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Lipids of *Sarcina lutea*

III. Composition of the Complex Lipids

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ABSTRACT

HUSTON, CHARLES K. (Fort Detrick, Frederick, Md.), PHILLIP W. ALBRO, AND GERALD B. GRINDEY. Lipids of *Sarcina lutea*. III. Composition of the complex lipids. J. Bacteriol. **89**:768–775. 1965.—The complex lipids from a strain of *Sarcina lutea* were isolated and separated into fractions on diethylaminoethyl cellulose acetate and silicic acid columns. These fractions were monitored in several thin-layer chromatography systems. The various lipid types were characterized by their behavior in thin-layer systems and by an analysis of their hydrolysis products. The fatty acid composition of the column fractions was determined by gas-liquid chromatography. A number of components (13) were separated by thin-layer chromatography and characterized. The major components were polyglycerol phosphatide (17.0%), lipoamino acids (15.1%), phosphatidyl glycerol (13.8%), and an incompletely characterized substance (15.0%). Minor constituents included phosphatidyl inositol (5.5%), phosphatidic acid (4.2%), phosphatidyl serine (2.0%), and phosphatidyl choline (1.0%). No phosphatidyl ethanolamine was observed.

Few bacterial phospholipids have been studied thoroughly by modern analytical techniques. Lovern (1957) described many bacterial phospholipids as simple fatty acid esters of phosphorylated carbohydrates. Ethanolamine is the most commonly reported nitrogen-containing component, whereas choline is found in some species (Asselineau and Lederer, 1960). Recent reports have also described the isolation of lipoamino acid complexes from the complex lipids of bacteria (MacFarlane, 1962*a*, *b*; Hunter and James, 1963; Ikawa, 1963).

We have reported (Huston and Albro, 1964) that *Sarcina lutea* contains a complex mixture of highly polar lipids comprising approximately 23% of the total extractable lipid. The present communication is on the composition of this polar fraction.

MATERIALS AND METHODS

Whatman diethylaminoethyl (DEAE) cellulose was obtained from Scientifica, Clifton, N.J., and converted to the acetate form, according to Rouser et al. (1961). Silicic acid was obtained from Mallinckrodt Chemical Works, St. Louis, Mo., and sieved to 100/200, 100/140, and 160/200 mesh. Inorganic reagents, solvents, and Hyflo Super-Cel were obtained from Fisher Scientific Co., Silver Spring, Md., and were the highest grades available. Chloroform, hexane, and propionic acid were redistilled before use. The methanol, acetone, benzene, and diisobutyl ketone were gas-chromatographically pure. Synthetic phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl choline (all dipalmitoyl) were A-grade from Calbiochem. Lecithin and cephalin standards were isolated from soy phosphatides by silicic acid column chromatography, according to Fleischer et al. (1962), and examined for homogeneity by thin-layer chromatography (TLC) according to Horrocks (1963). All other lipid standards used were the highest grades available from Mann Research Laboratories, New York, N.Y., or Applied Science Laboratories, Inc., State College, Pa. Rhodamine 6G and ninhydrin were obtained from Eastman Organic Chemicals, Rochester, N.Y. TLC apparatus and materials were obtained from Brinkmann Instruments, Inc., Great Neck, N.Y.

Culture conditions. S. lutea ATCC 533 was cultured at 25 C for 24 hr in tryptic soy broth (Difco) under forced aeration. The prepared medium was found to contain less than 0.009% lipid.

Cells were harvested, as previously described (Huston and Albro, 1964), immediately after the growth; all operations were performed below 5 C.

Lipid extraction. Extraction of lipids and isolation of the "complex lipid" fraction was accomplished as previously described (Huston and Albro, 1964). Nonlipid contaminants that were not removed by the Folch washing procedure were separated on Sephadex (Wells and Dittmer, 1963) and on DEAE cellulose columns (Rouser et al., 1961). These contaminants amounted to 8 to 14%

of the complex lipid fraction from silicic acid chromatography and consisted of glycerol, nitrogenous bases, and peptides.

