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Unique Lipid Composition of *Treponema pallidum* (Nichols Virulent Strain)

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The lipid composition of *Treponema pallidum* (Nichols virulent strain) was determined after purification of the organisms from the infected testes of corticosteroid-treated rabbits by differential centrifugation, filtration through Nuclepore membranes, and sedimentation in Hypaque density gradients. The total lipids were comprised of 32.2% neutral lipids, mainly cholesterol, and 67.8% phospholipids consisting of phosphatidylcholine (32.1%), sphingomyelin (14.8%), cardiolipin (13.0%), phosphatidylethanolamine (6.2%), phosphatidylinositol-serine (1.2%), and lysophosphatidylcholine (0.4%). Monoglycosyldiglyceride, a glycolipid comprising 25 to 50% of the total lipid of all *Treponema* previously examined, was not detected. The fatty acid composition was similar but quantitatively distinct from that of the infected testes tissue.

Attempts to cultivate *Treponema pallidum*, the causative agent of syphilis, in vitro have failed since its discovery almost 75 years ago. Largely due to this, little is known about the organism's lipid metabolism or composition. Many cultivable *Treponema* require long-chain fatty acids for growth (9, 16, 22) since they can neither synthesize, β-oxidize, nor desaturate fatty acids (1, 10, 19). Some intestinal and oral strains, however, can utilize short-chain fatty acids (26). Recent studies suggest that *T. pallidum* may also have lesions in lipid metabolism similar to many cultivable treponemes (9, 18, 21, 25).

Lipids comprise 15 to 20% of the cellular dry weight of the cultivable treponemes (10, 16). The major component is a glycolipid, monoglycosyldiglyceride (MGDG), containing galactose generally as the sugar moiety. MGDG comprises 25 to 50% of the total lipid of each of the approximately 20 treponemal strains of genital, oral, and intestinal origin so far examined (10, 16, 19) including *T. hyodysenteriae* (our unpublished data), the only cultivable pathogenic *Treponema* (12). Although Vaczi et al. (29) investigated the lipid composition of *T. pallidum* (Budapest strain), as well as four cultivable treponemes, they reported no quantitative data about the individual lipids or gave any indication of the glycolipid content of the organisms.

Since *T. pallidum* cannot be cultivated in vitro, the organism is obtained for laboratory studies from the testes of experimentally infected rabbits. A major impedance of research on *T. pallidum* is the difficulty of obtaining large yields of the organism free of rabbit testicular material. In recent years, however, the use of corticosteroid-treated rabbits (8), Nuclepore filters (25), and Hypaque gradients (2) has proven remarkably useful in reducing this problem. We have used all of these tools to obtain high numbers of purified *T. pallidum* for lipid analysis. Infected rabbit testicular tissue and *T. phagedenis* biotype *reiterii* were also examined as comparative controls. Our results have shown that *T. pallidum* appears to be unique among *Treponema* in that the major lipid component of other *Treponema*, namely MGDG, was not detected in this human pathogen.

MATERIALS AND METHODS

Chemicals. Fatty acids and fatty acid methyl ester standards were obtained from The Hormel Institute, Austin, Minn., and Nu-Chek Prep, Inc., Elysian, Minn.; neutral lipid and phospholipid standards were from Supelco, Inc., Bellefonte, Pa., and Avanti Biochemicals, Inc., Birmingham, Ala.; and Silica Gel H was from E. Merck AG, Darmstadt, Germany. A dye-binding protein assay (4) kit was purchased from Bio-Rad Laboratories, Richmond, Calif. All solvents were distilled before lipid analyses (28).

Propagation of treponemes. The Nichols virulent strain of *T. pallidum* was propagated by intratesticular inoculation of mature New Zealand and Dutch Belt rabbits (18). Infected rabbits were injected with 1 mg of dexamethasone (Beecham Laboratories, Bristol, Tenn.; Med-Tech, Inc., Elwood, Kan.) intramuscularly on day 3 postinfection and daily until sacrificed (8). *T. phagedenis* biotype *reiterii* was grown at 37°C in 165 ml of prereduced medium (16) containing 10% (vol/vol) newborn calf serum in stoppered 250-ml Erlenmeyer flasks with a gas atmosphere of 75% N_2 + 20% H_2 + 5% CO_2. Treponemes were collected during the early stationary phase (30 to 36 h of incubation)

by centrifugation at 12,000 × *g* for 30 min at 4°C, washed three times with phosphate-buffered saline (PBS, pH 7.3), and immediately used for lipid extraction.

