proportion of the trypanosome sample. Moreover, when IgG-negative *T. lewisi* cells from immunosuppressed hosts are treated in vitro with immune serum that shows only ablastic activity [after adsorption to remove trypanocidal antibodies (*10, 11*)], they become positive for IgG. Specific, nonablastic antibodies to coat antigens may also occur on circulating trypanosomes, but these have not yet been demonstrated.

Our results suggest that aggregation of surface coat components of *T. lewisi* may require less energy than membrane components of other eukaryotic cells require for capping, since capping occurs rapidly at 0°C. The loose fibrillar coat of the parasite may consist of components that have relatively weak interactions, thus facilitating antigenic mobility.

Others have suggested that antibody-induced capping in vivo might modulate parasite surface antigens, leading to antigenic variation in the causative agents of African trypanosomiasis (*3, 4*), or that it might provide a mechanism for escape from host antibody (*4*). However, host ablastic IgG does not appear to cause capping of *T. lewisi* antigens in vivo; only when this IgG is cross-linked by an additional ligand in vitro does capping occur. Electron microscopic studies of *T. lewisi* in which a double ligand technique with ferritin label is used, provide additional evidence for ligand-induced capping in vitro (*12*). However, in those experiments the primary ligand was multispecific gamma globulin (obtained from recovered hosts and containing ablastin plus trypanocidal antibodies) added in vitro, rather than naturally acquired host IgG. In contrast, our results indicate that specific, ablastic IgG acquired in vivo, despite its effects on cellular metabolism (*13*), causes no detectable modulation of surface antigens.

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References and Notes

1. R. B. Taylor, W. P. H. Duffus, M. C. Raff, S. DePetris, *Nature (London) New Biol.* **233**, 225 (1971); F. Loor, L. Forni, B. Pernis, *Eur. J. Immunol.* **2**, 203 (1972); S. DePetris and M. C. Raff, *ibid.*, p. 523; J.-C. Antoine, S. Avrameas, N. K. Gonatas, A. Stieber, J. O. Gonatas, *J. Cell Biol.* **63**, 12 (1974).
2. F. M. Kourilsky, D. Silvestre, C. Neauport-Sautes, Y. Loosfelt, J. Dausset, *Eur. J. Immunol.* **2**, 249 (1972).
3. J. J. Doyle, R. Behin, J. Mauel, D. S. Rowe, *J. Exp. Med.* **139**, 1061 (1974); *Ann. N.Y. Acad. Sci.* **254**, 315 (1975).
4. D. M. Dwyer, *J. Immunol.* **117**, 2081 (1976).
5. D. Barry, *J. Protozool.* **22**, 49A (1975); J. J. Doyle, in *Proceedings of the Fifth International Congress of Protozoology*, 26 June–July 1977, New York, p. 245.
6. P. A. d'Alesandro, *Exp. Parasitol.* **32**, 149 (1972); D. M. Dwyer, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1222 (1976).
7. F. A. Coventry, *Am. J. Hyg.* **5**, 127 (1925).
8. P. A. D'Alesandro, *J. Protozool.* **9**, 351 (1962).
9. K. Vickerman, in *Ecology and Physiology of Parasites*, A. M. Fallis, Ed. (Univ. of Toronto Press, Toronto, 1970) p. 58.
10. W. H. Taliaferro, *Am. J. Hyg.* **16**, 32 (1932).
11. P. A. D'Alesandro, *J. Infect. Dis.* **105**, 76 (1959).
12. P. V. Cherian and D. G. Dusanic, *Exp. Parasitol.* **43**, 128 (1977); *ibid.* **44**, 14 (1978).
13. P. A. D'Alesandro, *ibid.* **38**, 303 (1975).
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Absence of Glycerol Teichoic Acids in Certain Oral Streptococci

Abstract. *Glycerol teichoic acids were not detected immunochemically or chemically in phenol-water, hot saline (Rantz and Randall), or supernatant fluids of disrupted cells of* Streptococcus mitis. *Thus, teichoic acids do not appear to be found in most Gram-positive bacteria, as has been suggested.*

