SEPARATION OF TEICHOIC ACID OF STAPHYLOCOCCUS AUREUS INTO TWO IMMUNOLOGICALLY DISTINCT SPECIFIC POLYSACCHARIDES WITH α- AND β-N-ACETYLGLUCOSAMINYL LINKAGES RESPECTIVELY*

Antigenicity of Teichoic Acids in Man

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(Received for publication, March 16, 1964)

The finding of teichoic acids in bacterial cell walls (1, 2) has stimulated great interest in their chemical, biochemical, and immunochemical properties. The teichoic acids were identified chemically as polymers of glycerol (3, 4, and *cf.* reference 5) or ribitol (2) phosphate which contain glycosidically linked sugars and esterified D-alanine (*cf.* reference 6). Teichoic acid obtained from cell walls of *Staphylococcus aureus* was shown to be a polyribitol phosphate with α-, β- or α- and β-*N*-acetylglucosaminyl residues and D-alanine ester residues (7, 8). Immunochemical studies on teichoic acids of *S. aureus* have been carried out by several investigators using rabbit sera (9–14). Thus, Strominger and coworkers obtained sera from rabbits immunized with formalin-killed *S. aureus* strain Copenhagen, and the agglutination by these sera of cell wall preparations was inhibited by teichoic acids, by any degradation product of teichoic acid which contained the α-*N*-acetylglucosaminyl ribitol unit, by α-phenyl-*N*-acetylglucosaminide, and by *N*-acetylglucosamine. Thus, the sera were believed to contain antiteichoic antibody specific for α-*N*-acetylglucosaminyl residues (9, 12, 14). On the other hand, in the investigation of cross-reactions of teichoic acids from *S. aureus* strain H with *S. aureus* immune sera, Haukenes *et al.* (10) and Haukenes (11) found that their antisera reacted with teichoic acids which contained little or no α-*N*-acetylglucosaminyl linkages. Morse showed by techniques of hemagglutination inhibition and precipitation inhibition that the β-linked *N*-acetylglucosaminyl residue was the determinant group of the teichoic acid of *S. aureus* strain NYH-6 (13). Nathenson and Stro-

*Aided by a grant from the National Science Foundation (G-18727) and the General Research Support Grant, National Institutes of Health, United States Public Health Service.

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minger also reported β-*N*-acetylglucosaminyl specificity and attributed these discrepancies in the specificities of the antibodies to differences in the teichoic acids of the strains used for immunization (12).

In connection with the studies of this laboratory on the specificity of antigenic determinants of polysaccharides, teichoic acid Copenhagen was studied for its antigenicity in man. During these studies it was found that most human beings possess antibodies to teichoic acids of *S. aureus* probably as a consequence of prior contact with this microorganism; teichoic acid itself was found to be antigenic in man. The sera obtained on immunization permitted the demonstration that the teichoic acids from the Copenhagen and NYH-6 strains were each mixtures of two distinct polysaccharides, an α- and a β–teichoic acid depending upon the linkage of the *N*-acetylglucosaminyl residue to the ribitol. These two teichoic acids were separated immunochemically and studied.

Materials and Methods

Teichoic Acids.—Teichoic acid of *S. aureus* strain Copenhagen was kindly provided by Dr. J. L. Strominger and teichoic acids of *S. aureus* strain NYH-6 and *S. albus* strain Prengel were the gift of Dr. S. I. Morse.

Inhibitors of Specific Precipitation.—α-Methyl-*N*-acetylglucosaminide and β-methyl-*N*-acetylglucosaminide were provided by Dr. R. Kuhn, Dr. R. W. Jeanloz, Dr. G. F. Springer, and Dr. Y. Matsushima. The samples from Dr. Matsushima were recrystallized before use. Recrystallized materials gave $[\alpha]_D = +131°$ (c = 2 per cent in water) for the α-compound, −43.9° (c = 2 per cent in water) for the β-compound. β-Ethyl-*N*-acetylglucosaminide, α-methyl-*N*-acetylgalactosaminide, and β-ethyl-*N*-acetylgalactosaminide were furnished by Dr. R. W. Jeanloz. D-Ribitol-4-α-D-glucoside, D-ribitol-4-β-D-glucoside, and D-ribitol-4-α-D-glucosaminide were gifts from Dr. F. E. Hardy. Ribitol phosphate Ba salt was a gift from Dr. J. L. Strominger. Ribitol-4-α-glucosaminide was used as its *N*-acetyl derivative, and ribitol phosphate was used as its sodium salt.

Sera.—Blood was taken from several volunteers (Bl., Da., Ho., Is., and StJ.) before and after immunization with 3 injections of a total of 0.8 to 1.0 mg of teichoic acid Copenhagen given in 6 to 14 days. Immune sera were obtained 3 weeks after the last injection, and preserved with 0.25 per cent phenol and 1:10,000 merthiolate. Preimmunization serum samples are marked with subscript x and sera after immunization are marked with subscript 1 or 2. Sera from the first and second bleedings showed very similar precipitin curves.

Skin Test.—Skin tests were carried out with a sterile solution of teichoic acid (Copenhagen). The skin reaction was read 15 minutes after an intracutaneous injection of 0.05 ml of a solution (1 mg/ml).

Quantitative Precipitation Studies.—Precipitin curves for various sera with teichoic acids were obtained by the standard procedure (15), determining nitrogen by the ninhydrin method after digestion with sulfuric acid as described by Schiffman *et al.*(16). Generally 101 μl of serum was taken for each analysis; when larger amounts were taken they were recalculated to the smaller scale. This was done to obtain more precise values for the lower points on the precipitin curves.

