Utilization of [1-¹⁴C]Acetate in the Synthesis of Lipids by *Acholeplasmas*

PATRICIA K. HERRING and J. DENNIS POLLACK

Department of Medical Microbiology, The Ohio State University, Columbus, Ohio 43210, and
The Children's Hospital Research Foundation, Columbus, Ohio 43205

Fifteen *Acholeplasma* strains, including some belonging to *A. laidlawii*, *A. axanthum*, *A. granularum*, or *A. modicum*, incorporated radioactivity into their lipids when grown in a medium containing [1-¹⁴C]acetate. The majority of the radioactivity was found in the glycolipid (43%) and the polar lipid (55%) fractions. The amount of radioactivity incorporated into the lipids (disintegrations per minute per milligram of lipid) of strains of 6 *Mycoplasma* species was less than 1% of the amount found in the 15 *Acholeplasma* strains. The ability to synthesize lipids from acetate is a useful criterion for differentiating the genus *Acholeplasma* from *Mycoplasma*.

The order *Mycoplasmatales* Freundt contains the genera *Mycoplasma* Freundt and *Acholeplasma* Edward and Freundt. These genera are differentiated primarily by their cholesterol dependence. *Mycoplasmas* require cholesterol for growth; *acholeplasmas* do not (18, 29). Genome size (1, 2, 7), localization of reduced nicotinamide adenine dinucleotide oxidase activity (10, 11, 13, 20), and sensitivity to sodium polyanethol sulfonate (3), amphotericin B (21), or lysolecithin (5) are other criteria useful in making generic distinctions.

Previous investigations of *Acholeplasma* and *Mycoplasma* have suggested that these genera could also be differentiated by their ability to synthesize lipids from acetate (15, 22, 23, 30). We have investigated this possibility by examining 15 *Acholeplasma* strains and 6 *Mycoplasma* strains for their ability to incorporate acetate into their lipids.

MATERIALS AND METHODS

Bacterial strains. The *Acholeplasma* strains (see Table 1) were received from J. G. Tully (National Institutes of Health).

Mycoplasma hyorhina 7 was obtained from R. F. Ross (Iowa State University), and *M. pneumoniae* CL8, *M. gallisepticum* S6, *M. fermentans* PG18, *M. pulmonis* N3, and *M. neurolyticum* A were obtained from N. L. Somerson (Ohio State University).

The anaerobic *Acholeplasma bactoclasticum* JR described by Robinson and Hungate (19) was not included in our study of *acholeplasmas* because of a recent observation (M. Allison and I. M. Robinson, personal communication) that this strain has an absolute cholesterol requirement.

Media and growth conditions. All organisms were grown in SSR2 broth medium (12) and, where possible, in a modified Tryptose broth (MT) (15). The MT basal medium consisted of: Tryptose (Difco), 20 g; NaCl, 5 g; *N*-2-hydroxyethylpiperazine-*N'*-ethanesulfonic acid (Calbiochem, San Diego, Calif.), 9.77 g; and distilled water, 938 ml. The basal medium was adjusted to pH 8.2 with 10 N NaOH. Additions were as follows: 50% (wt/vol) sterile glucose solution, 20 ml; fresh yeast extract solution (Microbiological Associates, Inc., Bethesda, Md.), 50 ml; and PPLO Serum Fraction (Difco), 10 ml. The PPLO serum fraction used in MT or SSR2 media was from control lot 521051 or 525683.

Stock solutions of [1-¹⁴C]sodium acetate (738 μ Ci/mg, Amersham/Searle Corp., Arlington Heights, Ill.) were prepared in either MT or SSR2 medium at a concentration of 125 μ Ci/ml. These stock solutions were filter sterilized. Stock solutions of radioactive acetate were then added to their respective medium to give a final concentration of 0.25 μ Ci/ml in the complete medium. Penicillin G was also added to give a final concentration of 50 U/ml in MT medium or 100 U/ml in SSR2 medium.

For *acholeplasmas*, 3 to 6 ml of a 24-h-old MT or SSR2 culture was inoculated into 125 ml of the same medium containing radioactive acetate. *Acholeplasma* cells grown either in SSR2 or MT medium were harvested at 24 and 48 h, respectively. For *Mycoplasma* strains, 10 to 15 ml of a 24-h-old culture was inoculated into 250 ml of SSR2 containing radioactive acetate and harvested at 48 h, except for *M. gallisepticum* S6, which was harvested at 24 h. All organisms were incubated at 36 C.