Purification of *T. pallidum*. Treponemes were extracted aerobically from testes of corticosteroid-treated rabbits 11 to 14 days postinfection as previously described (18) until 35 to 50 ml of suspension was obtained from each testis. Serum-free Eagle medium containing 4 mg of fatty acid-free bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.) per ml and 2 mM glutathione (24) was used for extraction. Large particulate material was removed by centrifugation at 250 × *g* for 10 min at 22°C. The treponemes were concentrated by centrifugation at 18,800 × *g* (r_{max}) for 15 min at 15°C in a Sorval SS 34 rotor. The pellets of treponemes from one to four testes were suspended with the aid of a Vortex mixer and combined in 25 ml of PBS, pH 7.3, containing 2 mM glutathione (PBS-G). The suspension was centrifuged at 250 × *g* for 7 min at 22°C to remove aggregated tissue material resulting from the high-speed centrifugation. The supernatant fluid was filtered through an 0.8-μm Nuclepore filter to remove tissue cells (25). The treponemes were concentrated as before, suspended in PBS-G, and further purified by sedimentation at 100,000 × *g* (r_{max}) for 45 min at 15°C through discontinuous gradients of Hypaque-M (Winthrop Laboratories, New York, N.Y.) in Beckman SW 39L or SW 41 Ti rotors (2). After collection from the gradients, the treponemes were diluted with PBS, concentrated as before to remove the Hypaque-M, and then immediately used for lipid extraction. When protein analyses were also to be performed, the purified organisms were washed three times with PBS.

The portion of the gradients above the uppermost treponemal band was collected and treated like the treponemal fraction (2) in an effort to estimate the amount of tissue lipid contamination in the treponemal suspensions before the last purification step with Hypaque. Also, after extraction of *T. pallidum* from the testes, the remaining tissue was used for lipid extraction to compare with purified *T. pallidum*.

Lipid extraction. Lipids were extracted by a modification of the method of Bligh and Dyer (3). Methanol (7.5 ml) and chloroform (3.75 ml) were added, in order, to samples in 3.0 ml of water. The samples were blended with a Vortex mixer, extracted overnight at 4°C, and then centrifuged at 2,500 × *g* for 20 min at 4°C, and the supernatant fluids were saved. The pellets were extracted twice more with 14.25 ml of methanol–chloroform–water (2:1:0.8, by volume). The samples were centrifuged as before, and the supernatant fluids were combined with the first extract. Chloroform (11.25 ml), 7.5% (wt/vol) KCl (2.0 ml), and water (9.25 ml) were added to the extract, the resultant emulsion was broken by centrifugation, the chloroform layer was saved, and the methanol–water layer was washed with chloroform (7.5 ml). After the combined chloroform phases were evaporated to dryness under vacuum at 35°C, the lipid was collected with four 1.5-ml volumes of chloroform–methanol (19:1, vol/vol) and stored at −10°C in tubes sealed with Teflon-lined caps. Total lipid was estimated gravimetrically.

Analysis of fatty acids. Fatty acid methyl esters of the total lipid were prepared by transesterification. A portion of each lipid sample was dried under a stream of nitrogen, dissolved in 2 ml of 5% (wt/wt) HCl in anhydrous methanol, and heated at 110°C for 2 h. The methyl esters were extracted by addition of 2 ml of petroleum ether followed by 1 ml of water, removal of the petroleum ether layer, and reextraction with two additional 2-ml volumes of petroleum ether. The combined extracts were brought to a known volume before analysis. Methylation of the fatty acids of the individual polar and neutral lipid classes, separated by thin-layer and column chromatography, was performed similarly with heneicosanoic acid as an internal standard.

