An Animal Cell Mutant with a Deficiency in Acyl/Alkyl-dihydroxyacetone-phosphate Reductase Activity

EFFECTS ON THE BIOSYNTHESIS OF ETHER-LINKED AND DIACYL GLYCEROLIPIDS*

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In the accompanying paper (James, P. F., and Zoeller, R. A. (1997) J. Biol. Chem. 272, 23532–23539), we reported the isolation of a series of mutants from the fibroblast-like cell line, CHO-K1, that are deficient in the incorporation of the long chain fatty alcohol, hexadecanol, into complex lipids. All but one of these mutants, FAA.K1B, were deficient in long-chain-fatty alcohol oxidase (FAO) activity. We have further characterized this FAO* isolate. FAA.K1B cells displayed a 40% decrease in \([9,10-\text{H}]\)hexadecanol uptake when compared with the parent strain. Although incorporation of hexadecanol into the phospholipid fraction was decreased by 52%, the cells accumulated label in alkylglycerol (20-fold over wild type). The increase in 1-alkylglycerol labeling corresponded to a 4-fold increase in alkylglycerol mass. Short term labeling with \(\text{\textsuperscript{32}P}\) showed a 45–50% decrease in overall phospholipid biosynthesis in FAA.K1B. Both diacyl- and ether-linked species were affected, suggesting a general defect in phospholipid biosynthesis. Mutant cells were able to partially compensate for the decreased biosynthesis by decreasing the turnover of the phospholipid pools. The primary lesion in FAA.K1B was identified as a 95% reduction in acylalkyl-dihydoxyacetone-phosphate reductase activity. Whole cell homogenates from FAA.K1B were unable to reduce either acyl-dihydoxyacetate phosphate (DHAP) or alkyl-DHAP, supporting the notion that the reduction of these two compounds is catalyzed by a single enzyme. These data suggest that the biosynthesis of diacyl phospholipids, in Chinese hamster ovary cells, begins with the acylation of dihydoxyacetate phosphate as well as glycerol-3-phosphate and that the “DHAP pathway” contributes significantly to diacyl glycerolipid biosynthesis. Also, the severe reduction in acylalkyl-DHAP reductase activity in FAA.K1B resulted in only a moderate decrease in ether lipid biosynthesis. These latter data together with the observed increase in alkylglycerol levels support the existence of a shunt pathway that is able to partially bypass the enzymatic lesion.

The formation of phosphatidic acid is required for the formation of diacyl glycerolipids in both bacterial and animal cells. In bacterial systems, this is initiated with the reduction of dihydroxyacetone phosphate (DHAP),1 a product of glycolysis. The resulting sn-glycero-3-phosphate is acylated sequentially at the sn-1 and sn-2 positions to form phosphatidic acid. In animal cells, dihydroxyacetone phosphate (DHAP) can be acylated directly. In this “DHAP pathway,” the ketone at the sn-2 position of the resulting sn-1-acyl-DHAP must be reduced, by acyl/alkyl-DHAP reductase (1), prior to further acylation. Purified acyl/alkyl-DHAP reductase also catalyzes the reduction of the ether-linked sn-1-alkyl-DHAP (2), a reaction that is required in the synthesis of ether-linked glycerolipids, such as plasmalogen and glycerol ether diesters (1). While the acylation of DHAP is accepted as the first step in ether-linked glycerolipids, the importance of the DHAP pathway and acyl/alkyl-DHAP reductase in the synthesis of diacyl glycerolipids is in question. It has been estimated that this route is a significant contributor to diacyl glycerolipid synthesis (3, 4), and other studies suggest that this pathway plays a minor role (5–7).

Acyl/alkyl-DHAP reductase is a membrane-bound enzyme that has been localized to the cytosolic face of both the peroxisomal (8) and microsomal membranes (9). Interestingly, in both human (10, 11) and rodent (12, 13) cells that do not assemble peroxisomes properly, other activities associated with the peroxisomal membrane, such as DHAP acyltransferase and alkyl-DHAP synthase, are severely decreased, but acyl/alkyl-DHAP reductase activity remains unaffected. Although it has been purified to homogeneity (2), the amino acid sequence for this enzyme has not been determined, the gene has not been isolated, and there is no information concerning how this enzyme is targeted.