Lipoteichoic acids (LTA) are a class of amphipathic polymers composed of glycerol, phosphate, alanine, fatty acids, and variable quantities of hexoses that are found associated with the cell membranes in Gram-positive bacteria (*1*). In recent studies attempting to develop a serological grouping scheme for *Streptococcus mitis*, an ill-defined group of oral streptococci associated with dental plaque as well as with many cases of endocarditis, I noticed that extracts of many strains did not react with an antiserum against teichoic acids (polyglycerol phosphate or PGP antiserum). The failure to detect teichoic acid was surprising since it has generally been assumed that most Gram-positive bacteria with the exception of certain micrococci, such as *Micrococcus lysodeikticus* (now classified as *M. luteus*), contain these polymers, at least in the membrane-associated form (*1*). Since these teichoic acids have been associated with a number of diverse biologically important activities in Gram-positive bacteria, such as regulation of autolysins, ion transport, immunogenicity, virulence, and adherence of bacteria to mammalian cells and tooth surfaces (*1, 2*), the absence of these polymers in a large group of Gram-positive bacteria could have significant implications for general as well as for oral microbiology.

Although several extensive studies that have provided a better physiological basis for the classification of viridans streptococci have been reported (*3*), many laboratories still use the epithet *Streptococcus mitis* to designate α-hemolytic streptococci that do not fit more easily recognized groups, for example, *S. salivarius, S. mutans*, and *S. sanguis*. Indeed, the most common error appears to be confusion between *S. mitis* and *S. sanguis*. Although these species are similar physiologically, studies have shown that their genomes differ significantly (*4*); one reflection of this difference is the carbohydrate and peptidoglycan composition of their cell walls (*5*). I have observed that one of the simplest means of distinguishing *S. sanguis* from *S. mitis* biochemically is the determination of ammonia from arginine; *S. sanguis* is positive and *S. mitis* negative. Also, *S. sanguis* contains the Lancefield group H antigen whereas it is absent in *S. mitis*.

A large number of strains designated *S. mitis* (*S. mitior*) were assembled from various laboratories for a serological study; in addition, a culture collection of reference strains of *S. sanguis* were available from previous studies. All strains were subcultured on Mitis-Salivarius agar (Difco) in order to ensure purity of the cultures. In some cases, two or more colonial types were observed on this medium but no differences were detected on blood agar, the usual medium used for isolation of these bacteria. Individual colonies were picked for stock cultures, and subsequently biochemical tests were performed in which a modification of the physiological tests for speciation, proposed recently by Facklam, was used (*3*).

Antigen extracts were obtained from whole cells by use of the following procedures. Phenol-water (PW) extraction was carried out at 65° to 68°C with 70 percent phenol; this procedure solubilizes membrane LTA (*6*). The Rantz and Randall (RR) (*7*) method utilizes the supernatants of cells, suspended in 0.15*M* NaCl and heated at 121°C for 15 minutes, as a source of antigens. Studies of *S. sanguis* suggest that this procedure solubilizes primarily cell wall polymers including wall teichoic acids (*8*). Antigens were also obtained from supernatants of cells disrupted in a Ribi press at 55,000 pounds per square inch at 10° to 15°C; these preparations contain an admixture of cell wall, cytoplasmic components, and LTA (*1, 6*). All of the extracts were dialyzed against H_2O and were lyophilized prior to testing by Ouchterlony and

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Table 1. Minimum concentration of hemagglutinin detected in phenol-water extracts. Titers are expressed in micrograms per milliliter.

Test antigen (strain of organism)	Antiserums against			
	PGP*	72x41	6249	10557
72x41†	0	25		
ATCC 6249‡	0		12.5	
ATCC 10557†	0			0
72x35†	0			0
1D‡	<0.04			0.3§
6E‡	0.08			0
3G‡	50	25		

*Antiserum against teichoic acid. †Received as *Streptococcus sanguis*. ‡Received as *S. mitis*. §This reaction was slight at all concentrations of antigen.

immunoelectrophoretic (IEP) techniques (*8*). Antiserums against PGP were prepared in rabbits with the use of increasing doses (0.04 to 0.4 mg) of viable cells of *Lactobacillus casei*, strain L324M, injected intravenously. Animals were bled by cardiac puncture and the antiserums were tested by Ouchterlony and IEP for the presence of teichoic acid antibodies with the use of preparations of purified teichoic acids (*8*). Reactions were compared to a reference PGP antiserum (*1*) obtained from Kenneth Knox (Institute for Dental Research, Sydney, Australia). The antiserums to PGP could detect approximately 2.5 μg of teichoic acid in gel diffusion tests. Antiserums to strains 72x41 and ATCC 10557 (received as *S. sanguis*) and ATCC 6249 (received as *S. mitis*) were prepared in essentially the same manner as the PGP antiserums.

