Giese, A. C. (1962), Cell Physiology, 2nd ed, Philadelphia, Pa., Saunders, p 81.
King, E. J. (1932), *Biochem. J. 26*, 292.
Luft, J. H. (1956), *J. Biophys. Biochem. Cytol. 2*, 799.
Luft, J. H. (1961), *J. Biophys. Biochem. Cytol. 9*, 409.
Mackenzie, C. G., Mackenzie, J. B., and Beck, P. (1961), *J. Biophys. Biochem. Cytol. 9*, 141.
Mackenzie, C. G., Mackenzie, J. B., and Reiss, O. K. (1962), *J. Cell Biol. 14*, 269.
Mackenzie, C. G., Mackenzie, J. B., and Reiss, O. K. (1964), *Exptl. Cell Res. 36*, 533.
McQuilkin, W. T., Evans, V. J., and Earle, W. R. (1957), *J. Natl. Cancer Inst. 19*, 855.
Oyama, V. I., and Eagle, H. (1956), *Proc. Soc. Exptl. Biol. Med. 91*, 305.
Reynolds, E. S. (1963), *J. Cell Biol. 17*, 208.
Robertson, J. D. (1959), *in* The Structure and Function of Subcellular Components, Biochemical Society Symposia, No. 16, Crook, E. M., Ed., New York, N. Y., Cambridge University Press, p 3.
Sjöstrand, F. S. (1963), *J. Ultrastruct. Res. 9*, 561.
van Deenen, L. L. M., Houtsmuller, U. M. T., de Hass, G. H., and Mulder, E. (1962), *J. Pharm. Pharmacol. 14*, 429.

Lipid Alterations after Cell Wall Inhibition. Fatty Acid Content of *Streptococcus pyogenes* and Derived L-Form*

Charles Panos, Murray Cohen, and Geraldine Fagan

ABSTRACT: The fatty acid content of whole streptococcal and derived L-form (*i.e.*, cells without rigid cell walls) cells in the mid-logarithmic phase of growth, and their respective isolated membranes, was compared by capillary column gas chromatography. Oleic acid predominated in L-form whole cells and membranes, whereas *cis*-vaccenic acid was found to predominate within the cells and membranes of the parent *Streptococcus pyogenes*. This reversal of positional isomers was not noted in the hexadecenoic acid fractions. In addition, the per cent of total C_{18} acids was greater than the total C_{16} acids in the L-form, whereas the reverse was found in the coccus. Control studies with the streptococcus grown in L-form medium could not account for these changes in the L-form as due solely to an osmotic effect. It was observed that permanent loss of cell wall biosynthesis (*i.e.*, L-form) could be associated with fatty acid alterations in the relative proportions of the C_{18} positional isomers. The presence of minute amounts of: (a) straight chain fatty acids containing an odd number of carbon atoms; (b) monoenoic fatty acids containing odd and even numbers of carbon atoms; and (c) a series of branched methyl fatty acids from cells and membranes of both organisms was demonstrated. The presence of various positional isomers within the tetra-, hexa-, and octadecenoic acid fractions from the coccus, its derived L-form, and their respective membranes supports the validity of earlier concepts of the mode of lengthening of monounsaturated fatty acids in bacteria. These results on the content of long chain monoenoic acids, together with the observations of others on the content of cyclopropane ring containing fatty acids in L-forms, indicate that a plausible function for bacterial ring containing fatty acids may be as cell wall structural units. The high resolving capabilities of capillary column gas chromatography and its application to bacterial fatty investigations is demonstrated.

Some information has appeared concerning the lipid content of microbial forms lacking a rigid bacterial cell wall. More recently, the total lipid and nonsaponifiable lipid content of pleuropneumoniaelike (PPLO) and L-type organisms was compared (Smith and Rothblat, 1962). Of the various cell wall-less organisms examined (PPLO, salt requiring and nonrequiring L-forms), the salt-requiring L-forms contained the least amount of these two lipid classes. O'Leary (1962a) has determined the fatty acid content of a PPLO and noted certain over-all differences and similarities between it and those of many bacterial species. These included the presence of bacterial cyclopropane ring containing lipids but a lower proportion of unsaturated to saturated acids than found in bacteria. A comparison of the lipids and lipopolysaccharide from the bacillary and L-forms of a Proteus has also been documented (Nesbitt and Lennarz, 1965). The L-form was found to contain 1.5 times as much extractable lipid and considerably less

* From the Department of Biochemistry, Albert Einstein Medical Center, Northern Division, Philadelphia, Pa. *Received December 6, 1965; revised February 17, 1966.* This investigation was supported by a research grant (AI-4495) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service, and a contract (NR 103-576) from the Office of Naval Research. One of us (C. P.) is a Senior Career Development Awardee [U. S. Public Health Award (7-K3-GM 15,531)].

