JOURNAL OF BACTERIOLOGY, Apr., 1965

Vol. 89, No. 4
Printed in U.S.A.

Comparison of Lipids and Lipopolysaccharide from the Bacillary and L Forms of *Proteus* P18

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Received for publication 21 November 1964

ABSTRACT

NESBITT, J. A., III (The Johns Hopkins University School of Medicine, Baltimore, Md.), AND W. J. LENNARZ. Comparison of lipids and lipopolysaccharides from the bacillary and L forms of *Proteus* P18. J. Bacteriol. **89**:1020–1025. 1965.—Comparative studies on the L form of *Proteus* P18 and the parent bacterium grown in a defined medium showed that the L form contained 1.5 times as much extractable lipid (dry weight) as the bacillary form. The composition of the lipids extractable by chloroform-methanol was quite similar in the two bacterial forms. The occurrence of myristate and β-hydroxymyristate in the bound, nonextractable lipid was found to be a reflection of the presence of lipopolysaccharide (LPS) in each organism. The bacillary organism contains three to four times as much LPS as the L form. The LPS isolated from both organisms contains heptose, hexosamine, phosphorus, 3-deoxyoctulosonate, glucose, galactose, and fatty acids.

Vendrely and Tulasne (1952) showed that a stable L form of *Proteus* P18 contained four to five times as much lipid on a dry-weight basis as its parent bacterium. More recently, several workers investigated the lipids of the *Proteus* L form with various conditions of growth and extraction. Krembel (1963) confirmed the fourfold difference observed earlier, but investigations by other workers (Rebel et al., 1960; Rebel, Bader-Hirsch, and Mandel, 1963; Smith and Rothblat, 1961) showed that the L form contained twice as much total lipid as the parent bacterium. Evaluation of these studies is difficult because of use of undefined growth media and/or cells harvested after prolonged growth. Since variations in cultural conditions might markedly influence the lipid composition and content of the cells, we undertook to investigate in a comparative manner the lipid content of the bacillary and L form of *Proteus* grown under conditions of rapid growth in a chemically defined medium. Both the lipids extractable with chloroform-methanol and the lipopolysaccharide (LPS) of each bacterial form have been studied.

MATERIALS AND METHODS

Growth conditions. The bacillary and L form of *Proteus* P18 were obtained from C. Weibull, Central Bacteriological Laboratory of Stockholm City, Sweden. This strain of *Proteus* was reported to have biochemical properties similar to *Proteus mirabilis* (Morrison and Weibull, 1962). Both forms were maintained on the medium described by Abrams (1955) supplemented with 10% deactivated horse serum and 0.8% agar (Difco), and were subcultured every 2 to 4 weeks. Cells used in all lipid studies were grown on a rotary shaker (200 rev/min) at 30 C in the serum- and penicillin-free medium of Abrams (1955).

Large quantities of cells were obtained by growth in 500-ml Erlenmeyer flasks containing 200 ml of medium. In the case of the bacillary form, flasks were inoculated with 0.7 ml of a culture previously grown for 12 to 16 hr in a 125-ml Erlenmeyer flask. To grow the L form, 2 ml of medium were heavily inoculated from a plate and allowed to grow for 24 hr. The resulting culture was used to inoculate 50 ml of medium contained in a 125-ml Erlenmeyer flask. After 24 hr of growth, 8.0 ml of this culture were used to inoculate each 200-ml culture.

Growth was estimated turbidimetrically with a Klett photoelectric colorimeter equipped with a 660 filter. Cells were harvested by centrifugation at 9,000 × *g* for 10 min. No washing steps were performed. Cell dry weight determinations were accomplished by desiccation of the cells under high vacuum until a constant weight was achieved. In all experiments, cells in either middle logarithmic phase (turbidity of bacillary and L form, 240 and 130 Klett units, respectively) or late

logarithmic-early stationary phase (bacillary and L form, 425 and 225 Klett units, respectively) were used.

