The Glycerol Teichoic Acid from Walls of *Staphylococcus epidermidis* I 2

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1. Walls of *Staphylococcus epidermidis* I 2 contain 30% (w/w) of a glycerol teichoic acid containing phosphate, D-alanine and D-glucose in the molecular proportions 1:0.25:0.50. 2. The teichoic acid was isolated by extraction with trichloroacetic acid and with dilute aqueous *NN*-dimethylhydrazine at pH 7, and was shown to be a (1→3)-linked poly(glycerol phosphate) containing β -D-glucopyranosyl and D-alanyl ester substituents. 3. 2-O- β -D-Glucopyranosylglycerol was isolated and characterized as its crystalline hexa-O-acetate. 4. Unlike that of certain other bacteria, the peptidoglycan component of the wall is not solubilized by *NN*-dimethylhydrazine. 5. The membrane teichoic acid is also a (1→3)-linked poly(glycerol phosphate) but contains a smaller proportion of glucosyl substituents.

Although unsubstituted glycerol teichoic acids are antigenic (McCarty, 1959), the serological specificity of glycosylated teichoic acid is mainly determined by the nature of the glycosyl substituents. Thus the specificity of group D antigen from streptococci (membrane teichoic acid) is due to the kojibiosyl and kojitriosyl substituents (Wicken, Elliott & Baddiley, 1963; Wicken & Baddiley, 1963). The teichoic acid containing α -glucosylglycerol residues from walls of *Staphylococcus epidermidis* T1 (Davison & Baddiley, 1963, 1964) cross-reacts serologically with that from *Staph. epidermidis* 1254; the latter is believed to correspond to 'polysaccharide B' of Julianelle & Wieghard (1935) (Losnegard & Oeding, 1963). Morse (1963) found that the antigenic determinant of the glycerol teichoic acid from *Staph. epidermidis* (Prengel) was α -glucosyl. During a survey of the composition of walls of staphylococci (Davison & Baddiley, 1963), teichoic acids containing glucosylglycerol residues, but with no serological cross-reaction with the above polymers, were isolated from several strains of *Staph. epidermidis* (Oeding, Myklestad & Davison, 1967); they had negative optical rotations and contained β -linked glucose. Thus teichoic acid from strain T2 contained glucose and phosphate in the molecular ratio 1.61:1 and must therefore possess disaccharide substituents. On the other hand, teichoic acid from strain I2 contained less glucose (0.74:1), but both preparations gave a single identical precipitation line on agar diffusion with anti-(strain I2) serum. We report a more detailed examination of the teichoic acid from strain I2; it contained monoglucosylglycerol residues but diglucosylglycerol was absent.

EXPERIMENTAL

Materials

Calf intestinal phosphomonoesterase was purchased from Sigma Chemical Co. (St Louis, Mo., U.S.A.); β -glucosidase was from L. Light and Co. Ltd., Colnbrook, Bucks. Glucostat reagent was from Worthington Biochemical Corp. (Freehold, N.J., U.S.A.). Sephadex was from Pharmacia (Uppsala, Sweden). 1-Fluoro-2,4-dinitrobenzene was from British Drug Houses Ltd. (Poole, Dorset). Hydrazine hydrate was supplied by British Drug Houses Ltd., and anhydrous hydrazine was prepared by heating 60g. of aq. 85% (w/v) hydrazine hydrate solution with 60g. of NaOH at 113° for 2 hr. in a stream of O₂-free N₂. After 2 hr. the anhydrous hydrazine was recovered by distillation (Smith & Howard, 1944). *NN*-Dimethylhydrazine was purchased from British Drug Houses Ltd., and was distilled before use, the fraction boiling at 62–63° (1.03 kg./cm.²) being collected.