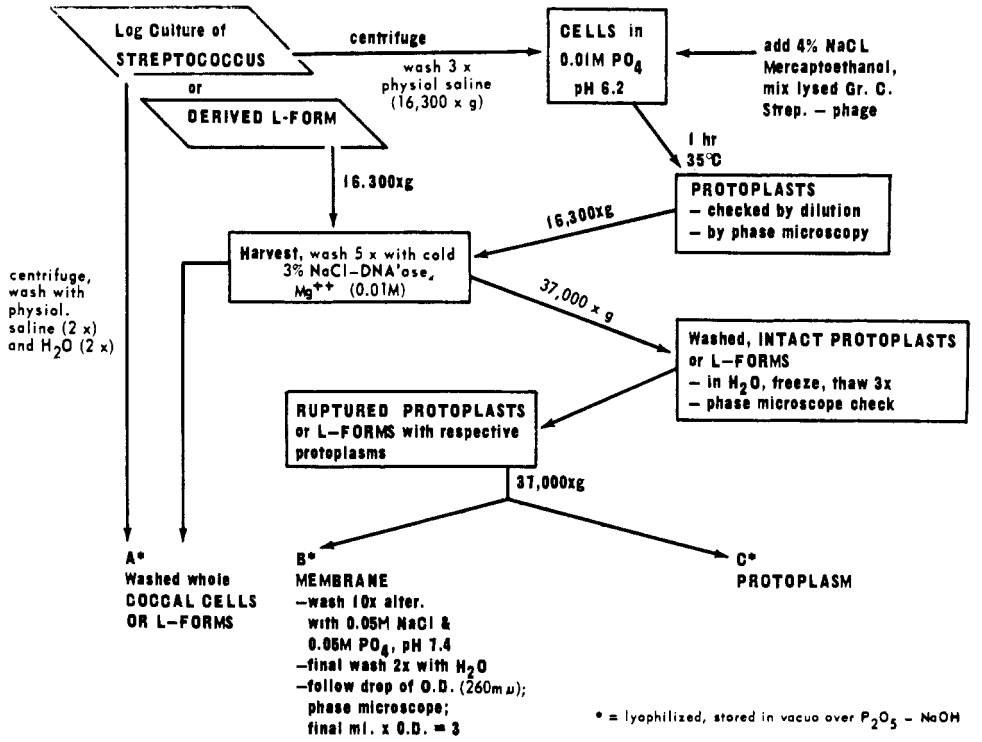

FIGURE 1: Scheme for preparation of whole cells and components.

lipopolysaccharide than did the bacillary form. The increase in the extractable lipid content of this L-form was attributed to a proportional increase of all the lipid components rather than to an increase in any one specific component. Although lipid studies have been performed with various anatomical components from certain streptococci (Ibbot and Abrams, 1964; Vorbeck and Marinetti, 1965), a detailed lipid investigation of a Gram-positive organism with its derived stable L-form has not appeared.

Diversified biochemical data have been gathered concerning a stable salt-requiring L-form derived from *Streptococcus pyogenes*. This report extends this comparison by dealing with the composition of the long-chain fatty acid content of these two intact organisms obtained from mid-logarithmically grown cells and of their respective isolated and purified cytoplasmic membranes. Part of these results have been reported in preliminary form (Panos and Cohen, 1965).

Experimental Section

Microorganisms and Cultivation Methods. The *S. pyogenes* and its stable salt-requiring L-form are the same as those used previously (Panos, 1965a). The complex growth medium (10 l.) contained lipid-free bovine albumin (0.8% w/v, Armour) in lieu of the necessary horse serum; otherwise both organisms were grown as has been described (Panos and Barkulis, 1959). Prior to use, the dried growth medium was extracted overnight at 4° with multiple changes of ethyl ether by continual shaking. This ethereal extract, upon evaporation, yielded 0.1% (w/w) of an oily lipidlike material which was not characterized further. The fatty acid content of the extracted growth medium was 0.05–0.07% of its dry weight. This medium was used for all large scale cultivations and for the maintenance of streptococcal and L-form stock cultures. Controls included fatty acid analyses of the streptococcus, grown for the first time, in the high osmotic environment of the L-form. For the study of positional isomers of octadecenoic acid, 900 ml of culture of intact coccal or L-form cells, contained in 2-l. flasks, was harvested at suitable time intervals (Figure 1). An actively growing streptococcal (5% v/v) or L-form (10% v/v) inoculum was used to initiate each flask. Doubling times were 56 and 87 min for the parent coccus and L-form, respectively.

Whole Cells and Membranes. A schematic diagram for the preparation of whole cells and membranes from each organism is illustrated in Figure 1. Unless specified otherwise, all centrifugations were performed at 4° with a total time elapsing per wash of *ca.* 25 min. Only cells collected at comparable stages of growth were employed. Streptococcal L-form growth was determined turbidimetrically, by viable counts and dry weight determinations. Coccal membranes were obtained after conversion of the streptococcus to the protoplast form by the group C phage-induced lysin (personal communication by Dr. S. S. Barkulis, Ciba Pharmaceuticals, Summit, N. J.). Protoplast membrane protein controls included protein determinations of

L-form membranes treated with lysin. Protoplast formation was measured by osmotic fragility (*i.e.*, dilution with water) and by phase microscopy. The washing procedures used were essentially those of Slade and Shockman (1963). By these methods, the yield of membranes (dry weight) from lyophilized whole L-form or protoplast cells was *ca.* 9%.