Extraction of lipids. Lipids were extracted from the cells with chloroform-methanol 2:1 (v/v) (CM) according to the procedure described by Folch, Lees, and Sloane-Stanley (1957). Cells were suspended in CM (20 ml per g of packed cells) and stirred at room temperature for 2 hr. The suspension was filtered through a Büchner funnel, and the cell residue was extracted two more times for 1 hr each, with 25% of the original volume of CM for each extraction. The combined CM extract was washed with 0.2 volumes of 0.9% NaCl solution, and then was concentrated to dryness in vacuo. The lipids so obtained were dissolved in chloroform, filtered through glass wool, and then concentrated to dryness. The insoluble, complex mixture remaining after CM extraction contained the "nonextractable lipid."

Isolation of lipopolysaccharide (LPS). Cells were ruptured by extrusion through a French pressure cell chilled to 0 C. The suspension was centrifuged for 60 min at 100,000 × *g*, and the resulting particulate fraction was washed three times with 0.3 M NaCl. The LPS was isolated from the washed pellet by the phenol extraction method of Westphal, Luderitz, and Bister (1952).

Characterization of lipids and fatty acids. Lipids isolated from cells grown in the presence of inorganic P^{32} were chromatographed on silicic acid with use of gradient elution with chloroform-methanol, as described by Lennarz (1964). The elution of neutral lipids was monitored gravimetrically. The radioactivity in the phospholipid was determined with a Packard Tri-carb liquid scintillation counter. The major peaks were checked for homogeneity with thin-layer chromatography on silicic acid with a solvent system containing $CHCl_3$-CH_3OH-7 N NH_4OH (60:30:5). Lipids were visualized with Rhodamine 6G, and with ninhydrin spray reagents. The water-soluble products of acid hydrolysis of the phospholipids were characterized by paper chromatography by the technique of Wheeldon, Brinley, and Turner (1962).

Fatty acids were liberated from the CM extractable lipids, the nonextractable lipids, and the purified lipopolysaccharide (LPS) by alkaline hydrolysis. Samples (1 to 50 mg) were heated at 75 C in the presence of 10% NaOH and 50% methanol (1 to 5 ml) for 3 hr, except in the case of preparations containing LPS, in which hydrolysis was carried out overnight. The hydrolysates were cooled, extracted twice with petroleum ether, and then adjusted to *p*H 1 to 2 with concentrated HCl. The acidified solutions were extracted three times with diethyl ether (1 to 5 ml). The combined ether extracts were then washed with water, and the sample was concentrated under nitrogen. The fatty acids were then methylated with diazomethane. The methyl esters were analyzed by means of gas chromatography on a 6-ft U-shaped column packed with diethyleneglycol succinate (column temperature, 175 C; argon tank pressure, 10 psi). Fatty acid methyl esters were identified by co-chromatography with authentic samples. Confirmation of the structural assignments was obtained by gas chromatographic analysis before and after separation of the total fatty acids into three classes: saturated, unsaturated, and polar (hydroxy) fatty acids. Silicic acid column chromatography was used to separate the polar from the nonpolar methyl esters according to the method of Light, Lennarz, and Bloch (1962). The nonpolar methyl esters were further separated into saturated and unsaturated methyl esters according to the technique described by Goldfine and Bloch (1961).

Other methods. Protein (Layne, 1957) and total phosphate (King, 1932) were determined by standard methods. Nucleic acid was determined by absorption at 260 mμ, with an extinction coefficient of 24 optical-density units per mg of nucleic acid. Heptose, hexosamine, 3-deoxyoctulosonate, glucose, galactose, and fatty acids in the LPS were determined by the methods described by Elbein and Heath (*in press*).

RESULTS

Growth characteristics. To obtain cells from both bacterial forms at comparable stages of growth, the growth curves of each form were determined (Fig. 1). The growth rate of the L form, measured on a cell dry weight basis, was approximately one-half that of the bacillary form, whereas the overall extent of growth was approximately two-thirds that of the bacillary form. In contrast, when measured by turbidity, the apparent rate and overall extent of growth was much lower in the L form. However, the L form lysed upon dilution, and the turbidity values obtained from undiluted samples of the L form during the later growth stages were erroneously low. With the bacillary form, the turbidity measurements were in good agreement with dry weight determinations.