Quantitative Inhibition of Precipitation.—The inhibition assays were performed as described in reference 15.

Analysis of Washed Specific Precipitates for Antigen.—Washed specific precipitates were extracted twice at room temperature for 15 minutes with 2 ml of 5 per cent trichloroacetic acid

(17, *cf.* reference 15). The combined extracts were extracted with 8 ml of ether 5 times to remove trichloroacetic acid. The extracted residues were concentrated to dryness and the resulting dried material was heated with 2 N hydrochloric acid at 100°C for 4 hours. Excess of hydrochloric acid and water were evaporated in a desiccator over P_2O_5 and NaOH *in vacuo*. The residues were taken up in water and acetylated with 5 per cent acetic anhydride and saturated bicarbonate (18), and resulting *N*-acetyl derivative was determined by the method described by Reissig *et al.* (19, *cf.* reference 15).

As controls, teichoic acid alone, teichoic acid plus human gamma globulin, and a dextran antidextran specific precipitate were used in amounts comparable to the teichoic acid and antiteichoic acid specific precipitates.

Time-hydrolysis studies of teichoic acid Copenhagen showed that hydrolysis for 2 to 4 hours at 100°C with 2 N HCl gave values in the secondary curve described by Sanderson *et al.* (8); *i.e.*, recoveries of hexosamine were 78.2, 81.6, and 83.0 per cent for 2, 3, and 4 hours respectively based on the accepted formula weight for teichoic acid (8). All analyses were done with 4 hours hydrolysis except for one which was 2 hours. The quantities of teichoic acid recovered from the specific precipitates were calculated from the hexosamine values by comparing them with hexosamine values obtained from teichoic acid controls. Antibody control (dextran plus antidextran) did not give any significant hexosamine value. Teichoic acid plus gamma globulin control gave 80 and 97 per cent recovery.

Agar Diffusion Techniques for Specific Precipitation.—The Preer method (20) was used for a one dimensional diffusion study, using 0.3 per cent agar. For diffusion in two dimensions, the Ouchterlony technique (21) was used with some modification (*cf.* reference 15).

RESULTS

Skin Test—Four volunteers were injected intracutaneously with 0.05 ml of the teichoic acid Copenhagen solution (1.0 mg/ml saline). Minor skin reactions were observed before immunization in three (Bo., 8/30 mm wheal/erythema; Da., 4/35; StJ., 10/40), and two of them showed somewhat stronger reactions after immunization (Bo., 8/18; Da., 10/30; StJ., 14/60). One subject (Is.) who had a history of severe staphylococcal infection about 3 years previously showed a very strong skin reaction on initial test with the teichoic acid (20/60) and was not immunized.

Quantitative Precipitation of Human Sera by Teichoic Acids.—Sera from three subjects taken before and after immunization were tested with teichoic acid Copenhagen which had been used for immunization. The results are shown in Fig. 1. Before immunization all three subjects showed detectable amounts of antiteichoic acid and after immunization all showed a rise in precipitable antibody N. Bo.$_1$ and Da.$_1$ showed typical precipitin curves, but StJ.$_{1 \text{ and } 2}$ showed a peculiar curve consisting of a small curve followed by an almost straight line region between 0.2 and 4 μg of antigen. This type of curve was repeatedly obtained with postimmunization samples.

Results of quantitative precipitin studies with the various sera and teichoic acids are given in Fig. 2. Serum samples from Ho. and Is. showed very high precipitin levels against teichoic acid Copenhagen and NYH-6 without any immunization. Da.$_x$, Da.$_{1 \text{ or } 2}$, StJ.$_x$, and StJ.$_{1 \text{ or } 2}$ also precipitated with teichoic acid NYH-6. Generally teichoic acid NYH-6 precipitated much more nitrogen