We have isolated a mutant, FAA.K1B, that displays a general defect in phospholipid biosynthesis and an increase in 1-alkylglycerol levels. Further characterization showed this cell line to be defective in acylalkyl-DHAP reductase activity. The observation that these mutants were reduced in the biosynthesis of diacyl phospholipids suggests that the DHAP pathway plays a significant role in the production of these lipids in

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1 The abbreviations used are: DHAP, dihydroxyacetone-phosphate; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary; AG, sn-1-alkylglycerol.

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CHO cells. These cells will be useful in investigating the inter-
relationship between diacyl and ether glycerolipid biosyn-
sis. This cell line may serve as a tool to isolate a gene that is
responsible for acyl/alkyl-DHAP activity and to examine intra-
cellular targeting of this enzyme.

EXPERIMENTAL PROCEDURES

Materials—[1-1H]Ethanolamine (28.8 Ci/mmol) was purchased from
Amersham Corp. [methyl-3H]Thymidine (84 Ci/mmol) was purchased from
ICN Radiochemicals (Irvine, CA). [9,10-3H]Hexadecanoic acid (60
Ci/mmol), [32P]ATP (9000 Ci/mmol), [γ-32P]ATP (30 Ci/mmol), [1-32P]
GTP, [3H]cholesterol, [1-3H]ether lipid standards, [1-3H]glycero-
phosphocholine, [1-3H]phosphatidylethanolamine, and [9,10-
3H]hexadecanol were purchased from the American Type
Culture Collection (Rockville, MD). FAA.K1B is a variant of CHO-K1 that was isolated from a population of cells defec-
tive in the ability to take up long chain fatty alcohol.

Cells and Culture Conditions—CHO-K1 (wild-type) cells were ob-
tained from the American Type Culture Collection (Rockville, MD).

FAA.K1B is a variant of CHO-K1 that was isolated from a population of cells that were defective in the ability to take up long chain fatty alcohol.

Acyl-DHAP and alkyl-DHAP (hexadecyl-DHAP) were prepared as
described by Hajra et al. (19). All other biochemicals were purchased
from Sigma unless otherwise specified.

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described by Hajra et al. (19). All other biochemicals were purchased
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Alkyl-DHAP synthase activity was assayed by measuring the incorporation of \([1-\text{H}]\)hexadecanol into alkyl-DHAP in the presence of 1-acyl-DHAP, as described by Davis and Hajra (14). Acyl/alkyl-DHAP reductase was monitored by measuring the incorporation of label from B-[4-\text{H}]NADPH into chloroform-soluble counts in the presence of 1-O-hexadecyl-DHAP (30) or 1-palmitoyl-DHAP (2).

Ethanolamine Labeling of Phospholipids—Cells were plated into sterile glass scintillation vials at 3 \times 10^5 cells/vial and allowed to attach overnight at 37 °C. The cells were then incubated for 2 or 4 h at 37 °C in growth medium containing [1-\text{H}]ethanolamine (approximately 1 \muCi/\muL). Labeling medium was removed, and the cellular lipids were recovered from the monolayers, as described above, for fatty alcohol uptake studies. The labeled phospholipids were separated using a double-development, single-dimension TLC system (31). This separated the labeled phospholipids into three bands as follows: band 1, plasmenylethanolamine; band 2, phosphatidylethanolamine; and band 3, and minor, unidentified lipid that traveled near the solvent front. The bands of interest were localized by fluorography and quantitated by liquid scintillation spectrometry as described above. The majority (>95%) of the label was found in bands 1 and 2. When the cells were supplemented with 1-hexadecylglycerol (alkylglycerol; AG), this compound was added to the labeling medium from a 20 mM stock (in ethanol). The medium was sonicated for 5 min in a sonicating bath just prior to addition of the labeling medium.