From Fig. 1 it is evident that lysis of the L form ensues after the cells enter the stationary

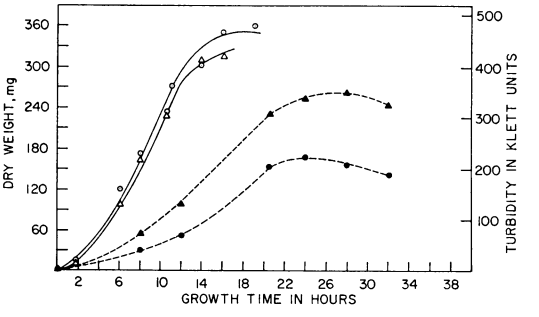

FIG. 1. *Growth of the L form (broken lines) and bacillary form (solid lines) as measured by turbidity (circles) and by dry weight (triangles).*

TABLE 1. *Lipid content of the L form and the bacillary form of Proteus P18*

Determination	Growth phase			
	Middle logarithmic		Late logarithmic	
	Bacillary	L form	Bacillary	L form
	%	%	%	%
Extractable lipids	11.4*	15.6	10.3	14.5
Total phosphorus of extractable lipids	0.40	0.55	0.35	0.50
Extractable lipid/total cellular protein	21.7	—	17.8	30.3
Fatty acids of nonextractable lipid	1.7	0.5	1.7	0.6

* All percentages are based on dry weight of cells, unless indicated otherwise. Values are the average of duplicates agreeing within 10%.

phase. This lysis is evidenced by a decrease in the dry weight determination values, a decrease in the turbidity values, and an increase in compounds absorbing ultraviolet light at 260 mμ in the supernatant fluid obtained after centrifugation of cells. This sensitivity of the L form to lysis was even more evident when attempts were made to wash cell pellets obtained by centrifugation; neither water nor fresh culture medium could be used for washing without concomitant lysis. These findings agree with those of Weibull (*personal communication*) but are in contrast to those of Fleck (1963) who, using a different defined medium, was unable to observe lysis of L-form cells.

Comparison of the lipids in the two forms. The results of a study of the amount of lipid measured on a dry-weight basis are presented in Table 1. It is clear that total extractable lipid constitutes approximately 10 to 11% of the total dry weight of the bacillary form and 14 to 16% of the dry weight of the L form. This 50% increase in the L form is also evident if total lipid phosphorus is measured, or if comparison is made on the basis of total cellular protein instead of dry weight. The growth phase from which the cells are harvested appears to have little effect on the amount of total lipid per cell. Also shown in Table 1 is the percentage of total fatty acids isolated from the nonextractable lipids of both forms (see below).

To ascertain whether the 50% greater amount of extractable lipid in the L form was due to an increase in one particular component of the total lipid or to a general increase in all the lipids, the lipid composition of the two forms was compared. Cells were grown in the presence of inorganic P^{32}, and the lipids were extracted and examined by thin-layer chromatography and by silicic acid column chromatography with gradient elution. The qualitative and quantitative distribution of the various lipids in both forms was approximately the same by either chromatographic method. No attempt was made to identify all the lipids. In both forms of *Proteus*, neutral lipids and fatty acids comprised only 4 to 5% of the total lipids. The fatty acid composition of the neutral lipids has been reported by Krembel (1964). The major phospholipid (ca. 70%), was ninhydrin-positive and, upon thin-layer chromatography, migrated with the same R_F as authentic phosphatidylethanolamine. The presence of phosphatidylethanolamine in the L form of *Proteus* has previously been reported by Rebel et al. (1960). Two minor phospholipids, together constituting approximately 20 to 25% of the total phospholipids, were detected. Both migrated more rapidly than phosphatidylethanolamine upon column or thin-layer chromatography, were ninhydrin-negative, and yielded only glycerol and fatty acids upon acid hydrolysis. These phospholipids therefore appear to be phosphatidic acid and glycerol derivatives of phosphatidic acid. In contrast to the findings briefly reported by Rebel et al. (1963), we were unable to demonstrate the presence of glycolipids when the total extractable lipids were subjected to analysis by the anthrone method of Radin (1959) or when thin-layer chromatograms of the lipids were treated with the benzidine reagent (Harrison and MacWilliam, 1954).

