Effects of small amounts of pentadecan-2-one on the growth of *Clostridium butyricum*

J. R. Gilbertson, R. A. Gelman,[1] Teh-Hsing Chiu, L. I. Gilbertson,[2] and T. E. Knauer[3]

Department of Pharmacology and Physiology and Department of Microbiology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA 15261

Abstract Primary alcohols occur as trace lipids and are the only long-chain alcohol species present in *Clostridium butyricum.* Secondary alcohols do not occur physiologically in this microorganism. Exposure of these cells to the methyl ketone, pentadecan-2-one, results in a marked decrease in the primary alcohol content with the secondary alcohol, pentadecan-2-ol, becoming the major alcohol present. This change in lipid composition is associated with a significant decrease in growth rate that is proportional to the log of the pentadecan-2-one concentration of the incubation medium. When these cells are incubated with pentadecan-2-ol alone, growth is unaffected. Simultaneous exposure of the bacteria to pentadecan-2-one and a mixture of primary alcohols results in a partial relief of the growth inhibition observed with the ketone alone. These observations indicate that pentadecan-2-one inhibits the formation of primary alcohols that are important for normal growth of this bacterium.

Supplementary key words hexadecan-1-ol · octadecan-1-ol · octadecen-1-ol · pentadecan-2-ol · alk-1-enyl phosphoglyceride · lipid phosphorus

The presence of enzymes that catalyze the reduction of a fatty acyl CoA to a long-chain aldehyde and the aldehyde to the corresponding primary alcohol has been demonstrated in mammalian tissues (1–4). In mammals, long-chain primary alcohols play a unique metabolic role as the precursor of the ether moiety of the alkyl and alkenyl phosphoglycerides (5, 6). The methyl ketone, pentadecan-2-one, has been shown to be an effective competitive inhibitor of the mammalian long-chain aldehyde reductase (2) and to inhibit the growth of mammalian cells significantly if included in trace amounts in the culture medium (7).

Cl. butyricum also contains ether phosphoglycerides and readily incorporates fatty acids and long-chain aldehydes into the ether moiety of these phospholipids (8–11). In addition, enzymatic reduction of a long-chain acyl CoA to the corresponding primary alcohol through an aldehyde intermediate has been observed in this bacterium (9). Considering the biochemical similarity of the mammalian and bacterial systems, we investigated the effect of pentadecan-2-one on the growth rate and lipid metabolism of *Cl. butyricum.*

EXPERIMENTAL PROCEDURES

Chemicals

All solvents were reagent grade and were redistilled before use. The sources for the reagents used in the chromatographic procedures and the lipid standards have been published (1, 12). Bovine serum albumin, fraction V, was purchased from Sigma Chemical Co., St. Louis, MO.

Lipid analysis

The methods employed in the isolation, quantitation, and identification of individual lipid types have been described (1, 12). The total lipid extract was resolved into a neutral and a phospholipid fraction (13). The phospholipid fraction was analyzed for total lipid phosphorus and alkenyl phosphoglycerides (13). Total long-chain alcohols were isolated from the neutral lipid fraction and converted to acetoxy alkanes prior to gas–liquid chromatography. Identification of individual acetoxy alkanes was based on retention times relative to that of the acetoxy alkane of 1-nonadecanol and confirmed by mass spectrometry (12, 14).

Purification and solubilization of pentadecan-2-one and long-chain alcohols

The methyl ketone was purified as described (7). Pentadecan-2-ol, hexadecan-1-ol, octadecan-1-ol, and *cis*-9,10-octadecen-1-ol each gave only one spot on thin-layer chromatography and was at least 96% pure as judged by gas–liquid chromatography. Pentadecan-2-one was solubilized by sonication in 0.16 M NaCl

[1] Present address: Battelle Northwest Laboratories, Richland, WA.
[2] Present address: Rockefeller University, New York, NY.
[3] Present address: Medical College of Virginia, Richmond, VA.

Downloaded from www.jlr.org by guest, on June 19, 2012

containing 30 mg of bovine serum albumin per ml; it was then centrifuged and filtered through a 0.22 μM millipore filter prior to use (7). In other experiments the long-chain alcohols and pentadecan-2-one were dissolved in 95% ethanol and then rapidly injected into the culture medium.