Initially the extracts obtained by the disruption of cells were employed in serological tests because they were easier to prepare than PW and RR extracts. In addition, the number of antigens detected in these extracts was greater when tested against homologous antiserums. As I indicated previously, extracts of disrupted cells would normally contain LTA (*1*). To date, 51 strains have been tested by Ouchterlony and IEP methods; 23 of these strains did not react with antiserum to PGP. Controls of similar preparations from Lancefield's group D and group H streptococci, known to contain LTA, were positive. Representative results shown in Fig. 1a indicate that extracts (40 mg/ml) of a group of strains received as *S. mitis* reacted with antiserum to PGP; Fig. 1b shows another group of strains labeled *S. mitis* that did not react with the antiserum. The concentrated extract was used to ensure that a failure to detect teichoic acid in the extract was not due to its low concentration (*8*). Physiological tests showed that none of the teichoic acid–negative strains produced ammonia from arginine; in contrast, only two of the teichoic acid–positive strains failed to produce it. Thus a clear-cut relationship appeared to exist between the presence or absence of teichoic acid and the ability to hydrolyze arginine, a property that, as I indicated earlier, helps distinguish *S. mitis* and *S. sanguis*. The RR extracts of ten of the teichoic acid–positive strains were tested against reference group H antiserum (*8*) and were positive, whereas extracts of the *S. mitis* strains did not react. Thus, both serological and physiological criteria suggested that the teichoic acid–positive strains were predominantly *S. sanguis*, whereas the teichoic acid–negative strains appeared to be *S. mitis*.

Lipoteichoic acids can also be detected in very small quantities by use of the hemagglutination technique (*6*). Indeed, this method can be used to detect many amphipathic antigens because of their ability to sensitize erythrocytes, presumably due to lipid content (*1*). For this study the PW extracts were used, since this is the standard method for obtaining acylated LTA (*1*). The extracts were treated with deoxyribonuclease, ribonuclease, and trypsin to reduce contaminating nucleic acids and proteins (*8*). The extracts were boiled for 10 minutes and centrifuged to remove the enzymes. Sheep erythrocytes were sensitized with a portion of serial dilutions of the extracts for 24 hours at 4°C. Diluted PGP antiserum (1:4) as well as antiserums against homologous strains were used in the assay. The antiserums were absorbed with the erythrocytes to remove natural hemagglutinins prior to use in the tests. The results for the strains tested are shown in Table 1. Controls of unsensitized erythrocytes were performed for each absorbed antiserum and all were negative. Strains 72x41, 6249, 10557, and 72x35 (which was received as *S. sanguis*) did not react with PGP antiserum. The latter two strains were presumed to derive from the same culture (NCTC 7864) (*4, 9*), albeit they were received from different sources. It is of interest that despite divergent histories the cultures appear to be serologically the same. Strain 3G (received as *S. mitis*) is of interest because it gives a strong reaction in gel precipitation and gives an identity with the other teichoic acids shown in Fig. 1a but did not sensitize erythrocytes very well (titer, 50 μg/ml). This observation suggested that the 3G teichoic acid contained little lipid or that the lipid was removed during extraction with PW. Although the latter method has been reported to remove some labile fatty acid esters in both lipopolysaccharides and in some teichoic acids (*8, 10*), it is not generally thought to cause deacylation of LTA. Further study is required to determine precisely the effect of the PW extraction procedure on these preparations (*11*). In any case, the PW procedure solubilizes a teichoic acid that does not sensitize erythrocytes, which is interesting because this method is the standard for extraction of erythrocyte-sensitizing LTA. Also, these *S. mitis* strains did not contain a major hemagglutinin in PW extracts when tested against homologous antiserums. It is not yet clear whether other nonimmunogenic amphipathic molecules may be present.

Table 2. Chemical composition of phenol-water extracts. Values are expressed as percentages; ND, not detected.

Components	Strains tested			
	72x41*	72x35*	1D†	3G†
Phosphorus	2.2‡	2.1	4.7	6.5
Glycerol	4.9‡	6.0	19.9	24.8
Anhydroribitol	1.1	1.5	0.2	ND
Rhamnose	6.0	1.2	2.9	5.2
Ribitol	5.2	3.6	2.3	0.8
Glucose	14.9	21.0	25.0	21.5
Galactose	12.6	22.8	16.1	10.2

*Received as *Streptococcus sanguis*. †Received as *S. mitis*. ‡Molar ratios of glycerol to phosphate are: for strain 72x41, 1:0.7; strain 72x35, 1:1; strain 1D, 1:1; and strain 3G, 1:1.2.