Fatty acid composition. Since the L form contained more extractable lipid than the bacillary form, but less fatty acid derived from nonextractable lipids, the fatty acid composition of the lipids of the two forms was studied (Table 2). With regard to the fatty acid derived from the extractable lipids, it is clear that the fatty acid composition of the two forms is qualitatively identical, except that an unidentified fatty acid, constituting 7.2% of the total fatty acids, was found in the L form. Also noteworthy is the absence of β-hydroxymyristate acid (see below). The major fatty acid (ca. 50%) in both forms is palmitic acid. The presence of cyclopropane fatty acids, which is typical of gram-negative bacteria, has previously been reported in the L form of *Proteus* P18 by Krembel (1964). Pronounced differences in the proportion of the C16 unsaturated and C17 cyclopropane acids are evident in the two forms. It has been demonstrated in other organisms that there is a precursor-product relationship between these two acids (Chung

TABLE 2. *Fatty acid composition of the extractable and nonextractable lipids*

Fatty acid[b]	Percentage by weight[a]			
	Extractable lipid		Nonextractable lipid	
	Bacillary	L form	Bacillary	L form
14:0	1.4	4.4	41.0	16.0
Unknown[c]	—	—	2.0	1.2
Unknown[d]	—	—	10.2	4.7
16:0	49.9	46.7	8.2	23.2
16:1	3.9	17.6	2.5	12.9
Unknown[e]	—	—	1.7	1.6
17:Cyclo	22.2	6.9	—	1.0
18:0	4.5	2.9	—	3.3
18:1	10.0	10.4	—	—
19:Cyclo	7.2	3.9	—	—
Unknown[f]	—	7.2	—	—
14:β-OH	—	—	34.7	35.4

[a] Per cent composition was determined by tracing the curves on paper, cutting out the peaks, and determining their weight (Janek, 1960). Components constituting less than 1% of the total are not listed.

[b] Fatty acids listed in order of increasing retention time. Abbreviations: numbers before the colon represent chain length; those after the colon represent number of double bonds; cyclo represents the cyclopropane moiety, and β-OH represents β-hydroxy.

[c] An unsaturated nonhydroxy acid with retention time (RT) = 0.67 (RT of palmitate = 1.00).

[d] An unsaturated nonhydroxy acid; RT = 0.874.

[e] A polar acid; RT = 1.17.

[f] A polar acid; RT = 3.35.

and Law, 1964) and that the proportion of each is strongly dependent on the physiological age of the culture (Croom, McNeil, and Tove, 1964; Norris and Bloch, *unpublished data*). In the present study, however, cells of both forms were obtained at the middle logarithmic stage of growth; nevertheless, a distinct difference in the proportion of the C16 unsaturated to C17 cyclopropane fatty acids in the two forms is evident. This suggests that factors other than age may operate in control of the conversion of the unsaturated acid to the cyclopropane acid.

The fatty acids obtained from the nonextractable lipids of the two forms are qualitatively identical, although there are a number of marked quantitative differences (Table 2). Even more striking, however, are the differences between these fatty acids and those derived from the extractable lipids. β-Hydroxymyristate acid, which is not present in the extractable lipid, constitutes more than one-third of the total fatty acid, whereas cyclopropane fatty acids are virtually absent. In both forms myristic, stearic, and β-hydroxymyristic acids constitute together more than 70% of the total fatty acids derived from the nonextractable lipids.