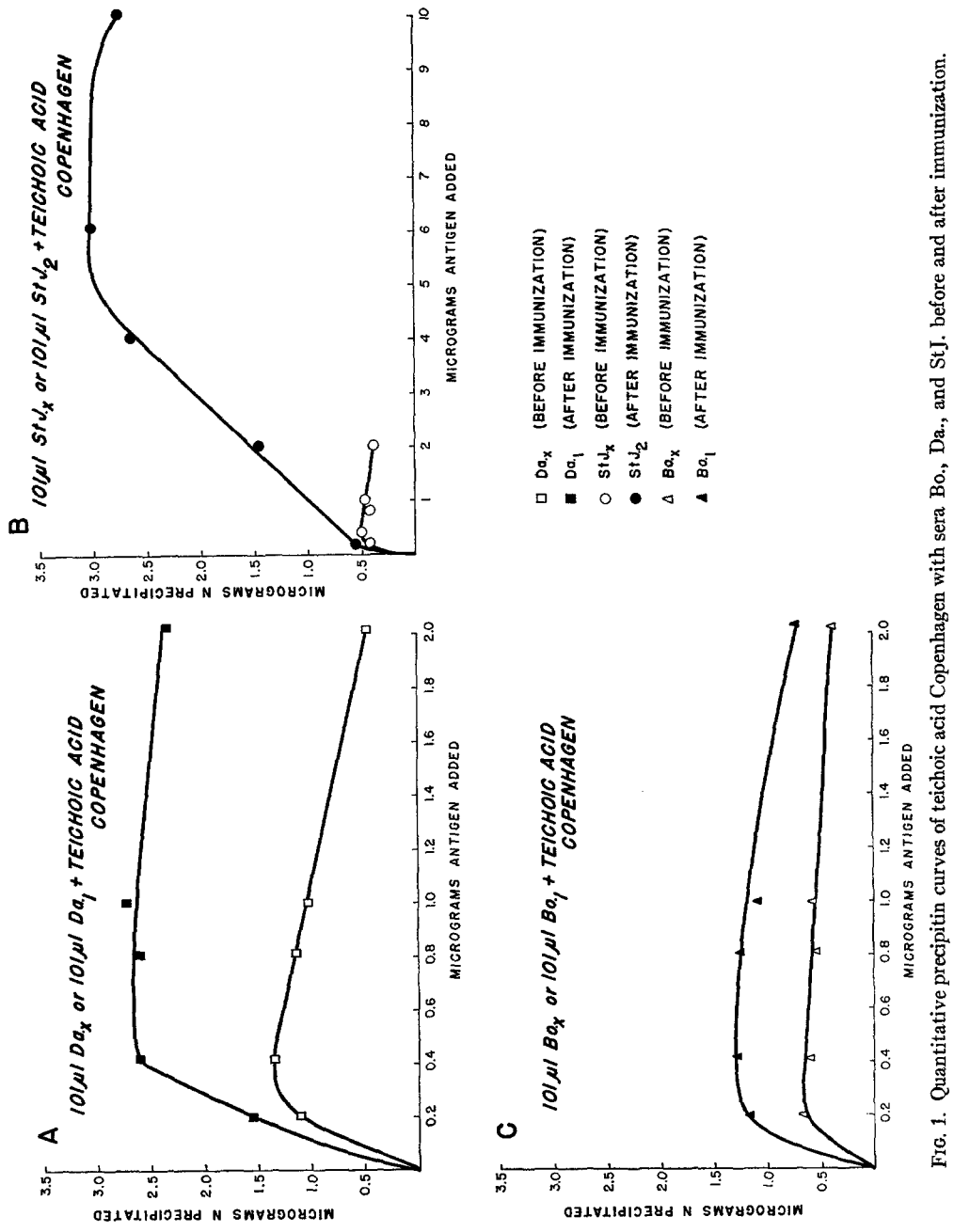


FIG. 1. Quantitative precipitin curves of teichoic acid Copenhagen with sera Bo., Da., and St.J. before and after immunization.