Column chromatography. Four classes of lipids were isolated on DEAE cellulose acetate columns, by the method of Rouser et al. (1961). "Dipolar ionic" and neutral lipids were eluted with chloroform-methanol (7:1, v/v), "basic" lipids with chloroform-methanol (7:3, v/v), "acidic" lipids with glacial acetic acid, and "highly acidic" lipids with chloroform - methanol - concentrated ammonium hydroxide (80:20:1, v/v/v). The fraction eluted with chloroform-methanol (7:1) was resolved into neutral lipids, lecithins, and lysolecithins on a silicic acid-silicate column (Rouser et al., 1961). Free fatty acids were removed from the "acidic" lipid fraction on silicic acid (Fleischer et al., 1962).

Phosphatidic acid and polyglycerol phosphatide (cardiolipin type) were isolated by elution from an acid-washed silicic acid column with 5% methanol in chloroform. Phosphatidic acid travelled with the solvent front, whereas the polyglycerol phosphatide eluted much later. After rechromatography, each of the fractions gave a single spot on thin-layer plates. None of the other components could be isolated in pure form by column chromatography.

TLC. Silica gel G plates prepared according to Stahl (1958), or silica gel G plates impregnated with 10% sodium acetate (Horrocks, 1963), were developed in the ascending manner in unlined tanks to resolve the various complex lipids and to monitor column fractions. Chloroform-methanol-water (65:25:4, v/v/v; Horrocks, 1963), chloroform-methanol-14% aqueous ammonia (17:7:1, v/v/v), and diisobutyl ketone-acetic acid-water (8:5:1, v/v/v; Lepage, 1964) were used as solvent systems. The spots were detected with (i) aqueous Rhodamine 6G, (ii) iodine vapors, (iii) a molybdate reagent (Dittmer and Lester, 1964), (iv) Dragendorff's reagent for choline and quaternary ammonium compounds, (v) 0.25% ninhydrin in acetone buffered to *p*H 5.5 with acetate, (vi) iodine-sodium azide reagent for sulfolipids (Block, Durrum, and Zweig, 1958), and ammoniacal silver nitrate for phosphatidic acid, phosphatidyl glycerol, polyglycerol phosphatide, and inositides.

In some cases, the silicic acid-impregnated filter-paper system of Marinetti (1962) was used to aid in the identification of lipids for which no standards were available.

Quantitation of TLC-resolved material was performed according to Amenta (1964).

Hydrolysis and paper chromatography. Water-soluble products, produced by hydrolysis of the various fractions and individual components according to Ikawa (1963), were analyzed by ascending chromatography in *n*-butanol-acetic acid-water (2:1:1, v/v/v; Fink, Cline, and Fink, 1963), or in phenol–*n*-butanol–formic acid–water (50:50:3:10, v/v/v/v; Goldfine, 1962); and by descending chromatography in *n*-butanol-acetic acid-water (4:1:5, v/v/v; Block, Le Strange, and Zweig, 1952), or *n*-butanol-propionic acid-water according to Benson and Maruo (1958). Phosphate esters were detected with Hayes-Isherwood reagent, amino compounds with 0.4% ninhydrin in water-saturated *n*-butanol, carbohydrates with 2.5% aniline hydrogen phthalate, polyhydroxy compounds with ammoniacal silver nitrate, and quarternary ammonium salts with Dragendorff's reagent. Whatman no. 1 paper was used for ascending chromatography and Whatman no. 4 paper for descending chromatography.

Acetonation of vicinal hydroxyl groups. Samples suspected of containing either phosphatidyl glycerol or polyglycerol phosphatide were acetonated by the procedure of Benson and Maruo (1958). Samples before and after acetonation were analyzed by TLC on silica gel G in chloroform-methanol-water (65:25:4, v/v/v). Detection was as described above.

Chemical methods. Inorganic phosphorus was determined by the procedure of Fiske and Subba-Row (1925), total phosphorus by the Allen (1940) modification, amino nitrogen according to Lea and Rhodes (1955), and total nitrogen according to Miller and Miller (1948). Acetals and aldehydes were qualitatively sought with azobenzenepbenylhydrazine-sulfonic acid (Feigl, 1954). Fatty acids were determined by saponification, methanolysis, and weighing. Fatty acid methyl esters were produced for gas chromatography as previously described (Huston and Albro, 1964).

Gas-liquid chromatography (GLC). Gas-liquid chromatography of fatty acid methyl esters was carried out as described previously (Huston and Albro, 1964) after separation of the normal fatty acid and hydroxy acid esters on silicic acid columns (Kishimoto and Radin, 1963).