Lipopolysaccharide of the bacillary and L form. The similarity of the fatty acids in the nonextractable lipids to those in the lipopolysaccharide (LPS) of *Escherichia coli* (Burton and Carter, 1964) prompted a qualitative and quantitative study of the LPS of the L and bacillary form of *Proteus*. It was found that the level of fatty acids in nonextractable lipid (Table 1) was, in fact, a manifestation of the level of LPS in each organism. The LPS isolated from the bacillary form by the phenol extraction method constituted 4 to 5% of the dry weight of cells, whereas that found in the L form made up only approximately 1.5% of the dry weight. Thus, it appears that the nonextractable lipid present in the residue obtained after CM extraction is, for the most part, LPS. Analysis of the LPS of each form revealed the presence of a number of components that are typical of the LPS of several gram-negative organisms (Osborn et al., 1964; Table 3). Upon paper chromatography of the

TABLE 3. *Analysis of lipopolysaccharide*

Component[a]	Lipopolysaccharide prepared from	
	Bacillary form	L form
	%[b]	%[b]
Heptose	8.3, 11.5[c]	8.5, 7.2
Hexosamine	17.0, 15.9	17.5, 23.2
Phosphorus[d]	0.8, 0.9	1.00, 0.5
3-Deoxyoctulosonate[e]	3.3	2.0
Glucose	7.1, 7.1	0.8, 0.8
Galactose	0.6, 0.3	1.4, 1.0
Fatty acid	8.8, 10.0	6.0, 4.6
Protein	2.4, 0.5	5.6, 3.6
Nucleic acid	1.6, 0.8	2.3, 1.8
Ash	<2.0	<1.0

[a] The following compounds were used as standards: heptose, D-glyceromannoheptose; hexosamine, D-glucosamine hydrochloride; fatty acid, β-hydroxylauric acid.

[b] Based on dry weight of LPS.

[c] Each value is the average of duplicates. The two values represent the result of analyses on different batches of LPS.

[d] Calculated as phosphate.

[e] These values are considered minimal, as there is a small degree of decomposition under the conditions of hydrolysis.

TABLE 4. *Fatty acid composition of lipopolysaccharide*

Fatty acid	Percentage by weight[a]	
	Bacillary	L form
14:0	29.2	10.1
Unknown[b]	1.5	1.3
Unknown[c]	9.5	8.5
16:0	6.8	2.4
Unknown[d]	5.0	6.6
14:β-OH	48.0	70.6

[a] See footnote *a*, Table 2.
[b] An unsaturated, nonhydroxy acid; RT = 0.67.
[c] An unsaturated, nonhydroxy acid; RT = 0.87.
[d] An uncharacterized acid, RT = 1.17

hydrolysis products of the LPS, only two neutral sugars, glucose and galactose, were found. The major quantitative difference in the LPS of the bacillary and L form was found to reside in the amounts of these two sugars. Glucose constituted 7.1% and galactose ca. 0.45% of the dry weight of LPS in the bacillary form, whereas the values for these compounds in the L form were 0.8 and ca. 1.2%, respectively. In Table 4 the results of a gas chromatographic analysis of the fatty acids of the LPS of the two forms are summarized.

DISCUSSION

Numerous studies on the chemical composition of bacterial L forms have revealed that one major biochemical alteration that occurs upon transition from the bacillary form is the loss of a number of compounds normally found in the mucopeptide component of the cell wall (Smith, 1964). Furthermore, it is clear that rather specific effects on other components of the cell envelope can result upon conversion to the L form. For instance, in the case of the *Proteus* L form, certain specific phage receptor sites are lost (Bloss, 1963), whereas the O antigen is retained (Minck and Kirn, 1960; Von Prittwitz und Gaffron, 1955). Since lipids are thought to be an integral part of the cell envelope of gram-negative bacteria, it seemed possible that the transition to the L form might result in quantitative or qualitative alterations in the lipid components of the cell. Earlier reports had suggested that there were pronounced quantitative differences in the extractable lipids; these observations have been confirmed in the present study. When the L form of *Proteus* P18 and its parent bacterium are grown on the same defined growth medium under conditions of rapid growth, the L form contains 1.5 times as much lipid on a dry weight basis as the bacillary form. Others (see Introduction) have found increases varying from two- to fourfold. It is likely that differences in growth conditions or lysis occurring during growth or collection of cells can explain these reported variations in lipid content. The major increase in lipid content occurs in the lipid fraction extractable with chloroform-methanol. The composition of these lipids, primarily phospholipids, is qualitatively and quantitatively similar in both L and bacillary form. The increased lipid content of the L organism thus appears to be due to a proportional increase of all the lipid components rather than to a specific increase of any one component.