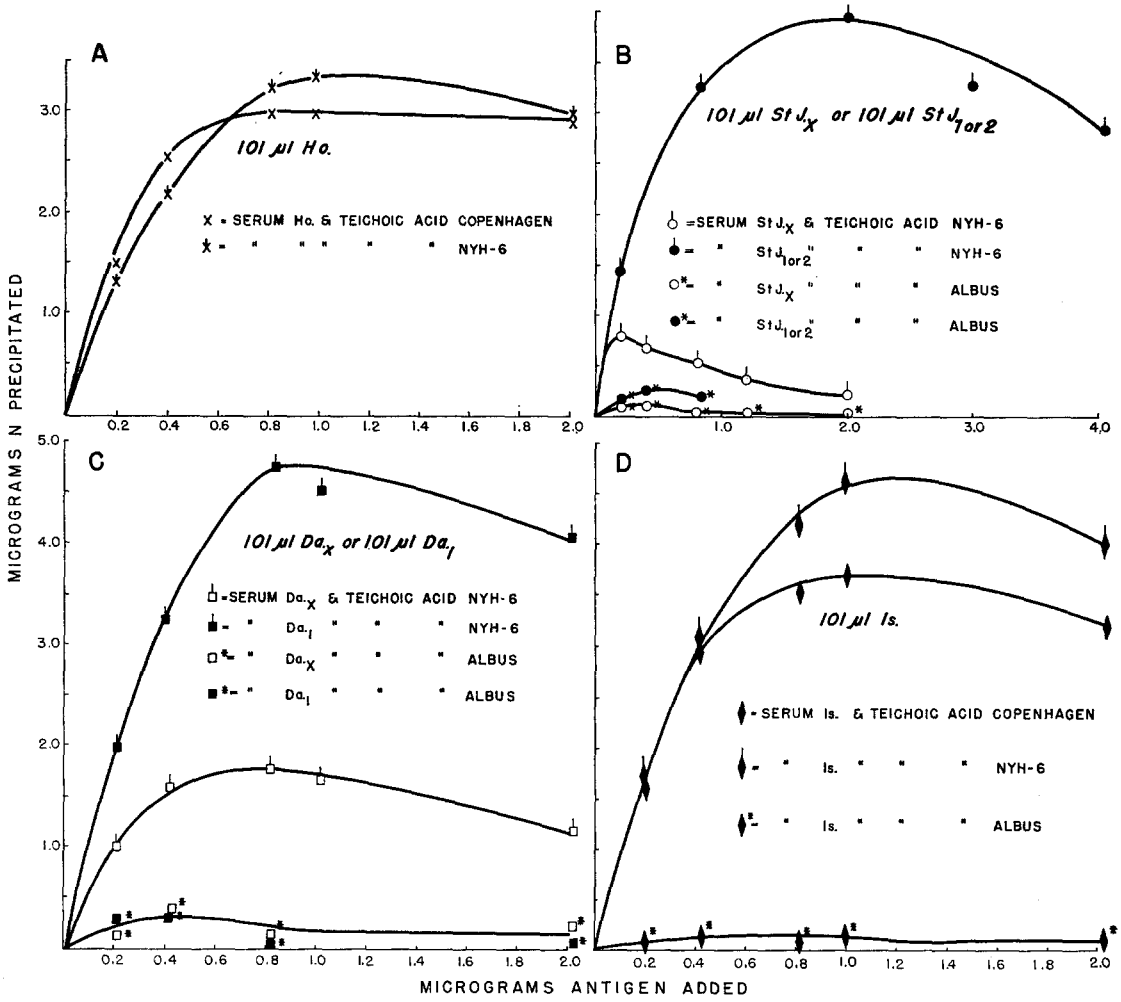
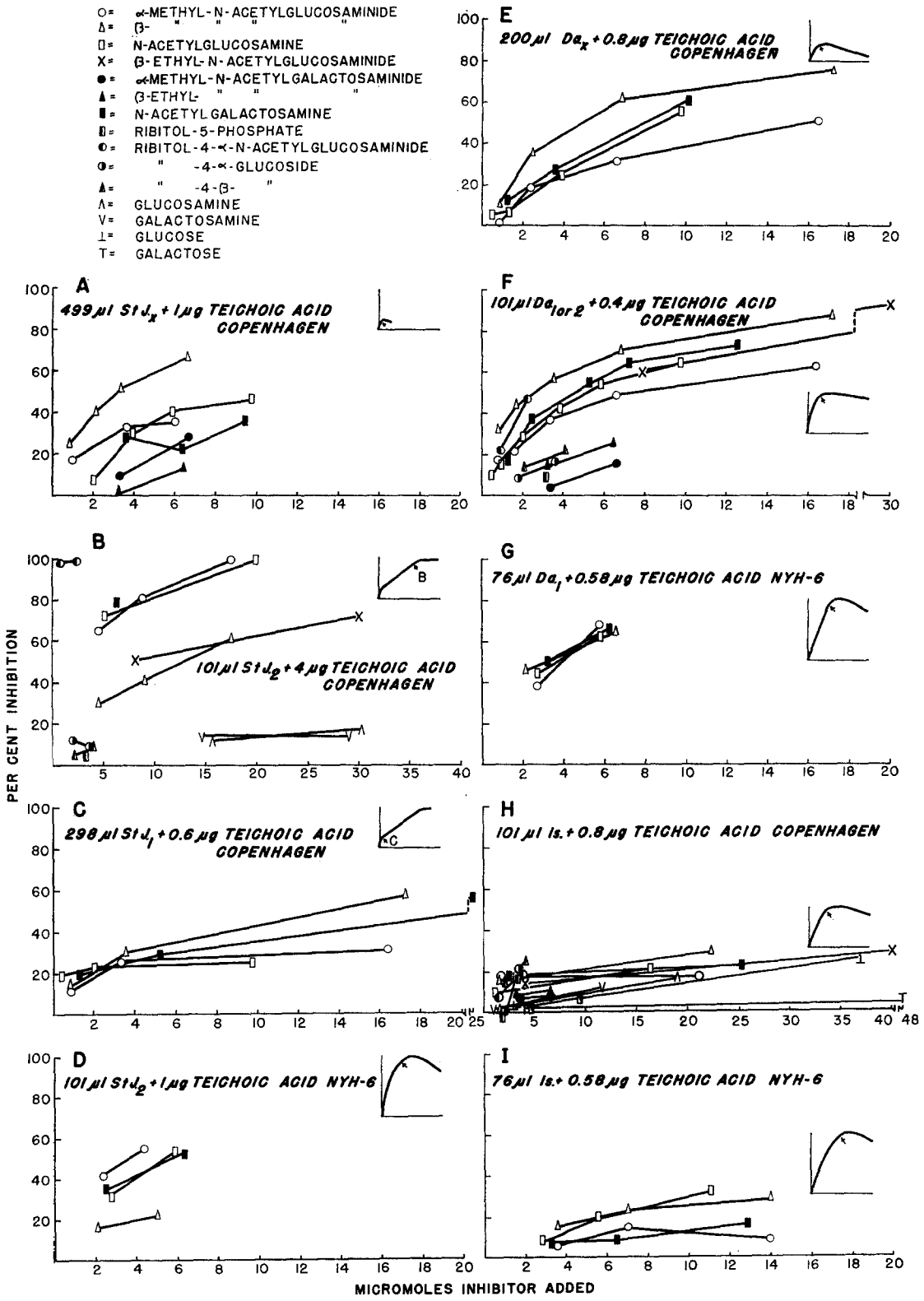


FIG. 2. Quantitative precipitin curves of various sera with teichoic acids from *S. aureus* Copenhagen and NYH-6 and *S. albus* Prengel.



from the same antisera than did the Copenhagen material. On the other hand, teichoic acid from *S. albus* did not precipitate any significant amount of nitrogen from the sera tested.

Quantitative Inhibition of Precipitation.—Quantitative inhibition curves with various antisera and teichoic acids are shown in Fig. 3. In the system of $StJ._x$–teichoic acid Copenhagen, β-methyl-*N*-acetylglucosaminide was a better inhibitor than α-methyl-*N*-acetylglucosaminide. With antiserum $StJ._{1\text{ or }2}$ which gave the peculiar curve in Fig. 1 *B* with the Copenhagen teichoic acid, two sets of inhibition experiments were performed at the points indicated in the insert graphs in Figs. 3 *B* and 3 *C*. At the point used in Fig. 3 *C* β-methyl-*N*-acetylglucosaminide was a better inhibitor than the α-compound, but at the point used in Fig. 3 *B* the α-compound was better than the β, suggesting that the peculiar curve is due to two types of antibodies in serum $StJ._{1\text{ or }2}$. One is anti-

TABLE I

Recoveries of Teichoic Acids from the Specific Precipitates

Experiment	Antisera		Antigens				Recovery
	ml		*μg*				*per cent*
1	6	$Da._2$	21	Teichoic acid	Copenhagen		70
2	4	"	30	"	"	NYH-6	93
3	6	Is.	21	"	"	Copenhagen	78
4	4	"	30	"	"	NYH-6	72
5	0.5*	$StJ._2$	21	"	"	Copenhagen	9
6	10‡	"	21	"	"	"	30
7	3	"	30	"	"	NYH-6	39

* This corresponds approximately to point *B* in Fig. 3 *B*.

