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Lipid Composition of Growing and Starving Cells of *Arthrobacter crystallopoietes*

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The lipid composition of growing and starving cells of *Arthrobacter crystallopoietes* was compared. Although the lipid composition of the two cell types was similar, the amount of total lipids recovered from the starving cells was 30.4% less than that recovered from the growing cells. The loss of lipids, as compared to the loss of total cell mass during starvation, was (i) proportional to the loss of the cell mass (phosphatidylinositol, phosphatidylglycerol-2, and cardiolipin), (ii) greater than the loss in cell mass (neutral lipids, "glycophospholipids," and phosphatidic acid), or (iii) less than the loss in cell mass (coenzyme Q, glycolipids, and phosphatidylglycerol-1).

When subjected to the stress of starvation, bacteria degrade specialized reserve materials such as glycogen and poly-β-hydroxybutyric acid (PHB), and intracellular polymeric constituents such as ribonucleic acid (8, 40). The utilization of lipids as endogenous reserves of carbon or energy, or both, has not been investigated extensively. The work of Stephenson and Whetham (49) demonstrated that the lipids of *Mycobacterium phlei* serve as endogenous reserves and are utilized after the exhaustion of glucose in the growth medium. Loss of lipids was also observed in washed suspensions of *Bacillus cereus*, during a 10-hr period of nutrient depletion (22). Lipid utilization was not detected in studies with *Sarcina lutea* (42) and *Pseudomonas aeruginosa* (53).

Several other studies measured a variety of cellular components during starvation but did not follow the changes in the lipids (5, 7, 8). In addition, there are no reports in the literature on the lipid composition of microorganisms as influenced by a significant environmental change such as starvation. The influence of such environmental factors as temperature and medium constituents on microbial lipids is well documented (14, 20, 21, 23, 30, 33, 37, 38, 50).

Arthrobacters, the most numerous single group of bacteria in soils (17, 34, 35), are capable of survival under such environmental stresses as desiccation and nutrient depletion. Zevenhuizen (57) showed that 75% of the cells of an *Arthrobacter* soil isolate remained viable after 18 days of starvation. Boylen and Ensign (4) reported that *A. crystallopoietes* cells remained completely viable when shaken for 30 days in phosphate buffer. These viability limits are the longest ever reported for starving bacteria (4). This study was undertaken to determine the effect of starvation on the lipid content and composition of this organism.

MATERIALS AND METHODS

Bacteria. Two morphological forms of *Arthrobacter* are recognized: the spherical and the rod-shaped. In *A. crystallopoietes* (ATCC 14581), the change from one form to the other has been shown to be nutritionally controlled (12). Although the physiological reactions occurring in both cell forms during starvation are different (5), both forms remain viable for similar periods of time (4). Only the spherical form was used in this study.

Growth and starvation conditions. *A. crystallopoietes* was grown as spheres at 30 C on a glucose-mineral salts medium for 36 hr (three cell generations) as described (4). The population was growing exponentially when the cells were harvested. Starvation conditions were imposed for a period of 2 weeks, since previous studies (4, 5) showed that by this time the events which differentiate a growing state from a starving state have occurred and have reached a steady state. During starvation, the cells were incubated in 0.03 M potassium phosphate buffer (pH 7.0) at 30 C in a water bath shaker. After 1 day of starvation, the cells were aseptically centrifuged and resuspended in fresh, sterile starvation medium, because it has been shown (5) that during the initial hours of starvation the cells, under these conditions, excrete organic material. The lipids were labeled by growing

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the cells in the presence of uniformly labeled ^{14}C-glucose (20 μCi/liter of medium).

Materials. Phosphatidylglycerol, phosphatidic acid, and standard fatty acid methyl esters were obtained from Supelco, Inc., Bellefonte, Pa. All other standard phospholipids, 1,2- and 1,3-diglycerides, and mono- and digalactosyldiglycerides were purchased from Applied Science Laboratories, Inc., State College, Pa. Uniformly labeled ^{14}C-glucose was obtained from New England Nuclear Corp., Boston, Mass. Other materials were of the best grade available commercially.

