# Sjögren-Larsson Syndrome

## Deficient Activity of the Fatty Aldehyde Dehydrogenase Component of Fatty Alcohol:NAD$^+$ Oxidoreductase in Cultured Fibroblasts

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## Abstract

**Sjögren-Larsson syndrome (SLS) is an inherited disorder associated with impaired fatty alcohol oxidation due to deficient activity of fatty alcohol:NAD$^+$ oxidoreductase (FAO). FAO is a complex enzyme which consists of two separate proteins that sequentially catalyze the oxidation of fatty alcohol to fatty aldehyde and fatty acid. To determine which enzymatic component of FAO was deficient in SLS, we assayed fatty aldehyde dehydrogenase (FALDH) and fatty alcohol dehydrogenase in cultured fibroblasts from seven unrelated SLS patients. All SLS cells were selectively deficient in the FALDH component of FAO, and had normal activity of fatty alcohol dehydrogenase. The extent of FALDH deficiency in SLS cells depended on the aliphatic aldehyde used as substrate, ranging from 62% of mean normal activity using propionaldehyde as substrate to 8% of mean normal activity with octadecanal. FALDH activity in obligate SLS heterozygotes was partially decreased to 49±7% of mean normal activity using octadecanal as substrate. Differential centrifugation studies in fibroblasts indicated that this FALDH enzyme was largely particulate; soluble FALDH activity was normal in SLS cells. Intact SLS fibroblasts oxidized octadecanol to fatty acid at < 10% of the normal rate, but oxidized free octadecanal normally, suggesting that the FALDH affected in SLS is chiefly involved in the oxidation of fatty alcohol to fatty acid. These results show that the primary enzymatic defect in SLS is the FALDH component of the FAO complex, which leads to deficient oxidation of fatty aldehyde derived from fatty alcohol. (*J. Clin. Invest.* 1991. 88:1643–1648.) Key words: ichthyosis • mental retardation • lipid metabolism • genetic disease • neurological disease**

## Introduction

Sjögren-Larsson syndrome (SLS)[1] is an autosomal recessive disorder characterized by the presence of congenital ichthyosis, mental retardation, and spastic di- and tetraplegia (1). The disorder was first described more than 30 years ago in a group of Swedish patients, but additional cases have been reported worldwide (2–4).

SLS is one of several ichthyotic syndromes associated with abnormal lipid metabolism. Cultured skin fibroblasts and leukocytes from SLS patients were recently found to have deficient activity of fatty alcohol:NAD$^+$ oxidoreductase (FAO), an enzyme that catalyzes the oxidation of fatty alcohol to fatty acid (5, 6). This biochemical defect results in accumulation of fatty alcohol in cultured fibroblasts (5) and the plasma (6) of most SLS patients.

FAO is a complex enzyme consisting of separate proteins that sequentially metabolize fatty alcohol to fatty aldehyde and fatty acid, reactions which are catalyzed by fatty alcohol dehydrogenase (FADH) and fatty aldehyde dehydrogenase (FALDH), respectively (7). Consequently, the biochemical defect in SLS could involve either component of the FAO complex. We now report that SLS patients are specifically deficient in the FALDH component of FAO.

## Methods

*Chemicals.* [1-$^{14}$C]Palmitate (58 mCi/mmol), [1-$^{14}$C]stearate (56 mCi/mmol), and other $^{14}$C-labeled fatty acids were obtained from ICN Radiochemicals, Irvine, CA. [1-$^{14}$C]-labeled fatty alcohols were synthesized from the corresponding radioactive fatty acids by reduction with $Li(Al)H_4$ as described (8). Nonradioactive fatty alcohols were obtained from Sigma Chemical Co., St. Louis, MO. [1-$^{14}$C]-labeled fatty aldehydes and unlabeled octadecanal and hexadecanal were synthesized from the corresponding radioactive or nonradioactive fatty alcohols by reaction with 1-chlorobenzotriazole as described (9). Octanal and tetradecanal were obtained from Aldrich Chemical Co., Inc., Milwaukee, WI; other aldehydes were purchased from Sigma Chemical Co. Nonradioactive fatty aldehydes were quantitated by gas-liquid chromatography (6). All fatty aldehydes were stored in ethanol under a nitrogen atmosphere at −20°C. Solvents were either reagent grade or HPLC grade from J. T. Baker Chemical Co., Phillipsburg, NJ. All other chemicals were from Sigma Chemical Co.

*Cells.* Human cultured skin fibroblasts were derived from normal subjects or SLS patients by skin punch biopsy after obtaining informed consent. Fibroblast cell lines were grown from seven unrelated SLS homozygotes from the following countries of origin: United States (AB, CB); Chile (AZ); New Zealand (AC); Australia (DE); Sweden (LN); France (YL). Fibroblast cultures were also obtained from five obligate SLS heterozygotes, who were parents of the SLS homozygotes studied.