DNA and Protein Synthesis—The rate of DNA and protein synthesis was assayed by measuring the rate of incorporation of [methyl-\text{H}]thymidine and [\text{35S}]methionine into trichloroacetic acid-insoluble material (32). Cells were plated into 24-well plates at 5 \times 10^5 cells/well and allowed to attach overnight at 37 °C. Either [\text{35S}]methionine (6 \muCi) or [methyl-\text{H}]thymidine (2.5 \muCi) were added to the wells, and the cells were incubated at 37 °C. After 3 h, medium was removed, and 1 ml of ice-cold 10% trichloroacetic acid was added. The cell monolayer was washed three times with ice-cold trichloroacetic acid followed by four washes with ice-cold ethanol:ether (3:1). After drying, 0.5 ml of 0.5 N NaOH was added to solubilize the cell material at 37 °C for 2 h. Aliquots were used for liquid scintillation counting.

RESULTS

Uptake and Distribution of \([9,10-\text{3H}]\)Hexadecanol into Complex Lipids—FAA.K1B was isolated from a mutagenized population of CHO-K1 that had survived a tritium suicide selection protocol designed to select cells that were less able to take up \([9,10-\text{H}]\)hexadecanol in stable pools of lipid (20). All, except FAA.K1B, were characterized as having a defect in fatty-alcohol oxidase activity.

FAA.K1B was significantly reduced in its ability to take up hexadecanol from the medium (Fig. 1), displaying a 40% decrease when compared with CHO-K1. The metabolic fate of hexadecanol that was taken up by FAA.K1B was examined. Following 3 h of labeling the cells with \([9,10-\text{H}]\)hexadecanol, the lipids were extracted, and the individual lipid species were separated using thin layer chromatography (Fig. 2). Quantitation (Table I) showed that labeling of every phospholipid class was reduced in FAA.K1B, particularly in the choline phospholipids (phosphatidylcholine). Incorporation of hexadecanol into the sn-1 position of the ether-linked phospholipid, plasmenylethanolamine (observed as a fatty aldehyde upon \text{HgCl}_2 treatment), was also greatly reduced. Within the neutral lipids, cholesterol ester and triglyceride labeling was similar in FAA.K1B and wild-type cells, but the incorporation of label into glycerol ether diesters was severely reduced in these mutants.

The most striking anomaly in the labeling pattern of FAA.K1B was the appearance of a labeled lipid species that traveled with the neutral lipids (Fig. 2). The labeling in this lipid species was increased in FAA.K1B by 21–22-fold over wild-type cells and represented approximately 40% of the label incorporated into complex lipids in FAA.K1B (Table I). Based on several criteria, we identified this lipid species as alkylglycerol. These criteria included the following: 1) co-migration of the labeled compound with authentic sn-1-hexadecanoylglycerol on TLC using four solvent systems (see "Experimental Procedures"); 2) resistance to mild-base hydrolysis; and 3) labeling with hexadecanol but not hexadecanoic acid or \text{32P}_1 (not shown). Isopropylidene derivatization of the neutral lipid fraction followed by analysis by gas-liquid chromatography showed a 4-fold increase in alkylglycerol levels in FAA.K1B over wild-type cells (Table II). There was only a slight increase in the level of monoacylglycerols.

Phospholipid Biosynthesis, Turnover, and Composition—Short term labeling of cells, using \text{32P}_1, showed a 45–50% decrease in the rate of phospholipid biosynthesis in FAA.K1B compared with wild-type cells (Fig. 3). Examination of the individual phospholipid species showed that the labeling of all phospholipid species, except phosphatidylethanolamine, was...
followed by a second development in isopropyl ether:acetic acid (96:4). Plasmenylethanolamine (Plasmenyleth.) aldehyde is the result of HgCl₂ ether:acetic acid (80:20:1). To isolate AG a fraction of the extracted lipids were developed first, using two-dimensional thin layer chromatography as shown in Fig. 2. All neutral lipids, except alkylglycerol (AG), were separated using (21), the individual lipid species were isolated using three different systems on thin layer chromatography. Phospholipids were separated using phospholipid composition revealed significant, although minor, decreases in phosphatidylcholine, plasmenylethanolamine, and phosphatidylinositol and an increase in the relative amounts of sphingomyelin and phosphatidylethanolamine. 

