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# Isolation and characterization of a Chinese hamster ovary cell line deficient in fatty alcohol:NAD$^+$ oxidoreductase activity

(Sjögren–Larsson syndrome/somatic cell mutant/fatty alcohol metabolism)

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ABSTRACT **We have isolated a mutant Chinese hamster ovary cell line that is defective in long-chain fatty alcohol oxidation. The ability of the mutant cells to convert labeled hexadecanol to the corresponding fatty acid *in vivo* was reduced to 5% of the parent strain. Whole-cell homogenates from the mutant strain, FAA.1, were deficient in long-chain fatty alcohol:NAD$^+$ oxidoreductase (FAO; EC 1.1.1.192) activity, which catalyzes the oxidation of hexadecanol to hexadecanoic acid, although the intermediate fatty aldehyde was formed normally. A direct measurement of fatty aldehyde dehydrogenase showed that the FAA.1 strain was defective in this component of FAO activity. FAA.1 is a two-stage mutant that was selected from a previously described parent strain, ZR-82, which is defective in ether lipid biosynthesis and peroxisome assembly. Because of combined defects in ether lipid biosynthesis and fatty alcohol oxidation, the ability of the FAA.1 cells to incorporate hexadecanol into complex lipids was greatly impaired, resulting in a 60-fold increase in cellular fatty alcohol levels. As the FAO deficiency in FAA.1 cells appears to be identical to the defect associated with the human genetic disorder Sjögren–Larsson syndrome, the FAA.1 cell line may be useful in studying this disease.**

In animal cells, long-chain fatty alcohols are generated primarily through the reduction of fatty acids by fatty acyl-CoA reductase (EC 1.2.1.42) (1). They can also be generated after the breakdown of sphingomyelin (2) and ether lipids (3) (from fatty aldehyde) or obtained from the surrounding milieu (diet). As they are generated, long-chain fatty alcohols must be metabolized or removed. They can be incorporated as the alcohol into ether lipids (4) and, to a minor extent, wax esters (5, 6), or they can be oxidized to fatty acid and incorporated into complex lipids (7).

Reports have described the oxidation of fatty alcohols (8, 9) and fatty aldehydes (10–13) in animal cells with an emphasis on the oxidation of shorter chain substrates (2–12 carbons). An activity capable of converting hexadecanol (16 carbons) to the corresponding fatty acid, called fatty alcohol:NAD$^+$ oxidoreductase (FAO; EC 1.1.1.192), has been described in rat liver (14) and human fibroblasts (7). The rat liver FAO activity described by Lee (14) displayed a rather broad substrate specificity (with roughly equal activities observed with 10- to 16-carbon substrates). This activity was characterized in a crude microsomal fraction; it is possible that more than one enzyme or enzyme system was responsible for the oxidation of the various substrates.

The number of activities catalyzing the conversion of long-chain fatty alcohol to fatty acid is not known, and the importance of fatty alcohol metabolism with respect to other aspects of cellular function in mammalian cells is unclear. To address some of these issues, we have devised a selection procedure using a combination of "tritium suicide" (15) and colony autoradiography (16) to isolate a Chinese hamster ovary (CHO) cell line that is unable to oxidize hexadecanol to the fatty acid. This mutant is biochemically similar to human fibroblasts from patients with a genetic disorder, Sjögren–Larsson syndrome (17, 18).

## EXPERIMENTAL PROCEDURES

**Materials.** [9,10-$^3$H]Hexadecanoic acid (28 Ci/mmol; 1 Ci = 37 GBq) and EN$^3$HANCE spray were purchased from DuPont/NEN). The tritiated hexadecanoic acid was used to chemically synthesize [9,10-$^3$H]hexadecanol by the method of Davis and Hajra (19). Fatty aldehydes were prepared from the fatty alcohols by the method of Ferrell and Yao (20). Unless specified, all other biochemicals were purchased from Sigma. Polyester cloth (17-$\mu$m mesh) was purchased from Tetko (Elmsford, NY).