Several workers have reported the presence of the O antigen (presumably a LPS) in L forms of *Proteus* (Minck and Kirn, 1960; Von Prittwitz and Gaffron, 1955). The present work demonstrates the presence of a LPS in the L form, although the amount found is only one-third to one-fourth that in the parent bacterium. It is possible that this decrease reflects a uniform reduction in the LPS content of the various bodies of the L form. On the other hand, it is equally possible that in the L-form culture, only some structural elements contain LPS. Several workers (Mandel et al., 1959; Weibull and Beckman, 1961; Rebel et al., 1963) have demonstrated the morphological and chemical heterogeneity of the L form.

It appears that the LPS from *Proteus* P18 contains many of the components found in the LPS of other gram-negative bacteria (Osborn et al., 1964). The composition of the fatty acids isolated from the nonextractable lipid is qualitatively similar in the two organisms studied here and appears to reflect the composition of the fatty acids found in the purified LPS. β-Hydroxymyristic acid, found in the nonextractable lipids, as well as in the purified LPS preparations of both forms, has been shown by Burton and Carter (1964) to be a typical component of the Lipid A portion of the lipopolysaccharide of *E. coli*. Since, in both organisms, compounds typical of the LPS of *E. coli* (Elbein and Heath, *in press*) account for only 50% of the dry weight of the LPS, it is possible that the LPS in the *Proteus* species contains several components not found in the LPS of *E. coli*. The LPS of the bacillary form contains slightly more fatty acid and about 10 times as much glucose as the L form, whereas the L form contains slightly more galactose. However, more detailed studies of the LPS preparations are necessary before any definite conclusions can be drawn as to whether this difference in the glucose levels is a true indication of differences in the structure of LPS of the two forms.

In any event, it is clear that conversion of the bacillary form into the L form results in marked,

opposite quantitative changes in cellular lipids and LPS. Since the LPS and, at least in part, the cellular lipid are components of the cell envelope, it seems likely that these quantitative changes are closely related to the pronounced morphological changes that occur upon conversion of the bacillary form to the L form.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI-04948 from the National Institute of Allergy and Infectious Diseases. One of us (W. J. L.) is the recipient of a Clayton scholarship.

It is a pleasure to acknowledge the valuable advice of Edward C. Heath on the analysis of lipopolysaccharide.

LITERATURE CITED

ABRAMS, R. Y. 1955. A method for the cultivation of L forms in liquid media. J. Bacteriol. **70**:251.

BLOSS, L. 1963. The relationship between two *Proteus mirabilis* L forms and bacteriophages. Arch. Mikrobiol. **45**:1–6.

BURTON, A. J., AND H. E. CARTER. 1964. Purification and characterization of the lipid A component of the lipopolysaccharides from *E. coli*. Biochemistry **3**:411–418.

CHUNG, A., AND J. H. LAW. 1964. Cyclopropane fatty acid synthetase: partial purification and properties. Biochemistry **3**:967–974.

CROOM, J. A., J. J. MCNEILL, AND S. B. TOVE. 1964. Biotin deficiency and the fatty acids of certain biotin-requiring bacteria. J. Bacteriol. **88**:389–394.

FLECK, J. 1963. Etude de la croissance en milieu liquide d'une souche de *Proteus* et de sa form L. Compt. Rend. **250**:183–185.

FOLCH, J., M. LEES, AND G. H. SLOANE-STANLEY. 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. **266**:497–509.

GOLDFINE, H., AND K. BLOCH. 1961. On the origin of unsaturated fatty acids in clostridia. J. Biol. Chem. **236**:2596–2601.

HARRIS, G., AND I. C. MACWILLIAM. 1954. A dipping technique for revealing sugars on paper chromatograms. Chem. Ind. (London), p. 249.