Cells were grown at 37°C in an atmosphere of 5% $CO_2$, 95% air in Dulbecco's minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). All experiments were performed on confluent cells at passage 2 through 12.

*Enzyme assays.* Confluent fibroblasts from one 75-cm$^2$ culture flask were collected by trypsinization and washed three times with PBS. The cell pellet was homogenized with a glass Teflon motor-driven homogenizer in 1 ml of 25 mM Tris-HCl, pH 8.0, 0.25 M sucrose.

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1. *Abbreviations used in this paper:* FADH, fatty alcohol dehydrogenase; FALDH, fatty aldehyde dehydrogenase; FAO, fatty alcohol:NAD$^+$ oxidoreductase; SLS, Sjögren-Larsson syndrome.

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FAO activity was measured as described (6). To measure the radioactive hexadecanal accumulated during the FAO assay, fibroblast homogenates containing 10–25 μg protein were incubated under standard FAO assay conditions (6) in the presence of 12 μM [$^{14}$C]-hexadecanol for 30 min at 37°C. Reactions were terminated by the addition of 2 ml each of hexane, water, and 0.3 M NaOH in ethanol. After vortexing the mixture for 1 min, the upper hexane layer containing radioactive hexadecanal was removed and the lower phase was reextracted with 2 ml hexane. The hexane extracts were combined, dried under nitrogen, and radioactive hexadecanal was purified by thin-layer chromatography on silica gel G plates using a solvent system consisting of hexane/chloroform/methanol (73/25/2). After addition of 1 ml of 2 N HCl to the remaining lower phase of the reaction mixture, radioactive hexadecanoic acid was extracted twice with hexane and isolated by chromatography on plates using a solvent system consisting of hexane/diethyl ether/acetic acid (60/40/0.9). Hexadecanal, hexadecanol, and hexadecanoic acid standards (25 μg) were included on each chromatography plate. The plates were stained with rhodamine G and the lipid spots were visualized under UV light. The spots containing radioactive hexadecanoic acid and hexadecanal were collected by scraping the appropriate area of silica gel from their respective thin-layer chromatography plates and quantitating by scintillation spectroscopy.

FALDH was assayed fluorometrically by measuring the fatty aldehyde-dependent production of NADH. The reaction was monitored in a fluorometer (111; Turner Designs, Sunnyvale, CA) equipped with a heated microcuvette chamber maintained at 37°C. The excitation wavelength was 365 nm and the emission wavelength was monitored at 460 nm. Reaction tubes contained 50 mM glycine-NaOH buffer, pH 9.5, 1.5 mM $NAD^+$, 0.2 mg of fatty acid–free BSA, 10 mM pyrazole, and 10–40 μg homogenate protein in a final vol of 0.4 ml. Reaction tubes were preheated for 2 min at 37°C, and the assay was initiated by the addition of 3 μl of a 21.5-mM hexadecanal (or octadecanal, tetradecanal, or dodecanal) solution in ethanol to achieve a final fatty aldehyde concentration of 160 μM. Control incubations consisted of identical reaction mixtures, but the reaction was initiated by addition of ethanol lacking fatty aldehyde. The reactions were typically monitored for 15 min using a chart recorder. The fatty aldehyde–dependent activity was calculated by subtracting the change in fluorescence measured in the absence of fatty aldehyde from that measured in the presence of fatty aldehyde.

Aldehyde dehydrogenase activity using propionaldehyde, hexanal, and octanal as substrates could not be assayed under the same reaction conditions used for hexadecanal, because these shorter substrates caused an increase in reaction fluorescence even without added cell homogenate. Instead, activity was measured using 25 mM Tris-HCl, pH 8.8 in place of the glycine-NaOH buffer, and the final substrate concentrations were 0.70 mM, 0.70 mM, and 1.3 mM for hexanal, octanal, and propionaldehyde, respectively.