**Cells and Culture Conditions.** The wild-type CHO cell line CHO-K1 was obtained from the American Type Culture Collection. ZR-82 is a derivative of CHO-K1 that lacks functional peroxisomes and does not synthesize ether lipids (21). All cell lines were incubated at 37°C in 5% $CO_2$/95% air. Cells were grown in Ham's F-12 medium (Flow Laboratories) containing 10% (vol/vol) fetal bovine serum (GIBCO) supplemented with 1 mM glutamine (designated F12c medium).

**Tritium-Suicide Selection for Long-Chain Fatty Alcohol-Uptake Mutants.** Mutagenized (16) ZR-82 cells ($2 \times 10^6$) were plated in a 75-cm$^2$ tissue culture flask and allowed to attach overnight. Two milliliters of F12c medium containing [9,10-$^3$H]hexadecanol was added to the culture to achieve a final concentration of 2 $\mu$M [9,10-$^3$H]hexadecanol at 2 $\mu$Ci/ml. After 3 hr at 37°C, the labeled medium was removed, the cells were washed once with 10 ml of F12c medium, and then incubated for 1 hr at 37°C in 15 ml of F12c containing no additions. The medium was removed, the cells were washed once with F12c, harvested with trypsin, centrifuged at 600 × *g*, and resuspended in F12c containing 10% (wt/vol) glycerol at $5 \times 10^5$ cells per ml; this suspension was placed in vials (0.75 ml per vial) and frozen in liquid nitrogen.

**Autoradiographic Detection of Long-Chain Fatty Alcohol-Uptake Mutants.** Cells that survived tritium suicide selection were plated out at a density of ≈100 cells per 60-mm-diameter tissue culture dish. After the cells were allowed to attach and adjust overnight, they were overlayed with a sterile polyester cloth and glass beads (22). After 10 days at 37°C, the polyester cloth containing immobilized colonies was removed and placed in 5 ml of serum-supplemented medium containing 2 $\mu$M [9,10-$^3$H]hexadecanol (1 $\mu$Ci/nmol). After 3 hr, the polyester was removed, briefly blotted again, placed in 5 ml of serum-supplemented medium containing no additions, and

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Abbreviations: FAO, fatty alcohol:NAD$^+$ oxidoreductase; CHO, Chinese hamster ovary.

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incubated for 1 hr. The polyester was then removed and washed once in F12c medium and twice in phosphate-buffered saline (PBS), with blotting between each wash. The colonies were fixed by placing the polyester in ice cold 10% $CCl_3COOH$, followed by a rinse in 3% $CCl_3COOH$. The polyesters were then dried, sprayed with EN$^3$HANCE, and exposed at −70°C to Kodak X-AR film for 3 days.

**Fatty Alcohol and Fatty Acid Uptake Measurements.** Cells were plated out in sterilized 20-ml glass scintillation vials at $2.5 \times 10^5$ cells per vial in 1.5 ml of F12c medium and allowed to attach overnight. F12c (0.5 ml) containing 8 μM [9,10-$^3$H]-hexadecanol (2 μCi/0.5 ml) or 8 μM [9,10-$^3$H]hexadecanoic acid (2 μCi/0.5 ml) was then added to each vial and cells were harvested after incubation for 1.5 or 3.0 hr. Some samples were subjected to a "wash cycle" after 3 hr of labeling to remove unmetabolized fatty alcohol or fatty acid from the cells. In such cases, the medium containing radioactive ligand was removed, the cells were gently washed twice with 2 ml of F12c and incubated for an additional hour in 2 ml of this medium at 37°C. In either case, medium was then removed, the cells were washed twice with 3 ml of F12c and once with 3 ml of PBS, and the lipids were extracted from the cell monolayer with 3.8 ml of chloroform/methanol/PBS, 1:2:0.8 (vol/vol), after the addition of 300 μg of carrier lipid (from rat liver). One milliliter of $CHCl_3$ and 1 ml of PBS were then added to form a two-phase system (23), and the lipids were recovered in the lower phase after a brief centrifugation. The upper phase was washed once with 2 ml of a preequilibrated lower phase from a similar two-phase system. An aliquot of the combined lower phases was used for liquid scintillation counting to quantitate the uptake of radioactive ligand. Vials containing no cells were incubated with the radioactive medium under similar conditions and analyzed as controls. Duplicate vials containing unlabeled cells were used for protein determinations. Protein was quantitated by the method of Lowry *et al.* (24) in all experiments.