Fatty Acid Extractions, Standards, and Chromatographic Methods. The alkaline hydrolysis fatty acid extraction methods of Hofmann *et al.* (1957) were used throughout. Available chromatographic standards were obtained from Applied Science Laboratories, University Park, Pa., or the Hormel Institute, Austin, Minn. Esterified microbial fatty acid mixtures were resolved with a Perkin-Elmer Model 801 gas chromatograph equipped with a Leeds and Northrup 1–5 mv recorder and containing a 6 ft by 1/8 in. butanediol succinate column. The operating parameters for this column are essentially as described elsewhere (Ettre *et al.*). Each fatty acid was identified by comparing its position of elution with an authentic sample and/or from relative retention plots determined in these laboratories. Confirmation was also obtained by comparing retention time data before and after hydrogenation of a given methylated fatty acid mixture. In addition, purity of each peak, reconfirmation of identity, and resolution of probable posi ional isomeric mixtures within a particular monoethenoid fraction was achieved by the use of a 150-ft Carbowax (Golay) capillary column as has been recently detailed (Panos, 1965b). Unless noted otherwise, the designated abbreviations ($C_{15}T$, $C_{16}T$, $C_{17}T$) of the probable branched methyl fatty acids are based upon their total carbon atom content.

Chemical Methods. Methylation of free fatty acids was achieved using boron trifluoride in methanol. Hydrogenations were performed with 5% palladium on charcoal in methanol at room temperature. Protein was determined by the Lowry procedure. Infrared spectra were obtained with a Perkin-Elmer Model 337 grating spectrophotometer equipped with a scale expansion accessory as thin films between KBr crystals. Spectra of minute fatty acid mixtures were obtained with the aid of a beam (4×) condenser attachment.

Results

All of the cells employed for these studies were obtained at their respective mid-logarithmic growth phases. The optical densities corresponding to mid-physiological youth of each organism are tabulated in Table I. It had been established that the cell walls of group A streptococci comprise *ca.* 25% of the total dry weight of the intact coccus (Barkulis and Jones, 1957). It is apparent from these data (Table I) that, on an equivalent weight basis, no significant difference was discernible in the total fatty acid content of the whole streptococcus, its L-form, or the coccus grown in L-form medium. The fatty acid content of L-form membranes, however, was 56.3% greater than that of membranes isolated from the parent streptococcus, a difference that remained consistent. The protein anomaly, *i.e.*, a greater protein content in coccal membranes than that of L-form membranes, was partially resolved as due to the action of the phage-induced lysin. Treatment of whole L-forms with lysin, as done for protoplast formation, resulted in an increase in their protein content corresponding more closely to that within streptococcal membranes.

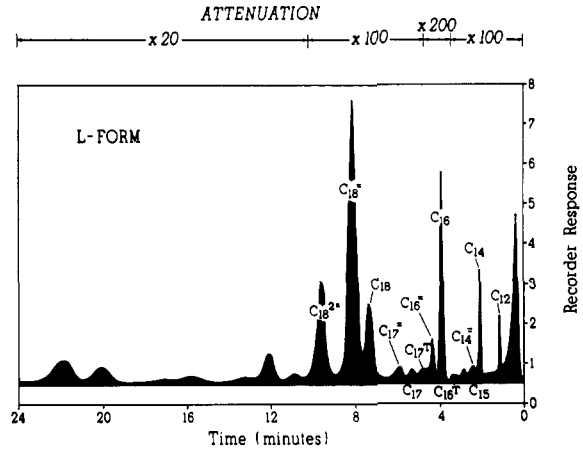

FIGURE 2: Typical packed column (butanediol succinate) gas chromatographic pattern of total fatty acid methyl esters from mid-log whole L-form cells. Cells grown in extracted Brucella broth–albumin medium.

TABLE I: Whole Cell and Membrane Composition.

Cellular Material	Whole Cells Harvest (OD_{650}) at	Content (%) Non-sapon.	Total Fatty Acids	Protein
Streptococcus	0.450	0.22	4.04	ND[a]
Streptococcal membrane	0.450	ND	14.83	75.2
L-Form	0.290	0.16	4.92	ND
L-Form membrane	0.250	ND	23.18	58.9
Controls				
Growth medium[b]	...	0.01	0.05–0.07	ND
Streptococcus in L-Form medium	0.330	0.11	4.01	ND
L-Form membrane, lysin treated	0.302	ND	ND	65.9

[a] ND = not done. [b] Extracted Brucella broth with albumin.