FADH activity was assayed in the reverse direction in a reaction mixture consisting of 50 mM glycine-NaOH, pH 9.5, 0.1 mg fatty acid–free BSA, 1 mM NADH, 14 μM [$^{14}$C]-hexadecanal (~ 260,000 cpm dissolved in 3 μl ethanol) and 10–25 μg homogenate protein in a total vol of 0.2 ml. Reactions were initiated by addition of the homogenate. Control incubations lacked homogenate. After 15 min at 37°C, reactions were terminated by addition of 2 ml each of hexane, 0.3 M NaOH in ethanol, and water. Reaction mixtures were agitated with a vortex mixer for 1 min and the upper hexane phase containing radioactive hexadecanol was removed. The lower phase was extracted again with 2 ml hexane. The hexane extracts were combined and dried under nitrogen. Radioactive hexadecanol was separated from [$^{14}$C]-hexadecanal by chromatography on silica gel G plates using a solvent system consisting of hexane/chloroform/methanol (73/25/2). Standard hexadecanal and hexadecanol were spotted on each plate. The fatty alcohol region was visualized under UV light after staining the plate with rhodamine G, and the radioactive hexadecanol was collected by scraping and quantitated by scintillation spectroscopy. FADH activity was calculated by subtracting the radioactivity measured in control reactions from that measured in reactions containing fibroblast homogenate, and specific activity was expressed as picomoles per minute per milligram protein.

*Fibroblast fatty aldehyde and fatty alcohol oxidation.* Cultured fibroblasts were grown to confluency in 35-mm diameter culture dishes. One day before study, the cells were fed with fresh DMEM containing 10% fetal bovine serum. The culture medium was removed on the day of the experiment and replaced with identical medium containing either 20 μM [1-$^{14}$C]octadecanal or 3.5 μM [1-$^{14}$C]octadecanol. After incubation for 20 min (octadecanal) or 40 min (octadecanol) at 37°C, the dishes were placed on ice and the medium was quickly removed. Cell monolayers were washed twice with ice-cold PBS and cells were collected by scraping in methanol. Lipids were extracted overnight with chloroform/methanol (1/1). Insoluble cellular material was pelleted by centrifugation and the organic solvent containing extracted lipids was dried under nitrogen. Lipids were resuspended in 2 ml of 0.3 M NaOH in 95% ethanol and saponified for 1 h at 80°C. 2 ml of water was added, and nonsaponifiable lipids were extracted with hexane and discarded. After addition of 1 ml of 2 N HCl, saponifiable lipids were extracted into hexane and dried. Radioactive fatty acids were isolated by thin-layer chromatography on silica gel plates using a solvent system consisting of hexane/ether/acetic acid (60/40/1). Nonradioactive palmitate served as a standard. Fatty acid spots were visualized by spraying the plate with rhodamine G and examined under UV light. Fatty acid spots were scraped and radioactivity was determined by scintillation spectroscopy.

*Fibroblast subcellular fractionation by differential centrifugation.* Confluent fibroblasts from three 75-$cm^2$ culture flasks were collected by trypsinization and washed twice with PBS. Cells were resuspended in 5.8 ml of 25 mM Tris-HCl, 0.25 M sucrose, pH 8.0, and homogenized with 15 strokes in a motor-driven glass-Teflon homogenizer. All subsequent procedures were performed at 4°C. The homogenate was centrifuged for 10 min at 500 *g* in a refrigerated centrifuge (RC2B; Sorvall Instruments Div., DuPont Co., Newton, CT) using an SS-34 rotor. The 500 *g* pellet was resuspended in 1 ml of the same homogenization buffer. The 500 *g* supernatant was sequentially centrifuged for 30 min at 33,000 *g* and 120,000 *g* in an ultracentrifuge (L5-50; Beckman Instruments, Inc., Palo Alto, CA) using an SW50.1 swinging bucket rotor. In each case, the pellets and aliquots of the supernatants were removed at each step for enzyme assay.

## Results

The oxidation of fatty alcohol to fatty acid proceeds through a fatty aldehyde intermediate, and requires the sequential action of the FADH and FALDH components of the FAO complex. To determine which component of FAO was deficient in SLS fibroblasts, we reasoned that a defect in the FADH component would prevent the formation of fatty aldehyde intermediate, whereas a deficiency in FALDH activity would lead to accumulation of the fatty aldehyde intermediate. We, therefore, quantitated the amount of radioactive hexadecanal that accumulated when cell homogenates were incubated with [1-$^{14}$C] hexadecanol under standard FAO assay conditions. As shown in Table I, production of radioactive hexadecanoic acid was deficient in cell homogenates from five unrelated SLS patients, but [1-$^{14}$C] hexadecanal accumulated almost fivefold more in the mutant cells than in normal homogenates. These results pointed to a defect in the FALDH component of FAO.