Chromatography

Paper chromatography was carried out on Whatman no. 1, no. 4 and 3MM papers, previously washed with 2*N*-acetic acid solution and then water. Thin-layer plates of silica gel (Kieselgel G; E. Merck A.-G., Darmstadt, Germany) were prepared. The following solvent systems were used: *A*, propan-1-ol-aq. NH₃ (sp.gr. 0.88)-water (6:3:1, by vol.); *B*, butan-1-ol-ethanol-water-aq. NH₃ (sp.gr. 0.88) (40:10:49:1, by vol.) (organic phase); *C*, butan-1-ol-pyridine-water (6:4:3, by vol.); *D*, toluene-2-chloroethanol-pyridine-aq. 25% (w/v) NH₃ (10:7:3:1, by vol.); *E*, chloroform-benzyl alcohol-acetic acid (70:30:3, by vol.).

Products were detected by the periodate-Schiff reagents for α -glycols (Baddiley, Buchanan, Handschumacher & Prescott, 1956), the molybdate reagents for phosphoric esters (Hanes & Isherwood, 1949), the aniline phthalate

reagent for reducing sugars (Partridge, 1949), the alkaline AgNO_3 reagent for reducing compounds (Trevelyan, Procter & Harrison, 1950) and the ninhydrin reagent for amino compounds (Consden & Gordon, 1948).

Analytical methods

Phosphorus was determined by the method of Chen, Toribara & Warner (1956), amino compounds were determined by the method of Rosen (1957) and by the Technicon AutoAnalyzer and reducing sugars were determined by the method of Park & Johnson (1949). Hexose was determined by the phenol- H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956) and D-glucose with the Glucostat reagents. Periodate was determined spectrophotometrically (Dixon & Lipkin, 1954; Aspinall & Ferrier, 1957) and formaldehyde by the method of Hanahan & Olley (1958).

Isolation of walls

Staph. epidermidis 12 was grown in 10l. batches with forced aeration for 16hr. at 37° in a medium with the following composition: Oxoid nutrient broth no. 2, 25g.; glucose, 10g.; Difco yeast extract, 5g.; K_2HPO_4 , 3g.; water (demineralized), 1l. Olive oil was added as antifoam and cells were harvested in a refrigerated Sharples centrifuge. The yield was 70g. wet wt. A suspension of cells (70g.) in water (290ml.) was disrupted by shaking with Ballotini no. 11 beads in an International centrifuge (shaker head) at 1300rev./min. for 40min. Beads were removed by filtration through a no. 1 sintered-glass funnel and walls isolated by differential centrifugation, washed with M-sodium phosphate buffer, pH 7.0, followed by water and then freeze-dried. The yield was 2g. The walls were examined by electron microscopy and were free from visible contaminants.

Isolation of teichoic acid

With trichloroacetic acid. Freeze-dried walls (1.0g.) containing 3.0% of phosphorus were stirred with 10% (w/v) trichloroacetic acid (20ml.) at 4° for 24hr. After centrifugation, the supernatant solution was mixed with cold ethanol (100ml.) and kept at 2°. The wall residue was extracted with a further portion of trichloroacetic acid (20ml.) for 48hr. at 4°. The supernatant solutions were separately mixed with cold ethanol (100ml.); the precipitates were

recovered by centrifugation, combined and dissolved in 10% (w/v) trichloroacetic acid (5ml.). The small amount of insoluble material was removed by centrifugation, and addition of cold ethanol (25ml.) precipitated teichoic acid (96mg.) as a fine white powder. The residual walls were washed with ethanol and ether.