Methyl esters of the fatty acids were analyzed with a Fisher-Victoreen model 4000 gas-liquid chromatograph equipped with flame ionization detectors. Methyl esters were separated isothermally at 195°C in an aluminum column (0.32 by 244 cm) packed with 15% EGSS-X on 100/120 mesh Gas-Chrom P (Applied Science Laboratories, Inc., State College, Pa.). Fatty acid methyl esters were identified by comparisons of relative retention times to those of reference standards. Graphs of log relative retention times versus carbon numbers were used to tentatively identify fatty acids not present in standard mixtures. Confirmation of the identification of unsaturated fatty acids was determined by catalytic hydrogenation of the methyl esters followed by rechromatography and examination for peak shifts. Quantitation of each fatty acid was achieved by comparison of peak heights times retention times to that of the internal standard.

Fractionation, identification, and quantitation of lipid classes. The total lipid extracts were separated into individual lipid classes by thin-layer chromatography on glass plates coated with a 500-μm thickness of Silica Gel H. Neutral lipids were separated with solvent systems of petroleum ether–diethyl ether–acetic acid (90:15:2 and 50:70:1, by volume); glycolipids were separated with chloroform–methanol–concentrated ammonium hydroxide (60:35:5, by volume); phospholipids were separated with chloroform–methanol–acetic acid–water (25:15:4:2, by volume); and phosphatidylethanolamine was separated from phosphatidylglycerol with chloroform–methanol–ammonium hydroxide (65:37:4, by volume).

The lipid classes were first separated and identified by comparison of their mobilities with those of lipid standards in the various solvent systems. Identification was further confirmed by using specific color reagents (11): α-naphthol for glycolipids, molybdenum reagent for phospholipids, and ninhydrin for lipids having free amino groups. When recovery of the individual classes was not necessary, separated lipid components were located by spraying the plates with 50% (wt/vol) sulfuric acid and heating until charred.

For quantitative estimation of the individual lipid classes, preparative thin-layer chromatography on glass plates (20 by 20 cm) coated with 500-μm layers of Silica Gel H was used. Except for cardiolipin which migrated with the neutral lipids, the phospholipids were first separated from the neutral lipids with chloroform–methanol–acetic acid–water (25:15:4:2, by volume). After the position of each phospholipid component was located by exposure to iodine vapor, the

Silica Gel H was scraped from the individual areas into screw-capped tubes containing heneicosanoic acid as an internal standard and 5% (wt/wt) HCl in methanol. The methyl esters of the fatty acids were prepared and analyzed as described above. The neutral lipids were separated from cardiolipin by scraping the Silica Gel H into a glass column containing 2 g of silicic acid in chloroform and eluting with 5 bed volumes of chloroform. Cardiolipin was then eluted with 5 bed volumes of methanol-chloroform-water (2:1:0.8, by volume). The solvent ratio was adjusted to 1:1:0.9, the chloroform layer was recovered and dried under a stream of nitrogen, and cardiolipin content was determined by fatty acid analysis. The neutral lipids were concentrated by evaporation, separated into individual components by preparative thin-layer chromatography, and the fatty acid-containing classes analyzed like the phospholipid components.

Quantitation of the individual lipid classes was calculated from the average chain length of the fatty acids in each class and the known weight of heneicosanoic acid added as an internal standard to each sample before methylation.

The sterol and sterol ester fractions of the neutral lipids were quantitated by gas-liquid chromatography of the trimethylsilyl derivatives (7). Free sterols were first obtained from the sterol esters by hydrolysis for 2 h at 80°C in a mixture of 0.1 ml of diethyl ether, 0.5 ml of ethanol, and 0.1 ml of 12 N aqueous KOH followed by extraction of the free sterols with diethyl ether. The sterol samples were combined with 100 μg of cholestane used as an internal standard, dried, and reacted for 15 min with 50 μl of pyridine-hexamethyldisilazane-chlorotrimethylsilane (9:3:1, by volume) to form the trimethylsilyl derivatives. The derivatives were separated isothermally at 250°C in a glass column (0.2 by 183 cm) packed with 3% OV-17 on 100/120 mesh Gas-Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) with an F & M model 810 gas-liquid chromatograph equipped with a flame ionization detector. Quantitation was achieved by comparison of peak height times retention time to that of the cholestane standard.