To confirm these indirect results, we assayed both enzymatic components of FAO. FALDH was assayed fluorometrically by monitoring the hexadecanal-dependent production of NADH. Using normal fibroblast homogenates, hexadecanal-dependent NADH production increased linearly over time for at least 15 min. As shown in Fig. 1, the reaction rate was optimal at pH 9.5 and was linearly dependent on the amount of

*Table I. Radioactive Products Formed during Assay for Fatty Alcohol:NAD⁺ Oxidoreductase in Cultured Skin Fibroblasts Using [1-$^{14}$C] Hexadecanol as Substrate*

| Cell line | Radioactive products | | Ratio of hexadecanoic acid to hexadecanal |
|---|---|---|---|
| | Hexadecanoic acid | Hexadecanal | |
| Sjögren-Larsson syndrome ($n$ = 5) | 18±7* | 33±5 | 0.6±0.1 |
| Normal controls ($n$ = 5) | 106±24 | 5±1 | 19±9 |

* Radioactive products are expressed as picomoles per minute per milligram protein.

homogenate protein added. The enzyme activity was saturable with respect to hexadecanal and $NAD^+$ concentrations, with apparent $K_m$ values of 16 $\mu$M and 100 $\mu$M, respectively. Little or no activity was detected when $NAD^+$ was replaced with $NADP^+$.

FADH activity in fibroblasts could not be reliably measured in the forward direction by monitoring fatty aldehyde production from [$^{14}$C]-hexadecanol due to the large amount of FALDH activity, which tended to oxidize fatty aldehyde to fatty acid; conditions to selectively inhibit FALDH without losing FADH activity were not found. However, FADH could be measured in the reverse direction by monitoring the NADH-dependent production of [$^{14}$C]-hexadecanol when fibroblast homogenates were incubated with radioactive hexadecanal. Under these conditions, the reaction proceeded linearly over time for at least 40 min and was dependent on the amount of homogenate protein added (Fig. 2). FADH activity was saturated with increasing hexadecanal and NADH concentrations. The apparent $K_m$ for hexadecanal was 3 $\mu$M, and the enzyme displayed two apparent $K_m$'s for NADH at 36 $\mu$M and 100 $\mu$M. FADH activity showed a broad pH dependence between pH 8 and pH 10 (not shown). When NADH was replaced with NADPH, hexadecanal reduction was detected at 50–60% of that measured with NADH. The activity measured in the presence of both NADH and NADPH was additive, suggesting the presence of NADPH-dependent aldehyde reductase. Therefore, the FADH component was routinely assayed with NADH alone.

The FAO complex and its FALDH and FADH components were assayed in fibroblast homogenates from normal controls and seven unrelated SLS patients using 16-carbon substrates (Table II). In normal cells, FALDH activity was at least 30-fold greater than either the total FAO complex activity or FADH activity, suggesting that the initial step in fatty alcohol oxidation (to aldehyde) was rate-limiting for the FAO complex. As shown in Table II, FALDH activity was deficient in all SLS fibroblasts, whereas FADH activity was normal.

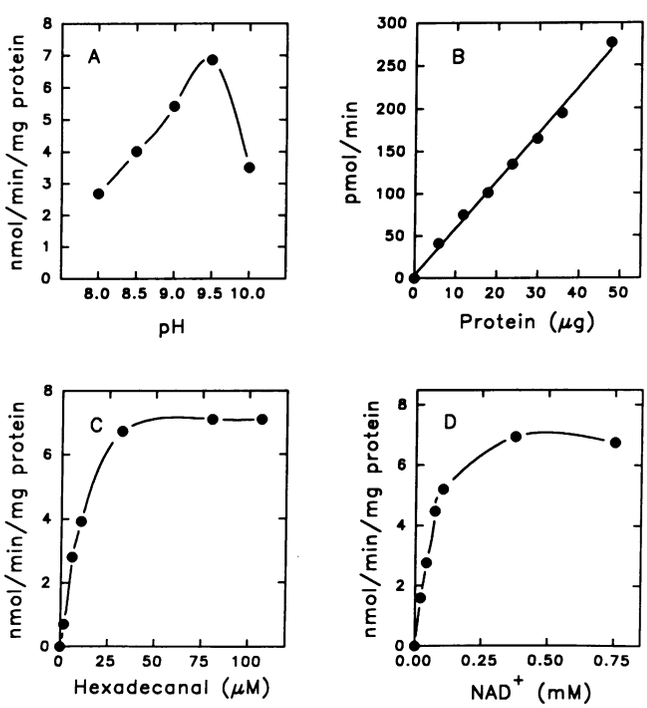


*Figure 1.* Reaction parameters for FALDH activity in normal fibroblast homogenates. Data points represent the mean of duplicate determinations. Except as noted, all reactions were performed at pH 9.5 for 15 min, and contained 160 $\mu$M hexadecanal and 1.5 mM $NAD^+$. (*A*) Reaction pH dependence. Each reaction was performed in glycine buffer and contained 29 $\mu$g homogenate protein. (*B*) Homogenate protein dependence. (*C*) Hexadecanal concentration dependence. Each reaction contained 29 $\mu$g homogenate protein. (*D*) Effects of varying $NAD^+$ concentration. Each reaction contained 22 $\mu$g homogenate protein.