Bacterial strains

Cl. butyricum (ATCC 6015) was maintained on Todd–Hewitt agar stabs supplemented with 0.17% L-ascorbic acid. *Escherichia coli* K-40 (a strain derived from K-12) was obtained from Dr. D. S. Feingold, Department of Microbiology, School of Medicine, University of Pittsburgh, Pittsburgh, PA.

Growth conditions

Cl. butyricum was grown anaerobically on L-ascorbic acid supplemented with Todd–Hewitt broth (Difco Laboratories, Detroit, MI) or fluid thioglycolate medium (Bio Quest Laboratories, Cockeysville, MD). For the large-scale propagation of cells, a 500-ml Erlenmyer flask containing 300 ml of nitrogen-equilibrated Todd–Hewitt broth was inoculated with two loopfulls of *Cl. butyricum* and incubated anaerobically at 37°C for 16 hr. Approximately 50 ml of this seed culture was then used to inoculate six 2-liter flasks containing 1.5 liters of culture medium either without (control cells) or with (treated cells) pentadecan-2-one (6.5 μg/ml). In all instances the same amount of albumin was present in the control and treated flasks. The cultures were incubated anaerobically at 37°C overnight and cells were harvested by centrifugation in a Sorvall RC-2B centrifuge at 8,000 *g* for 10 min. The cells (app. 1 g/liter) were washed twice with cold isotonic saline and collected by centrifugation prior to lipid analysis. For the acute growth experiments seed cultures were grown on fluid thioglycolate medium at 37°C overnight. Then, 0.2-ml aliquots were removed and added to a series of Klett tubes containing 7 ml of media with various concentrations of pentadecan-2-one. These cultures were incubated as before for different time intervals. Bacterial growth was assessed by measuring the change in transmittance with a Klett-Summerson colorimeter equipped with a #66 filter.

Escherichia Coli K-40 seed cultures were grown anaerobically at 37°C in nitrogen-equilibrated trypticase soy broth for 16 hr (12). At the end of this time 0.1-ml aliquots of the culture were added to Klett tubes containing the same medium. Bacterial growth was measured as above in the presence of 3.8, 9.9, and 19.9 μg of albumin-bound pentadecan-2-one. The growth response of the ketone-treated bacteria was compared to the growth of similar quantities of bacteria from the same culture grown in the presence of the same amount of albumin but in the absence of ketone.

The possibility that adding long-chain alcohols to the medium might reverse the growth inhibitory effects of the methyl ketone was evaluated by simultaneously incubating the bacteria in media containing 26.4 nmol of pentadecan-2-one per ml and different amounts of a mixture of primary long-chain alcohols. The molar ratio (35:50:15) of hexadecan-1-ol, octadecan-1-ol, and *cis*-9,10-octadecen-1-ol was similar to that observed naturally. The ketone and long-chain alcohols were dissolved in 95% ethanol and added by inserting the needle into the incubation medium and rapidly ejecting 40 μl of solution from a 100-μl syringe. A similar volume of 95% ethanol was added to the control cultures. The concentrations of ketone and long-chain alcohols present varied from 24.6 nmol per ml for pentadecan-2-one and 2.46 nmol per ml for long-chain alcohol, a molar ratio of 10:1, to a molar ratio of 1:4. At a molar ratio of 1:4 the pentadecan-2-one concentration was 24.6 nmol per ml.

RESULTS

As shown in **Fig. 1**, the growth of *Cl. butyricum* was inhibited when small amounts of pentadecan-2-one were present in the culture medium; a 4-fold decrease in growth rate was observed at a ketone concentration of 9.9 μg per ml. The insert in Fig. 1 is a plot of the growth response over the 4-hr interval (60–300 min) vs. the ketone concentration of the medium. From this plot it is apparent that the inhibition of growth is proportional to the log of the ketone concentration. The metabolic defect accounting for these results is not known. However, observed effects of methyl ketones on the metabolism of long-chain alcohols in mammalian tissues suggest a decrease in the long-chain alcohol and/or glycerol ether content of these cells as a contributing factor (7).