Carbohydrates were separated as their trimethylsilyl (TMS) derivatives (Bentley et al., (1963) on a column (121.92 by 0.635 cm) of 3% SE-52 on 60/80 mesh Chromosorb W at various temperatures.

Infrared analysis. Infrared spectra of various fractions and constituents were examined as thin films on KBr or AgCl plates with a Perkin-Elmer model 21 recording infrared spectrophotometer.

RESULTS

In the complex lipid fraction, 13 components were observed, as indicated on the TLC tracing shown in Fig. 1. The relative amount of each component and tentative identifications are summarized in Table 1. Table 2 provides data on the weight per cent of the complex lipid, phosphorus, nitrogen, fatty acid, and amino nitrogen content of the various column fractions.

Samples of the total complex lipid material were tested, before and after acid hydrolysis, for aldehyde, acetal, or sulfhydryl functions. All such tests were negative.

Chromatography of the various fractions on silicic acid-impregnated paper confirmed several of the identifications in Table 1, but the wide

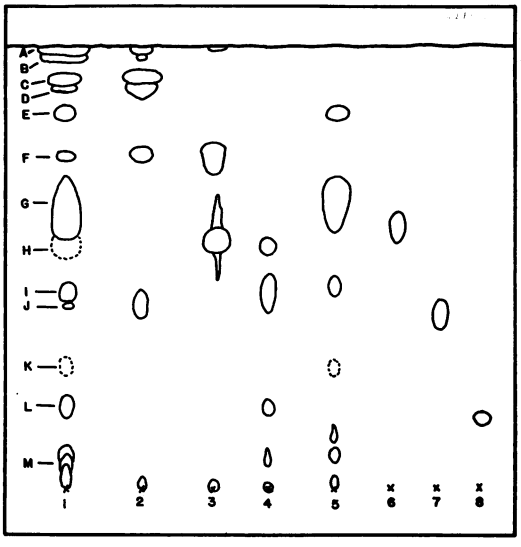

FIG. 1. *Thin-layer chromatography of Sarcina lutea complex lipids. Solvent system: chloroform-methanol-14% aqueous ammonia (17:7:1, v/v/v). Spots: (1) total complex lipid, (2) neutral-"dipolar ionic" fraction, (3) "basic" fraction, (4) "acidic" fraction, (5) "highly acidic" fraction, (6) authentic phosphatidyl ethanolamine, (7) soy lecithin, and (8) authentic phosphatidyl serine.*

TABLE 1. *Complex lipid content of Sarcina lutea*

Spot*	DEAE column fraction†	Total complex lipid	Tentative identification
		%	
A	N-D	3.0	Neutral lipid
B	N-D	1.0	Oxidized glyceride
C	N-D	8.4	Lipoamino acid
D	N-D	6.7	Lipoamino acid
E	HA	4.2	Phosphatidic acid‡
F	N-D, B	8.0	Free fatty acid (salt)
G	B, HA	17.0	Polyglycerol phosphatide‡
H	B, A	15.0	Unidentified
I	A, HA	13.8	Phosphatidyl glycerol‡
J	N-D	1.0	Lecithin
K	HA	5.5	Phosphatidyl inositol‡
L	A	2.0	Phosphatidyl serine‡
M	N-D, B, A, HA	14.4	Nonlipid

* See Fig. 1 and Results.

† N-D = neutral or dipolar ionic; B = basic; A = acidic; HA = highly acidic.

‡ Additional confirmation of identity provided by chromatography according to Marinetti (1962).

variety of lipoamino acid complexes made it impossible to resolve all the components by this technique.

The identification of spots in Fig. 1, total complex lipid, is as follows.

Spot A: neutral lipid. This spot occurred in the neutral-"dipolar ionic" lipid fraction and was eluted from silicic acid with the least polar solvent used. It moved with the solvent front in all TLC systems, gave a yellow color with Rhodamine 6G, and did not react with the other detection reagents. On this basis, the material was identified as neutral lipid carryover, and was primarily responsible for the high fatty acid-phosphorus (FA/P) ratio observed in the neutral-"dipolar ionic" lipid fraction.

Spot B: oxidized glyceride. This brown material was easily visible on the TCL plate without treatment. Since it was absent in preparations handled entirely under nitrogen, it was probably oxidized glyceride and not a pigment.