Extraction of lipids. The method used was a modification of that described by Klein (27). The harvested cells were divided into portions of approximately 100 mg (dry weight). Each portion was shaken with 25 ml of methanol, allowed to stand at room temperature for 10 min, and centrifuged; the sedimented cells were re-extracted with 25 ml of 95% ethanol. The extracts were combined. The residues were resuspended in 25 ml of absolute ethanol, a boiling chip was added, the ethanol was boiled down to about one-third of the original volume in a water bath at 80 C; this preparation was added to the combined extracts after centrifugation. The residues were then extracted twice with 25 ml of petroleum ether. The petroleum ether extracts were combined with all of the others and evaporated to dryness in vacuo. The lipids were resuspended in chloroform-methanol (2:1, v/v) and washed by the Folch method (15). The lipid yields were determined gravimetrically and counted for radioactivity. This method, as compared with that of Bligh and Dyer (3), yielded approximately 10% more total lipid phosphate. Subsequent saponification of the residue did not increase the total lipid yield. As controls for the method, mixtures of standard phospholipids were extractable quantitatively and the phospholipids remained structurally intact.

Column chromatography. Lipids were fractionated on activated silicic acid columns (Unisil, 100 to 200 mesh, Clarkson Chemical Co., Williamsport, Pa.) as described by Rouser (43). The fractions were analyzed by thin-layer chromatography.

Thin-layer chromatography. Commercially available Silica Gel G plates 250 μm thick (Analtech, Inc., Wilmington, Del.) were used without prior heat activation. Neutral lipids were separated in a solvent of petroleum ether-ether-acetic acid (70:30:1, v/v; reference 26). Phospholipids were chromatographed in a two-dimensional solvent system of chloroform-methanol-7 N ammonium hydroxide (60:35:5, v/v; reference 36) in the first dimension, and chloroform-methanol-acetic acid (65:25:8, v/v; reference 36) in the second dimension. Preparative thin-layer chromatography of phospholipids was accomplished by developing a plate that contained 5 to 7 μmoles of lipid phosphate in a solvent of chloroform-methanol-7 N ammonium hydroxide (60:35:5, v/v). Spots of individual phospholipids were scraped off and eluted as described by White and Frerman (55). They were then rechromatographed in the two-dimensional system described above.

Autoradiograms were made by placing Kodak X-ray films (Blue Sensitive, single coated) over the thin-layer chromatography plates. Recovery of radioactive components from the plates for counting was achieved by scraping off the gel directly into a scintillation vial. Radioactivity was determined in a Packard Tri-Carb liquid scintillation counter.

For preparative purposes, lipid bands were detected by exposure to iodine vapors. Neutral lipids were detected under ultraviolet light after spraying with 0.2% 2',7'-dichlorofluorescein in methanol. Phospholipids were detected with the spray reagent of Dittmer and Lester (10). Carbohydrate-containing lipids were detected with the phosphoric acid-phenol spray described by Brennan (6) and the diphenylamine reagent (51). Ninhydrin reagent was used to detect amino groups. Vicinal glycol groups were detected by periodate oxidation followed by the acetylacetone reagent of Schwartz (44).

Analysis of the lipids. Lipid phosphate was determined by the method of Ames (1). The presence of vicinal glycols in intact and deacylated lipids was assayed by measuring formaldehyde production according to the method described by Ames (2); glycerol was used as standard. Carbohydrates were estimated by the method of Dubois (11).

Phospholipids were deacylated by the mild alkaline methanolysis procedure described by White (54). Acid hydrolysis of phospholipids was carried out as described by Brennan (6).

Ascending paper chromatography was performed with Whatman no. 1 paper. The following solvents were used: A, isopropanol-ammonium hydroxide-water (7:1:2, v/v; reference 39); B, butanol-propionic acid-water (71:36:50, v/v; reference 31); C, phenol saturated with water-acetic acid-ethanol (10:1:1.2, v/v; reference 9); and D, ethyl acetate-acetic acid-formic acid-water (18:3:1:4, v/v; reference 6).