The turnover of phospholipid in FAA.K1B was also examined. When cells were labeled for only 12 h prior to chase, there was no loss of label from the newly formed phospholipids over the first 10 h (Fig. 4A). There was no significant difference between wild-type and mutant cells with respect to loss of label throughout the course of the 26 h following labeling. When cells were labeled for many generations to steady-state label the phospholipid pools, we observed a significantly slower turnover of phospholipid in the mutant cells (Fig. 4B).

**Identification of the Enzymatic Lesion in FAA.K1B, as a Deficiency in Acyl/Alkyl-dihydroxyacetone-phosphate Reductase**—The [32P] labeling patterns observed in FAA.K1B (Table III) suggested that the synthesis of both diacyl phospholipids and ether-linked phospholipids was affected in this cell line. The biosynthetic pathways for these two types of phospholipid share certain steps and they also contain steps that are unique to each. The first two steps in ether lipid biosynthesis are catalyzed by the peroxisomal enzymes, peroxisomal dihydroxyacetone-phosphate acyltransferase and alkyl-DHAP synthase (33). These two activities were normal in whole cell homogenates from FAA.K1B (Table V). DHAP acyltransferase was normal whether it was assayed at pH 5.5 or pH 7.4. The acylation of glycerol-3-phosphate, by glycerol-3-phosphate acyltransferase, was also normal in FAA.K1B homogenates. The third step in the pathway is catalyzed by alkyl/acyl-DHAP reductase, which reduces the ketone group at the sn-2 position of the backbone. This activity was reduced to 5% of wild-type values in whole cell homogenates of FAA.K1B. This reduction was observed using either acyl-DHAP or alkyl-DHAP as a substrate.

To examine steps downstream of the reductase, we bypassed the reductase lesion with supplementation of the medium with sn-1-hexadecylglycerol (alkylglycerol; AG). This compound readily enters cells and is phosphorylated to enter the ether lipid pathway as 1-alkyl-2-lyso-sn-glycerol-3-phosphate, the product of the reductase. Assuming steps downstream of the reductase are intact, AG supplementation should result in bypass of the reductase lesion and restoration of ether lipid biosynthesis. AG supplementation has been used previously (34) to identify lesions downstream in the pathway for plasmenylethanolamine biosynthesis in other mutants. As shown in Fig. 5, the labeling of plasmenylethanolamine was reduced by approximately 50% in FAA.K1B, whereas the labeling of phosphatidylethanolamine was comparable to wild type. These results agree well with the labeling pattern observed when using [32P] (Table III). The addition of AG to the medium restored the labeling of plasmenylethanolamine to near wild-type values, suggesting that the steps downstream of acyl/alkyl-DHAP re-

**TABLE I**

| Species | CHO-K1 | FAA.K1B | CHO-K1 | FAA.K1B |
|---------|--------|---------|--------|---------|
| SM      | 77 ± 8 | 215 ± 27| 21 ± 2 | 73 ± 22 |
| PC      | 1,289 ± 150 | 21 ± 2 | 18 ± 1 | 73 ± 22 |
| PI      | 66 ± 8 | 15 ± 3  | 32 ± 1 | 73 ± 22 |
| PS      | 197 ± 16| 73 ± 22| 21 ± 2 | 73 ± 22 |
| PE      | 432 ± 46| 127 ± 16| 127 ± 16| 73 ± 22 |
| Fatty alcohol | 24 ± 1 | 523 ± 12| 184 ± 2 | 161 ± 8 |
| AG      | 184 ± 2 | 387 ± 13| 73 ± 22| 73 ± 22 |
| TAG     | 125 ± 3 | 125 ± 3 | 125 ± 3| 73 ± 22 |
| GEDE    | 362 ± 8 | 102 ± 5 | 102 ± 5| 73 ± 22 |
| CE      | 787 ± 60| 56 ± 10 | 56 ± 10| 73 ± 22 |