To obtain pellets of *mycoplasmas*, the broth cultures were centrifuged at 16,000 $\times g$ for 20 min. The pellets were washed twice by resuspension in 200 ml of cold Kappa buffer (12) followed by centrifugation. The washed organisms were resuspended in 30 ml

of ammonium acetate (0.155 M, pH 7.5), immediately centrifuged at $27,000 \times g$ for 15 min, frozen at -25°C , and freeze-dried. The freeze-dried cells were stored in vacuo at -25°C .

Extraction and purification. The freeze-dried pellets (2 to 47 mg) were weighed to 10^{-4} g and were extracted three times with 5 ml of chloroform-methanol (2:1, Lipopure Reagents, Applied Science Laboratories, Inc., State College, Pa.). The pooled extract was filtered, dried under nitrogen, and purified by Sephadex chromatography as described by Rouser et al. and Siakotos and Rouser (24, 25). The chloroform-methanol (19:1, water-saturated) fraction was dried under nitrogen, and the residue ("purified lipid") was weighed to 10^{-5} g (Cahn G-2 Electrobalance, Ventron Inst. Corp., Paramount, Calif.). Data are reported as percent lipid recovered from whole cells, designated "% lipid."

Determination of acetate contamination. The final supernatant wash fluids contained about 340 dpm/ml, presumably all $[1\text{-}^{14}\text{C}]\text{acetate}$. Washed cell pellets never exceeded 1 ml in volume. By using these values, and assuming an intracellular space of 50%, we computed that harvested cells, prior to freeze-drying, were contaminated with a maximum of 170 dpm of $[1\text{-}^{14}\text{C}]\text{acetate}$. In other experiments, Sephadex columns were loaded only with labeled acetate and chromatographed to determine how much radioactivity would elute into the chloroform-methanol (19:1, water-saturated) fraction and, therefore, contaminate the purified lipid fraction. About 50% of the labeled acetate applied to the Sephadex columns was eluted in this fraction. With these data, we calculated that no more than 100 dpm in any of our experiments could be ascribed to acetate contamination. In experiments with *Acholeplasma* strains, the acetate contamination was computed to be 0.77% ($n = 127$, standard deviation 1.77%).

To determine if spots seen in thin-layer chromatography (TLC) radioautographs were contaminating $[1\text{-}^{14}\text{C}]\text{acetate}$, TLC plates were spotted with only radioactive acetate and chromatographed. These experiments showed there were no spots found on TLC of radioactive acetate that corresponded to any found in our radioautographs of purified lipid.

Distribution of radioactivity in lipid classes. Samples of purified lipid containing known amounts of radioactivity were placed on silicic acid columns, and the neutral lipid and glycolipid fractions were eluted (24, 32). These eluates were dried under nitrogen and assayed for radioactivity. The radioactivity in the non-eluted polar fraction was estimated by subtracting the sum of the recovered neutral lipid and glycolipid fraction radioactivity from the amount applied to the column.

Scintillation cocktail (13) was added to purified lipid of known weight and assayed for radioactivity in a liquid scintillation counter (model 3310S, Packard Instrument Co.). The samples were counted for 20 min and corrected for quenching and background, and radioactivity was reported as disintegrations per minute per milligram of lipid (13).

Fatty acid isolation. Fatty acid methyl esters were prepared from purified lipids with known amounts of radioactivity by using a modification of the procedure

of Metcalfe et al. (6). The samples were dried under nitrogen and assayed for radioactivity as for lipid classes.

TLC of lipids. Phospholipids were isolated by the two-directional TLC technique described by Turner and Rouser (31). In this procedure we used commercially prepared Silica Gel H plates, 250 μm thick, containing 7.5% magnesium acetate (Analtech Inc., Newark, Del.). Neutral lipids were resolved by four-directional-development TLC (4D-TLC) (9).

To visualize lipids, plates were sprayed with a sulfuric acid-dichromate spray (Applied Science Laboratories Inc., State College, Pa.) and charred. Phospholipids were also visualized by spraying with Phospray (Supelco Inc., Bellefonte, Pa.).