Products of deacylated lipids were demonstrated by use of the vicinal glycol spray (44) or the molybdenum spray of Hanes and Isherwood (19). The acid hydrolysis products were demonstrated by use of the ammoniacal silver nitrate spray (47) and the sodium periodate-benzidine dip (16).

Gas chromatography. Fatty acid methyl esters of total lipids and neutral lipids were prepared by saponification of the lipids, extraction of the fatty acids, and methylation with boron trifluoride (32).

Fatty acid methyl esters of phospholipids were recovered as such from the organic phase after the mild alkaline methanolysis procedure described by White (54).

The fatty acid methyl esters were analyzed on a Varian model 1500 gas chromatograph equipped with a hydrogen flame detector. Helium was used as the carrier gas at a flow rate of 10 ml/min. The injector and detector temperatures were 275 and 280 C, respectively. Chromatography was conducted on two columns: a polar column, 50 ft by 0.02 inch inner diameter (15.24 meters by 0.5 mm), S.C.O.T. (support coated open tubular column), coated with diethylene glycol adipate (DEGA), Perkin Elmer Corp., Norwalk, Conn.; and a nonpolar column, 50 feet by 0.02 inch inner diameter, S.C.O.T., coated with OV-1, Perkin Elmer Corp. The DEGA

column was operated isothermally at 130 C. The OV-1 column was temperature-programmed from 150 to 225 C at a rate of 2 C/min or was operated isothermally at 175 C.

RESULTS

Lipid composition. The per cent composition of the total lipids of starving and growing cells of *A. crystallopoietes* is summarized in Table 1.

Characterization of lipids. Total lipids were fractionated by silicic acid column chromatography according to the method of Rouser [43]. The fractions were assayed gravimetrically for the amount of lipid and colorimetrically for phospholipid phosphate (10), and were counted for radioactivity (Table 2).

The neutral lipids recovered in fraction 1 accounted for about 10% of the starved-cell lipids and 12% of the growing-cell lipids. Thin-layer chromatography in the solvent system petroleum ether-ether-acetic acid (70:30:1, v/v) revealed the presence of monoglycerides, 1,2- and 1,3-diglycerides, and free fatty acids. These were quantitated gravimetrically and by scraping individual spots into scintillation fluid and counting (Table 1).

The lipids recovered in fraction 2 (Table 2) accounted for about 46% of the growing-cell lipids and 49% of the starving-cell lipids. In each case, about 2% of the total phospholipid phosphorus was associated with this fraction. On thin-layer chromatography in chloroform-methanol-acetic acid-water (100:60:16:8, v/v), the trace phospholipid co-chromatographed with phosphatidylglycerol, and the major lipid ran with the solvent front. Because of the intense yellow color of this fraction, a spectrum of the material dissolved in ethanol was obtained, which showed a characteristic maximum at 275 nm. This peak disappeared after reduction with $NaBH_4$, and a new maximum appeared at 290 nm. The spectrum of authentic coenzyme Q (Calbiochem, Los Angeles, Calif.) displayed the same characteristics (29). The lipid was identified as coenzyme Q on the basis of its spectrum, and was quantitated gravimetrically and by its radioactivity. It is a major lipid in these cells, accounting for 48.3% of the total lipids in starving cells and for 44.6% of the total in growing cells. Others have reported the presence of coenzyme Q in other bacteria, but in much smaller quantities (2, 25).