**TABLE II**

| Species | Alkylglycerol levels | Monoacylglycerol levels |
|---------|----------------------|-------------------------|
| CHO-K1  | pmoles/10⁶ cells     | pmoles/10⁶ cells        |
| FAA.K1B |                      |                         |
| 14:0    | 17.1 ± 5.2           | 28.6 ± 10.0             |
| 16:0    | 353.7 ± 110.7        | 343.7 ± 98.6            |
| 18:0    | 232.1 ± 101.5        | 108.8 ± 12.4            |
| 18:1    | 50.1 ± 4.9           | 10.8 ± 12.4             |
| Total   | 436.8 ± 138.0        | 503.8 ± 138.0           |

**FIG. 3. Phospholipid biosynthesis in CHO-K1 and FAA.K1B.** Cells were labeled with [32P] at 37 °C followed by extraction of the lipids as described under “Experimental Procedures.” An aliquot of the chloroform-soluble material was used for scintillation spectrometry. All values represent the average ± S.D. of three separate samples.

**TABLE III**

| Radioactivity | CHO-K1 | FAA.K1B |
|---------------|--------|---------|
| SM            | 77 ± 8 | 215 ± 27| 21 ± 2 | 73 ± 22 |
| PC            | 1,289 ± 150 | 21 ± 2 | 18 ± 1 | 73 ± 22 |
| PI            | 66 ± 8 | 15 ± 3  | 32 ± 1 | 73 ± 22 |
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Somatic Cell Mutant Deficient in Glycerolipid Biosynthesis

Distribution of label from [9,10-²H]hexadecanol into individual phospholipid and neutral lipid species

Cells were labeled with 5 μM [9,10-²H]hexadecanol for 3 h at 37 °C as described under “Experimental Procedures.” After extraction of the lipids (21), the individual lipid species were isolated using three different systems on thin layer chromatography. Phospholipids were separated using two-dimensional thin layer chromatography as shown in Fig. 2. All neutral lipids, except alkylglycerol (AG), were separated using n-hexane:diethyl ether:acetic acid (90:20:1). To isolate AG a fraction of the extracted lipids were developed first, using n-hexane:diethyl ether:acetic acid (20:80:1) followed by a second development in isopropl ether:acetic acid (96:4). Plasmenylethanolamine (Plasmenyleth.) aldehyde is the result of HgCl₂ cleavage of the vinyl ether linkage between TLC runs during the two-dimensional TLC (see legend to Fig. 2). Abbreviations are: SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; AG, sn-1-alkylglycerols; TAG, triacylglycerol; GEDE, glycerol ether diester; CE, cholesterol ester. All values represent the average ± S.D. of three samples.
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**TABLE III**

Short term $^{32}$P, labeling of individual phospholipid species

Cells were labeled for 5 h with $^{32}$P, at 37 °C as described under "Experimental Procedures." After extraction of the lipids (21), the individual lipid species were isolated using two-dimensional thin layer chromatography. Abbreviations are: SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; pPE, plasmalogenethanolamine. All values represent the average ± S.D. of three samples. The values in parentheses represent the percent of the value for CHO-K1.

|          | Radioactivity |
|----------|---------------|
|          | cpm/μg protein |
| CHO-K1   |               |
| SM       | 23 ± 5        |
| PC       | 1,016 ± 107   |
| PI       | 186 ± 36      |
| PS       | 14 ± 2        |
| PE       | 184 ± 14      |
| pPE      | 134 ± 5       |
| FAA.K1B  |               |
| SM       | 8 ± 2 (35)    |
| PC       | 440 ± 59 (43) |
| PI       | 151 ± 2 (81)  |
| PS       | 9 ± 3 (64)    |
| PE       | 172 ± 17 (93) |
| pPE      | 59 ± 5 (44)   |