To test this proposal, bacteria were grown anaerobically in 2-liter flasks in the presence and absence of pentadecan-2-one. The long-chain alcohol, alkenyl phosphoglycerols, and lipid phosphorus content of the ketone-treated and untreated cells were determined (**Table 1**). From these results and the compositional data presented in Table 2, it is apparent that when pentadecan-2-one was added to the medium the pentadecan-2-ol content increased significantly but the alkenyl phosphoglyceride and total phospholipid contents were not affected.

Qualitative analysis of the long-chain alcohols from the control cells (**Table 2**) indicated that the major components were the primary alcohols hexadecan-1-ol,

Downloaded from www.jlr.org by guest, on June 19, 2012

ASBMB JOURNAL OF LIPID RESEARCH

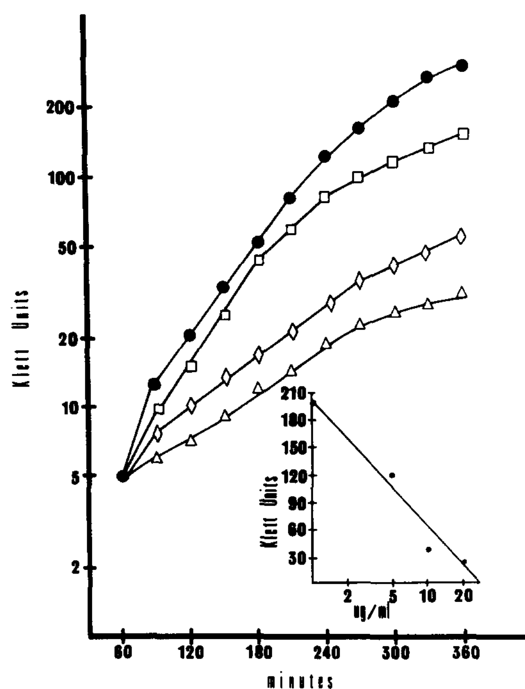

Fig. 1. Effect of albumin-bound pentadecan-2-one on the growth rate of *Cl. butyricum.* Klett tubes containing 7 ml of culture medium were innoculated with two loopfuls of *Cl. butyricum* and incubated overnight at 37°C. Aliquots, 0.2 ml, were removed from this seed culture and added to Klett tubes containing fresh medium having the following concentrations of pentadecan-2-one (µg per ml): ●, 0.0; □, 3.8; ◇, 9.9; and △, 19.9. The growth rate was assessed by the change in transmittance when the bacteria were incubated over a 7-hr interval at 37°C. Transmittance was measured with a Klett-Summerson colorimeter equipped with a #66 filter. The insert is a semi-log plot of the growth response in Klett units over the 4-hr interval, 60 to 300 min, vs. the pentadecan-2-one concentration of the medium.

octadecan-1-ol, and octadecen-1-ol. Secondary alcohols were not detected and cyclopropane alcohols were present only as minor components. In the ketone-treated cells, a marked change occurred in the long-chain alcohol composition. The secondary alcohol, pentadecan-2-ol, became the major alcohol present and accounted for at least 90% of the total. Since this moiety was detected only in cells exposed to the ketone, the cells apparently took up pentadecan-2-one from the medium and reduced it in situ to the corresponding secondary alcohol. The large decrease in the proportion of primary alcohols in the ketone-treated cells, as compared to the controls, indicated that the ketone had inhibited primary alcohol biosynthesis. Thus the increase in long-chain alcohols in the ketone-treated cells as compared to the control does not reflect a superimposition of secondary alcohols on a normal background of primary alcohols.

Since pentadecan-2-ol was present only in the ketone-treated cells, the possibility existed that the secondary alcohol was affecting cell growth. This was evaluated by incubating *Cl. butyricum* with pentadecan-2-ol at the same concentrations and under the same conditions as employed with pentadecan-2-one. Under these conditions the pentadecan-2-ol-treated cells grew at the same rate as cells grown in untreated media, indicating that the secondary alcohol did not affect cell growth.