TABLE 1. *Lipid content of starving and growing cells of A. crystallopoietes*

Lipid	Starving		Growing		Lipid loss (%)[c]	Lipid loss (%)/mass loss (%)[d]
	Amt (mg)[a]	Percentage of total lipids[b]	Amt (mg)	Percentage of total lipids		
Coenzyme Q	26.6	48.3	35.3	44.6	24.6	0.77
Neutral lipids (total)	5.4	9.8	9.7	12.3	44.3	1.40
Monoglycerides	0.99	1.8	1.57	2.0	36.9	1.16
1,2-Diglycerides	1.26	2.3	2.28	2.9	44.7	1.41
1,3-Diglycerides	2.37	4.3	4.26	5.4	44.3	1.40
Free fatty acids	0.77	1.4	1.57	2.0	50.9	1.61
Glycolipids (total)	5.5	10.0	7.7	9.8	28.6	0.90
Monogalactosyldiglyceride	2.75	5.0	4.0	5.1	31.2	0.98
Digalactosyldiglyceride	2.75	5.0	3.69	4.7	25.4	0.80
Phospholipids (total)	16.8	30.8	26.0	32.8	35.4	1.12
"Glycophospholipids"	0.0	0.0	0.77	0.98	100.0	3.16
Phosphatidylinositol	0.50	0.92	0.77	0.98	35.0	1.10
Phosphatidylglycerol-1	5.09	9.33	6.66	8.4	23.5	0.74
Phosphatidylglycerol-2	3.54	6.5	5.47	6.9	35.3	1.11
Cardiolipin	7.62	13.98	11.73	14.8	35.0	1.10
Phosphatidic acid	0.0	0.0	0.63	0.8	100.0	3.16
Total lipids	55.1		79.2		30.4	0.96

[a] Calculated on the basis of total lipids extracted from the total dry cell mass of starving (1002.2 mg) and growing (1466.7 mg) cells.

[b] Calculated on the basis of total lipids recovered from each cell type.

[c] Calculated: 100 (mg of lipid growing − mg of lipid starving/mg of lipid growing).

[d] Based on the dry cell mass given in *a*, the cell mass loss = 31.6%.

TABLE 2. *Fractionation of A. crystallopoietes lipids on silicic acid*[a]

Fraction	Solvent[b]	Radioactivity (counts/min)		Lipid (mg)[c]		Phosphate (mg)[d]		Components[e]
		Starving	Growing	Starving	Growing	Starving	Growing	
1	A	19.0×10^3	12.7×10^3	1.84	1.5	<0.001	<0.001	Neutral lipids
2	B	95.7×10^3	47.2×10^3	9.21	5.8	0.1	0.07	Coenzyme Q
3	C	8.1×10^3	4.5×10^3	0.67	0.5	0.05	0.04	Glycolipid
4	D	1.6×10^3	1.3×10^3	0.15	0.13	0.19	0.21	Phospholipids
5	E	69.2×10^3	37.5×10^3	6.71	4.61	5.35	3.5	Phospholipids

[a] The total quantities applied to columns were: 18.87 mg of starving-cell lipids, from about 342 mg of dry cells, 198×10^3 counts/min; and 12.82 mg of growing-cell lipids, from about 237 mg of dry cells, 108×10^3 counts/min. The percent recoveries of counts and milligrams of lipids ranged between 93 and 98%.

[b] The columns were eluted with the following solvents, each of which was collected separately: A, 500 ml of chloroform; B, 500 ml of chloroform-acetone (1:1); C, 500 ml of acetone; D, 500 ml of chloroform-methanol (4:1); E, 500 ml of methanol. The columns were washed with 200 ml of methanol and 200 ml of chloroform. The washes were pooled and monitored for counts and phosphate content. Not more than 0.5% additional counts and no phosphate were detected in these washes.

[c] Lipids were measured gravimetrically.

[d] Phospholipid phosphate was determined by the method of Ames (1).

[e] Components were identified by thin-layer chromatography as described in Materials and Methods.

The significance of its presence in *A. crystallopoietes* in such quantities was not investigated further.

Most of the radioactivity in fraction 3 of both growing- and starving-cell lipids was recovered in one spot when the lipids from this fraction were chromatographed in chloroform-methanol-7 N ammonium hydroxide (60:35:5, v/v). The spot had an R_F of 0.75, similar to that of standard monogalactosyldiglyceride, and could be detected with either the silver nitrate (47) or the diphenylamine spray (51), but not with the phosphate spray (10). The intact glycolipid and standard monogalactosyldiglyceride were rechromatographed in diisobutyl ketone-acetic acid-water (80:50:10, v/v), the solvent system described by LePage (28). The R_F value obtained for both was 0.50, similar to that reported (28).