JANAK, J. 1960. Statistiche Auswertung der Messmethoden zur quantitativen Bearbeitung von registrierten Differentialkurven in der Gaschromatographie. J. Chromatog. **3**:308–312.

KING, E. J. 1932. The colorimetric determination of phosphorus. Biochem. J. **26**:292–297.

KREMBEL, J. 1963. Etude des lipides de la forme L stable de *Proteus* P18: identification des acides gras de la fraction acetonosoluble. Pathol. Microbiol. **26**:592–600.

KREMBEL, J. 1964. Etude des lipides de la forme L stable de *Proteus* P18: determination de la structure des acides insatures et identification d'un acide a cycle propanique. Etude de l'hydrosoluble de saponification des graisses neutres. Bull. Soc. Chim. Biol. **46**:503–508.

LAYNE, F. 1957. Protein estimation with the Folin-Ciocalteu reagent, p. 448. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 3. Academic Press, Inc., New York.

LENNARZ, W. J. 1964. The chemical characterization and enzymic synthesis of a bacterial glycolipid. J. Biol. Chem. **239**:PC3110–3112.

LIGHT, R. J., W. J. LENNARZ, AND K. BLOCH. 1962. The metabolism of hydroxystearic acids in yeast. J. Biol. Chem. **237**:1793.

MANDEL, P., M. SENSENBRENNER, P. DEGREGORIO, AND A. M. BADER. 1959. Distribution of nucleic acids among different stable L forms of *Proteus* P18. Nature **184**:566.

MINCK, R., AND A. KIRN. 1960. Pleuropneumonia-like organisms and L forms of bacteria. Ann. N.Y. Acad. Sci. **79**:658–661.

MORRISON, T. H., AND C. WEIBULL. 1962. The occurrence of cell wall constituents in stable *Proteus* L forms. Acta Pathol. Microbiol. Scand. **55**:475–482.

OSBORN, M. J., G. M. ROSEN, L. ROTHFIELD, L. D. ZELEZNICK, AND B. L. HORECKER. 1964. Lipopolysaccharide of the gram-negative cell wall. Science **145**:783–789.

RADIN, N. 1959. Methods Biochem. Analy. **6**:163.

REBEL, G., A. M. BADER, M. SENSENBRENNER, AND P. MANDEL. 1960. Etude comparee des lipides du bacille *Proteus* et d'une souche stable de formes L qui en derive. Compt. Rend. **250**: 3516–3517.

REBEL, G., A. M. BADER-HIRSCH, AND P. MANDEL. 1963. Recherches sur les lipides des formes L derivees du *Proteus* P18. I. Presence et absorption du cholesterol. Bull. Soc. Chim. Biol. **45**: 1327–1343.

SMITH, P. F. 1964. Comparative physiology of pleuropneumonia-like and L-type organisms. Bacteriol. Rev. **28**:97–125.

SMITH, P. F., AND G. H. ROTHBLAT. 1962. Comparison of lipid composition of pleuropneumonia-like and L-type organisms. J. Bacteriol. **83**:500–506.

VENDRELY, R., AND R. TULASNE. 1952. Premieres observations sur la constitution chimique des formes L des bacteries. Bull. Soc. Chim. Biol. **34**:785–789.

VON PRITTWITZ UND GAFFRON, J. 1955. Das Verhalten eines Stammes von *Proteus* vulgaris und seiner L Phase gegenuber homologen Antiseren. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **163**:313–318.

WEIBULL, C., AND H. BECKMANN. 1961. Chemical and metabolic properties of various elements found in cultures of a stable *Proteus* L form. J. Gen. Microbiol. **24**:379–391.

WESTPHAL, O., O. LUDERITZ, AND F. BISTER. 1952. Über die Extraktion von Bakterien mit Phenol/Wasser. Z. Naturforsch. **7B**:148–155.

WHEELDON, L. W., M. BRINLEY, AND D. A. TURNER. 1962. Simultaneous determination of glycerol, inositol, serine and ethanolamine in a phospholipid hydrolysate. Anal. Biochem. **4**: 433–443.