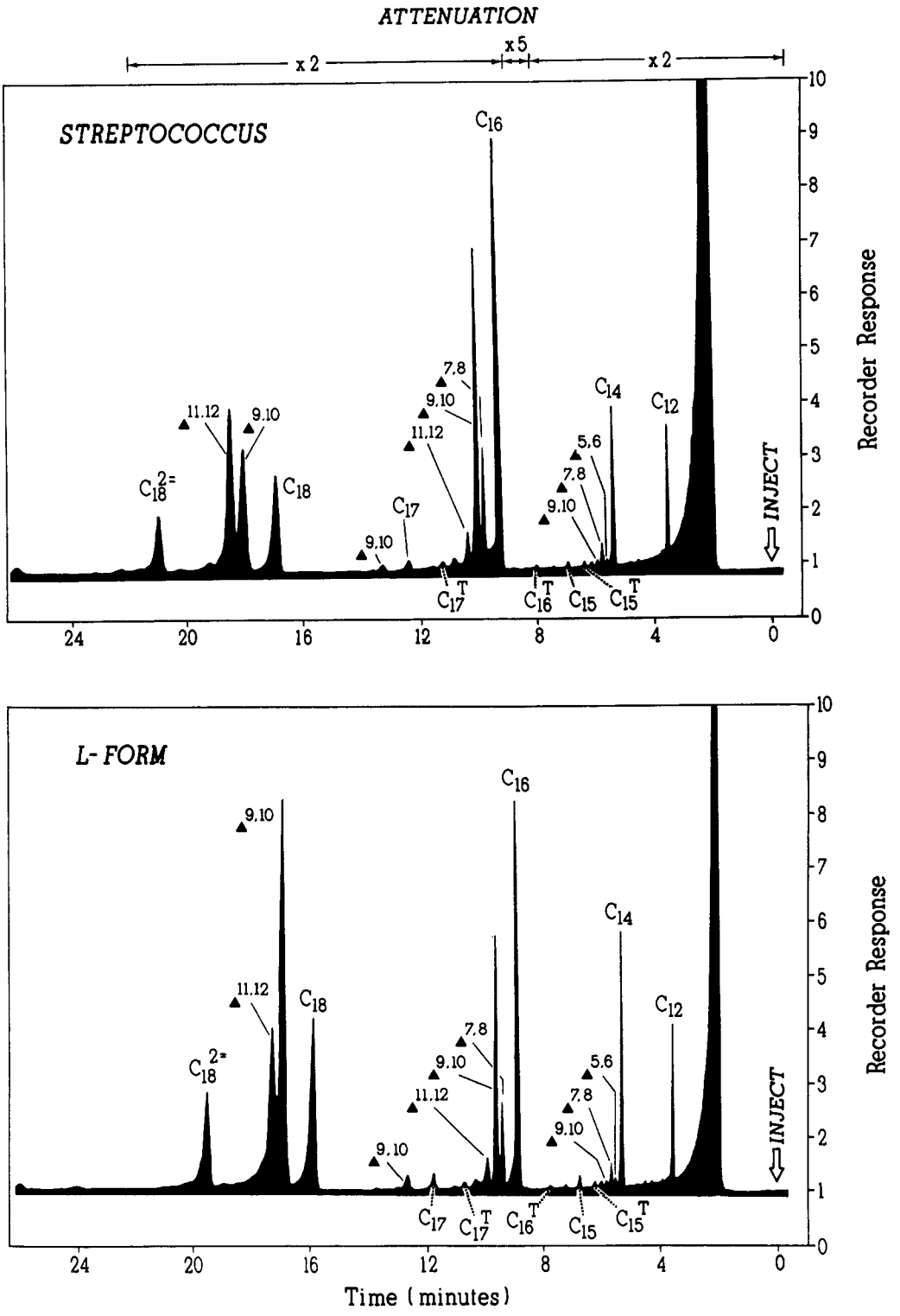

FIGURE 3: Typical capillary gas chromatographic pattern of total fatty acid methyl esters from mid-log cells. Cells grown in extracted Brucella broth–albumin medium; column: Golay, Carbowax 1540, 150 ft at 185°; total amount injected, 0.2–0.4 μl; split ratio *ca.* 100:1.

Infrared spectra of fatty acid mixtures from the parent coccus and its derived L-form, as well as from their respective membranes, were determined and yielded identical results.