Deacylation and acid hydrolysis of the glycolipid and standard monogalactosyldiglyceride yielded the same products: monogalactosylglycerol after deacylation; glycerol and galactose after acid hydrolysis. The glycolipid was concluded to be monogalactosyldiglyceride and, on the basis of radioactivity, accounted for 5% of the total lipids of both growing and starving cells (Table 1).

Another glycolipid was recovered in fraction 4. When the fraction was chromatographed in chloroform-methanol-7 N ammonium hydroxide, the glycolipid has the same R_F value as phosphatidylglycerol. The glycolipid could be separated from phosphatidylglycerol only by the two-dimensional chromatography described in Materials and Methods. Its elution pattern and affinity for phosphatidylglycerol is unexplained.

The unknown glycolipid was removed from the plate, eluted, and rechromatographed in the solvent system of LePage (28) with standard mono- and digalactosyldiglycerides. In this system, the intact glycolipid and standard digalactosyldiglyceride had an R_F value of 0.26. The unknown was further characterized as digalactosyldiglyceride on the basis of chromatography of the products of deacylation and acid hydrolysis. The molar ratio of glycerol to sugar for both the unknown and the standard was found to be 1:2. This glycolipid accounted for 4.7% of the total lipids of growing cells and for 5% of the total lipids of starving cells (Table 1).

The finding of only two glycolipids in this strain of *A. crystallopoietes* does not agree with the findings of others. It was reported that, besides the two glycolipids recovered here, *A. crystallopoietes* contains a dimannosyldiglyceride and traces of tri- and tetraglycosyldiglycerides (46). The dimannosyldiglyceride also has been found in *A. globiformis* (52). It is possible that in this strain of *A. crystallopoietes* the quantity of dimannosyldiglyceride is undetectable, or that the glycolipid was inadvertently discarded in the aqueous phase of the Folch washing procedure (45).

Characterization of phospholipids. The majority of phospholipids were eluted in fraction 5 (Table 2). Phospholipids were chromatographed in the two-dimensional system described in Materials and Methods and identified by the following criteria: (i) color reactions

of the prosthetic groups; (ii) comparison with the R_F values of standards in the two-dimensional system; (iii) chromatography of deacylated products; (iv) chromatography of acid hydrolysis products; and (v) estimation of phosphate to glycerol ratios.

Identification of intact phospholipids. Nine phospholipids were recovered from the growing cells and four from the starving cells. Their color reactions and R_F values are listed in Table 3. Phospholipids which had the same R_F values as standards were no. 5 (phosphatidylinositol), no. 6 (phosphatidylglycerol), no. 8 (cardiolipin), and no. 9 (phosphatidic acid). No available standards corresponded to phospholipids 1, 2, 3, 4, and 7.

Identification of deacylated phospholipids. Phospholipids were deacylated by mild alkaline methanolysis (56). The deacylated products were chromatographed as described in Materials and Methods. The R_F values of the deacylated lipids in three chromatographic systems were in good agreement with those published (2, 9). Lipid 5 gave a single, phosphate- and silver nitrate-reacting spot which co-chromatographed with standard deacylated phosphatidylinositol. Lipid 6 co-chromatographed with standard glycerolphosphorylglycerol (GPG) and reacted with the phosphate (19) and vicinal glycol spray (44). Lipid 8 gave a single spot which was phosphate-positive and co-chromatographed with diglycerolphosphorylglycerol. Lipid 9 co-chromatographed with α-glycerolphosphate and was phosphate-positive.