With *NN*-dimethylhydrazine. Walls (330mg.) were suspended in water (480ml.) in a 2l. conical flask and mixed with a freshly prepared solution (120ml.) of aq. 2% (w/v) *NN*-dimethylhydrazine, previously adjusted to pH 7 with formic acid. The suspension was stirred vigorously at 80° for 2hr. and samples were removed for analysis of phosphate and measurement of extinction at 550nm. The turbidity of the suspension remained constant whereas the amount of organic phosphate in solution was 69% (of the total) at 30min., 85% at 90min. and 90% at 120min. The residual walls (198mg.), containing 0.8% of phosphorus, were recovered by centrifugation. The supernatant solution was applied to a column of DEAE-cellulose (HCO_3^- form) (Archibald, Baddiley & Button, 1968) and teichoic acid was recovered by gradient elution with $(\text{NH}_4)_2\text{CO}_3$. Only one peak representing organic phosphate was observed and the teichoic acid was recovered by freeze-drying the combined appropriate fractions. A further portion of walls (330mg.) was shaken with the dilute *NN*-dimethylhydrazine at 37° for 72hr., when 52% of the phosphorus was solubilized; the teichoic acid was isolated by ion-exchange chromatography as described above.

Analysis of walls before and after extraction

Samples (3mg.) of walls were hydrolysed in 6N-HCl (3ml.) in evacuated sealed tubes at 100° for 16hr. The hydrolysates were evaporated to dryness *in vacuo* over KOH and analysed in the Technicon AutoAnalyzer (Table 1).

Action of *NN*-dimethylhydrazine on the carboxyl and amino groups in the wall

A sample of wall (20mg.) was treated for 2hr. with *NN*-dimethylhydrazine at 80° as described above. The mixture was freeze-dried to remove excess of reagent, and then suspended in aq. 1% NaHCO_3 (5ml.) and shaken with 5% (v/v) 1-fluoro-2,4-dinitrobenzene in ethanol (10ml.) for

Table 1. Composition of intact and extracted cell walls

Extraction procedures and amino acid analysis are described in the text.

	Amount in cell wall ($\mu\text{moles/g.}$)	Amount in residue after extraction with dimethylhydrazine ($\mu\text{moles/g.}$)	Amount in residue after extraction with trichloroacetic acid ($\mu\text{moles/g.}$)
Alanine	1060	1600	1800
Ammonia	350	500	600
Aspartic acid	70	34	190
Glutamic acid	380	600	870
Glycine	1200	3200	4300
Leucine	50	2	140
Lysine	420	630	900
Serine	150	150	290
Phosphate	970	260	260

Table 2. *N*- and *C*-Terminal amino acids in walls before and after treatment with *NN*-dimethylhydrazine

Procedures are described in the text.

	Amount in cell walls (μ moles/g.)	Amount in walls after treatment with dimethylhydrazine (μ moles/g.)
<i>N</i> -Terminal		
Alanine	46	21
Glycine	14	19
ϵ -Aminolysine	130	107
<i>C</i> -Terminal		
Alanine	225	223
Glycine	235	235

5 hr. in the dark at 22°. The walls were then washed with successive portions of water, ethanol and ether and transferred in 6*N*-HCl (5 ml.) to Pyrex tubes; these were evacuated, sealed and heated at 100° for 16 hr. The hydrolysate was diluted with water (25 ml.) and extracted with ether. The ether-soluble and water-soluble fractions were examined by two-dimensional chromatography on silica gel in solvents *D* and *E*. The amounts of the DNP-amino acids were determined from the extinction at 362 nm. of fractions eluted from the thin-layer plates. An unextracted sample of walls was dinitrophenylated and examined as above. Samples (20 mg.) of walls before and after treatment with *NN*-dimethylhydrazine were heated in Pyrex tubes containing hydrazine sulphate (81 mg.) at 100° for 2 hr. Anhydrous hydrazine (1.33 ml.) was added to each and the tubes were sealed and heated at 60° for 16 hr. Excess of hydrazine was removed *in vacuo* over H₂SO₄. The residues were dissolved in water (3 ml.) and shaken with benzaldehyde (1.5 ml.) for 2 hr. at room temperature. The water layers were removed and analysed for amino acids. Results are given in Table 2.