Radioautography. X-ray film (No-Screen Medical X-ray film NS-54T, Kodak, Rochester, N. Y.) was exposed to unsprayed TLC plates for 7 days and developed. The final prints showed labeled lipids as dark spots on a white background.

RESULTS

SSR2 medium supported the growth of all the strains tested. *Acholeplasmas*, but not mycoplasmas, grew in the MT medium (Table 1).

The percent lipid and the disintegrations per minute per milligram of lipid were determined for the *acholeplasmas* grown in the SSR2 and MT media. The same data were obtained for mycoplasmas grown in SSR2 medium (Table 1). The percent lipid was not significantly different ($P > 0.05$) between strains of the two genera grown in SSR2 medium. However, the amount of radioactivity from $[1\text{-}^{14}\text{C}]\text{acetate}$ incorporated into the lipids of these organisms clearly separated the two genera. *Acholeplasma* strains incorporated significantly more radioactivity into their lipids than did *Mycoplasma* strains ($P < 0.025$).

There was no significant difference ($P > 0.05$) in the percent lipid of *Acholeplasma* cells grown in either SSR2 or MT medium. There was significantly more ($P < 0.005$) radioactivity incorporated into the lipids of the *Acholeplasma* cells grown in the MT medium than was incorporated when grown in the SSR2 medium (Table 1).

Additional experiments demonstrated that almost all of the radioactivity was lipoidal and not contaminating $[1\text{-}^{14}\text{C}]\text{acetate}$.

The purified lipid from five *Acholeplasma* strains grown in the MT medium was fractionated on silicic acid columns (Table 2). Only 3% of the total radioactivity was found in the neutral lipid fraction. The balance of the radioactivity was distributed without a statistically significant difference ($P > 0.05$) between the glycolipid and polar lipid fractions.

TABLE 1. Comparison of *Acholeplasma* and *Mycoplasma* strains: the % lipid of whole cell (dry weight) and the incorporation of [$1\text{-}^{14}\text{C}$]acetate radioactivity into their lipids^a

Species	Strain	SSR2 medium		MT medium	
		% Lipid dry wt	dpm/mg of lipid	% Lipid dry wt	dpm/mg of lipid
<i>Acholeplasma laidlawii</i> A	PG8	12.1 ^b	41,300	13.1	300,404
<i>A. laidlawii</i> B	PG9	13.9 ^c	77,000	11.6	214,255
<i>A. laidlawii</i>	Flamm	16.0	37,200	12.7	329,055
<i>A. laidlawii</i>	Granoff	13.8	62,800	16.9	213,253
<i>A. granularum</i>	31B-1	19.6	1,000	17.1	111,559
<i>A. granularum</i>	BTS-39	20.7	32,989	12.4	365,541
<i>A. axanthum</i>	S743	15.1	9,500	14.2	14,053
<i>A. modicum</i>	Squire (=PG49)	15.6	2,500	15.3	20,357
<i>Acholeplasma</i> sp	2310-S	13.8	4,600	27.4	2,869
<i>Acholeplasma</i> sp	3M-152	15.2	20,500	14.3	48,914
<i>Acholeplasma</i> sp	Haig (=179L)	12.3	8,300	14.5	181,279
<i>Acholeplasma</i> sp	b3046	15.1	97,881	13.3	190,552
<i>Acholeplasma</i> sp	19L	13.3	147,451	14.7	297,309
<i>Acholeplasma</i> sp	Harris	15.6	14,689	19.1	77,567
<i>Acholeplasma</i> sp	H86N	11.0	14,433	9.0	63,244
<i>Mycoplasma pneumoniae</i>	CL8	29.7 ^d	77	ND ^e	ND
<i>M. gallisepticum</i>	S6	19.7 ^f	180	ND	ND
<i>M. fermentans</i>	PG18	14.7	48	ND	ND
<i>M. pulmonis</i>	N-3	13.9	61	ND	ND
<i>M. neurolyticum</i>	A	13.0 ^g	26	ND	ND
<i>M. hyorhinis</i>	7	20.1	75	ND	ND

^a Data are the means of three to eight different determinations.^b Razin et al. (17) found 12.8.^c Literature values (14, 17, 26) have ranged from 8.3 to 12.1.^d In the same medium, Pollack et al. (14) reported a value of 26.4; Prescott et al., using another medium (16), reported a value of 11.5.^e Not determined.^f Razin et al. (17) found 15.7.^g Razin et al. (17) reported 20.3, and Smith (28) reported 13.7.TABLE 2. Radioactivity in lipid fractions of *Acholeplasma* strains