Protein analysis. Assays were performed on sonicated suspensions of purified *T. pallidum* by modifications of the methods of Lowry et al. (17) and Bradford (4). Bovine gamma globulin and serum albumin, fraction V, were used as reference proteins.

Treponemal counts. Numbers of treponemes were determined by dark-field microscopy as previously described (24) from at least eight cover slips with duplicate samples. Treponemal enumeration with a Petroff-Hausser chamber yielded the same results as those obtained with the cover slip procedure.

RESULTS

Studies on the lipid composition of *T. pallidum* could be performed only if organisms were obtained free of rabbit tissue components. To accomplish this, we utilized an extensive purification scheme that included three of the best available tools for obtaining high yields of purified *T. pallidum* (2, 8, 25). We obtained 10^{10} to 4×10^{10} (mean = 2.3×10^{10}) treponemes per rabbit, with an average recovery of 45% after purification.

The last major step in the purification scheme used Hypaque gradients (2). Baseman et al. (2) reported that a heavy band of tissue material could be seen in the upper portion of the gradients above the treponemal bands after centrifugation. Our gradients contained no band or only a faintly visible band in this region, although particles were always detectable by dark-field microscopy. As a precautionary measure, we discarded the harvests from any rabbits resulting in a heavy band of tissue material: 2 of 35 harvests were discarded. The tissue fraction was always well separated from the treponemal bands and easily removed before recovery of the treponemal fraction. About 90.4% of the lipids and 96.4% of the fatty acids recovered from both the tissue and *T. pallidum* fractions of the gradients were found in the treponemal fraction. The final purified treponemal suspensions were virtually free of detectable tissue material as judged by dark-field microscopy. Baseman et al. (2) previously reported that *T. pallidum* from Hypaque gradients are unassociated with host components as determined by both dark-field and electron microscopy.

Protein and lipid contents of several purified treponemal suspensions containing different numbers of organisms were determined as an additional indication of the purity of our final preparations (Table 1). The amounts of lipid and protein were directly related to the number of organisms. The protein content of each sample was dependent, however, on the method and reference protein used for the assay. All data on protein values are presented since results obtained with the newer dye-binding assay of Bradford (4) cannot be properly compared with protein values determined by the procedure of Lowry et al. (17). The protein content of *T. pallidum* determined by the Lowry et al. (17) procedure with bovine serum albumin averaged 0.56 ± 0.07 (standard error of the mean) $\times 10^{-7}$ μg/treponeme.

The individual lipid classes identified in *T. pallidum* are shown in Table 2. Phospholipids comprised 67.8% of the total lipid and were the same as found in the infected testes tissue, although major quantitative differences were found. The ratio of the relative amounts of each phospholipid class in the total lipid or phospholipids of *T. pallidum* compared with the amounts quantitated by McMeans et al. (unpublished data) in normal and infected rabbit testes tissue were as follows: phosphatidylcholine, 1.0 to 1.3; sphingomyelin, 1.6 to 2.0; cardiolipin, 3.9

TABLE 1. *Protein and lipid content of purified T. pallidum[a] (Nichols virulent strain)*

Sample[b]	Total *T. pallidum* ($\times 10^7$)	Total lipid (μg)	Total protein[c] (μg)			
			Dye-binding assay		Lowry assay	
			BγG	BSA	BγG	BSA
A	380	286	390	182	216	256(0.67)[d]
B	824	396	528	245	243	312(0.38)
C	1,149	694	1,006	470	485	619(0.54)
D	1,528	960	1,700	812	812	1,001(0.65)
Mean ± SEM[e]						(0.56 ± 0.07)

[a] Virulent *T. pallidum* was purified from infected rabbit testes as described in the text. After removal from Hypaque gradients, the organisms were washed three times with 35 ml of phosphate-buffered saline (pH 7.3) before lipid and protein determinations.