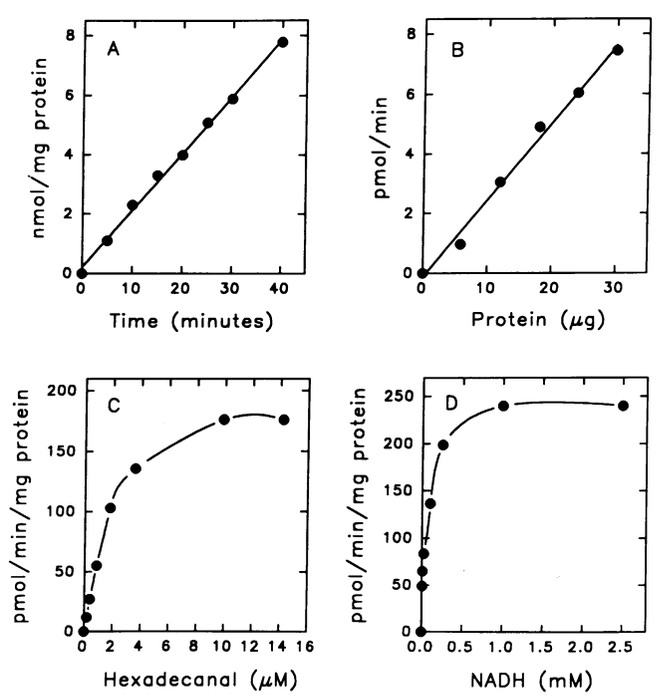


*Figure 2.* Reaction parameters for FADH activity in normal fibroblast homogenates. Data points represent the results of duplicate determinations. Except as noted, all incubations were for 15 min in the presence of 14 $\mu$M hexadecanal and 1 mM NADH. (*A*) Time course of the reaction. Each reaction contained 15 $\mu$g homogenate protein. (*B*) Homogenate protein dependence. (*C*) Hexadecanal concentration dependence. Each reaction contained 17 $\mu$g homogenate protein. (*D*) Effects of varying NADH concentration. Each reaction contained 20 $\mu$g homogenate protein.

*Table II. Enzyme Activities Associated with Fatty Alcohol Oxidation in Cultured Skin Fibroblasts from Normal Controls and Seven Unrelated SLS Patients. Cells Were Assayed for the FAO Complex, FALDH, and FADH Using 16-Carbon Substrates*

| Cell lines | FAO | FADH | FALDH |
|---|---|---|---|
| Normal controls | 101±29* | 268±46 | 9139±2578 |
| | ($n$ = 18) | ($n$ = 10) | ($n$ = 9) |
| Range | 71–172 | 175–333 | 5683–12,563 |
| Sjögren-Larsson syndrome | | | |
| AB | 15.5 | 274 | 1858 |
| AC | 21.0 | 227 | 2359 |
| AZ | 18.7 | 254 | 1144 |
| CB | 12.7 | 241 | 1334 |
| DE | 27.4 | 219 | 1186 |
| LN | 12.1 | 272 | 1068 |
| YL | 19.9 | 241 | 2623 |
| Mean±SD | 18.2±5.3 | 247±21 | 1653±633 |

* All enzyme activities are expressed as picomoles per minute per milligram protein (mean±SD).

The extent of enzyme deficiency in SLS fibroblasts was more profound with longer chain substrates than with shorter ones (Fig. 3). Mean FALDH activity in SLS ranged from 27% of mean normal activity using dodecanal as substrate to 8% of mean normal activity with octadecanal. A similar trend was seen with FAO complex activity, and there was a strong correlation ($r$ = 0.96) between FALDH and FAO activities using 18-carbon substrates. SLS fibroblasts were also deficient in FALDH activity using shorter substrates, such as octanal (25% of normal activity) and hexanal (30%), but were only mildly deficient using propionaldehyde as substrate (Table III). Disulfiram (10 mM), an inhibitor of acetaldehyde dehydrogenase (10), had little or no inhibitory effect on FALDH activity when octadecanal was used as substrate (data not shown).

FALDH activity was assayed in fibroblast homogenates from five unrelated obligate SLS heterozygotes using octadecanal as substrate. As shown in Table IV, mean FALDH activity was decreased in SLS heterozygotes to 49±7% of mean normal activity.