‡ This corresponds approximately to point *C* in Fig. 3 *C*.

body specific to the β-*N*-acetylglycosaminyl residue and the other to the α-*N*-acetylglucosaminyl residue. Ribitol-4-α-*N*-acetylglucosaminide was a quite powerful inhibitor in the latter system. When the $StJ._2$ was tested using teichoic acid NYH-6 as an antigen, the α-methyl-*N*-acetylglucosaminide was a better inhibitor than the β-compound. With both $Da._x$ and $Da._{1\text{ or }2}$ and teichoic acid Copenhagen, the β-compound was a better inhibitor than the α-compound, indicating that both of these sera contained mostly antibody specific to β. In the $Da._1$–teichoic acid NYH-6 system, the α- and β-compounds were equally active. *N*-acetylglucosamine and *N*-acetylgalactosamine were also good inhibitors when tested in all sera mentioned above. On the other hand with serum

FIG. 3. Quantitative inhibition by various sugars and sugar derivatives. Arrows on small inserts indicate points at which the inhibition assays were carried out.

Is. and teichoic acid Copenhagen or NYH-6 none of the compounds tested was a good inhibitor.

Analysis of Specific Precipitates for Antigen.—Known amounts of teichoic acids were mixed with sera Da.$_2$, Is., and StJ.$_2$ at proportions indicated in Table I, and kept at 37°C for 1 hour and in a refrigerator for 7 days with occasional mixing. The precipitates were washed twice with saline and once with distilled water in the cold. The specific precipitates were treated with trichloroacetic acid and ether as described, and the quantity of teichoic acid was determined. The results are summarized in Table I.

With sera Da.$_2$ and Is., the recoveries of teichoic acids from the specific precipitates ranged from 70 to 93 per cent. However, from the precipitates of StJ.$_2$–teichoic acid systems poor recoveries were obtained. The very poor recovery (9 per cent) in Experiment 5 indicated that only part of teichoic acid added to the system was precipitated by the antiserum, even though the determination was carried out in the antibody excess region.

TABLE II

Yield of α- and β-Teichoic Acids Obtained from Teichoic Acid Copenhagen

Experiment	α-Teichoic		β-Teichoic	
	Amount isolated	Over-all recovery	Amount isolated	Over-all recovery
	μg	*per cent*	*μg*	*per cent*
1	13.2	6.3	21.6*	43
2	13.5	6.4	17.6*	35

* These amounts were obtained from 2.4 ml of the supernatant. This volume is equivalent to 50.4 μg of the original teichoic acid.

Immunochemical Separation of Teichoic Acid Copenhagen into Two Fractions.—210 μg of teichoic acid Copenhagen was mixed with 5 ml of StJ.$_2$ serum and diluted to 10 ml with saline. This corresponds to point *B* on the curve in Fig. 3*B* or to Experiment 5 of Table I. The mixture was incubated at 37°C for 1 hour and kept in a refrigerator for 7 days with occasional mixing. The resulting precipitate was collected, washed, and treated with trichloroacetic acid and ether as described in the section on Analysis of Specific Precipitates for Antigen. The aqueous phase was then dialyzed against distilled water in the cold overnight and the non-dialyzable fraction lyophilized. The dried material was dissolved in 1.5 ml of saline and pH of the solution adjusted to 7. This fraction was designated as α–teichoic acid.

2.4 ml of the original supernatant was mixed with 40 ml of StJ.$_2$ serum, an amount to bring the proportions of antigen and antibody approximately to point *C* on the curve (Fig. 3 *C*), incubated, and treated as described for α–