[b] Samples A, B, C, and D contained the total number of treponemes collected and purified from four, one, two, and three rabbits, respectively. The total number of organisms recovered and analyzed in sample A was exceptionally low relative to the number of rabbits used. For unknown reasons all four animals had suboptimal infections at time of harvest, which undoubtedly contributed to the cause of the low treponemal yield. Similar problems have been reported by others (8).

[c] Protein content was estimated by a dye-binding (4) and the Lowry (17) procedures. Bovine gamma globulin (BγG) and serum albumin (BSA) were used as standard proteins in each assay.

[d] Values in parentheses denote micrograms of protein $\times 10^{-7}$ per treponeme.

[e] SEM, Standard error of the mean.

TABLE 2. *Lipid composition of purified T. pallidum (Nichols virulent strain)*

Component	Wt % of total lipid
Phospholipids	67.8 ± 1.0[a]
Phosphatidylcholine	32.1 ± 2.3
Sphingomyelin	14.8 ± 0.4
Cardiolipin	13.0 ± 1.0
Phosphatidylethanolamine	6.2 ± 0.5
Phosphatidylinositol/serine	1.2 ± 0.1
Lysophosphatidylcholine	0.4 ± 0.1
Neutral lipids	32.2 ± 1.0
Cholesterol	26.5 ± 0.8
Other sterols	1.1 ± 0.1
Free fatty acids	2.9 ± 0.4
Triglycerides	1.6 ± 0.1
Sterol esters	0.2 ± 0.0
Glycolipid	None detected

[a] Mean relative percentage ± standard error of the mean, $n = 3$.

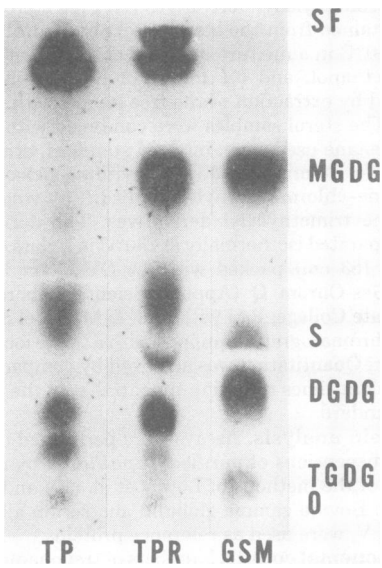

FIG. 1. *Thin-layer chromatogram comparing the presence and absence of monoglycosyldiglyceride (MGDG) in the total lipids (200 μg) from T. phagedenis biotype reiterii (TPR) and purified T. pallidum (TP), respectively. Lipids were resolved on glass plates coated with Silica Gel H with chloroform-methanol-ammonium hydroxide (60:35:5, vol/vol/vol) and detected by spraying with 0.5% α-naphthol in methanol-water (1:1, vol/vol) followed by 50% sulfuric acid and charring. Glycolipids were located as purple spots before charring. The only α-naphthol-positive spot detected in either of the two organisms was MGDG in T. phagedenis biotype reiterii. Abbreviations: GSM, glycolipid standard mixture; S, sulfatide; DGDG, digalactosyldiglyceride; TGDG, trigalactosyldiglyceride; O, origin; SF, solvent front.*

to 6.2; phosphatidylethanolamine, 0.4 to 0.6; phosphatidylinositol-serine, 0.3; and lysophosphatidylcholine, 1.0. Neutral lipids were comprised mainly of cholesterol and accounted for the remainder of the lipid. No glycolipid, specifically no MGDG, was detected in *T. pallidum* (Fig. 1), although it was easily extracted from *T. phagedenis* biotype *reiterii* by the same procedure used to extract the total lipids from *T. pallidum*.