Figure 2 is a typical pattern of a fatty acid methyl ester mixture from whole L-form cells and demonstrates the resolving capabilities achieved by the packed butanediol succinate column. We have only been con-

cerned with the identification of fatty acids up to and including the octadecadienoic acid region. These acids (*i.e.*, dodecanoic to octadecadienoic acid) account for at least 95% of the total long chain fatty acids found within whole cell or membrane preparations from both the coccus and its derived L-form. In addition to the predominating long chain fatty acids within such preparations, these patterns repeatedly indicated the presence of minute quantities of what appeared to be a series of straight chain fatty acids containing: (a) odd numbers of carbon atoms (C_{15}, C_{17}); (b) monoenoic fatty acids containing odd and even carbon atoms (C_{14}═, C_{16}═, C_{17}═); as well as (c) small quantities of branched methyl fatty acids (C_{15}T, C_{16}T, C_{17}T). The probable identity of these branched methyl fatty acids was deduced by comparing their log relative retention times with authentic standards and by their persistence in aliquots from these microbial fatty acid mixtures reexamined after hydrogenation. Infrared and gas chromatographic studies failed to reveal the presence of branched hydroxyl groups, the cyclopropane ring, and, as was expected, long chain fatty acids possessing the *trans* configuration in lipid mixtures from either organism.

Figure 3 illustrates the capillary (Golay) gas chromatographic separations of lipid mixtures from whole streptococcal and L-form cells. The use of this technique, its extremely high resolving capabilities, and its potential in the examination of microbial extracts have already been detailed (Panos, 1965b). The quantitation of minor fatty acid components was achieved by increasing their peak heights, *via* increased sensitivity settings, on duplicate aliquots. As is evident, this procedure is readily capable of separating positional isomers of methylated long chain fatty acids (C_{14}, C_{16}, and C_{18} monoethenoid regions). In addition to obviating the need of degradative procedures for determining the position of unsaturation, quantitation and identification of individual components in such mixtures may be readily performed with extremely minute sample sizes (order of micrograms). A relationship between the log relative retention time and position of double bond in esterified long chain fatty acids from this streptococcus has been presented (Panos, 1965b). Similarly, Figure 4 shows the log relative retention time of authentic methyl-branched fatty acids with this capillary column. Parallel lines have been drawn through the acids having identical branching positions counting from the methyl (ω) end of the molecule. Standards of saturated fatty acids are plotted for comparison. Table II compares the corrected relative retention times of available standards with those of the various branched methyl fatty acids found within coccal and L-form membranes and whole cells. The probable point of methyl branching is apparent; however, absolute confirmation must await isolation of sufficient quantities for detailed chemical analyses.

RELATIONSHIP BETWEEN LOG RELATIVE RETENTION TIME AND METHYL BRANCH POSITION OF ESTERIFIED BRANCHED LONG CHAIN FATTY ACIDS*

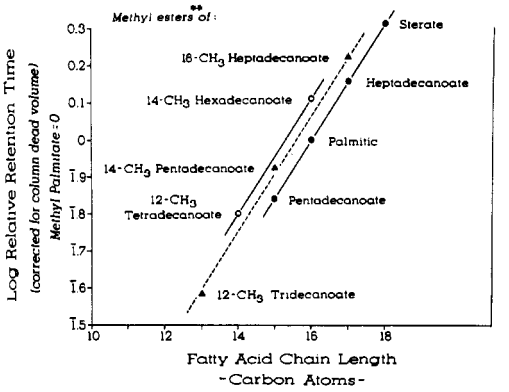

FIGURE 4: *Golary Carbowax 1540 column, 150 ft at 185°; **commercial standards; >95% purity; saturated straight-chain fatty acids plotted for comparison.

The corrected relative retention time (capillary gas chromatography) of the heptadecenoic acid found within all whole cell and membrane preparations was 1.555 (methylpalmitate = 1). The position of unsaturation of this acid has been tentatively identified from corrected log relative retention time charts as 9,10-heptadecenoic acid.

The fatty acid content of whole coccal and L-form cells, their respective membranes, and the streptococcus grown in L-form medium are tabulated in Table III. These analyses were performed with packed butanediol succinate columns. As is noted, the unsaturated fatty acids present disappeared after hydrogenation and were accompanied by corresponding increases in their

TABLE II: Corrected Retention Times of Long-Chain Branched Methyl Fatty Acid Esters by Capillary Column Gas Chromatography.

Methyl Esters of	Rel Retention Time[a] Streptococcus and L-Form	Standard
12-Methyltridecanoate		0.384
12-Methyltetradecanoate		0.634
14-Methylpentadecanoate		0.842
14-Methylhexadecanoate		1.269
16-Methylheptadecanoate		1.663
Methyltetradecanoate (C_{15}T)[b]	0.621	
Methylpentadecanoate (C_{16}T)[b]	0.835	
Methylhexadecanoate (C_{17}T)[b]	1.264	

[a] Methylpalmitate = 1; standards obtained commercially and of purities >95%. [b] Probable identity as determined by comparative packed and capillary gas chromatographic data with standards and from relative retention time plots of parallel lines correlating position of methyl branching.

TABLE III: Fatty Acid Composition[a] of *Streptococcus* and Derived L-Form.