**Oxidation of Fatty Alcohol to Fatty Acid in Intact Cells.** Cells were incubated for 3 hr with medium containing 2 μM [9,10-$^3$H]hexadecanol, and the cellular lipids were extracted as described for the uptake studies. The lipid fraction was dried with nitrogen, resuspended in 0.5 ml in 95% ethanol containing 0.5 M NaOH, and saponified at 80°C for 1 hr to release esterified fatty acids. After acidification by the addition of 0.5 ml of 1 M HCl, PBS (2 ml) and methanol (1 ml) were added, and lipids (primarily fatty alcohol and fatty acid) were extracted three times with 2 ml of *n*-hexane. The combined hexane extracts were dried, and fatty acids were isolated by TLC with silica gel 60 plates and *n*-hexane/diethyl ether/acetic acid, 60:40:1 (vol/vol), as the solvent system (solvent system I). The band corresponding to fatty acid was scraped and assayed as described in Table 1.

**FAO and Fatty Aldehyde Dehydrogenase Assays.** Cells were harvested by either scraping with a rubber policeman or by trypsinization (similar results were obtained by either method). The cells were pelleted by centrifugation and washed twice with PBS. The final cellular pellet was resuspended in 25 mM Tris·HCl, pH 8.0/0.25 M sucrose and homogenized with 15 strokes of a motor-driven, glass–Teflon homogenizer. Homogenates were assayed that day or stored at −20°C prior to assay. If frozen, the homogenates were thawed on ice and sonicated in a glass test tube for 1 min in a sonic water bath to assure even dispersion. FAO assays, which measure the complete conversion of radioactive fatty alcohol to the fatty acid, were performed by the method of Lee (14) with few modifications. The reaction mixture contained 50 mM Tes/glycine buffer (pH 8.25), 0.5 mg of fatty acid-free bovine serum albumin per ml, 2.5 mM $NAD^+$, 5 μM [9,10-$^3$H]hexadecanol at ≈$5 \times 10^5$ cpm per assay, and 25–50 μg of cell homogenate protein in a total volume of 0.2 ml. The reactions were initiated by the addition of $NAD^+$. After 20 min at 37°C, the reactions were stopped by the addition of 1 ml of 2% acetic acid in methanol. Control assays were held on ice, and the reactions were stopped immediately after the addition of the $NAD^+$. The lipids were extracted under acidic conditions (23), dried, spotted on silica gel H (Alltech Associates) plates, and chromatographed with solvent system I. The TLC plate was sprayed with EN$^3$HANCE and exposed to Kodak X-AR film at −70°C. The bands of interest were scraped into liquid scintillation vials containing 1 ml of methanol and counted in 10 ml of Liquiscint by liquid scintillation spectrometry. All products were identified by their comigration on TLC with authentic standards in three solvent systems: solvent system I; ethyl ether/benzene/ethanol/acetic acid, 40:50:2:0.2 (vol/vol); and benzene. Putative fatty aldehyde was further characterized by reaction with 2,4-dinitrophenylhydrazine in methanol (25), which resulted in all of the radioactivity cochromatographing with the 2,4-dinitrophenylhydrazone derivative of hexadecenal. Further characterization of the putative fatty acid involved mild base hydrolysis (0.1 M NaOH at 60°C for 2 hr) and reaction with boron trifluoride (26). The putative fatty acid was resistant to alteration by base hydrolysis, but boron trifluoride treatment resulted in the formation of a radioactive product which cochromatographed with methyl palmitate. Base hydrolysis of the putative wax ester resulted in 95% of the radioactive cochromatographing with hexadecanol on TLC.