Deacylated lipid 7 behaved like the standard GPG in all three solvent systems. It gave a single phosphate- and vicinal glycol-reacting spot. It co-chromatographed with standard GPG, although the intact lipid behaved differently from standard phosphatidylglycerol and standard lysophosphatidylglycerol. Recently, it was reported (56) that *A. simplex* contained two species of phosphatidylglycerol which chromatographed differently because one species contained hydroxylated fatty acids. Thin-layer chromatography of the fatty acids (56), followed by gas chromatography, did not reveal the presence of hydroxylated fatty acids in the phospholipids of *A. crystallopoietes*. Others have reported that phosphatidylglycerols have different chromatographic mobilities owing to different salt forms (24). On that basis, lipid 7 was tentatively identified as another salt form of phosphatidylglycerol and labeled phosphatidylglycerol-2.

Phospholipids 1, 2, 3, and 4 were detected in the growing cells, but not in the starving cells. Elution of each phospholipid did not yield sufficient material for structural studies; attempts at such studies resulted in loss of material. Therefore, these sugar-containing phospholipids were pooled for determination of phospholipid phosphorus and qualitative examination of their acid hydrolysis products. They contributed only 3% of the total phospholipid phosphorus (Table 4). Acid hydrolysates, chromatographed on Whatman no. 1 paper in solvent D and demonstrated by the periodate-benzidine dip (16), contained innositol, mannose, and glycerol. Further characterization could not be carried out. On the basis of the color reactions (Table 3) and the acid hydrolysis products, these phospholipids are referred to as "glycophospholipids."

Except for the finding of trace amounts of "glycophospholipids" and a 20% lower total phospholipid content, the data on the phos-

TABLE 3. *Color reactions and R_F values of phospholipids from starving (S) and growing (G) cells*

Spot	R_F[a]		Color reactions[b]					Phospholipid
	Solvent I	Solvent II	Nin	PO_4	Ph-Ph	$AgNO_3$	ViG	
1 (G)	.018	.018	−	+	+	+	−	"Glycophospholipids"
2 (G)	.082	.10	−	+	+	+	−	"Glycophospholipids"
3 (G)	.26	.16	−	+	+	+	−	"Glycophospholipids"
4 (G)	.18	.34	−	+	+	+	−	"Glycophospholipids"
5 (G & S)	.16	.15	−	+	+	+	−	Phosphatidylinositol
6 (G & S)	.44	.52	−	+	−	−	+	Phosphatidylglycerol-1
7 (G & S)	.32	.67	−	+	−	−	+	Phosphatidylglycerol-2
8 (G & S)	.45	.87	−	+	−	−	+	Cardiolipin
9 (G)	.68	.85	−	+	−	−	±	Phosphatidic acid

[a] Solvent I was chloroform-methanol-7 N ammonia (60:35:5, v/v) in the first dimension; solvent II was chloroform-methanol-acetic acid (65:25:8, v/v) in the second dimension.

[b] The abbreviations are: Nin, ninhydrin; PO_4, phosphate spray (10); Ph-Ph, phosphoric-phenol spray (6); $AgNO_3$, ammoniacal silver nitrate spray (47); ViG, vicinal glycol spray (44).

TABLE 4. *Quantitation of phospholipids*

Phospholipid[a]	Phosphate (μmoles)[b]		Percentage of total phosphate[c]	
	Starving	Growing	Starving	Growing
"Glycophospholipids"		0.25		3.0
Phosphatidylinositol	0.25	0.25	3.0	3.0
Phosphatidylglycerol-1	2.50	2.12	30.3	25.6
Phosphatidylglycerol-2	1.75	1.75	21.1	21.1
Cardiolipin	3.75	3.75	45.4	45.2
Phosphatidic acid		0.2		2.4

[a] For developing solvents and R_F values, see Table 3.

[b] A total of 10 μmoles of lipid phosphate from each cell type was chromatographed on five plates. Each lipid was removed from the plate and its phosphate content was determined (1). The amounts reported are averages of five determinations. The average recovery of phosphate was 83%.

[c] Calculated on basis of total phosphate recovered from spots.

pholipid composition of *A. crystallopoietes* reported here generally agrees with that reported recently (46).