Potentiometric titration

Teichoic acid (36 mg.) was treated with *N*-NH₃ (3 ml.) for 3 hr. at room temperature and the solution was evaporated to dryness *in vacuo*. The residue was dissolved in water (2 ml.) and passed through a column (5 cm. $\times$ 1 cm.) of Dowex 50 (H⁺ form) resin to remove alanine, alanine amide and NH₄⁺ ions. The column was washed with water and the combined eluate and washings were adjusted to 10 ml. and titrated against carbonate-free 0.10*N*-NaOH, the pH being recorded continuously on a meter. The graph of $\Delta pH/\Delta v$ against *v* (volume of NaOH added) showed maxima at 1.37 and 1.43 ml., corresponding to the points of neutralization of primary and secondary acidic groups of phosphate.

Incubation with phosphomonoesterase

Teichoic acid (1 mg.) was incubated for 12 hr. at 37° in 0.01*M*-(NH₄)₂CO₃, pH 9.3 (2 ml.), containing 0.2 mg. of phosphomonoesterase. After 12 hr. the ratio inorganic phosphate:total phosphate was 1:17. After similar treatment of teichoic acid extracted by *NN*-dimethylhydrazine the ratio was 1:19.

Hydrolysis in alkali

Teichoic acid (4 mg.) prepared by extraction from walls with trichloroacetic acid was heated with *N*-NaOH (0.2 ml.) for 3 hr. at 100° in a sealed Pyrex tube. The hydrolysate was passed through a column (2 ml.) of Dowex 50 (NH₄⁺) form resin and the eluate was freeze-dried and examined chromatographically in solvent *A*. Small proportions of glycerol and a glycerol glucoside were present, together with substantial amounts of organic phosphates. A further sample (4 mg.) was hydrolysed as above and incubated for 16 hr. at 37° in 0.01*M*-(NH₄)₂CO₃, pH 9.3 (5 ml.), containing phosphomonoesterase (0.2 mg.); the products were examined chromatographically. Small proportions of glycerol and the glycerol glucoside were again detected. After the hydrolysis 1.9% of the phosphorus was present as inorganic phosphate; potentiometric titration showed that only 50% of the phosphodiester bonds had been hydrolysed, and on incubation with phosphomonoesterase the yield of inorganic phosphate was 50%. A sample of the hydrolysate was passed through a column (2 ml.) of Dowex 2 (acetate form) resin; all of the phosphorus and 80% of the glucose were retained. The eluate contained 20% of the total glucose and this was present as glucosylglycerol; free glycerol was also detected on paper chromatography.

Degradation by hydrofluoric acid

Teichoic acid (280 mg.) was dissolved in *N*-NH₃ (10 ml.) and kept at room temperature for 5 hr. The solution was evaporated to dryness on a rotary evaporator, and the residue was dissolved in water (2 ml.) and then passed through a column of Dowex 50 (H⁺ form) resin (10 ml.). The teichoic acid was recovered from the eluate and water washings (100 ml.). Alanine hydrochloride was recovered by elution with *N*-HCl and rotary evaporation of the eluate. Incubation with *D*-amino acid oxidase in 0.02*M*-sodium pyrophosphate buffer, pH 7.5, showed that 82% of the alanine had the *D*-configuration.

The solution of teichoic acid was neutralized with NH₃ solution, concentrated by rotary evaporation and then freeze-dried in a polyethylene tube. The residue was incubated with aq. 60% (w/v) HF (1 ml.) at 0° for 16 hr. The acid was neutralized with LiOH and Li₂CO₃, the solution was kept at 4° overnight and LiF was removed by centrifugation. The supernatant solution was combined with the water washings of the LiF, concentrated and applied to a column (3 cm. $\times$ 1.6 cm.) of charcoal (Norit) (3 g.) and Celite (Hyflo Super-Cel) (3 g.) on a bed of Celite (1 g.). The column was washed with water (200 ml.) and aq. 25% (v/v) ethanol (200 ml.). The aqueous eluate contained glycerol; the ethanol eluate, which contained glucosylglycerol and a mixture of organic phosphates, was evaporated to dryness, and the residue was dissolved in water (5 ml.) and applied to a column (50 cm. $\times$ 2 cm.) of DEAE-cellulose (HCO₃⁻ form). Water (500 ml.) was passed through the column and linear gradient elution was carried out with water (500 ml.) in the mixing vessel and 0.15*M*-(NH₄)₂CO₃, pH 8.4 (500 ml.), in the reservoir. Fractions (8 ml.) were collected automatically and analysed for phosphorus. Three peaks representing organic phosphate were obtained. Paper-chromatographic examination showed that each peak represented a mixture of products. The first corresponded to two compounds that were separated