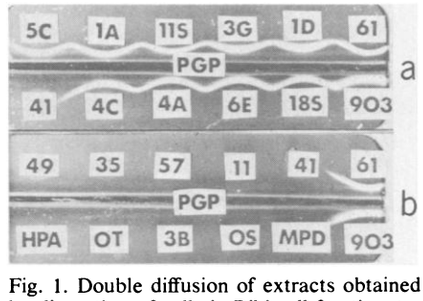

Fig. 1. Double diffusion of extracts obtained by disruption of cells in Ribi cell fractionator at 55,000 pounds per square inch. Whole cells and cell walls were sedimented by centrifugation. Peripheral wells contain cell extracts and center trough contains antiserum against teichoic acid (PGP). Wells labeled 61 and 903 contain extracts of Lancefield's group D and group H, respectively. (a) Strains received as *Streptococcus mitis* that were positive for teichoic acid; 41 represents negative control. (b) Wells labeled 35 and 57 represent extracts of strains 72x35 and 10557 received as *S. sanguis*. Other strains were received as *S. mitis*; all were negative for teichoic acid. 61 and 903 were positive controls.

Although the immunochemical evidence definitely suggested the absence of LTA, chemical evidence for the absence of teichoic acids was sought. Phenol-wa-

ter extracts of strains that did and did not react with the PGP antiserum were hydrolyzed for chemical analysis in 1*N* HCl for 8 hours; hydrolysis was carried out in evacuated tubes at 100°C. The samples were dried to remove HCl and treated with alkaline phosphatase prior to reduction and acetylation for analysis of the polyol and sugar alditol acetates by gas-liquid chromatography (*4*). A comparative chemical analysis of strains that contain LTA and those that do not is shown in Table 2; strains 1D (received as *S. mitis*) and 3G in which glycerol teichoic acid was detected immunochemically contained 20 and 25 percent glycerol, respectively; strains 72x41 and 72x35, in which no teichoic acid was detected immunochemically, contained 5 and 6 percent glycerol. In general, the glycerol:phosphate ratio hovered around 1:1, but the presence of ribitol in all extracts indicated that these extracts still contained nucleic acid derivatives; this was expected since the extraction procedure and enzyme treatment is only a first step in the purification. It is clear, however, that teichoic acid–positive strains have significantly higher quantities of glycerol.

The failure to detect glycerol teichoic acid among these strains confirms the observation of McCarty (*12*). In his original paper concerning the occurrence of the polymer in bacteria, he observed that more than 50 percent of *S. sanguis* and *S. viridans* (an older synonym sometimes used for *S. mitis* strains and other α-hemolytic streptococci) did not contain the antigen. However, these observations apparently have been forgotten in the many recent publications concerning the location and function of these interesting polymers. In *M. lysodeikticus*, which also does not have a teichoic acid, a lipomannan has been found that is speculated to serve the same function as the lipoteichoic acid (*13*). It has been suggested that such amphipathic molecules may be essential for growth of Gram-positive bacteria (*1*). The presence of small amounts of glycerol and phosphate in *S. mitis* suggests a lipid may be present that could be part of an amphipathic molecule, and studies aimed at isolating such a polymer are under way. However, the results of my studies indicate clearly that LTA is not present in *S. mitis*, and thus the polymer is not universally found in all Gram-positive bacteria. These observations suggest that this species has adapted to existence without LTA and, therefore, teichoic acids may not be as essential for all Gram-positive bacteria as had been speculated (*1*). On the other hand, the most notable feature of *S. mitis* is the apparent lack of physiological properties often associated with most other streptococci. For example, most *S. mitis* strains ferment only a few sugars and their cell walls contain little, if any, rhamnose; thus, as suggested earlier in this report, identification is often by exclusion. Could it be that the absence of teichoic acids, although not preventing growth, does act to limit the physiological activity of this species? Such limitation may occur through effects on permeability; this in turn may be related to autolysin activity which may be regulated by LTA (*2*). Further study of these ubiquitous oral bacteria may eventually provide the clues for a better understanding of the role of LTA in Gram-positive bacteria.