Identification of the fatty acid methyl esters. Methyl esters of total lipids, neutral lipids, and phospholipids were analyzed on both polar and nonpolar columns. Analysis of the methyl esters on the OV-1 column with the use of temperature programming indicated the absence of fatty acid methyl esters greater in chain length than 16 carbon atoms. There was no indication of the presence of C_8 and C_{10} fatty acid methyl esters.

The fatty acid methyl esters were identified by comparing their retention times with those of standard mixtures of saturated, unsaturated, branched, and hydroxylated fatty acid methyl esters. In addition, the unknown mixtures were co-chromatographed with standards isothermally on both columns.

A typical chromatogram of the fatty acid methyl esters from the total lipids of starving and growing cells is reproduced in Fig. 1. The per cent distribution of the fatty acids in neutral lipids and phospholipids of both types of cells is summarized in Table 5. The results show that the fatty acid composition of starving and growing cells is very similar. The major fatty acids are anteiso-C_{15} and anteiso-C_{17}, accounting for about 90% of the total fatty acids. The absence of unsaturated acids is striking. These results are in good agreement with the other reports on the fatty acid composition of arthrobacters (46, 52, 56).

Lipid content. The total lipids extracted from growing cells of *A. crystallopoietes* amounted to 5.4% of the dry weight. The total lipids extracted from cells starved for 2 weeks accounted for 5.5% of the dry weight. The counts/minute recovered in the lipids of growing and starving cells compare well with the gravimetric measurements: 5.6 ± 0.5% of the initial counts were recovered as lipid.

However, it has been shown that starvation resulted in a decrease of total cell mass with no loss in viability (4). Therefore, it was of interest to establish whether the loss in total cell mass was accompanied by loss of total lipids.

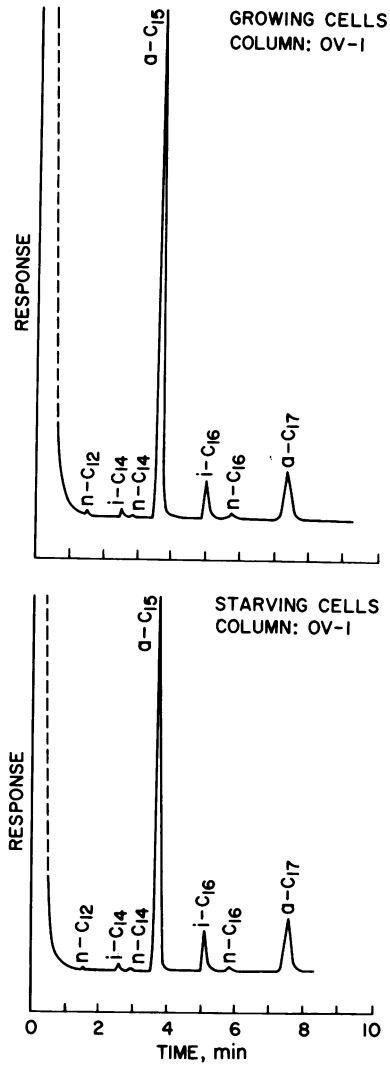

FIG. 1. *Gas chromatographs of fatty acid methyl esters from total lipids of growing and starving cells of A. crystallopoietes.*

In this experiment, the total cell mass decreased by 31.6% after 2 weeks of starvation. The total lipids decreased by 30.4%. Lipid loss, therefore, was almost proportional to the decrease in total cell mass (Table 1).

To determine whether the loss of any lipid component exceeded, equaled, or was less than the loss of the cell mass, the total quantities of each lipid recovered from starving and growing cells were compared with the loss in total cell mass.

The results summarized in Table 1 are based on gravimetric measurements and were confirmed by colorimetric and radioactive measurements. They show that the loss of neutral lipids, phosphatidic acid, and "glycerophospholipids" exceeded the loss in cell mass. The quantities of the other lipids either decreased proportionally with the cell mass, or to a lesser extent than the cell mass.