by preparative paper chromatography; the remaining fractions were not examined further.

Identification of degradation products

Glycerol. The aqueous eluate from the charcoal column was evaporated to dryness and the product run as a band on Whatman 3MM paper in solvent *A*. The area containing glycerol was cut out and the glycerol was recovered by elution with water and evaporation *in vacuo* over P_2O_5 . The resulting syrup was dissolved in anhydrous pyridine (1ml.) and mixed with benzoyl chloride (0.2ml.), heated at 50° for 30 min. and then mixed with crushed ice (1g.). The product was crystallized from ethanol as needles, m.p. 73–74°. The yield was 69.5mg. The infrared spectra of the sample and authentic tri-*O*-benzoyl glycerol were identical.

2-*O*- β -D-Glucopyranosylglycerol. Rotary evaporation of the aqueous eluate from the DEAE-cellulose column gave a single glycerol glucoside that was unaffected by incubation with α -glucosidase but completely hydrolysed to glycerol and glucose with β -glucosidase. On hydrolysis with 2N-HCl for 6 hr. at 100° equimolar amounts of glycerol and glucose were produced.

The glucoside (0.25mg.) was dissolved in mm-NaIO₄ (5ml.) and the solution was kept in the dark at room temperature. Oxidation was complete after 48 hr., 1.9mol.prop. of periodate being reduced/mol.prop. of glucose, and formaldehyde was not formed. The solution of oxidized material was passed through a column of Dowex 1 (acetate form) resin (1ml.) and freeze-dried, and the residue was hydrolysed with 2N-HCl. Paper chromatography (solvent *A*) showed a single product (periodate-Schiff spray reagents) with the mobility and colour reaction of glycerol.

The glucoside (16mg.) was acetylated with acetic anhydride in pyridine. Recrystallization from ethanol gave long needles, m.p. and mixed m.p. 127°. The yield was 15mg. The infrared spectra of the sample and authentic hexa-*O*-acetyl-2-*O*- β -D-glucopyranosylglycerol were identical.

Glucosylglycerol glycerol phosphate. This compound contained equimolar amounts of glucose and phosphate, and on acid hydrolysis it gave glucose, glycerol and glycerol monophosphates. On hydrolysis in N-NaOH at 100° for 3 hr. the only products were glucosylglycerol and glycerol monophosphates.

Di(glucosylglycerol) phosphate. This compound slowly gave a colour with the periodate-Schiff spray reagents and was stable towards alkali. Glucose and phosphate were present in the molecular ratio 2:1. Acid hydrolysis gave glucose, glycerol and glycerol monophosphates.

Membrane teichoic acid

The supernatant fraction from the centrifugation of walls of disrupted cells was centrifuged at 100000g for 30 min., and the resulting gel was freeze-dried and extracted with 10% (w/v) trichloroacetic acid at 2° for 48 hr.; teichoic acid was recovered by precipitation with ethanol and acetone in the usual manner. The precipitate contained 11.4% of phosphorus and the phosphate:glucose molecular ratio was 1:0.15. Samples were hydrolysed with acid and alkali as described above and the products, detected by paper chromatography, are given in Table 3. Hydrolysis with alkali and incubation with phosphomonoesterase as described above for the wall teichoic acid converted 90% of the phosphorus into inorganic phosphate. Glycerol and glucosylglycerol were the only products detected on paper after the removal of salts from the hydrolysate; the glucosylglycerol had chromatographic mobility and colour reactions identical with those of 2-*O*- β -D-glucopyranosylglycerol.