	Compn of Total Fatty Acids (%)													
	C_{12}	C_{14}	$C_{14}{=}$	$C_{15}T$	C_{15}	$C_{16}T$	C_{16}	$C_{16}{=}$	$C_{17}T$	C_{17}	$C_{17}{=}$	C_{18}	$C_{18}{=}$	$C_{18}{}^{2}{=}$	Recovery
L-Form Whole Cells	1.06	3.58	0.48	TR[b]	0.37	TR	26.39	6.92	0.31	0.58	0.82	8.34	34.87	13.12	96.84
							33.31						56.33		
Hydrogenated[c]	0.81	4.16	...	TR	0.39	TR	33.75	...	0.23	1.49	...	56.30	...	...	97.13
Membrane	1.59	3.71	0.83	TR	0.38	TR	26.43	8.74	0.71	0.77	0.90	8.43	31.85	12.28	96.62
Streptococcus Whole Cells	0.92	2.37	1.15	0.25	0.17	0.17	34.84	18.05	0.45	0.67	0.55	6.74	25.10	5.42	96.85
							52.89						37.26		
Hydrogenated[c]	0.56	3.62	...	0.24	0.21	0.17	53.60	...	0.47	1.04	...	37.61	...	...	97.52
Membrane	0.79	1.27	1.06	TR	0.23	0.20	31.18	19.39	0.73	0.51	0.50	7.20	26.11	6.58	95.75
Streptococcus in L media	0.91	2.97	0.42	TR	0.32	0.18	34.01	11.70	1.46	0.63	0.94	5.86	25.10	10.26	94.76
							45.71						41.22		

[a] At mid-log, growth; cells grown in Brucella broth with albumin; analyses by packed column gas chromatography. [b] TR = trace. [c] Identical sample after hydrogenation.

saturated homologs. The excellent agreement obtained before and after hydrogenation with the monoethenoid C_{14}, C_{16}, C_{17}, and C_{18} fatty acids from whole cell preparations confirmed their probable identity as given initially from log relative retention time plots. The total C_{16} fatty acid content of L-form cells and membranes was found to be lower than the combined C_{18} content; the opposite was found to be true in the fatty acids from coccal whole cells and membranes.

Table IV quantitates the isomeric content of the various monounsaturated fractions after separation by capillary column gas chromatography. It was observed that the monoethenoid ratios of total tetradecenoic, hexadecenoic, and octadecenoic acids were 1:14:73; 1:16:22, and 1:28:60 for whole L-form, streptococcal, and coccal cells grown in L-form medium, respectively. The L-form contained 62% less hexadecenoic isomers but 39% more octadecenoic acids than the parent coccus from which it was derived. Similarly, L-form membranes contained 45% less hexadecenoic isomers and 22% more octadecenoic acids than coccal membranes. A greater percentage of oleic acid ($C_{18}{}^{9,10}$; 81%) was consistently found in the L-form and its isolated membranes (78%) than *cis*-vaccenic acid ($C_{18}{}^{11,12}$; 19%). The opposite isomeric distribution was found within the bacterium; *i.e.*, oleic 40% and *cis*-vaccenic 60% in both whole cells and membranes. In the streptococcus from L-form medium, the content of oleic and *cis*-vaccenic acids was 66 and 34%, respectively. By comparison, the total octadecenoic acid content of the coccus was identical with that of the streptococcus, obtained at an equivalent growth phase, from L-form medium. However, both were appreciably lower than the total C_{18} monoenoic content of the L-form. In the total C_{16} monoenoic fraction, the streptococcus in L-form medium, while lower than the coccus, was 40% greater than the L-form.

In separate experiments, the fatty acid content of whole coccal and L-form cells was determined over the entire growth cycle of each organism (positional isomer study). Table V lists the doubling time of each of the parameters studied. A study of the increase of oleic and *cis*-vaccenic acids within each organism over their respective logarithmic growth phases proved that these observed isomeric differences were not due to the larger inoculum size necessary for initiation of L-form growth (L. Hynes and C. Panos, unpublished data).

Discussion

As has been mentioned or apparent in relatively recent microbial lipid reviews (O'Leary, 1962b; Kates, 1964), detailed fatty acid analyses of bacterial L-forms are conspicuous by their absence from the literature. In these investigations attempts were made, using cells of comparable physiological youth, to obtain such information as well as to ascertain whether loss of cell wall biosynthetic capabilities (*i.e.*, resulting stable L-form) are accompanied by lipid alterations at the membrane level. It was observed that membrane fatty acid analyses mimicked those of whole cell findings and

TABLE IV: Monoethenoid Fatty Acid Positional Isomer Content.[a]

Source	C_{14}[b] Total (%)	C_{14} Position of Double Bond 5,6	7,8	9,10	C_{16} Total (%)	C_{16} Position of Double Bond 7,8	9,10	11,12	C_{18} Total (%)	C_{18} Position of Double Bond 9,10	11,12
L-form	0.48	0.07	0.21	0.20	6.92	1.21	5.20	0.51	34.87	28.14	6.73
L-form membranes	0.83	Trace	0.57	0.26	8.74	1.80	6.20	0.74	31.85	24.91	6.94
Streptococcus	1.15	0.19	0.77	0.19	18.05	4.62	12.06	1.37	25.10	10.24	14.86
Streptococcal membranes	1.06	0.14	0.71	0.21	19.39	5.51	12.17	1.71	26.11	10.65	15.46
Streptococcus, in L-form medium	0.42	Trace	0.19	0.23	11.70	2.05	7.94	1.71	25.10	16.57	8.53

[a] Average of two determinations. Per cent of each isomer contained in total monoethenoid fraction as determined by capillary gas chromatographic analyses. Total per cent of each fraction determined by packed column chromatographic analyses. [b] Carbon atom chain length.