TABLE 5. *Per cent distribution of fatty acid methyl esters in the lipids of growing and starving cells of A. crystallopoietes*[a]

Fatty acid methyl ester	Starving cells		Growing cells	
	Phospholipids	Neutral lipids	Phospholipids	Neutral lipids
n-12:0	T	3.9	T	T
n-13:0		1.9		T
i-14:0	T	T	T	T
n-14:0	T	2.0	T	2.0
a-15:0	74.0	71.5	79.0	81.0
n-15:0	T		T	
i-16:0	8.4	5.3	7.7	2.9
n-16:0	1.2	6.7	T	2.7
a-17:0	15.6	7.9	11.9	9.3

[a] Percentages calculated from the total of all peak areas. Column: OV-1. T denotes values between 0.1 and 1%.

DISCUSSION

Two questions were asked in this study on the lipids of starving and growing cells of *A. crystallopoietes*. First, can the cell lipids of this organism serve as substrates for cellular metabolism during starvation? Second, does the organism, in response to the change in environment and in order to survive, modify its lipid composition when it enters a period of starvation?

That lipids do serve as energy reserves in eukaryotic organisms is well established. Their role as energy-storage materials in bacteria is not as clear. The available evidence indicates that lipids can serve as endogenous substrates in *M. phlei* (49) and *B. cereus* (22), but not in *S. lutea* (42) or *P. aeruginosa* (53). Many bacteria utilize PHB, a lipid-related storage compound, as an endogenous source of carbon (7, 8). Since *A. crystallopoietes* does not produce PHB (5), it was conceivable that the organism might utilize its lipids during prolonged starvation.

With respect to the second question, it is well recognized that the lipid composition of microorganisms is affected by such environmental factors as temperature, medium constituents, and *p*H (14, 30, 33, 50). The changes in the lipids are a reflection of the organism's adjustment to the environment. For example, studies on the effect of temperature on the fatty acid composition of microorganisms (14, 25, 30, 33, 41, 50), and on the fatty acids of both synthetic and natural membranes (18, 48) have demonstrated that the physicochemical properties of membrane lipids play an important role in membrane structure and function, and that the changes in lipids reflect the organism's need to maintain those properties for functional purposes (13, 18, 41, 48).

Possibly for the same reasons, the phospholipid composition of certain gram-positive bacteria is altered in response to changes in medium constituents and *p*H (25). Cells of *B. megatherium* (37), *B. subtilis* (38), *Staphylococcus aureus* (20), and *Streptococcus faecalis* (20), grown at neutral *p*H, contained appreciable amounts of phosphatidylglycerol, which decreased when the organisms were grown in an acidified medium. The decrease was accompanied by an accumulation of certain dipolar ionic phospholipids (20, 37). The same changes were observed in the membrane lipids of protoplasts prepared from cells grown in media with different *p*H (37, 38).

The imposition of starvation conditions on an organism is an environmental change which differs from the above examples in that further cell growth is prevented. Lipids might be structurally altered under these conditions.

The question of lipid utilization during starvation is not answered directly by this study. The results show that: (i) during starvation the total lipid content decreases proportionally with the decrease in total cell mass; (ii) the loss of individual lipid components such as neutral lipids, phosphatidic acid, and "glycophospholipids" exceeds the loss in total cell mass; (iii) most of the phospholipids, except phosphatidylglycerol-1, decrease almost proportionally with the cell mass; and (iv) coenzyme Q, phosphatidylglycerol-1, and glycolipids decrease to a lesser extent than the total cell mass. These results suggest a preferential

depletion of certain lipids, be it by catalytic processes, lack of precursors for synthesis, or utilization of precursors for other processes. The findings, although not completely explainable at this time, do point to an involvement of lipids in the cellular changes which occur during starvation.

In answer to the second question, the findings indicate that, structurally, the lipid constituents are not affected by such an important environmental change as starvation. It should be pointed out that during both growth and starvation, the other environmental variables (temperature and *p*H) were not altered. It is conceivable that starvation in this controlled environment contributed to the structural stability of the lipid components.

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