Spot C: lipoamino acid. This spot was ninhydrin-positive, yellow with Rhodamine 6G, and, upon hydrolysis, yielded glycerophosphate, phenylalanine, and a material that moved faster than any of the natural quaternary ammonium bases, but which reacted with Dragendorff's reagent. The Dragendorff-positive substance may be an artifact of the hydrochloric acid hydrolysis (De Koning, 1963). The spot was thus considered to be lipoamino acid, the exact structure of which has not been determined. However, since it was of relatively low polarity and nonacidic, we may suppose that the linkage was through the carboxyl group of the amino acid.

Spot D: lipoamino acid. Serine, threonine, and a trace of phenylalanine were found in the hydrolysate of this material. The presence of the two hydroxy constituents, serine and threonine,

TABLE 2. *Analysis of column fractions*

DEAE column fraction*	Complex lipids	Phosphorus	Molar ratios†		
			FA/P‡	Total N/P	Amino N/P
	%	%			
N-D	23.0	0.78	7.56	2.20	2.00
B	17.4	0.54	1.98	2.00	1.86
A	18.8	2.22	1.97	0.12	0.12
HA	26.4	3.56	1.97	2.18	1.94
Phosphatidic acid	4.2	4.87	1.98	0.10	0
Polyglycerol phosphatide	17.0	4.49	1.99	0.12	0

* N-D = neutral or dipolar ionic; B = basic; A = acid; HA = highly acidic.

† Most fractions contained significant amounts of nitrogenous nonlipid, resulting in many of the high N/P ratios.

‡ FA = fatty acid; P = phosphorus; and N = nitrogen.

in view of the failure of this material to reduce ammoniacal silver nitrate, suggests the possibility that the ester linkage occurs through the OH group of the amino acids. Since this material moved much faster than ordinary phosphatidyl serine, a structure other than the usual acylated glycerol phosphate skeleton is postulated. And, since this spot was ninhydrin-positive, the linkage was probably not through the NH_2 group. The amino acid-containing lipids of *S. lutea* gave indications of great diversity in structure, but complete characterization of each type was beyond the scope of this study.

Spot E: phosphatidic acid. This material was highly acidic, easily eluted from silicic acid with 5% methanol in chloroform, and contained no nitrogen. It had a FA/P molar ratio of 1.98 and a phosphorus content of 4.87%, which corresponds almost exactly with the calculated value for diisopentadecanoyl phosphatidic acid. It gave a weak reaction with the molybdate reagent and a strong reaction with a modified Hayes-Isherwood reagent, thus reflecting its oxidizing nature. Only glycerophosphate and fatty acids were found in the hydrolysis products. These data, plus the observed R_F values in various TLC systems, identified this spot as phosphatidic acid.

Spot F: salt of free fatty acid. The fatty acid nature of this material was ascertained by its negative reaction with the two molybdate sprays and with ninhydrin when developed in the acid or neutral TLC systems, and by its extraction from the complex lipid mixture with aqueous 0.1 M borax. When developed in the ammoniacal solvent system, this spot held sufficient ammonia to give a transient ninhydrin reaction. Qualitative analysis of this material (Wiig, Line, and Flagg, 1954) indicated the absence of calcium, magnesium, and ammonium salts. Sodium ions were found, but could easily be contaminants. The relative size of the spot was larger in older preparations, indicating its formation by slow autohydrolysis of more complex lipid compounds.

Spot G: polyglycerol phosphatide. On the basis of TLC data and column chromatographic properties, as well as its reaction with various of the detection reagents, spot G could have been either a bis-phosphatidic acid salt or a cardiolipin type of polyglycerol phosphatide. Hydrolysis of this material, after isolation on silicic acid, gave diphosphoglycerol (R_F 0.13 in butanol-acetic acid-water, 2:1:1, v/v/v), and diglycerol phosphate [R_F 0.17 in the descending system of Benson and Maruo (1958)]. The spot had a phosphorus content of 4.49%, which would be expected for tetraisopentadecanoyl cardiolipin of the structure described by MacFarlane and Gray (1957). The FA/P molar ratio of 1.99 indicates