The fatty acid compositions of the total lipid of *T. pallidum* and infected rabbit testicular tissue remaining after extraction of *T. pallidum* are compared in Table 3. The fatty acids from

TABLE 3. *Fatty acid composition of the total lipid from purified T. pallidum (Nichols virulent strain) and infected rabbit testes tissue*[a]

Fatty acid	*T. pallidum*	Testis
14:0[b]	2.3 ± 0.1[c]	0.6 ± 0.1[d]
15:0	0.9 ± 0.1	2.0 ± 0.0
16:0	42.2 ± 0.2	22.6 ± 1.6
16:1	2.1 ± 0.0	2.0 ± 1.0
17:0	1.0 ± 0.1	1.4 ± 0.0
18:0	10.1 ± 0.2	11.4 ± 0.8
18:1	13.2 ± 0.8	10.7 ± 0.4
18:2	10.3 ± 0.2	9.3 ± 0.4
20:3	0.2 ± 0.0	2.9 ± 0.2
20:4	2.4 ± 0.2	13.1 ± 2.2
22 series and above	12.6 ± 0.3	22.1 ± 0.4
Minor components[e]	2.7 ± 0.5	2.0 ± 0.2

[a] Virulent *T. pallidum* was purified from infected rabbit testes as described in the text. The fatty acid compositions of the total lipid extracted from the purified organisms and the testes tissue remaining after elution of the organisms were determined.

[b] Number of carbon atoms:number of double bonds.

[c] Mean relative percentage ± standard error of the mean, $n = 3$.

[d] Mean relative percentage ± difference from the mean, $n = 2$.

[e] Sum of individual fatty acids comprising less than 0.6% of the total fatty acids from both *T. pallidum* and the testes.

the organism were the same as found in the tissue. Palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids comprised about 76% of the treponemal fatty acids. Prominent differences between *T. pallidum* and the tissue were that the organism contained a greater proportion of palmitic acid (16:0), whereas the tissue was comprised of greater amounts of fatty acids of 20 carbon chain lengths and above. Differences in the amounts of 14:0 and 15:0 fatty acids in the organism and the tissue were also found.

DISCUSSION

Major problems in research on *T. pallidum* are the acquisition of sufficient treponemes and their purification from infected tissue. We have obtained high yields of purified organisms for studies of lipid composition by using several well-documented tools: Hypaque gradients (2), Nuclepore membranes (25), and corticosteroid-treated rabbits (8). Baseman et al. (2) introduced the use of Hypaque gradients to obtain purified *T. pallidum*, but heavy bands of tissue material were present in their gradients above the treponemal fractions. We were able to reduce the host material above the *T. pallidum* bands in our Hypaque gradients so that no tissue band or only a slightly macroscopically visible band was evident. This decreased the possibility of the organisms being contaminated with material from the tissue fraction during recovery of the treponemal fraction. The comparatively small amount of host material present in the tissue fraction of our gradients can be attributed to the preliminary steps of purification employing differential centrifugation and filtration through Nuclepore membranes (25) and to the use of corticosteroid-treated rabbits. Hardy and Nell (8) have reported that harvests from corticosteroid-treated animals are much cleaner than those from nontreated animals. They reported that suspensions of *T. pallidum* purified by differential centrifugation from treated rabbits contained an average of 1.9×10^{-7} μg of protein per treponeme as determined by the Lowry method with bovine serum albumin as a standard. We obtained an average protein concentration in our final purified suspensions about 70% lower than this.

Vaczi et al. (29) compared the lipids of *T. pallidum* (Budapest strain) purified from corticosteroid-treated rabbits by differential centrifugation with those of several cultivable treponemes. Each strain contained 11 to 13 components including phosphatidylcholine, phosphatidylethanolamine, cardiolipin, and the corresponding lyso-compounds, but no quantitative data on these components or the identity of the other lipids were reported. The types of phospholipids that we found in *T. pallidum* were similar to the cultivable treponemes (10, 16, 19) and rabbit testicular tissue as well, but the relative amounts of individual phospholipids were different. Vaczi et al. (29) reported that the acetone-insoluble lipids which contained the bulk of the phospholipids comprised 37.4% of the total lipids of *T. pallidum*, or about one-half the phospholipid content that we determined. Smibert (27) used the Beckman Diagnostic electrophoretically purified fluorescent treponemal antibody antigen as a source of *T. pallidum* for lipid analysis. The total lipid contained 41% phospholipid comprised of 35% phosphatidylcholine, 33% phosphatidylinositol, and 31% phosphatidylserine (C. B. Walker and R. M. Smibert, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, Abstr. no. P40, p. 151). We found the amount of phosphatidylcholine in *T. pallidum* was about 27 times the combined amounts of phosphatidylinositol and phosphatidylserine rather than about one-half as reported for the fluorescent treponemal antibody antigen. We also found that 50% of the phospholipid consisted of components other than phosphatidylcholine, phosphatidylinositol, and phosphatidylserine.

