, and HMG-CoA synthase as well as SS itself along with marked suppression of cholesterol synthesis (49). Our results, however, strongly indicate that inhibition of SS activities by 50% is not sufficient to reduce the

plasma LDL cholesterol levels with or without the functional LDL receptor at least in mice (Table II).

Note Added in Proof—Bile acids have been found to be natural ligands for the FXR (50–52).

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