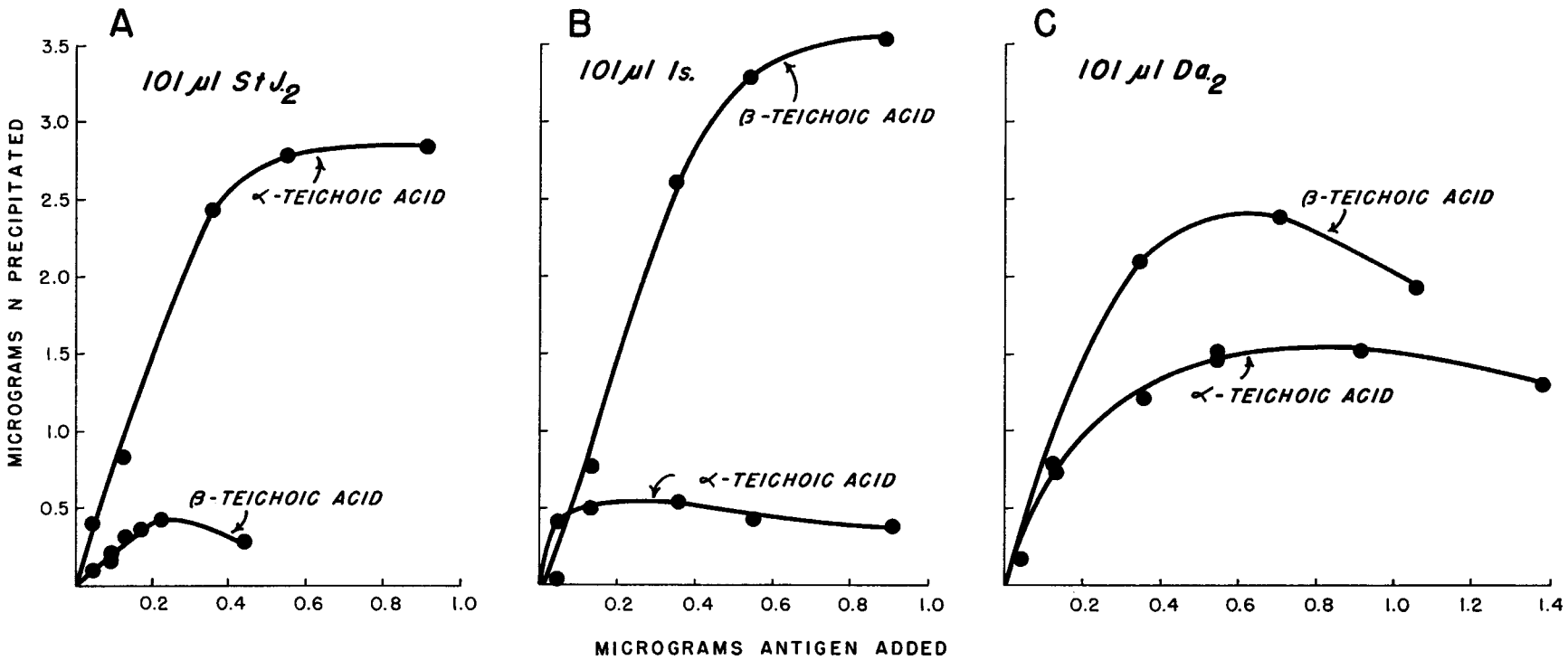


FIG. 4. Quantitative precipitin curves with immunochemically separated α– and β–teichoic acids.

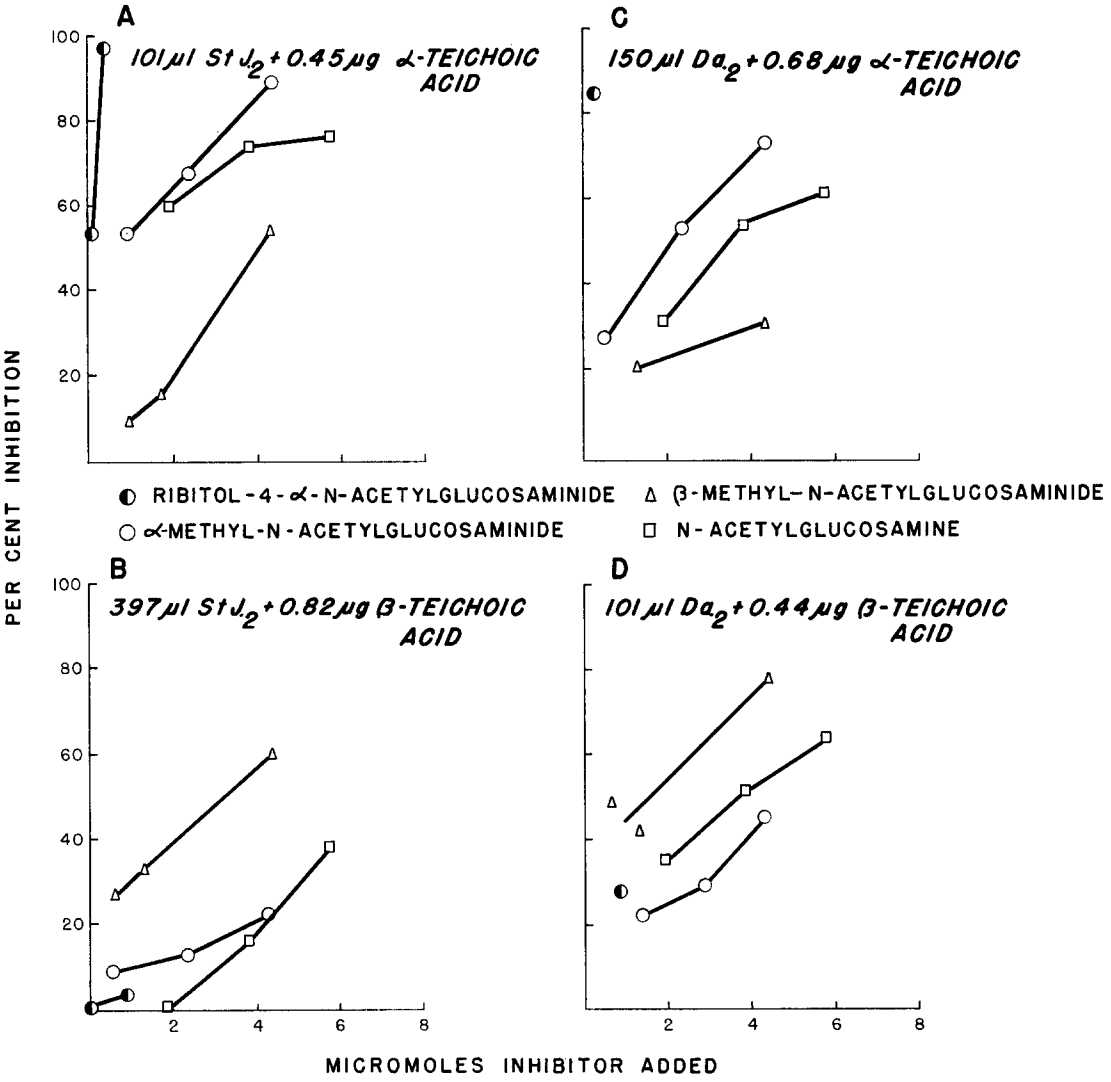
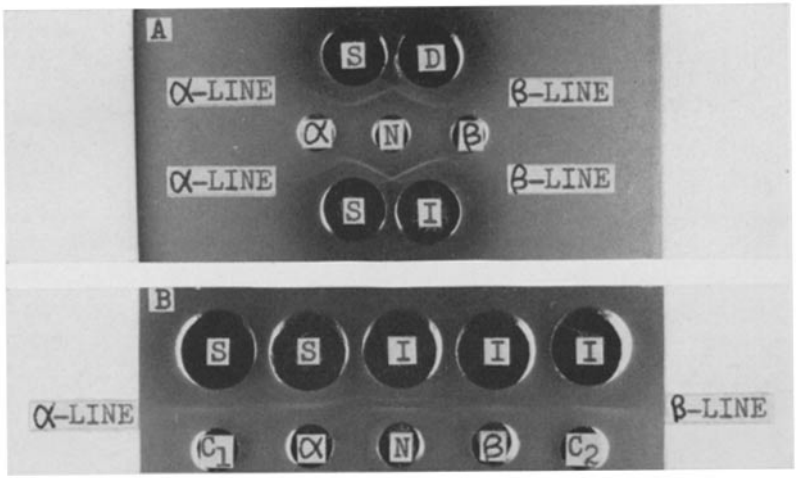