Fatty aldehyde dehydrogenase activity was assayed fluorometrically by measuring the fatty aldehyde-dependent production of NADH. The reaction was monitored in a Turner 111 fluorometer equipped with a heated microcuvette chamber maintained at 37°C. The excitation wavelength was 365 nm (primary filter 7–60) and emission wavelength at 460 nm (secondary filters 2A and 48). Assays contained 50 mM glycine adjusted to pH 9.0 with NaOH, 0.5 mM $NAD^+$, 0.25 mg of fatty acid-free bovine serum albumin per ml, 10 mM pyrazole, and 20–60 μg of cell homogenate protein in a final volume 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 solution in 95% ethanol to achieve a final fatty aldehyde concentration of 160 μM.

**Cellular Fatty Alcohol Levels.** Cells were grown in serum-supplemented medium, washed three times with ice-cold PBS, and scraped into 1 ml of methanol. After transfer into a glass, screw-capped test tube, 2 ml of chloroform was added, the cellular lipids were extracted as described (23), and an internal standard (pentadecanol) was added. Fatty alcohols were isolated, and the individual fatty alcohol species were quantitated after separation of the acetate derivatives on capillary gas–liquid chromatography by a method described previously for cultured human fibroblasts (7, 18).

## RESULTS

**Isolation of Fatty Alcohol Oxidase Mutants: Tritium Suicide.** Mutagenized cells were exposed to [9,10-$^3$H]hexadecanol and then frozen in liquid nitrogen. While frozen, cells that accumulate the labeled hexadecanol are damaged by radiolysis and should not survive upon thawing and recovery. We thought that mutants unable to convert the fatty alcohol to the fatty acid should accumulate less tritium and, thus, survive longer freezing periods. To insure that unoxidized fatty acids would not be diverted into ether lipids, we used an ether lipid-deficient derivative of CHO-K1, ZR-82, as the parent strain (21, 27).

A mutagenized population of ZR-82 cells treated with [9,10-$^3$H]hexadecanol accumulated an average of 7.5 dpm per cell. When cells in one vial ($3.75 \times 10^5$ tritium-treated cells) were thawed after 7 days in liquid nitrogen, few survived. Approximately 25 colonies developed 10 days after revival. These

surviving cells were harvested and replated, and the resulting colonies were screened for their ability to take up [9,10-$^3$H]hexadecanol by using colony autoradiography (16). Three colonies were identified that gave little or no signal upon autoradiography (Fig. 1). We were able to recover and clonally purify one of the putative mutants (designated FAA.1).

**Fatty Alcohol Uptake in FAA.1 Cells.** The uptake of [9,10-$^3$H]hexadecanol was quantitated in FAA.1 cells under conditions similar to those used for the selection and screening process. Over the initial 3-hr labeling period, both FAA.1 and ZR-82 cells incorporated less hexadecanol than CHO-K1 cells (Fig. 2*A*). The reduced fatty alcohol uptake in the parent strain (ZR-82) was likely due to the inability of ZR-82 cells to synthesize ether lipids. Values for fatty alcohol incorporation in FAA.1 cells over the initial 3 hr were only slightly lower than values for ZR-82 cells; however, during the 1-hr "wash cycle," <50% of label was lost from ZR-82 and CHO-K1 cells, while almost all of the label was lost from FAA.1 cells (Fig. 2*A*). After the complete 4-hr labeling protocol (which included the "wash cycle"), FAA.1 cells had taken up 82% and 93% less fatty alcohol compared with the parent strain, ZR-82, and the CHO-K1 strain, respectively. At three different temperatures (33°C, 37°C, and 40°C) and with a 10-fold higher concentration of hexadecanol, similar results were obtained (data not shown).

FAA.1 cells were impaired in their ability to incorporate hexadecanol into complex lipids (Table 1). After 3 hr (prior to the "wash cycle"), most of the radioactivity from [9,10-$^3$H]hexadecanol was associated with complex lipids in CHO-K1 and the parent strain, but the majority remained as the free fatty alcohol in FAA.1 cells. The amount of radioactivity found as the free alcohol in the FAA.1 cells was 3-fold more than in the other strains even though the overall accumulation of radioactivity was greatly reduced. Little change in the labeling pattern occurred during the "wash cycle" in any of the cell lines, with the noticeable exception of the loss of most of the free fatty alcohol (not shown).