Species	Strain	Radioactivity (%) ^a		
		Neutral lipid	Glyco-lipid	Polar lipid
<i>A. laidlawii</i> A	PG8	3	37	61
<i>A. laidlawii</i> B	PG9	1	42	57
<i>A. granularum</i>	31B-1	5	47	48
<i>A. granularum</i>	BTS-39	4	53	43
<i>A. axanthum</i>	S743	2	34	65

^a Mean: 3, 43, and 55 for neutral lipid, glycolipid, and polar lipid, respectively.

Radioactive purified lipid and non-radioactive lipid standards were chromatographed (Fig. 1) by 4D-TLC. The upper right plate of Fig. 1 is a chromatogram, visualized by charring, of lipid standards. The upper left plate of Fig. 1 shows *A. laidlawii* A strain PG8 lipids,

which were also visualized by charring. In this latter plate, the spots of the *A. laidlawii* A strain PG8 lipid near the origin (lower left corner of plate) correspond to phospholipid and glycolipid areas. There are also fainter spots in the diglyceride area (upper right quadrant). The lower plate of Fig. 1 shows a reproduction of a radioautograph of a similar sample of *A. laidlawii* A strain PG8 lipids. The radioactivity appears to be located in the same areas as the lipids in the upper left plate of Fig. 1. These localized areas as well as the entire TLC plate were assayed for radioactivity. The percent of the total plate radioactivity in the diglyceride and phospholipid plus glycolipid spots was 6.3% and 93%, respectively. Only 0.7% of the radioactivity was found on the remainder of the plate. This agrees closely with the data from the silicic acid column, which show 3% of the radioactivity in the neutral lipid and 97% in the polar plus glycolipid fractions.

To confirm further that the unresolved

radioactivity at the origin of the TLC plates in Fig. 1 was lipid, additional TLC was conducted. Figure 2 shows radioautographs of *A. axanthum* S743, *A. granularum* BTS-39, and *A. laidlawii* A strain PG8 chromatographed for resolution of phospholipids. These radioactive lipids have not been completely identified.

Earlier work showed that *A. laidlawii* B strain PG9 incorporated acetate radioactivity into fatty acids (15). We isolated at least 50% of all the radioactivity in unfractionated purified lipids from the organisms listed in Table 2 in a methyl ester fatty acid fraction.

DISCUSSION

MT medium was originally chosen for our work because of the low serum content (1%),

but growth of the *Mycoplasma* strains in this medium was negligible or absent. Therefore, SSR2 medium (containing 3% serum), which supported growth of all of the strains studied, was used as the test medium.

As shown in Fig. 1, there was an increase in the disintegrations per minute per milligram of lipid of the *Acholeplasma* strains grown in MT medium when compared to SSR2 medium ($P < 0.005$). This increase is attributed to a greater biosynthetic use of acetate as a lipid precursor in the lipid-poor MT medium compared to the SSR2 medium.

Some of the *Acholeplasma* strains, especially b3046, showed a wide variation in replicate experiments in disintegrations per minute per milligram of lipid. This variation may be due in part to different lots of medium ingredients.

Only about 50% of the radioactivity was

FIG. 1. 4D-TLC (9) of lipid standards and *A. laidlawii* A strain PG8-purified lipids. Upper left plate, *A. laidlawii* A strain PG8-purified lipids (0.50 mg) visualized by sulfuric acid-dichromate spray and charring. Lower plate, Radioautograph of *A. laidlawii* strain PG8-purified lipids (0.20 mg). Upper right plate, Lipid standards (25 to 50 µg each): tetracosane, cholesteryl palmitate, methyl palmitate, tripalmitin, 1,3-dipalmitin, 1,2-dipalmitin, palmitic acid, cholesterol, 1-monopalmitin, 2-monopalmitin, phosphatidic acid, phosphatidyl glucose, and lecithin.