As there was a marked decrease in the concentration of primary long-chain alcohols in the ketone-

TABLE 1. Effect of the inclusion of pentadecan-2-one in the incubation medium on the long-chain alcohol, alkenyl phosphoglyceride, and lipid phosphorus content of *Clostridium butyricum*

		Phospholipid	
Experimental Condition[b]	Total Long Chain Alcohols	Alkenyl Ethers	Lipid P
	mmol/g bacteria[a]	*µmol/g bacteria*[a]	
Control			
I	39.3	0.24	5.30
II	27.3	0.35	5.79
III	25.6	0.17	3.47
IV	46.2	0.44	3.64
Ketone-treated			
I	82.5	0.29	5.49
II	116.2	0.41	4.24
III	131.3	0.32	3.77
IV	105.5	0.35	3.57

[a] Wet weight.
[b] The numbers I, II, III, and IV represent results from separate experiments. The ketone-treated cells were grown in the presence of 6.5 µg pentadecan-2-one per ml over a 16-hr interval at 37°C. Control cells were grown in the same Todd-Hewitt broth without ketone. In all flasks the same amount of albumin was present.

TABLE 2. Effect of exposure to pentadecan-2-one on the qualitative composition of the long-chain alcohols from *Clostridium butyricum*[a]

	Control				Ketone-Treated			
Designation[b]	I	II	III	IV	I	II	III	IV
12:0	12:0	4.9	2.0	1.5	3.5	1.2		
15:sec					95.5	90.0	92.6	93.7
14:0	6.2	4.8	4.0	6.4				
15:0	3.2							
16:0	30.5	39.0	38.9	37.3		7.0	3.4	1.0
17:cyc	7.4		4.2	5.1				
18:0	29.2	40.1	41.1	39.7			4.0	5.0
18:1	12.2	10.5	8.8	10.4				

[a] Long-chain alcohol content (wt%) of the control and pentadecan-2-one-treated cells represents qualitative composition of the total alcohols from the separate experiments (I-IV) whose quantitative values are reported in Table 1.
[b] Designation is carbon number: number of double bonds; cyc refers to the cyclopropane moiety and sec denotes the secondary alcohols. The designation of the long-chain alcohols was based on the chromatographic mobility of the corresponding acetoxy alkane relative to standards on GLC using a polar, SP2340, and nonpolar column OV-101 and confirmed by mass spectrometry of the acetoxy alkanes.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 3. Effect of pentadecan-2-one and long-chain alcohols on the growth of *Clostridium butyricum*[a]

Experiment	Incubation Time (hr)				
	1	3	5	7	24
Control (10)	3 ± 1	14 ± 6	51 ± 15	82 ± 9	171 ± 17
Ethanol-treated (9)	3 ± 1	11 ± 4	49 ± 14	94 ± 9	173 ± 19
Pentadecan-2-one nmol/ml					
24.6 (8)	2 ± 1	9 ± 2	29 ± 8	49 ± 6	113 ± 17
Long-chain alcohols nmol/ml					
16.4 (8)	3 ± 1	9 ± 2	52 ± 12	76 ± 14	188 ± 22
49.3 (8)	3 ± 1	9 ± 2	53 ± 15	71 ± 14	182 ± 25
Pentadecan-2-one to long-chain alcohols molar ratio					
10:1 (7)	5 ± 1	10 ± 1	38 ± 5	68 ± 4	
1:4 (4)	4 ± 1	17 ± 2	58 ± 2	81 ± 2	140 ± 8

[a] The incubation conditions are described in the text. Control cultures contained only thioglycolate media. Forty μl of 95% ethanol was added to the ethanol-treated cultures. The 40-μl volume of ethanol injected into the other cultures served as the vehicle to deliver pentadecan-2-one or the mixed long-chain alcohols (hexadecan-1-ol, octadecan-1-ol, and octadecen-1-ol).

treated cells, the importance of this lipid class to the growth of *Cl. butyricum* was evaluated. This was accomplished by growing the cells in the presence of only pentadecan-2-one, a mixture of only long-chain primary alcohols and pentadecan-2-one, and a mixture of primary alcohols. Growth was compared to that observed in untreated media and in media containing 40 μl of 95% ethanol, the solvent utilized to deliver the lipid moieties (**Table 3**). The data indicate that addition of 95% ethanol or primary alcohols had no affect on cell growth. However, bacteria grown in the presence of 24.6 nmol per ml of pentadecan-2-one had a growth response that was significantly less than that of the control cells ($P > 0.05$) at all incubation intervals after 1 hr.