The subcellular distribution of FALDH was investigated in normal and SLS fibroblasts by fractionating cells by differential centrifugation. In normal fibroblasts ($n$ = 4), FALDH activity was found in all fractions, including a 500 $g$ pellet, 33,000 $g$ pellet, 120,000 $g$ pellet, and a 120,000 $g$ supernatant with a mean distribution of 29±13%, 53±11%, 11±2%, and 7±2% of total activity, respectively. In SLS cells ($n$ = 3), the residual FALDH activity showed an abnormal distribution with 21±13%, 53±7%, 1±1%, and 25±7% of total activity found in the 500 $g$ pellet, 33,000 $g$ pellet, 120,000 $g$ pellet, and a 120,000 $g$ supernatant fractions, respectively. Except for the 120,000 $g$ supernatant fraction which showed no decrease in FALDH activity, the specific activity of FALDH (measured as picomoles per minute per milligram protein) was decreased in all subcellular fractions in SLS cells compared to normal controls (Fig. 4). The greatest deficiency of FALDH was seen in the 120,000 $g$ pellet fraction (2% of mean normal activity). These results indicate that the FALDH which is deficient in SLS is a particulate or membrane-bound enzyme.

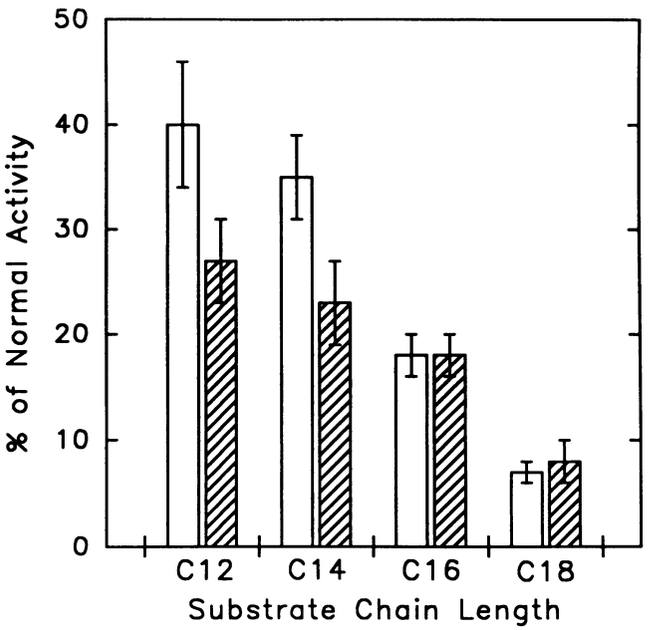


*Figure 3.* Residual activity of FAO and FALDH in crude homogenates of SLS fibroblasts using different chain length saturated fatty alcohols and fatty aldehydes as substrates. Seven SLS cell lines were assayed and data are expressed as the percentage±SEM of the mean specific activities measured in normal fibroblasts. FAO activities measured in normal fibroblasts were 142±35 ($n$ = 8), 129±30 ($n$ = 8), 101±29 ($n$ = 18), and 75±13 ($n$ = 14) pmol/min/mg protein using dodecanol, tetradecanol, hexadecanol, and octadecanol, respectively, as substrate. FALDH activities measured in normal fibroblasts were 10,622±2,045 ($n$ = 9), 9,510±2,326 ($n$ = 9), 9,139±2,578 ($n$ = 9), and 8,880±1,084 ($n$ = 12) pmol/min/mg protein using dodecanal, tetradecanal, hexadecanal, and octadecanal, respectively, as substrate. *Open bars,* FAO; *cross-hatched* bars, FALDH.

The presence of such a profound deficiency of FALDH activity in SLS fibroblasts raised the possibility that this FALDH functioned not only as a component of FAO but also to oxidize free fatty aldehydes. To determine whether free fatty aldehyde oxidation was impaired under more physiological conditions, intact fibroblasts were incubated with [$^{14}$C]-octadecanal and the production of radioactive octadecanoic acid was measured. As shown in Fig. 5, oxidation of free octadecanal was not impaired in SLS fibroblasts. In contrast, oxidation of

*Table III. Aldehyde Dehydrogenase Activity in Normal and SLS Fibroblasts Using Various Aldehyde Substrates*

| | | Aldehyde dehydrogenase activity* | | |
|---|---|---|---|---|
| Substrate | Concentration | Normal cells | SLS cells | Percent of normal activity in SLS |
| | *mM* | | | |
| Propionaldehyde | 1.3 | 5062±1972 | 3162±989 | 62 |
| Hexanal | 0.7 | 13,203±1379 | 3967±2189 | 30 |
| Octanal | 0.7 | 14,379±1970 | 3646±1557 | 25 |

* Enzyme activity is expressed as picomoles per minute per milligram of protein (mean±SD) for five normal and five SLS cell lines.