In contrast to fatty alcohol, uptake of [9,10-$^3$H]hexadecanoic acid was equivalent in all three cell lines (Fig. 2*B*). A 1-hr "wash cycle" removed very little radioactivity from any of the cell lines.

***In Vivo* Conversion of Fatty Alcohol to Fatty Acid Is Defective in FAA.1 Cells.** The amount of fatty acid formed from the [9,10-$^3$H]hexadecanol during the course of the 3-hr labeling period could be quantitated after saponification of the lipids (to free the esterified fatty acids). A comparison of fatty acid formation in CHO-K1 and ZR-82 showed that, even though less fatty alcohol was taken up by ZR-82 cells (Fig. 2*A*), identical amounts of fatty acid were produced in the two cell lines (Table 2). FAA.1 cells, which incorporated approximately equal amounts of hexadecanol compared with ZR-82 cells over the initial 3-hr labeling period, produced very little fatty acid (7% of either CHO-K1 or ZR-82).

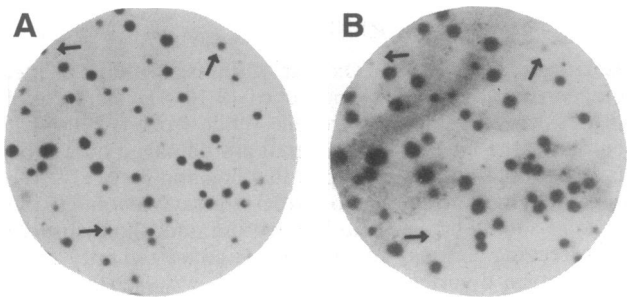


FIG. 1. Colony autoradiography for the identification of fatty alcohol uptake mutants. Colonies formed by cells surviving tritium suicide were prepared for autoradiography as described, and colonies were visualized with Coomassie blue (16). The pattern of colonies from this staining (*A*) was compared with the pattern produced on the x-ray film (*B*). Arrows indicate cell colonies that incorporated little or no radioactivity.