The growth response of the control cells was also compared to that of cells grown in the presence of both pentadecan-2-one and a mixture of long-chain primary alcohols. Cells cultured in media containing both ketone and primary alcohol at a molar ratio of 10:1 had a growth response significantly less than the controls ($P < 0.05$) after a 3-hr incubation. When the molar ratio was 1:4, a significant decrease in growth was not noted until after 24 hr.

The growth response of cells exposed to only pentadecan-2-one was also compared to that of cells simultaneously treated with both the ketone and the mixture of primary alcohols. In each instance, after a 5-hr incubation, the growth response of the cells treated with the mixture of primary alcohols was significantly greater ($P > 0.05$) than that of cells exposed to only the ketone, indicating that the long-chain primary alcohols can partially reverse the growth inhibition induced by pentadecan-2-one.

In the previous study a mixture of primary alcohols had been used; thus it was impossible to evaluate the effect of an individual primary alcohol. To determine whether one primary alcohol was more active than another, *Cl. butyricum* was grown in the presence of each alcohol with and without pentadecan-2-one (**Table 4**). Again, treatment with pentadecan-2-one (24.6 nmol per ml) effectively inhibited the growth of *Cl. butyricum* ($P < 0.05$), while the presence of the individual primary alcohols alone had no affect on bacterial growth. When pentadecan-2-one was added to the culture medium along with only one primary alcohol, the growth response varied with the primary alcohol used. Treatment with hexadecan-1-ol and pentadecan-2-one gave the same response as that observed with the ketone alone. However, in the presence of the two 18-carbon alcohols the bacterial growth rate after 5 hr was significantly greater ($P < 0.05$) than that of the cells treated with pentadecan-2-one. This response indicates that the 18-carbon alcohols are the moieties effective in partially restoring bacterial growth.

That the primary alcohol could not completely reverse the inhibition caused by pentadecan-2-one treatment is indicated by the observation that growth of the control cultures and those treated with only primary alcohols are significantly different ($P < 0.05$)

TABLE 4. Effect of hexadecan-1-ol, octadecan-1-ol, and octadecen-1-ol on the growth response of *Clostridium butyricum* treated with pentadecan-2-one[a]

	Incubation Time (hr)				
	1	3	5	7	24
Control	4 ± 4[b]	33 ± 6	74 ± 2	100 ± 2	182 ± 9
Pentadecan-2-one	6 ± 3	23 ± 3	50 ± 4	64 ± 4	94 ± 8
Hexadecan-1-ol	7 ± 5	31 ± 3	70 ± 5	98 ± 6	180 ± 9
Octadecan-1-ol	5 ± 3	32 ± 5	74 ± 7	100 ± 5	188 ± 8
Octadecen-1-ol	6 ± 4	30 ± 7	68 ± 15	99 ± 9	175 ± 14
Pentadecan-2-one plus					
Hexadecan-1-ol	7 ± 4	19 ± 5	40 ± 9	60 ± 5	87 ± 9
Pentadecan-2-one plus					
Octadecan-1-ol	6 ± 4	27 ± 4	59 ± 11	82 ± 7	132 ± 12
Pentadecan-2-one plus					
Octadecen-1-ol	5 ± 4	23 ± 6	57 ± 15	83 ± 10	141 ± 12

[a] The incubation conditions and the method of adding the long-chain alcohol and pentadecan-2-one are described in Table 3.

[b] The values given in the table are the average Klett units ± SD from six separate analyses. In this instance the concentration of pentadecan-2-one in the final culture medium was 24.6 nmol per ml and that of each long-chain alcohol was 98.4 nmol per ml. The molar ratio of pentadecan-2-one to long-chain alcohols in each instance was 1:4.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

from the cultures treated with both pentadecan-2-one and the primary alcohol.

The effect of other methyl ketones varying in chain length from 12 to 16 carbon atoms on the growth of *Cl. butyricum* was also evaluated (**Fig. 2**). Of the five methyl ketones tested, tridecan-2-one and tetradecan-2-one were the most effective, producing a 50% inhibition of growth at concentrations of 17 and 19 nmol per ml. Pentadecan-2-one and dodecan-2-one were much less effective, while hexadecan-2-one caused no inhibition at any concentration used.