*Table IV. Fatty Aldehyde Dehydrogenase Activities in Cultured Fibroblasts from SLS Homozygotes, Obligate SLS Heterozygotes, and Normal Controls using Octadecanal as Substrate*

| Cell line | FALDH Activity* | Percent of mean normal activity |
|---|---|---|
| SLS Homozygotes | | |
| AB | 1054 | 12 |
| AC | 787 | 9 |
| AZ | 736 | 8 |
| CB | 591 | 7 |
| DE | 270 | 3 |
| LN | 213 | 2 |
| YL | 1167 | 13 |
| | | 8±4 |
| Obligate SLS Heterozygotes | | |
| BB | 4948 | 56 |
| EN | 4673 | 53 |
| HA | 4517 | 51 |
| LL | 3284 | 37 |
| PZ | 4069 | 46 |
| | | 49±7 |
| Normal controls ($n$ = 12) | 8880±1084 | 100 |
| Range | 6950–10,772 | |

* FALDH activity is expressed as picomoles per minute per milligram protein (mean±SD). Each cell line was tested on two to four separate occasions.

radioactive octadecanol to fatty acid was decreased in intact SLS cells to < 10% of mean normal activity. Unlike the assays with fibroblast homogenates, however, there was no concomitant cellular accumulation of free radioactive octadecanal in the intact SLS cells (data not shown).

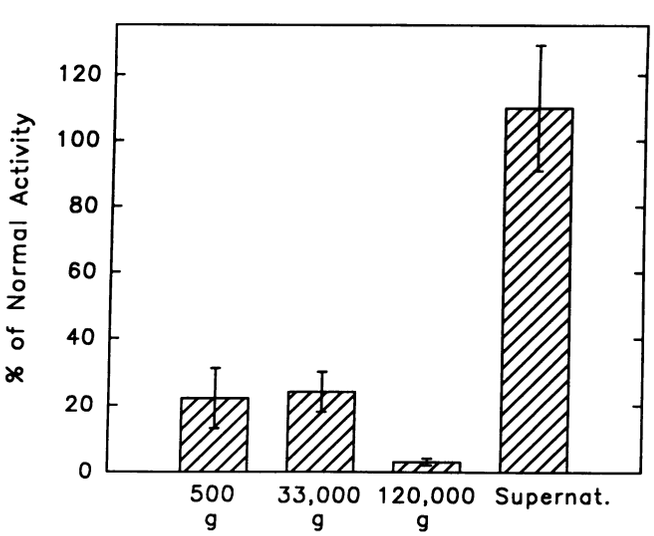


*Figure 4.* Differential centrifugation studies in normal and SLS cultured fibroblasts. Cultured fibroblasts were fractionated as described in Methods, and FALDH activity was assayed in each fraction. Data are expressed as the enzyme specific activities measured in SLS cells as a percentage of the mean activities in normal fibroblasts. Results shown are the mean±SEM for five experiments using four normal cell lines and three SLS cell lines.

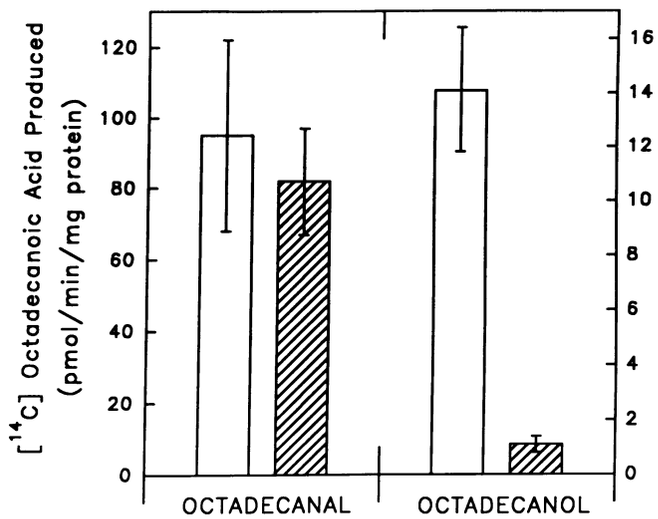


*Figure 5.* Oxidation of free fatty aldehyde (*octadecanal*) and fatty alcohol (*octadecanol*) to fatty acid by intact fibroblasts. Data represent the results of two experiments and are the mean±SD using three normal and four SLS cell lines. *Open bars,* normal controls; *cross-hatched bars,* SLS.

## Discussion

Our results indicate that the primary defect in SLS involves the FALDH component of FAO, which leads to impaired oxidation of fatty aldehyde derived from fatty alcohol metabolism. This FALDH component was deficient in SLS patients from seven unrelated kindreds, each patient presumably the result of independent mutations. No patient was found to be deficient in the FADH component of FAO. Thus, our SLS patients showed no evidence of biochemical heterogeneity. The observation that SLS heterozygotes have a partial deficiency in FALDH activity is consistent with this enzyme being the primary genetic defect in SLS.