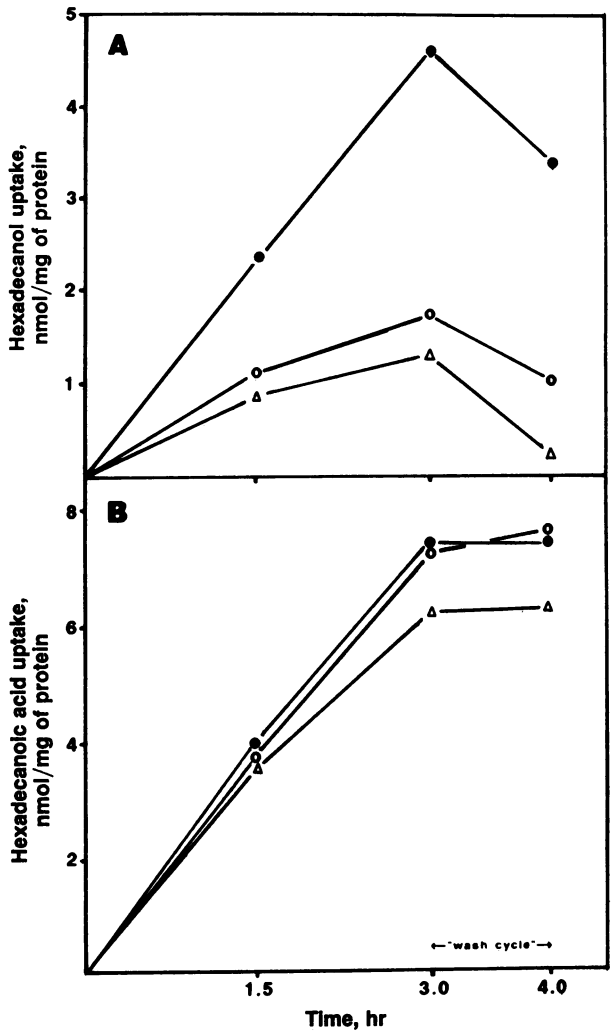


FIG. 2. Uptake of hexadecanol and hexadecanoic acid in CHO-K1, ZR-82, and FAA.1 cells. Cells were plated out and exposed to either 2 μM [9,10-$^3$H]hexadecanol or 2 μM [9,10-$^3$H]hexadecanoic acid as described. The amount of radioactivity taken up in each cell line was determined and normalized to the protein content. (*A*) Hexadecanol uptake. (*B*) Hexadecanoic acid uptake. Cell lines: ●, CHO-K1; ○, ZR-82; △, FAA.1. All values represent duplicate samples and did not vary by more than 10%.

**FAO Activity Is Reduced in FAA.1 Cells.** When whole-cell homogenates from CHO-K1 and ZR-82 cells were incubated with [9,10-$^3$H]hexadecanol and $NAD^+$, three radioactive products resulted (Fig. 3). These were identified as fatty acid, fatty aldehyde, and wax ester. The FAO activity in CHO cell homogenates displayed traits similar to those described for the FAO activity in rat liver (14): (*i*) fatty acid and fatty aldehyde were not formed in the absence of $NAD^+$ (although the putative wax ester was still formed); (*ii*) $NADP^+$ would not serve as a cofactor; (*iii*) the formation of the two oxidation products was inhibited by the sulfhydryl binding reagent *N*-ethylmaleimide; (*iv*) their formation was unaffected by sodium azide; and (*v*) the pH optimum for FAO activity was dependent upon the buffer used (data not shown). With our buffer system, the pH optimum was 8.25, in agreement with Lee's results (14).

The formation of wax esters was somewhat surprising. A cosubstrate for wax ester formation, acyl-CoA (28), was not added to the incubation mixtures. It is possible that enough endogenous acyl-CoA was present to allow for the formation

Table 1. Distribution of radioactivity in cells treated with [9,10-$^3$H]hexadecanol

| Lipid species | $^3$H incorporated per μg of protein, cpm | | |
|---|---|---|---|
| | CHO-K1 | ZR-82 | FAA.1 |
| Phospholipids | 462.1 | 137.2 | 31.2 |
| Fatty alcohol | 57.7 | 66.2 | 173.7 |
| Free fatty acid | 5.0 | 3.3 | 1.0 |
| Triglyceride | 24.8 | 15.1 | 3.3 |
| Fatty aldehyde | <0.1 | <0.1 | <0.1 |
| Ether-triglyceride | 15.7 | 3.3 | 1.3 |
| Cholesterol ester | 13.1 | 13.0 | 5.7 |
| Total | 578.4 | 238.2 | 216.3 |

Total lipids were extracted from cells that had been labeled for 3 hr (no "wash cycle") with 2 μM [9,10-$^3$H]hexadecanol (see Fig. 2). Lipids were separated by TLC with *n*-hexane/ethyl ether/acetic acid, 80:20:1 (vol/vol), as the solvent system. The individual neutral lipid species were localized by comigration with known standards. Neutral lipid bands and the phospholipid band (origin) were individually scraped from the TLC plate into a scintillation vial containing 1 ml of methanol, and the radioactivity was determined by scintillation spectrometry after adding 10 ml of scintillation fluid. In the solvent system described above, triglyceride and fatty aldehyde comigrated. To separate the two lipid classes, additional samples were run on TLC in *n*-hexane, followed by a second development (in the same dimension) in benzene. Data are from a representative experiment, and each value is the average of duplicate determinations, which did not vary by >10%.

of this product. Alternatively, another acyl donor, such as that described for retinyl ester formation (29), may be involved.

Regardless of the assay conditions, FAA.1 cell homogenates showed greatly reduced levels of FAO activity (Fig. 3). Conversion of hexadecanol to the fatty acid was reduced by 84%–88% in FAA.1 homogenates (Table 3). Wax ester formation was unaltered, and there was typically a 2-fold increase in aldehyde labeling (10% of the radioactive products in CHO-K1 cells). Mixing of homogenates from FAA.1 and ZR-82 cells resulted in intermediate FAO activities, suggesting that a trans-acting inhibitor was not present in FAA.1 cells (not shown).