The ability of the methyl ketone to inhibit cell growth is not characteristic of all bacteria. When *E. coli* K-40 was grown in the presence of pentadecan-2-one under the same conditions as *Cl. butyricum*, no change was noted in its growth rate. However, *E. Coli*, in contrast to *Cl. butyricum*, normally contains long-chain secondary alcohols as physiological constituents (12).

DISCUSSION

In a previous report concerning the long-chain alcohol composition of *Cl. butyricum*, hexadecan-1-ol and octadecan-1-ol accounted for 70% of the total alcohols present (7). Our results again indicate that these are the major long-chain alcohols present in this bacterium.

Addition of small amounts of pentadecan-2-one to the incubation medium results in a marked decrease in the growth rate of *Cl. butyricum*. This decrease is proportional to the log of the methyl ketone concentration of the medium. While the reason for this response is not known, several observations can be noted. First, the addition of pentadecan-2-ol to the incubation medium had no effect on the growth rate of *Cl. butyricum*, indicating that the secondary alcohol was not the moiety affecting growth. Second, inhibition was proportional to the log of the pentadecan-2-one concentration. This, in addition to the observed chain length specificity of the methyl ketones, suggests that the effect of the ketone was on a specific enzyme system. Finally, the 5-fold decrease in the long-chain primary alcohol content of the ketone-treated cells, coupled with the observation that adding primary alcohols to the incubation medium along with the ketone partially reversed the growth inhibition seen with ketone alone, suggests that long-chain primary alcohols play a significant role in the growth of *Cl. butyricum.*

In mammalian tissues, biosynthesis of the alkyl glycerols involves condensation of a long-chain alcohol with acyl dihydroxyacetone phosphate (5, 6). The corresponding alkenyl glycerol is formed by oxidation of the alkyl glycerol by a mixed-function oxidase (15).

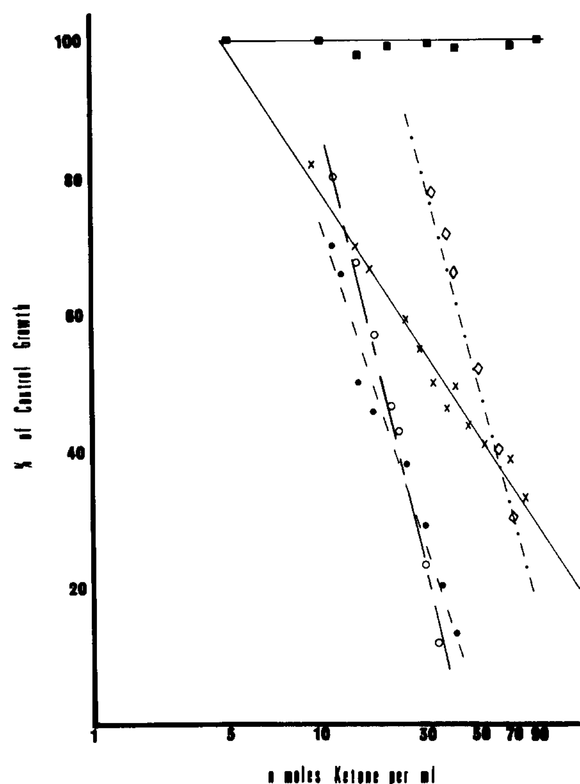

Fig. 2. Effect of methyl ketones of different chain lengths on the growth response of *Cl. butyricum.* Seed cultures were prepared as described in Fig. 1. Aliquots, 0.2 ml, were removed from the seed cultures and added to Klett tubes containing fresh medium having different concentrations of methyl ketones of different chain lengths. Growth was assessed by determining the change in transmittance of the culture medium. Key: Dodecan-2-one, ◇· —— ·◇; tridecan-2-one, ○ —— ○; tetradecan-2-one, ● —— ●; pentadecan-2-one, × —— ×; and hexadecan-2-one, ■ —— ■.