DISCUSSION

Walls of *Staph. epidermidis* I 2 contain 30% (w/w) of a glycerol teichoic acid that can be extracted with trichloroacetic acid or with dilute *NN*-dimethylhydrazine at pH 7 (Archibald & Baddiley, 1965). Preparations isolated by both procedures contain glucose and phosphate in the molecular ratio 0.5:1 and give similar products on acid hydrolysis. Teichoic acid isolated with *NN*-dimethylhydrazine contains small amounts of peptidoglycan, but most of this latter component in the walls remains as an insoluble residue [representing 60% (w/w) of the original wall] containing 0.8% of phosphorus and retaining the shape of the original cell wall. Under similar conditions dilute solutions of *NN*-dimethylhydrazine effect rapid and complete dissolution of the walls of several other bacteria (J. Anderson, A. R. Archibald, J. Baddiley & M. J. Curtis, unpublished work), but the nature of the action of dilute solutions of this reagent on walls is not known and so the reasons for the resistance of the peptidoglycan from *Staph. epidermidis* are uncertain. The amino acid composition of intact and extracted walls is given in Tables 1 and 2; about 30% of the

Table 3. *Products of hydrolysis of membrane teichoic acid (identified by paper chromatography)*

	Acid hydrolysis	Alkali hydrolysis	Alkali hydrolysis and enzymic dephosphorylation
Glycerol	+	+	+
Glucose	+	—	—
Glucosylglycerol	—	+	+
Glycerol monophosphates	+	+	—
Glycerol diphosphates	+	+	—
Alanine	+	+	+
Diglycerol phosphate	—	—	+

lysine residues have free ϵ -amino groups and 22% of the alanine and 20% of the glycine are *C*-terminal. It was shown that the number of *N*- and *C*-terminal groups was not appreciably altered on treatment of walls with *NN*-dimethylhydrazine.

Teichoic acids isolated by either procedure give similar products on hydrolysis with acid and alkali, except that small amounts of components from peptidoglycan were observed in the hydrolysis of material isolated with *NN*-dimethylhydrazine. The phosphorus content (7%) of this material was less than that of the teichoic acid that had been obtained by the trichloroacetic acid method (this contains 10% of phosphorus), and it appears that the small amount of peptidoglycan that is solubilized on treatment of walls with *NN*-dimethylhydrazine is attached to the teichoic acid. Structural studies were therefore carried out on teichoic acid that had been extracted by the trichloroacetic acid method. Such material contains phosphate, glucose and alanine in the molecular proportions 1:0.5:0.25. Acid hydrolysis gave alanine, glucose, glycerol and its mono- and di-phosphates; the glycerol and its phosphates are characteristic of teichoic acids containing a poly(glycerol phosphate) chain. On treatment with alkali only half of the phosphodiester linkages were hydrolysed, and small proportions of glycerol and glucosylglycerol were produced. The major products were more complex organic phosphates; these contained 80% of the total glucose, and no increase in the amount of glucosylglycerol was obtained by treatment of an alkali hydrolysate

with phosphomonoesterase, i.e. no simple glucosylglycerol phosphates were present.