FAO has not been purified and little is known about its subunit structure. At least four aldehyde dehydrogenases that act on propionaldehyde have been identified in human tissues (11), and it is possible that multiple FALDH enzymes also exist, perhaps with overlapping substrate specificities. One or more of these may function as a component of the FAO complex. Fatty aldehyde dehydrogenases that are active against dodecanal and shorter chain substrates have been purified from rat liver (12, 13) and rabbit intestine (14). The rabbit FALDH is capable of functioning in the complete oxidation of dodecanol to fatty acid when it is reconstituted with FADH (7). However, enzyme activity using substrates longer than 12-carbons was not reported, and it is unclear whether this same enzyme is analogous to the one deficient in SLS. Nevertheless, a single FALDH enzyme may function as a component of the FAO complex and catalyze the oxidation of a variety of aliphatic aldehydes. The finding that SLS fibroblasts showed considerable deficiency in FALDH activity using aldehydes from 6 to 18 carbons long is most consistent with a single FALDH acting as a component of FAO. The residual FALDH activity measured in crude homogenates and subcellular fractions of SLS cells may reflect the contribution of other aldehyde dehydrogenases that preferentially act on different aldehyde substrates or free fatty aldehydes. The presence of normal cytosolic FALDH activity but decreased particulate enzyme activity in SLS is

consistent with the existence of multiple enzymes that react on long-chain aldehydes. Cytosolic aldehyde dehydrogenases are known to have broad substrate specificities (15).

FALDH is thought to be a microsomal enzyme in rat liver (12, 13) and rabbit intestine (14). Although a small amount of FALDH activity appeared to be cytosolic, our differential centrifugation studies indicated that most FALDH activity is particulate in normal fibroblasts. Density gradient separations of cellular organelles will be necessary to determine the subcellular localization of FALDH and FAO.

Intact SLS fibroblasts oxidized free fatty aldehyde normally, which suggests the presence of more than one fatty aldehyde oxidizing enzyme. It is possible that another FALDH or a long-chain aldehyde oxidase exists, which is primarily responsible for oxidizing free fatty aldehyde and is not deficient in SLS.

The role of the FAO complex in recycling fatty alcohol is most apparent in SLS patients who are deficient in this enzyme activity and exhibit symptoms involving the skin and nervous system. With FAO deficiency, it is expected that long-chain alcohols would accumulate in SLS patients. The finding that FAO and FALDH activities in SLS were more severely decreased as the substrate chain length increased from 12 to 18 carbons is consistent with the previous report (6) that octadecanol and hexadecanol accumulate in plasma from SLS patients, but tetradecanol does not. Presumably, SLS patients possess sufficient residual FAO activity (35% of normal) with tetradecanol to prevent accumulation of this alcohol, whereas residual enzyme activity is decreased to < 20% of normal with longer chain alcohols. The FALDH deficiency against hexanal and octanal raises the possibility that SLS patients may accumulate medium-chain fatty alcohols. Judge et al. (16) have recently demonstrated decreased activity of hexanol oxidation in the epidermis and jejunal mucosa of SLS patients using a histochemical staining method.

Our finding that all SLS patients were deficient in FALDH and none were deficient in the FADH component of FAO raises the possibility that the SLS phenotype results partly from fatty aldehyde accumulation. Under FAO assay conditions, SLS fibroblast homogenates accumulated fatty aldehyde derived from fatty alcohol, whereas free aldehyde accumulation could not be demonstrated in intact SLS fibroblasts. In the intact SLS cell which possesses some NADH (and NADPH), it is possible that the fatty aldehyde is reduced back to fatty alcohol. Alternately, the fatty aldehyde may covalently interact with proteins or other molecules to form an aldehyde derivative, or be further converted to other metabolites.

The biological consequences of either long-chain alcohol or aldehyde accumulation are unknown. Alcohols less than 14-carbons long are known anesthetic agents, whereas fatty alcohols longer than 14-carbons have little or no anesthetic properties (17). Long-chain alcohols have been shown to partition into artificial lipid bilayers (17, 18) and synaptic vesicles (19). If fatty alcohol accumulates in the skin of SLS patients, it could modify the epidermal water barrier, which is critically dependent on the lipid composition of the stratum corneum (20), and lead to increased transepidermal water loss and ichthyosis. Fatty aldehydes would also be expected to partition into cell membranes, where the chemically reactive aldehyde group may form covalent bonds with a variety of adducts and interfere with the action of membrane-bound enzymes or disturb membrane function. Acetaldehyde, for example, has been shown to react with lysine residues in tubulin protein and inhibit microtubule assembly in vitro (21).

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