The cardiolipin content of *T. pallidum* is of

historical interest since the Wassermann antibody formed during the early course of syphilis is an antibody to this phospholipid. Although Kumar et al. (13) were unable to detect cardiolipin in 10^{12} *T. pallidum,* we easily found it in 5 $\times$ 10^9 organisms. An unanswered question is whether the Wassermann antibody in cases of syphilis is formed against cardiolipin of treponemal or tissue origin. Since *T. pallidum* contained about 13% cardiolipin, the antibody could be formed at least in part against the treponemal component. In false-positive, nonsyphilitic cases this, of course, would not be true. It should be noted that cardiolipin is a component of other treponemes (16).

Cholesterol was a major constituent of the lipids of *T. pallidum* but whether or not it is required for growth remains unknown. *T. phagedenis* contains cholesterol when grown with serum (19) but can be grown without serum and then contains no sterol (10, 16). On the other hand, *T. refringens* biotypes require cholesterol as an essential nutrient for growth (14, 23).

Evidence is gradually accumulating to indicate that *T. pallidum* may be similar to many cultivable treponemes regarding fatty acid metabolism. Long-chain fatty acids are required for growth by many strains of *Treponema* (9, 16, 22) since the organisms are unable to synthesize, β-oxidize, or desaturate fatty acids (1, 10, 19). The fatty acids in these organisms are the same as those found in the growth medium. Similarly, the fatty acid composition of *T. pallidum* was comparable to that of the infected tissue from which the organism was obtained. No fatty acids were present in the organism which were not available from the tissue. *T. pallidum* has also been shown to incorporate ^{14}C-labeled oleic and palmitic acids (25) but was unable to degrade them to $^{14}CO_2$ (21, 25) due to lack of the enzymes of the β-oxidation pathway (25). The effects of fatty acids on the survival of *T. pallidum* in vitro have also been found to parallel results obtained in growth studies with cultivable treponemes (9, 18).

The glycolipid MGDG is a major component of all *Treponema* examined (10, 16, 19; our unpublished data). It has therefore been proposed as a taxonomic marker to aid in differentiation of *Treponema* from other members of *Spirochaetales* (14, 16) and as a diagnostic tool to indicate the presence of treponemes in tissue (15). We did not detect MGDG in purified suspensions of *T. pallidum.* Vaczi et al. (29) did not report on the glycolipid content of either *T. pallidum* or the cultivable treponemes that they examined. Smibert (27) stated, however, that the Beckman purified fluorescent treponemal antigen preparation of *T. pallidum* contained 37% glycolipid-containing monosaccharide residues identified as arabinose, sedoheptulose, mannose, and glucose (C. B. Walker and R. M. Smibert, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, Abstr. no. P40, p. 151). Dupouey and Betz (6) isolated a glycolipid antigen from *T. phagedenis* biotype *reiterii* identified as MGDG (5). MGDG was easily detected by complement fixation reactions in *T. phagedenis* biotypes *reiterii* and *phagedenis* but not in *T. pallidum* (6). The absence of MGDG in *T. pallidum* is interesting in relation to evidence indicating the lack of genetic relatedness between *T. pallidum* and five cultivable *Treponema* (20). It would also be interesting to determine whether *T. pertenue* and *T. carateum,* treponemes pathogenic for humans which are morphologically and immunologically similar to *T. pallidum,* might resemble *T. pallidum* in lacking MGDG.

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