To obtain sufficient glucosylglycerol for its full characterization, teichoic acid was degraded by treatment with hydrofluoric acid under conditions (Glaser & Burger, 1964) that remove phosphate groups without affecting glycosidic linkages. Substantial amounts of glycerol and a monoglucosylglycerol were obtained, together with small proportions of organic phosphates. Glycerol was separated from the other products by chromatography on charcoal and was characterized as its crystalline tri-*O*-benzoate. Aqueous ethanol eluted glucosylglycerol, together with small amounts of organic phosphates that were separated by passage through a column of DEAE-cellulose. The glucosylglycerol was readily hydrolysed with β -glucosidase, giving only glycerol and glucose. Oxidation of the glycoside with periodate resulted in the reduction of 2mol.prop. of oxidant and subsequent acid hydrolysis of the product gave glycerol; it follows that, as the glycerol residue is unaffected by the oxidation, the glucosyl substituent must be at the 2-position on glycerol. Confirmation of the structure was obtained by preparation of the crystalline hexa-*O*-acetate with melting point, mixed melting point and infrared spectrum identical with those of synthetic 2-*O*- β -D-glucopyranosylglycerol.

Small amounts of several organic phosphates were eluted from the DEAE-cellulose column with ammonium carbonate; two of these were isolated by preparative paper chromatography. Glucosylglycerol glycerol phosphate (I) contained glucose and phosphate in the molecular ratio 1:1. On acid hydrolysis it gave glycerol, glucose and glycerol monophosphates, and with alkali the products were glucosylglycerol and glycerol monophosphates. Compound (I) gave a rapidly developing purple colour with the periodate-Schiff reagents; this is characteristic of compounds that form formaldehyde on oxidation with periodate (Roberts, Buchanan & Baddiley, 1963). The other phosphate (II) slowly gave a blue colour typical of a glycoside with the periodate-Schiff spray reagents, and contained glucose and phosphate in the molecular ratio 2:1. With acid it gave glucose, glycerol and glycerol monophosphates and it was stable towards alkali. These properties indicate that the compound is di(glucosylglycerol) phosphate (II).

After removal of the D-alanyl ester residues, potentiometric titration of the teichoic acid showed that almost all of the phosphate was present as diester. The proportion of monoester groups, indicated by titration and from the amount of inorganic phosphate produced on incubation with phosphomonoesterase, corresponds to a chain of 20 units in the extracted teichoic acid. The results described above are consistent with a structure in

(I)

(II)

which chains of (1→3)-linked glycerol phosphate units bear glucosyl substituents at the 2-position of about half of the glycerol residues, D-alanyl groups being attached in ester linkage to some of the glycerol residues that are not glucosylated. The ready removal of the alanyl residues during chromatography in ammoniacal solvents suggests that they are attached to hydroxyl groups on glycerol rather than to sugar hydroxyl groups.

The membrane teichoic acid of *Staph. epidermidis* I2 is a (1→3)-linked poly(glycerol phosphate) containing alanyl and glucosyl substituents. Enzymic dephosphorylation of an alkali hydrolysate converts 90% of the phosphorus into inorganic phosphate and gives glycerol, together with a glucosylglycerol that is probably identical with that from the wall teichoic acid. This polymer is thus similar in structure to that in the wall, but contains less glucose (phosphate:glucose ratio 1:0.15). Contamination of membrane teichoic acid (glycerol) with that from the wall (ribitol) has been encountered in the isolation of the former from other organisms, and it is likely that similar contamination may occur where both membrane and wall contain glycerol teichoic acids. Where these are of closely related structure it is difficult to recognize such contamination and it is possible that at least some of the glucose in the preparation of membrane teichoic acid represents wall material. Similarly *N*-acetylgalactosaminyl substituents in preparations of membrane teichoic acid from *Staphylococcus lactis* 7944 may actually represent contamination by the wall teichoic acid in which this substituent occurs in substantial amounts (Ellwood, Kelemen & Baddiley, 1963; Ellwood, 1962).

Although there is a close immunological similarity between the wall teichoic acid from *Staph. epidermidis* I2 and that from *Staph. epidermidis* T2 (Oeding *et al.* 1967), we obtained no evidence for the occurrence of diglucosylglycerol residues in the former; on the other hand, the glucose content of the strain T2 teichoic acid (glucose:phosphate molecular ratio 1.6:1) indicates that such units are probably present in this latter polymer.

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