The ability of FAA.1 homogenates to form aldehyde but not the fatty acid suggested a defect in a long-chain fatty aldehyde dehydrogenase, a component of FAO activity. Indeed, long-chain fatty aldehyde dehydrogenase activity in FAA.1 cell homogenates was reduced to 5–7% of the activity measured in the parent strain homogenates (Table 3).

**Fatty Alcohol Levels Are Increased in FAA.1 Cells.** The inability of FAA.1 cells to metabolize fatty alcohols resulted in marked increases in the cellular levels of this lipid (Table 4). When fatty alcohol levels were quantitated in cells grown in serum-supplemented medium (F12c), FAA.1 cells showed a 40- to 90-fold increase in the content of both hexadecanol and octadecanol compared with CHO-K1 cells, while octadecenol (18:1) levels were increased >300 times wild-type levels. Tetradecanol (14:0) levels were increased only 4-fold

Table 2. Oxidation of fatty alcohol to fatty acid in intact CHO-K1, ZR-82, and FAA.1 cells

| Cell line | Fatty acid formed from [9,10-$^3$H]hexadecanol, cpm/μg of cell protein |
|---|---|
| CHO-K1 | 1368 ± 35 |
| ZR-82 | 1354 ± 56 |
| FAA.1 | 95 ± 6 |

Cellular lipid extracts were prepared, and fatty acids were isolated by TLC as described. Each value is the average ± SD of three samples.

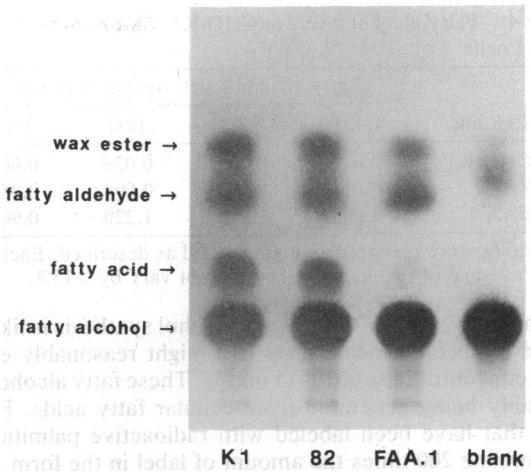


FIG. 3. Production of fatty aldehyde and fatty acid by whole-cell homogenates from CHO-K1, ZR-82, and FAA.1 cells. Preparation of assay mixtures, extractions, TLC analysis, and identification of products are described in *Experimental Procedures*.

in FAA.1 cells. Cumulatively, this represents a 60-fold increase in free fatty alcohol levels over levels in CHO-K1 cells. ZR-82 cells displayed a more moderate increase in fatty alcohol levels when compared with CHO-K1 cells (≈3.5-fold).

## DISCUSSION

We have isolated a mutant CHO cell line that is unable to take up and metabolize exogenous hexadecanol because of combined defects in ether lipid biosynthesis (21) and long-chain fatty alcohol oxidation. The mutant cells were defective in the ability to convert fatty aldehyde to fatty acid, yet fatty aldehyde was formed, suggesting that the oxidation of fatty alcohol to fatty acid is the result of two separate enzymes or enzyme systems, with an aldehyde as an intermediate. This is consistent with work by Ichihara *et al.* (12, 13), who, using a 12-carbon substrate, have been able to separate two activities from rabbit mucosa: a fatty alcohol dehydrogenase and a fatty aldehyde dehydrogenase. Our ability to obtain long-chain fatty alcohol oxidation mutants from the parent strain using only one round of mutagenesis and selection suggests that only one enzyme or enzyme system is responsible for the conversion of hexadecanol to fatty acid in CHO cells. FAA.1 cells may be used to define the importance of FAO activity in the metabolism of other substrates.