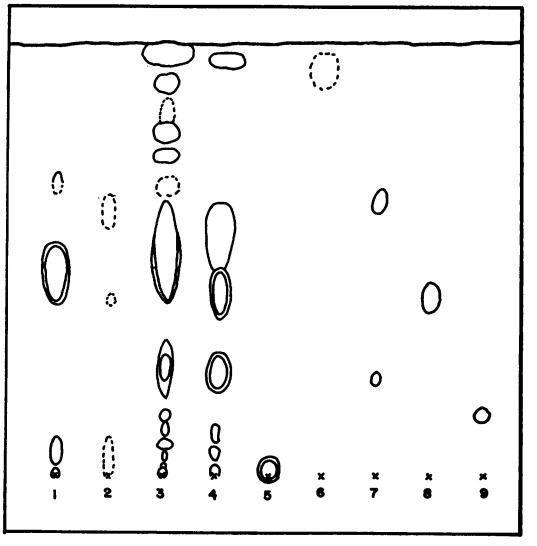

FIG. 2. *Thin-layer chromatography of phosphatidyl glycerol and phosphatidyl inositol. Solvent system: chloroform-methanol-water (65:25:4, v/v/v). Spots: (1) phosphatidyl glycerol fraction from silicic acid column, (2) material from spot 1 after acetonation, (3) total complex lipids, (4) "highly acidic" fraction, (5) inositol, (6) oleic acid, (7) authentic phosphatidyl ethanolamine, (8) soy lecithin, and (9) authentic phosphatidyl serine. Solid lines outline spots detected with molybdate reagent; dotted lines outline spots visible only after exposure to iodine vapors; double lines outline spots detected with alkaline silver nitrate. Spots 1 and 2 are the same concentration.*

that the structure is probably of the cardiolipin type rather than a bis-phosphatidic acid, which would have a FA/P ratio of 4:1. Further evidence for the cardiolipin structure was provided by the nearly negligible nitrogen content, failure of the material to reduce ammoniacal silver nitrate (bis-phosphatidic acid reacts), and the extreme tailing on silicic acid columns.

Spot H: unidentified. This material, upon hydrolysis, produced a wide variety of constituents, including glycerophosphate, serine, the methyl ester of alanine, methyl ethanolamine, and an as yet unidentified fast-moving Dragendorff-positive substance (De Koning, 1963). The appearance of this material primarily in the "basic" lipid fraction suggests that the nitrogenous moieties are linked through their carboxyl functions. It has, however, not been structurally characterized because it has been impossible to isolate it in a pure form.

Spot I: phosphatidyl glycerol. This material was identified by its acidic character, R_F values in the various TLC systems [especially the two-dimensional system of Lepage (1964)], loss of ability to

react with alkaline silver nitrate after acetonation (Fig. 2), and its response to the various detection reagents. It strongly resisted hydrolysis, probably because of the stability of the glycerol-phosphate bond (Olley, 1956). Hydroxyl absorption at 2.87 and at 9.43 μ was very prominent in the infrared spectrum of this material.

Spot J: lecithin. Spot J was identified as lecithin by its nonretention on DEAE cellulose acetate, R_F values in the TLC systems, reaction with Dragendorff's reagent, and the production of glycerophosphate and what appears to be (2-chloroethyl) trimethylammonium chloride (De Koning, 1963) upon hydrolysis.

Spot K: phosphatidyl inositol. Although this material is reported as comprising 5.5% of the complex lipid (Table 1), this value must be taken as a maximal one. GLC of the TMS derivative of inositol released by a 48 hr hydrolysis of this material in 6 N aqueous HCl at 110 C indicated a phosphatidyl inositol content of less than 1%. However, complete hydrolysis to free inositol is seldom achieved (Hanahan, 1960), and the higher value may be more nearly correct. Identification was based on the material's acidic nature, R_F values in various TLC systems, ability to reduce ammoniacal silver nitrate, and recovery of inositol from hydrolysates. The entire fraction in which this material appeared had a FA/P ratio of 1.96, and the R_F values were fairly high. It is therefore probable that this material was a monophospho-, monoinositol structure.

Spot L: phosphatidyl serine. The concentration of this material varied widely between batches of phospholipid material. It behaved as authentic phosphatidyl serine in various TLC systems, in its reactions with the detection reagents, and in producing serine upon hydrolysis. The reason for its variable quantitation is unknown.