TABLE V: Doubling Time of the Various Parameters.

	Minutes	
	Streptococcus	L-Form
Dry wt	53	90
OD (650 mμ)	53	86
Total fatty acid content	49	94

that, in each organism, the apparent major areas of change were reflected in the C_{16} and C_{18} chromatographic regions.

A comparison of the fatty acid content of *S. pyogenes* and its derived L-form illustrates that permanent cell wall inhibition (*i.e.*, the L-form) is associated with major shifts in the total C_{16} and C_{18} lipid content. While there is a significant increase in the total octadecenoic acid content of the L-form, as compared with that of the parent coccus, a decrease results in the L-form's *cis*-vaccenic acid content. The earlier work of Hofmann and co-workers (Hofmann, 1962) had established the fact that the predominating monoethenoid C_{18} fatty acid of bacteria is *cis*-vaccenic, not oleic, and that both of these positional isomers are present in the lipids of a group C streptococcus. Repetitive studies with these two organisms have shown that the predominating octadecenoic acid of this L-form is oleic acid while its isomer, *cis*-vaccenic acid, prevails in the parent streptococcus. These C_{18} positional isomeric differences persisted over the logarithmic growth phase of each organism (L. Hynes and C. Panos, unpublished results). Although Krembel (1964) had reported that oleic acid was the only octadecenoic acid present within a stable non-salt-requiring Proteus L-form, comparative data of the C_{18} monoenoic acid content of the parent bacillus was not presented. It should also be pointed out that: (a) a similar rearrangement of positional isomers was not noted in the isomeric hexadecenoic acids (Table IV) of each organism; and (b) as opposed to the C_{18} monoenoic content, no increase of total monoenoic hexadecanoic acids was observed upon conversion to the L-form or after growth of the coccus in L-form medium. On the contrary, a decrease in hexadecenoic acids in the coccus from L-form medium and in the stable L-form occurred. These data suggest that the C_{18} monoenoic region is more informative of fatty acid alterations.

The decrease in the *cis*-vaccenic acid content of the streptococci grown in L-form medium, similar to that found in the L-form, requires further consideration with regard to a possible osmotic effect. Three salient points must be made clear, however, before considering this aspect. They are that: (a) to date it has not been possible to grow L-forms derived from the group A streptococci in a salt-free medium; (b) that higher sodium chloride concentrations are necessary in conjunction with penicillin for production of these forms; and (c) the observed bizarre morphological alterations of the group A streptococci, when grown in L-form medium, correlate with and are reminiscent of intermediate stages observed during the development of an L-form. The streptococci grown in L-form medium contained the same percentage of total fatty acids as when grown in the usual medium. However, while the coccus had a higher total C_{16} (52.9%) than total C_{18} content (37.2%) the reverse was found in the L-form. In addition, the coccus had an identical total fatty acid and C_{18} monoethenoic content when grown in the absence or presence of NaCl; this was not so with the L-form. These inconsistent variations, seen in the presence of salt, indicate that the differences in C_{16} and C_{18} fatty acid content between the parent coccus and its L-form cannot be due primarily to a salt effect. The oleic and *cis*-vaccenic acid concentrations observed are difficult to explain in terms of merely an osmotic effect; *e.g.*, calculations illustrate that *cis*-vaccenic acid accounts for 19% of the total C_{18} monoenoic acids

in the salt-requiring L-form. In addition, the *cis*-vaccenic acid of the coccus grown in L-form medium represents a significant difference (34%) from that of the coccus grown in the usual manner (57%). Finally, the intermediate value for *cis*-vaccenic content of the coccus grown in salt may reflect an intermediate stage in the conversion of the coccus to an L-form.

We have recently shown that both L-forms and protoplasts from this group A *streptococcus* synthesize the postulated cell wall precursor, TDP[1]–rhamnose, even though unable to form the rigid streptococcal cell wall. However, protoplast membranes retain the ability to transfer rhamnosyl units from this nucleotide precursor to a suitable acceptor site while L-form membranes do not (Panos and Cohen, 1966). These facts, together with current concepts implicating lipid intermediates in cell wall biosynthesis, tempt speculation of the importance of membrane alterations in the L-form. These changes may be reflected, biochemically, in this L-form's (a) increased octadecenoic acid content, (b) elevated membrane fatty acid content, and (c) morphologically, by the fact that this L-form lacks an orderly division process (Panos, 1965a).