FIG. 5. Quantitative inhibition by various sugars and sugar derivatives of specific precipitation by separated α- and β-teichoic acids.

teichoic acid. This fraction was designated as β-teichoic acid and dissolved in 2 ml saline. Aliquot portions of each fraction were analyzed for teichoic acid. Yields of the separated teichoic acid are shown in Table II.

Quantitative Precipitin Studies with α- and β-Teichoic Acids.—Quantitative precipitin curves with α- and β-teichoic acids are shown in Fig. 4. The α-teichoic acid precipitated about 2.8 μg N with 0.1 ml of serum StJ.$_2$ which corresponded almost exactly to that precipitated by the parent teichoic acid at point *B* on the curve, while β-teichoic precipitated only 0.4 μg N. With 0.1



FIGS. 6 *A* and 6 *B*. Precipitation in agar with teichoic acids and sera Da.$_2$, Is., and StJ.$_2$. Note the crossing of the α- and β-lines in Fig. 6 *A*, the different positions of the α- and β-lines and their crossing between *N* and β, as well as the existence of two sharp lines for α- and β-teichoic acid in Fig. 6 *B*.

D, Da.$_2$; I, Is.; *S*, StJ.$_2$; *N*, teichoic acid NYH-6 (0.8 μg in *A*, 0.24 μg in *B*); C_1, teichoic acid Copenhagen (1.6 μg); C_2, teichoic acid Copenhagen (0.18 μg); α, α-teichoic acid (0.25 μg in *A*, 0.3 μg in *B*); β, β-teichoic acid (0.25 μg).

ml of serum Is., however, α-teichoic acid precipitated only 0.5 μg N but β-teichoic precipitated 3.5 μg N. With 0.1 ml of serum Da.$_2$ α- and β-teichoic acids precipitated 1.5 μg N and 2.4 μg N respectively.

Inhibition by Various Sugars of Specific Precipitation with α- and β-Teichoic Acids.—Inhibition experiments were carried out using antisera Da.$_2$ and StJ.$_2$. Fig. 5 shows that precipitation by α-teichoic acid with Da.$_2$ and StJ.$_2$ was highly inhibited by α-methyl-*N*-acetylglucosaminide and precipitation by β-teichoic acid was highly inhibited by β-methyl-*N*-acetylglucosaminide. Ribitol-4-α-*N*-acetylglucosaminide showed a tremendously high inhibition in α-system but not in β-system. From these results it appears that the α-teichoic acid contained a teichoic acid at least mostly and possibly entirely composed of

α-*N*-acetylglucosaminyl linkages, and that the β–teichoic acid was a teichoic acid mostly or perhaps entirely composed of β-*N*-acetylglucosaminyl linkages.

Precipitation in Agar.—Specific precipitation by teichoic acid Copenhagen and NYH-6 in agar was tested by the double diffusion technique. By the Preer method, with a series of antigen dilutions, two very distinct bands were observed with both the Copenhagen and NYH-6–Da.$_2$ systems and the NYH-6–StJ.$_2$ system. But with both NYH-6 or Copenhagen and Is. and with Copenhagen-StJ.$_2$ one distinct band and another very faint or doubtful band were observed.

To establish the relationship of these bands, the Ouchterlony technique was applied, and separated α- and β–teichoic acids were used as well. Fig. 6 shows a representative result.

Fig. 6 *A* shows that all three sera, Da.$_2$, Is., and StJ.$_2$, contained two distinct antibodies. One was antibody-specific for α–teichoic acid and the other antibody-specific for β–teichoic acid. The two lines produced by α- and β–teichoic acids respectively showed distinct crossing, indicating that these two teichoic acids are antigenically quite different. Crossing of the two lines is also shown in Fig. 6 *B*. Teichoic acid NYH-6 is evidently composed of at least two antigenic substances, because this teichoic acid gave two lines with all of the sera; these two antigenic substances are clearly associated with the α- and β–teichoic acids separated immunochemically from the Copenhagen sample. Attempts to demonstrate two lines with teichoic acid Copenhagen in a single system were unsuccessful. However, in the separated systems the α-line and the β-line could be obtained using suitable concentrations of teichoic acid Copenhagen (Fig. 6 *B*).