In *Cl. butyricum* the biosynthetic pathway for the glycerol ethers appears different from that observed in mammalian systems. Here, dihydroxyacetone phosphate does not provide the glycerol backbone of the ether moiety (16). Also, the large decrease in primary alcohol content noted here in the ketone-treated cells was not associated with an accompanying quantitative decrease in alkenyl phosphoglycerides. A decrease in the content of this phospholipid type would be expected if the long-chain alchols were direct precursors of the glycerol ethers in this bacterium.

This investigation was supported by Public Health Service Research Grants AM16837, DE03774, and DE04225 from the National Institutes of Health.

Manuscript received 8 August 1977; accepted 22 February 1978.

REFERENCES

1. Johnson, R. C., and J. R. Gilbertson. 1972. Purification and properties of an NADH dependent fatty acyl CoA reductase in bovine cardiac muscle. *J. Biol. Chem.* **247:** 6991–6998.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 19, 2012

2. Kawalek, J. C., and J. R. Gilbertson. 1976. Partial purification of the NADPH-dependent aldehyde reductase from bovine cardiac muscle. *Arch. Biochem. Biophys.* **173:** 649–657.
3. Tabokoff, B., and V. G. Erwin. 1970. Purification and characterization of a reduced nicotinamide adenine dinucleotide phosphate-linked aldehyde reductase from brain. *J. Biol. Chem.* **245:** 3263–3268.
4. Vignais, P. V., and I. Zabin. 1958. Formation d'aldehyde plamitque dans le cerveau rat. 5th Internat. Conf. Biochem. Lipids (Vienna). p. 78–84.
5. Hajra, K. 1970. Acyl dihydroxyacetone phosphate: precursor of alkyl ethers. *Biochem. Biophys. Res. Commun.* **39:** 1037–1044.
6. Snyder, F., B. Malone, and M. L. Blank. 1970. Enzymic synthesis of *O*-alkyl bonds in glycerolipids. *J. Biol. Chem.* **245:** 1790–1799.
7. Gilbertson, J. R., R. D. Fletcher, J. C. Kawalek, and B. Demcisak. 1976. Effects of pentadecan-2-one on the growth of cells in culture. *Lipids* **11:** 172–178.
8. Baumann, N. A., P-O. Hagen, and H. Goldfine. 1965. Phospholipids of *Clostridium butyricum*: Studies on plasmalogen composition and biosynthesis. *J. Biol. Chem.* **240:** 1559–1567.
9. Day, J. I. E., H. Goldfine, and P-O. Hagen. 1970. Enzymic reduction of long-chain acyl CoA to fatty aldehydes and alcohols by extracts of *Clostridium butyricum. Biochim. Biophys. Acta* **218:** 179–182.
10. Goldfine, H. 1964. Composition of the aldehydes of *Clostridium butyricum* plasmalogens. *J. Biol. Chem.* **239:** 2130–2134.
11. Hagen, P-O., and H. Goldfine. 1965. Phospholipids of *Clostridium butyricum*: Further studies on the origin of the aldehyde chains of plasmalogens. *J. Biol. Chem.* **242:** 5700–5708.
12. Naccarato, W. F., R. A. Gelman, J. C. Kawalek, and J. R. Gilbertson. 1972. Characterization and metabolism of free fatty alcohols from *Escherichia coli. Lipids* **7:** 275–281.
13. Gilbertson, J. R., and M. L. Karnovsky. 1963. Nonphosphatide fatty acyl esters of alkenyl and alkyl ethers of glycerol. *J. Biol. Chem.* **238:** 893–897.
14. Naccarato, W. F., J. R. Gilbertson, and R. A. Gelman. 1974. In vivo and in vitro biosynthesis of free fatty alcohols in *Escherichia Coli* K-12. *Lipids* **9:** 419–428.
15. Snyder, F., M. L. Blank, and R. L. Wykle. 1971. The enzymatic synthesis of ethanolamine plasmalogens. *J. Biol. Chem.* **246:** 3639–3645.
16. Hill, E. E., and W. E. M. Lands. 1970. Formation of acyl and alkenyl glycerol derivatives in *Clostridium butyricum. Biochim. Biophys. Acta* **202:** 209–211.

ASBMB

JOURNAL OF LIPID RESEARCH

JLR

Downloaded from www.jlr.org by guest, on June 19, 2012