FIG. 2. Radioautographs of purified lipids of: *A. axanthum* S743 (1.06 mg), upper left plate; *A. granularum* BTS-39 (0.24 mg), upper right plate; and *A. laidlawii* strain PG8 (0.25 mg), lower plate. TLC plates were chromatographed by the method of Turner and Roussell (31) to separate phospholipids.

found in the fatty acid methyl esters prepared from the lipids of *A. laidlawii* A strain PG8, *A. axanthum* S743, and *A. granularum* BTS-39. We do not know in what lipid moiety the remainder of the radioactivity is located. We postulate that it might be located in the glycerol backbone or the carbohydrate components of the glycolipids and phospholipids.

The radioautographs of 4D-TLC separation of lipids from *A. laidlawii* A strain PG8 show small amounts of radioactive components in the diglyceride area. The presence of these apparent diglycerides suggests a source for the intermediates in the synthesis of glycolipids and possibly phospholipids. Smith has shown that *A. laidlawii* B can synthesize monoglycosyl diglyceride (MGD) from 1,2-diglyceride and uridine-5'-diphosphoglucose (UDPG) and the diglucosyl diglyceride from MGD and UDPG (27). Plackett et al. (8) have found that *A. axanthum* S743 is capable of utilizing glycerol in the synthesis of phospholipids. The relatively

small amounts of the radioactive diglyceride-like compounds found in our work suggests a possible source of these compounds for more complex lipid synthesis.

Mårdh and Taylor-Robinson (5) found that the growth of strains of 31 *Mycoplasma* species was inhibited by lysolecithin; strains of *Acholeplasma* species were less sensitive. These workers used *Acholeplasma* sp. strain Squire (=PG49), which was recently established as *A. modicum* (4), and found that it was as sensitive to lysolecithin as the majority of the *Mycoplasma* strains. From our work, this strain is included in the genus *Acholeplasma* because it incorporates radioactivity into lipid.

Recently, Rottem et al. (22) detected acyl carrier protein activity in acholeplasmas and relatively less activity in mycoplasmas. Their findings are compatible with the relative amounts of acetate radioactivity incorporated into the lipids of the acholeplasmas and mycoplasmas that we have tested.

It is proposed that the ability of acholeplasmas to incorporate significant amounts of [1^{14}C]acetate into their lipid is a useful criterion for differentiating them from mycoplasmas, which incorporate only small amounts of [1^{14}C]acetate.

REPRINT REQUESTS

Address reprint requests to: Dr. J. Dennis Pollack, 215 Ross Hall, The Children's Hospital, 561 South 17th Street, Columbus, Ohio 43205.