**TABLE IV**

Phospholipid composition of CHO-K1 and FAA.K1B

Phospholipids were extracted and the individual lipid species were isolated by two-dimensional TLC as described under "Experimental Procedures." Phospholipid mass was determined using the method of Rouser et al. (25). All values represent the average ± S.D. of three separate samples. For abbreviations, see legend to Table III.

| Percent of total phospholipid | CHO-K1 | FAA.K1B |
|-------------------------------|--------|---------|
| SM                            | 9.6 ± 1.1 | 14.4 ± 2.6 |
| PC                            | 54.9 ± 2.4 | 49.4 ± 5.1 |
| PI                            | 7.3 ± 0.9  | 4.9 ± 0.7  |
| PS                            | 4.6 ± 1.1  | 5.8 ± 0.5  |
| PE                            | 12.3 ± 2.4 | 5.4 ± 2.0  |
| pPE                           | 8.2 ± 0.4  | 16.7 ± 2.1 |
| Other                         | 3.1 ± 0.9  | 6.0 ± 0.8  |
| PL content †                  | 3.81 ± 0.15 |

- Values are presented as μg of phosphorous (P)/mg of cellular protein.

**DISCUSSION**

FAA.K1B is a novel mutant that displayed a severe defect in acyl/alkyl-DHAP reductase activity. Lesions in peroxisomal DHAP acyltransferase activity (34) and alkyl-DHAP synthase (12), which catalyze the first and second steps, respectively, in ether lipid biosynthesis, resulted in much more dramatic effects on ether lipid biosynthesis. The observed accumulation of alkylglycerol in FAA.K1B suggests that these cells were able to partially bypass the reductase lesion through a salvage pathway. 1-Alkyl-DHAP, not reduced by the acyl/alkyl-DHAP reductase, would be dephosphorylated, followed by reduction of the resulting 1-alkyl dihydroxyacetone, by a separate reductase, to form 1-alkylglycerol. This could then be phosphorylated by a kinase to re-enter the biosynthetic pathway downstream of the acyl/alkyl-DHAP reductase as 1-alkyl-sn-glycero-3-phosphate. All of these activities have been demonstrated in animal cells (33). This salvage pathway, by generating alkylglycerols, could partially bypass the reductase lesion. The addition of high levels of exogenous alkylglycerol to the cells (Fig. 5) was able to more completely bypass the lesion.

Acyl/alkyl-DHAP reductase can also be used during the synthesis of diacyl glycerolipids (33). The biosynthesis of glycerolipids begins by the acylation of either glycerol-3-phosphate or dihydroxyacetone phosphate at the sn-1 carbon of the backbone. If glycerol-3-phosphate is the initial acyl acceptor, the sn-2 carbon is acylated next, to form phosphatidic acid. If DHAP is acylated (the DHAP pathway) the ketone at the sn-2 carbon must be reduced by the acyl/alkyl-DHAP reductase, prior to acylation. Although the role of the reductase in ether lipid biosynthesis is known, there has been some question concerning the importance of the reductase and the DHAP pathway for diacyl glycerolipid biosynthesis (3–7). The $^{32}$P labeling data (Table III) suggested that, for CHO cells, this pathway is significant. Overall, the rate of phospholipid biosynthesis was reduced by 45%. A decrease in ether lipid biosynthesis, alone, could not account for this decrease. In other ether lipid−mutants (12, 13), the decrease in ether lipid biosynthesis had no effect on the biosynthetic rates of the head group classes. In fact, the biosynthesis of the diacylated species was increased to compensate for the loss of the ether-linked species.