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References and Notes

1. A. J. Wicken and K. W. Knox, *Science* **187**, 1161 (1975); K. W. Knox and A. J. Wicken, *Bacteriol. Rev.* **37**, 215 (1973); P. A. Lambert *et al.*, *Biochim. Biophys. Acta* **472**, 1 (1977).
2. R. F. Cleveland, J. V. Holtje, A. J. Wicken, A. Tomasz, L. Daneo-Moore, G. D. Shockman, *Biochem. Biophys. Res. Commun.* **67**, 1128 (1975); A. R. Archibald, *Adv. Microb. Physiol.* **11**, 53 (1974); E. Hausman, O. Luderitz, K. Knox, A. J. Wicken, N. Weinfeld, *J. Dent. Res.* **54**, B94 (1975); C. A. Miller and R. W. Jackson, *Infect. Immun.* **13**, 1408 (1976); G. Rolla, Proceedings of a symposium on Microbial Aspects of Dental Caries, *Microb. Abstr.* (special suppl.) **2**, 309 (1976). For recent review of biological properties, see A. J. Wicken and K. W. Knox, *Microbiology* (American Society for Microbiology, Washington, D.C., 1977), pp. 360–365.
3. J. Carlsson, *Odontol. Revy* **19**, 137 (1968); R. F. Facklam, *J. Clin. Microbiol.* **5**, 184 (1977); G. Colman and R. E. D. Williams, in *Streptococci and Streptococcal Diseases*, L. W. Wanamaker and J. M. Maken, Eds. (Academic Press, New York, 1972), pp. 281–299.
4. A. L. Coykendall and P. A. Specht, *J. Gen. Microbiol.* **91**, 92 (1975).
5. B. Rosan, *Infect. Immun.* **13**, 1144 (1976).
6. M. J. Hewett, K. W. Knox, A. J. Wicken, *J. Gen. Microbiol.* **60**, 315 (1970).
7. L. A. Rantz and E. Randall, *Stanford Med. Bull.* **13**, 290 (1955).
8. B. Rosan, *Adv. Exp. Med. Biol.* **107**, 791 (1978); *Infect. Immun.* **7**, 205 (1973).
9. R. M. Cole, G. B. Calandra, E. Hutt, K. Nugent, *J. Dent. Res.* **55**, A142 (1976).
10. J. C. Tsang, C. S. Wang, P. Alapovic, *J. Bacteriol.* **117**, 786 (1974).
11. H. C. Cooper, F. W. Chorpenning, S. Rosen, *Infect. Immun.* **19**, 462 (1978). These investigators suggest that lipid is not necessary for erythrocyte sensitization. However, Wicken examining the same or a similar preparation found fatty acids present in the sensitizing antigen (A. J. Wicken, personal communication).
12. M. McCarty, *J. Exp. Med.* **109**, 361 (1959).
13. D. D. Pless, D. Schmidt, W. J. Lennarz, *J. Biol. Chem.* **250**, 1319 (1975); A. D. Powell, M. Duckworth, J. Baddiley, *Biochem. J.* **151**, 387 (1975).
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Ultraviolet Radiation–Induced Chromosomal Abnormalities in Fetal Fibroblasts from New Zealand Black Mice

Abstract. *Fibroblasts from New Zealand Black mouse fetuses manifest increased frequency of chromosomal breaks and interchanges after exposure to ultraviolet radiation when compared with cells from BALB/c fetuses. This chromosomal instability is similar to what has been reported in cells from patients with xeroderma pigmentosum and may be related to the chromosomally abnormal clones and malignancy previously reported in adult New Zealand Black mice.*

Several inherited diseases in man are characterized by chromosomal fragility usually manifest as breaks, fragments, and interchanges and by increased frequency of malignancy (*1*). These chromosomal instability syndromes include as prototypes, Bloom's syndrome, Fanconi's anemia, ataxia telangiectasia, and xeroderma pigmentosum. In the first three diseases, chromosomal aberrations occur spontaneously. In contrast, in xeroderma pigmentosum, which is characterized by defective repair of ultraviolet radiation–induced alterations in DNA (*2*), the cytogenetic abnormalities are found only after exposure to ultraviolet radiation (*3*).

Understanding of the relation between chromosome abnormalities and malignancy in these syndromes could be advanced by the discovery of suitable ani-

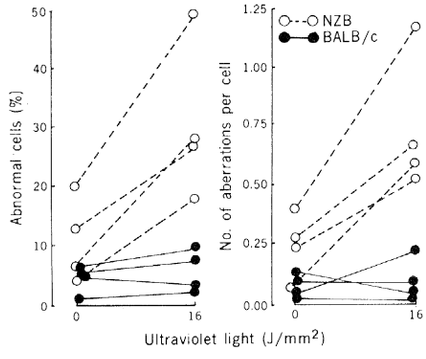

Fig. 1. Chromosomal abnormalities in cultured NZB and BALB/c fetal fibroblasts before and after exposure to ultraviolet radiation.

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