Spot M: water-soluble nonlipid. Much, but not all, of this material was removed from the complex lipid mixture in the methanol fraction from DEAE cellulose acetate. Paper chromatography of this material prior to hydrolysis revealed the presence of choline, serine, phenylalanine, alanine, glycerol, and inorganic phosphate. No fatty acids were recovered from acid hydrolysates of this material.

Lipoamino acid components. Cultures (24-hr) of *S. lutea* were quite rich in amino acid-containing lipids. Alanine, phenylalanine, serine, proline, leucine, isoleucine, valine, arginine, methyl alanine, tyrosine, and threonine were detected in various batches of the complex lipids. Whereas some of these may be nonlipid contaminants, there was evidence that serine, alanine, phenylalanine, leucine, tyrosine, and threonine were structural components of the lipid constituents examined. Phosphoryl-serine, -choline, and -threonine have been observed on paper chromatograms of various hydrolysates. No positive biuret reaction was observed with any of the preparations.

Cells allowed to stand at 4 C for weeks to months yielded a complex lipid fraction essentially free of amino nitrogen and extremely low in total nitrogen. In these samples, almost all of the nitrogenous material was removed by the Folch washing procedure. The only complex lipids found in significant amounts in these preparations were phosphatidic acid, polyglycerol phosphatide, and phosphatidyl glycerol.

Fatty acid composition. The fatty acid composition of the various complex lipid fractions is summarized in Table 3.

Spectral data. The infrared spectra of the various complex lipid fractions differed substantially in only two regions. The "basic" lipids absorbed in the 6 to 6.8 μ region, characteristic of the NH group of amino acids (Silverstein and Bassler, 1964), whereas the "acidic" lipids did not. The "acidic" lipids, on the other hand, absorbed conspicuously at 8.25 μ, characteristic of secondary amides (Silverstein and Bassler, 1964), but the "basic" lipids did not.

DISCUSSION

The complex of lipids of *S. lutea* contains fairly large quantities of lipoamino acids, phosphatidyl glycerol, and polyglycerol phosphatide (Table 1). Lecithin, phosphatidyl serine, and phosphatidyl inositol, common among plant and animal phospholipids, were present only in small amounts, whereas phosphatidyl ethanolamine was not detected at all. Akashi and Saito (1960) have previously reported that *Sarcina* phospholipids consist principally of phosphatidic acid. Phosphatidyl ethanolamine has been reported as the major phospholipid of *Serratia marcescens* (Kates, Adams, and Martin, 1964), *Escherichia coli* (Law, 1961), *Azotobacter agilis*, and *Agrobacterium tumefaciens* (Kaneshiro and Marr, 1962). The phospholipids of some lactic acid bacteria, however, have been described by Ikawa (1963) as containing no serine, ethanolamine, or choline. Instead, the principal ninhydrin-positive lipids of these organisms yielded lysine and alanine upon hydrolysis.

Lipoamino acid complexes amounted to 15.1% of the total complex lipids from *S. lutea*. These complexes are very labile to enzymatic degradation and may be lost from cells unless enzymatic processes are stopped immediately after harvesting (MacFarlane, 1962*a*). Gaby, Wolin, and Zajac

TABLE 3. *Fatty acid composition of Sarcina lutea complex lipids*[a]