LITERATURE CITED

1. Bak, L. B., F. T. Black, C. Christiansen, and E. A. Freundt. 1969. Genome size of mycoplasmal DNA. *Nature* (London) **224**:1209-1210.
2. Bode, H. R., and H. J. Morowitz. 1967. Size and structure of the *Mycoplasma hominis* H39 chromosome. *J. Mol. Biol.* **23**:191-199.
3. Kunze, M. 1971. Natrium-Polyanethol-Sulfonate als diagnostisches Hilfsmittel bei der Differenzierung von Mykoplasmen. *Zentralbl. Bakteriologie. Parasitenk. Infektionskr.* **216**:501-505.
4. Leach, R. H. 1973. Further studies on classification of bovine strains of Mycoplasmales, with proposals for new species, *Acholeplasma modicum* and *Mycoplasma alkalescens*. *J. Gen. Microbiol.* **75**:135-153.
5. Mårdh, P.-A., and D. Taylor-Robinson. 1973. The differential effect of lysolecithin on mycoplasmas and acholeplasmas. *Med. Microbiol. Immunol.* **158**:219-226.
6. Metcalfe, L. D., A. A. Schmitz, and J. R. Pelka. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. *Anal. Chem.* **38**:514-515.
7. Morowitz, H. J., H. R. Bode, and R. G. Kirk. 1967. The nucleic acids of mycoplasma. *Ann. N. Y. Acad. Sci.* **143**:110-114.
8. Plackett, P., P. F. Smith, and W. R. Mayberry. 1970. Lipids of a sterol-nonrequiring mycoplasma. *J. Bacteriol.* **104**:798-807.
9. Pollack, J. D., D. S. Clark, and N. L. Somerson. 1971. Four-directional-development thin-layer chromatography of lipids using trimethyl borate. *J. Lipid Res.* **12**:563-569.
10. Pollack, J. D., S. Razin, and R. C. Cleverdon. 1965. Localization of enzymes in *Mycoplasma*. *J. Bacteriol.* **90**:617-622.
11. Pollack, J. D., S. Razin, M. E. Pollack, and R. C. Cleverdon. 1965. Fractionation of mycoplasma cells for enzyme localization. *Life Sci.* **4**:973-977.
12. Pollack, J. D., N. L. Somerson, and L. B. Senterfit. 1969. Effect of pH on immunogenicity of *M. pneumoniae*. *J. Bacteriol.* **97**:612-619.
13. Pollack, J. D., N. L. Somerson, and L. B. Senterfit. 1970. Isolation, characterization, and immunogenicity of *Mycoplasma pneumoniae* membranes. *Infect. Immunity* **2**:326-339.
14. Pollack, J. D., N. L. Somerson, and L. B. Senterfit. 1973. Chemical composition and serology of *Mycoplasma pneumoniae* lipids. *J. Infect. Dis.* **127**:32-35.
15. Pollack, J. D., and M. E. Tourtellotte. 1967. Synthesis of saturated long chain fatty acids from sodium acetate- 1^{14}C by *Mycoplasma*. *J. Bacteriol.* **93**:636-641.
16. Prescott, B., G. Caldes, W. D. James, and R. M. Chanock. 1967. Phospholipid hapten of *Mycoplasma pneumoniae*. *Ann. N. Y. Acad. Sci.* **143**:497-500.
17. Razin, S., M. Argaman, and J. Avignan. 1963. Chemical composition of mycoplasma cells and membranes. *J. Gen. Microbiol.* **33**:477-487.
18. Razin, S., and J. G. Tully. 1970. Cholesterol requirement of mycoplasmas. *J. Bacteriol.* **102**:306-310.
19. Robinson, J. P., and R. E. Hungate. 1973. *Acholeplasma bactoclasticum* sp. n., an anaerobic mycoplasma from the bovine rumen. *Int. J. Syst. Bacteriol.* **23**:171-181.
20. Rodwell, A. W. 1967. The nutrition and metabolism of mycoplasma: progress and problems. *Ann. N. Y. Acad. Sci.* **143**:88-109.
21. Rottem, S. 1972. Differentiation of sterol-requiring from sterol-nonrequiring mycoplasmas by amphotericin B. *Appl. Microbiol.* **23**:659-660.
22. Rottem, S., O. Muhsam-Peled, and S. Razin. 1973. Acyl carrier protein in mycoplasmas. *J. Bacteriol.* **113**:586-591.
23. Rottem, S., and S. Razin. 1967. Uptake and utilization of acetate by mycoplasmas. *J. Gen. Microbiol.* **48**:53-63.
24. Rouser, G., G. Kritchevsky, and A. Yamamoto. 1967. Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids, p. 99-162. *In* G. V. Marinetti (ed.), *Lipid chromatographic analysis*. Marcel Dekker, Inc., New York.
25. Siakotos, A. N., and G. Rouser. 1965. Analytical separation of nonlipid water soluble substances and gangliosides from other lipids by dextran gel column chromatography. *J. Amer. Oil Chem. Soc.* **42**:913-919.
26. Smith, P. F. 1968. The lipids of *Mycoplasma*, p. 69-105. *In* R. Paoletti and D. Kritchevsky (ed.), *Advances in lipid research*. Academic Press Inc., New York.
27. Smith, P. F. 1969. Biosynthesis of glucosyl diglycerides by *Mycoplasma laidlawii* strain B. *J. Bacteriol.* **99**:480-486.
28. Smith, P. F. 1972. Lipid composition of *Mycoplasma neurolyticum*. *J. Bacteriol.* **112**:554-558.
29. Smith, P. F., and G. H. Rothblat. 1960. Incorporation of cholesterol by pleuropneumonia-like organisms. *J. Bacteriol.* **80**:842-850.
30. Tully, J. G., and S. Razin. 1969. Characteristics of a new sterol-nonrequiring *Mycoplasma*. *J. Bacteriol.* **98**:970-978.
31. Turner, J. D., and G. Rouser. 1970. Precise quantitative determination of human blood lipids by thin-layer and triethylamino ethylcellulose column chromatography. II. Plasma lipids. *Anal. Biochem.* **38**:437-445.
32. Vorbeck, M. L., and G. V. Marinetti. 1965. Separation of glucosyl diglycerides from phosphatides using silicic acid column chromatography. *J. Lipid Res.* **6**:3-6.