FAA.1 cells are unique in that they are almost completely unable to metabolize long-chain fatty alcohols, resulting in a 60-fold increase in cellular levels of this lipid. When using the values for fatty alcohol levels from Table 4 and assuming a value of 356 nmol of phospholipid per mg of cell protein for the CHO cells (30), the fatty alcohol/phospholipid ratio

Table 3. FAO and fatty aldehyde dehydrogenase activities in whole-cell homogenates

| Cell line | Specific activity, pmol/min per mg of protein | |
|---|---|---|
| | FAO | Fatty aldehyde dehydrogenase |
| CHO-K1 | 23.7 ± 5.0 | 1567 ± 159 |
| ZR-82 | 17.8 ± 2.1 | 1155 ± 74 |
| FAA.1 | 2.9 ± 0.4 | 48 ± 48 |

Reaction mixtures were prepared and assays were conducted as described. Each value is the average ± SD from three separate experiments.

Table 4. Fatty alcohol levels in CHO-K1, ZR-82, and FAA.1 cells

| Cell line | Fatty alcohol level, μg/mg of protein | | | |
|---|---|---|---|---|
| | 14:0 | 16:0 | 18:0 | 18:1 |
| CHO-K1 | 0.019 | 0.021 | 0.029 | 0.003 |
| ZR-82 | 0.013 | 0.089 | 0.094 | 0.054 |
| FAA.1 | 0.068 | 1.970 | 1.220 | 0.962 |

Samples were prepared and quantitated as described. Each value is the average of two samples and did not vary by >15%.

reaches ≈4.7 mol %. Since fatty alcohol synthesis is likely to occur in specific membranes, we might reasonably expect local concentrations of 10–15 mol %. These fatty alcohols are probably being generated from cellular fatty acids. FAA.1 cells that have been labeled with radioactive palmitic acid accumulate 200 times the amount of label in the form of the free fatty alcohol (4% of the total cellular radioactivity) when compared with CHO-K1 cells (P.F.J. and R.A.Z., unpublished data). The FAA.1 cells are apparently able to reduce fatty acids to fatty alcohols but are unable to metabolize the latter. Worth noting is our inability to detect significant accumulations of fatty aldehyde in FAA.1 cells (Table 1). This is not surprising when one considers the reactive nature of the aldehyde moiety. Any excess fatty aldehyde is probably reduced back to fatty alcohol (1) or reacts with protein side chains. Covalent modification of proteins by aldehydes has been demonstrated (31, 32).

FAA.1 cells are biochemically similar to fibroblasts from human patients suffering from a genetic disorder, Sjögren–Larsson syndrome (33). Patients with this disorder are characterized by mental retardation, spasticity, and severe congenital ichthyosis (17, 33). Recently, Rizzo *et al.* (18) have shown that fibroblasts from Sjögren–Larsson patients are defective in FAO activity; the specific lesion appears to be the inability to oxidize the fatty aldehyde (W.B.R., unpublished data). Also, cultured fibroblasts (18) and blood plasma (34) from patients suffering from Sjögren–Larsson syndrome have higher levels of fatty alcohol (3- to 6-fold more than controls). The fatty alcohol accumulation observed in the Sjögren–Larsson fibroblasts (18) is much less severe than that observed in FAA.1 cells, probably because they can form ether lipids (34). With the exception of blood plasma (34), fatty alcohol levels have not been determined in these patients' tissues. It is possible that, under certain dietary regimes, long-chain fatty alcohols could accumulate to very high levels in tissues, especially in tissues that do not synthesize much ether lipid (35). A buildup of fatty alcohols in the cellular membranes may alter membrane function. Alternatively, covalent modification of proteins by fatty aldehydes could play some role.

The relationship between decreased FAO activity and the symptoms associated with Sjögren–Larsson syndrome is not known. Also unknown is the number of genetic loci (complementation groups) associated with this disorder. The FAA.1 somatic cell mutant or other cell lines isolated by this selection protocol should be useful in identifying genetic lesions involved in Sjögren–Larsson syndrome and elucidating the roles that fatty alcohol and fatty aldehyde metabolism play in the disease.

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