Fatty acid carbon chain[b]	Composition (per cent)						
	Total complex lipid	Column fractions[c]				Phosphatidic acid	Polyglycerol phosphatide
		N-D	B	A	HA		
<12	1.0	1.9	0.6	0.2	1.1	4.8	—
12:0 + 12:1	0.3	0.6	0.4	0.3	Trace[d]	3.4	Trace
br-13:0 + 13:1	0.3	0.5	0.3	0.3	Trace	2.6	2.7
br-14:0	1.3	0.6	0.4	0.8	2.9	1.3	0.6
n-14:0	1.7	2.3	0.6	0.6	2.6	4.4	1.0
n-14:1	1.3	3.1	0.7	0.6	0.6	1.3	Trace
br-15:0 (iso)	18.4	11.9	23.5	22.4	17.7	3.3	20.3
br-15:0 (anteiso)	41.7	28.1	53.5	52.3	38.3	16.6	43.7
n-15:0	2.1	2.5	0.6	1.7	3.0	Trace	1.6
br-16:0	1.6	1.1	0.6	1.4	2.7	1.8	1.5
n-16:0	5.1	11.6	4.5	1.8	2.1	23.5	3.4
n-16:1	7.3	7.7	2.8	3.6	12.5	4.4	6.2
br-17:0	1.5	1.0	0.6	2.0	2.0	Trace	2.1
Unidentified	0.4	—	0.2	—	0.3	3.8	—
n-17:0	0.5	1.0	0.4	—	0.4	2.8	0.6
n-17:1	0.2	0.3	0.1	0.8	0.4	0.2	0.9
br-18:0	0.7	2.2	Trace	0.2	0.2	—	0.2
n-18:0	11.2	18.8	8.5	6.3	9.8	16.1	5.7
n-18:1 (Δ^{11})	2.8	4.2	1.0	2.0	3.3	8.3	2.5
br-19:0 (iso)	0.5	1.2	Trace	0.7	Trace	—	3.0
n-19:0	Trace	—	0.2	—	—	1.1	Trace
br-20:0 (anteiso)	Trace	—	Trace	—	Trace	—	0.9
n-20:0	Trace	—	Trace	—	—	—	2.7
br-22:0 (anteiso)	Trace	—	Trace	—	—	1.5	—

[a] Quantitation from chromatograms made with an 243.84 × 0.635 cm copper column containing 10% butanediol succinate on 80/100 mesh Chromosorb W. Column maintained at 190 C.
[b] Number of carbon atoms in acid:number of double bonds; br = branch.
[c] N-D = neutral or dipolar ionic; B = basic; A = acidic; HA = highly acidic.
[d] Trace = less than 0.1%.

(1960) and Hunter and James (1963) suggested that these complexes are involved in amino acid transport processes. The wide range of amino acids found associated with these lipids in the present study would seem to confirm this view. However, as has been suggested, these complexes in *S. lutea* apparently contain both carboxyl and hydroxyl linkages, and possibly (spot H) even amino linkages. Moreover, the amino acids appeared to be bound to other moieties in addition to phosphatidyl glycerol.

Hunter and Godson (1961) suggested that some lipoamino acid complexes may be transient intermediates in the transfer of amino acid residues from a nucleic acid template in the formation of lipoproteins. Serine, threonine, hydroxylysine, tyrosine, and other amino acids possessing a hydroxyl function may conceivably interchange during or after formation of the classical acidic cephalins. This could account for the variable content of phosphatidyl serine found in different batches of the *S. lutea* complex lipids.

The possibility exists, then, that the different lipoamino acid types found in *S. lutea* may serve different functions; some are primarily structural components, some are involved in amino-acid transport, and some are intermediates in lipoprotein synthesis.

The fatty acid composition of the "total complex lipid" (Table 3) differs in several respects from that reported previously (Huston and Albro, 1964). The 24-hr culture used in this study was selected to maximize the yield of lipoamino acid complexes, and the culture medium differed slightly in lipid composition from the Trypticase Soy Broth (BBL) used in earlier studies.

The fatty acid composition of the phosphatidic acid fraction differed appreciably from that of all the other fractions. If we suppose phosphatidic acid to be an intermediate in the synthesis of other phospholipids (Hanahan, 1960), or a product of the enzymatic removal of a nitrogenous base from glycerophosphatides (Kates, 1960), then this difference is difficult to explain. It

suggests that phosphatidic acid may be synthesized independently of the other phospholipids. Of the various fractions shown in Table 3, only the "basic" lipid and the "acidic" lipid fractions closely resemble each other in fatty acid composition. They also constitute similar percentages of the total complex lipid (17.4 and 18.8%, respectively). It is not unlikely, therefore, that these materials are formed from similar starting materials, e.g., diglycerides, and differ primarily only in the nature and linkage of the nitrogenous constituent.

The polyglycerol phosphatide of *S. lutea* appears to be a cardiolipin. This type has been reported in *Staphylococcus aureus* (MacFarlane, 1962*b*), whereas the bisphosphatidic acid type has been reported in *Bacillus polymyxa* (Matches, Walker, and Ayres, 1964).

Further discussion on the possible metabolic roles of the various complex lipids of *S. lutea* will have to await studies on the distribution of these components within the cell and characterization of the individual metabolic pathways for each component.

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