Credence in a possible correlation of cell wall inhibition with lipid alterations is strengthened by the earlier findings of Nesbitt and Lennarz (1965). They observed that a nonsalt-requiring, mid-logarithmically grown L-form of Proteus P18 displayed a drastic drop of its C_{17} and C_{19} cyclopropane ring containing fatty acid content as compared with that of the bacillary form from which it was derived. The fact that (a) as yet no known function has been ascribed to cyclopropane ring containing bacterial lipids, (b) that their precursors are known to be monoethenoid fatty acids of corresponding chain lengths, and (c) that upon cell wall inhibition a drop of the predominating bacterial octadecenoic acid (*cis*-vaccenic) in this streptococcal L-form was observed while a decrease in ring-containing fatty acids occurred in a Proteus nonsalt-requiring L-form strongly suggests that a plausible function of bacterial cyclopropane-containing fatty acids may be as cell wall structural units (see also Kates, 1964). In this streptococcus, which is devoid of cyclopropane ring containing fatty acids, as well as of lipids in its cell wall, the concentration of membrane long chain unsaturated fatty acids may be affected upon loss or alteration of the cell wall.

Earlier proposed schemes concerned with the bacterial synthesis of long chain monoethenoid fatty acids had postulated the presence of shorter monoethenoid members of the octadecenoic acid series (Scheuerbrandt and Bloch, 1962; Bishop and Still, 1963). More recently, O'Leary (1965) has detected the presence of decenoic, dodecenoic, and tetradecenoic acids in the lipids of the *Lactobacteriaceae*. We have been able to confirm the presence of his Δ^5-tetradecenoic acid and, in addition, have resolved other positional isomers in tetradecenoic and hexadecenoic acid mixtures from this streptococcus and its L-form. The finding of these various positional isomers substantiates the validity of earlier concepts of bacterial monounsaturated long-chain fatty acid synthesis.

Noteworthy is the presence of an uneven carbon chain length lipid, heptadecenoic acid, and what appears to be a series of long chain branched methyl fatty acids in all whole cells and membrane preparations. The biochemical significance of these acids is currently unknown. Finally, as is apparent, both whole cells and membrane preparations contained an octadecadienoic acid which, by packed column chromatography, possessed the same relative retention time as that of linoleic acid. This component was also prevalent in the lipids of the extracted growth medium. True bacteria do not synthesize polyunsaturated long chain fatty acids (Erwin and Bloch, 1964). Nevertheless, the streptococcus and its L-form apparently possess the ability of incorporating this preformed component into their membranes as a structural entity.

[1] Abbreviation used: TDP, thymidine diphosphate.

References

Barkulis, S. S., and Jones, M. F. (1957), *J. Bacteriol. 74*, 207.

Bishop, D. G., and Still, J. L. (1963), *J. Lipid Res. 4*, 81.

Erwin, J., and Block, K. (1964), *Science 143*, 1006.

Ettre, L. S., Averill, W., and Kabot, F. J., Gas Chromatographic Analysis of Fatty Acids, Perkin-Elmer, Applications No. GC-AP-001.

Hofmann, K. (1962), Fatty Acid Metabolism in Microorganisms, New York, N. Y., Wiley.

Hofmann, K., Henis, D. B., and Panos, C. (1957), *J. Biol. Chem. 228*, 349.

Ibbot, F. A., and Abrams, A. (1964), *Biochemistry 3*, 2008.

Kates, M. (1964), *Advan. Lipid Res. 2*, 17.

Krembel, J. (1964), *Bull. Soc. Chim. Biol. 46*, 503.

Nesbitt, J. A., and Lennarz, W. J. (1965), *J. Bacteriol. 89*, 1020.

O'Leary, W. M. (1962a), *Biochem. Biophys. Res. Commun. 8*, 87.

O'Leary, W. M. (1962b), *Bacteriol. Rev. 26*, 421.

O'Leary, W. M. (1965), *Biochemistry 4*, 1621.

Panos, C. (1965a), *J. Gen. Microbiol. 39*, 131.

Panos, C. (1965b), *J. Gas Chrom. 3*, 278.

Panos, C., and Barkulis, S. S. (1959), *J. Bacteriol. 78*, 247.

Panos, C., and Cohen, M. (1965), *Bacteriol. Proc.*, P124.

Panos, C., and Cohen, M. (1966), *Biochim. Biophys. Acta* (in press).

Scheuerbrandt, G., and Bloch, K. (1962), *J. Biol. Chem. 237*, 2064.

Slade, H. D., and Shockman, G. D. (1963), *Iowa State J. Sci. 38*, 83.

Smith, P. F., and Rothblat, G. H. (1962), *J. Bacteriol. 83*, 500.

Vorbeck, M. L., and Marinetti, G. V. (1965), *Biochemistry 4*, 296.