Although the synthesis of phosphatidylcholine was dramatically reduced in FAA.K1B, there was little effect on the biosynthesis of phosphatidylethanolamine (Table III). It is well

**FIG. 4. Phospholipid turnover.** The cellular phospholipids were labeled for 12 h (A) or 72 h (B) with $^{32}$P. Medium was removed and replaced with unlabeled growth medium. The lipids were extracted at the indicated times following fluid change, and the radioactivity associated with the lipid (chloroform-soluble counts) was determined as described under "Experimental Procedures." The data are expressed as the percent of lipid-associated radioactivity found at time = 0. All values represent the average ± S.D. of three separate samples. ●, CHO-K1; □, FAA.K1B.
Somatic Cell Mutant Deficient in Glycerolipid Biosynthesis

Whole cell homogenates were prepared and assays were performed as described under “Experimental Procedures.” Unless otherwise noted, all values represent the average ± S.D. of three homogenates.

![Table V](image)

| Strain     | DHAP acyltransferase pH 5.5 | pH 7.4 | G3P Acyltransferase | Alkyl-DHAP synthase | Acyl/Alkyl-DHAP reductase Alkyl-DHAP<sup>a</sup> | Acyl-DHAP<sup>b/c</sup> |
|------------|-----------------------------|--------|---------------------|---------------------|---------------------|----------------------|
| CHO-K1     | 0.66 ± 0.06                 | 1.44 ± 0.17 | 2.98 ± 0.17         | 0.112 ± 0.004       | 0.57 ± 0.06          | 0.65                 |
| FAA.K1B    | 0.59 ± 0.05                 | 1.20 ± 0.11 | 2.92 ± 0.12         | 0.121 ± 0.008       | 0.03 ± 0.01          | 0.04                 |

<sup>a</sup> Alkyl-DHAP used as the substrate.<br> <sup>b</sup> Acyl-DHAP used as the substrate.<br> <sup>c</sup> Values represent the averages of determinations using two separate samples and did not vary by more than 10%.

![Figure 5](image)

**TABLE VI**

Protein and DNA synthesis in CHO-K1 and FAA.K1B

Protein and DNA synthesis were monitored by measuring the incorporation of [35S]methionine and [3H]thymidine, respectively, into trichloroacetic acid (TCA)-insoluble material as described under “Experimental Procedures.” All values represent the average ± S.D. of three samples.

| Incorporation of label into TCA-insoluble material | [35S]Methionine cpm/mg protein | [3H]Thymidine cpm/mg protein |
|---------------------------------------------------|-------------------------------|-----------------------------|
| CHO-K1                                            | 12.6 ± 0.4                    | 2.89 ± 0.70                 |
| FAA.K1B                                           | 11.9 ± 1.6                    | 3.55 ± 0.25                 |

Also, the phospholipid content was only slightly affected. The mutant cells appear to have, at least partially, compensated for the biosynthetic lesion by decreasing phospholipid turnover (Fig. 4B). Decreased phospholipid turnover has been reported in other phospholipid biosynthesis mutants (38, 42). To some extent, the cells may have also been able to utilize phospholipid from the medium. Conditionally lethal mutants, displaying severe decreases in the biosynthesis of phosphatidylcholine and phosphatidylserine, could be rescued by supplementation of the medium with the affected phospholipids (38, 39, 43). Enough phospholipid may be found in the serum used to supplement the medium to compensate for a partial loss in phospholipid biosynthesis in FAA.K1B. We have observed that FAA.K1B is unable to grow in serum-free medium, but we have been unable to rescue this cell line with lipid supplementation.<sup>2</sup>

We cannot, therefore, identify any lipid or lipids as the crucial ingredient in the serum.

FAA.K1B is the first animal cell mutant to be described that is defective in a step in diacyl glycerolipid biosynthesis prior to the formation of phosphatidate, and it is defective in the first common step in the pathways for ether-linked and diacyl glycerolipid biosynthesis. Mutants such as these should help to examine the relationship between these two classes of lipid.

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<sup>2</sup> R. A. Zoeller, unpublished data.
Somatic Cell Mutant Deficient in Glycerolipid Biosynthesis

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