DISCUSSION

In 1935 Wieghard and Julianelle (22) isolated type-specific carbohydrates A and B from pathogenic and non-pathogenic strains of staphylococci respectively. These carbohydrates A and B which contained 6.3 and 6.4 per cent phosphorus and 26 and 39 per cent reducing sugar respectively, failed to induce formation of antibody in rabbits (23), but precipitating antibody to the carbohydrate A was found in patients with severe, prolonged, or generalized staphylococcal infection, but not in normal individuals (24). All patients with staphylococcal infection showed cutaneous hypersensitivity to the A carbohydrate (24). Recent studies on bacterial teichoic acids suggested that these type-specific carbohydrates might be teichoic acid because of the similarity in phosphorus and reducing sugar values.

Haukenes *et al.* (10) and Haukenes (11) studied polysaccharide A obtained from strains of *S. aureus* different from that of Julianelle and Wieghard, and found that this polysaccharide contained a teichoic acid moiety serologically identical with teichoic acid derived from *S. aureus* strain H. However, antibody

to this polysaccharide could not be demonstrated in human sera, and attempts to produce such antibody in mice and rabbits with polysaccharide A were not successful (25).

Recently Strominger reported (26) that intradermal injection of his preparation of teichoic acid Copenhagen reproduced wheal and erythema reactions in laboratory personnel which were originally observed by Julianelle and Hartman (24) with carbohydrate A in hypersensitive human subjects. The skin tests in the present study also confirmed their observations that the skin reaction to teichoic acid is of the immediate wheal and erythema type.

The present data using microquantitative precipitation techniques showed that all sera tested contained significant amounts of antibodies to teichoic acid. Thus, one subject with a history of staphylococcal infection had a very high antibody level while the others had the antibody without any recognized staphylococcal infection. From these observations it is probable that individuals develop such antibody in their sera as a result of contact or minor infections with *S. aureus*. Numerous other sera also contained antibody to teichoic acid. In two individuals, with antibody prior to injection, teichoic acid evoked a secondary response. Moreover with subject StJ., who had only antibody to β–teichoic acid prior to immunization, the response to α–teichoic acid may have been a primary one. Since teichoic acid from *S. albus* did not precipitate appreciably with these sera and since the precipitations by teichoic acid Copenhagen or NYH-6 were specifically inhibited, it may be inferred that these precipitations were not due to non-specific precipitation between acidic antigen and basic proteins in sera as described by Leonard and Thorne (27). Antistaphylococcal antibodies in normal human sera were also reported by numerous workers (28–33 *cf.* reference 34), and Mudd *et al.* noted that absorption with teichoic acid reduced the effectiveness of normal human serum in promoting phagocytosis of staphylococci of the same strain (35).

Inhibition experiments showed that both $Da._{x}$ and $StJ._{x}$ contained mostly antibody specific to the β-*N*-acetylglucosaminyl residue but immune sera $Da._{1 \text{ and } 2}$ and $StJ._{1 \text{ and } 2}$ were rich in anti-β and in anti-α respectively. Another interesting observation is that the precipitation of Is. by teichoic acid Copenhagen or NYH-6 was not greatly affected by any inhibitors tested. The antibodies in this serum may differ in the nature of their combining sites from those in Da. and StJ. Two possible explanations may be considered. One would involve a specificity to a grouping different from that of *N*-acetylglucosaminyl-ribitol structure and the other that the antibody in Is. is directed towards a determinant larger than the *N*-acetylglucosaminyl-ribitol linkage. Antibodies with combining sites for large and small oligosaccharides have already been fractionated from whole antidextran sera by Schlossman and Kabat (36).

It is also well known that antisera vary substantially in their ability to be inhibited by small mono- and oligosaccharides (37, 38). In the system

of StJ.$_{1 \text{ or } 2}$-teichoic acid Copenhagen at point *B* (Fig. 3 *B*) and also of StJ. or Da.-α-teichoic acid, ribitol-4-α-*N*-acetylglucosaminide showed very high inhibitory activity, indicating that the combining site of the antibody molecule has a specificity directed at least towards the α-*N*-acetylglucosaminyl-ribitol residue. Since ribitol-4-β-*N*-acetylglucosaminide was unfortunately not available no more detailed information can be given for the antibody in β-system.

The findings with the separated α- and β-teichoic acids show conclusively, in support of the quantitative precipitin and inhibition data and the agar diffusion studies, that there are two distinct polymers of teichoic acid Copenhagen rather than a single polymer with 15 per cent α- and 85 per cent β-*N*-acetylglucosaminyl residues as was believed to be the case by Sanderson *et al.* (8). Over-all recoveries of 6.4 per cent for α-teichoic acid and 39 per cent (average) for β-teichoic acid were reasonable considering possible losses during precipitation, extraction, and dialysis; moreover the ratio of 6.4 to 39 for the weights of the isolated α- and β-teichoic acids is very close to the ratio of 15 to 85. If the precipitin curves with α- and β-teichoic are plotted on adequately adjusted scales the sum of the curves for each teichoic acid gives a curve similar, but not exactly the same, as that obtained by the parent teichoic acid providing further proof for the existence of two polymers. However there is no evidence indicating that the α-teichoic acid is exclusively composed of α-linkages, and the β-polymer exclusively of β-linkages. For this purpose chemical and enzymatic studies on the separated teichoic acids are needed. β-*N*-Acetylglucosaminidase obtained from *Clostridium tertium* (39) did not act on teichoic acid Copenhagen, although it hydrolyzed β-methyl-*N*-acetylglucosaminide.

With respect to the NYH-6 strain, the finding of two precipitin lines in agar plates one fusing with α- and the other with β-teichoic acid indicates that it too is composed of two different antigenic polymers an α- and a β-teichoic acid; the proportions of each could not be determined precisely but are probably close to equal parts of each since in all cases the NYH-6 gave higher maximum precipitation than did Copenhagen teichoic acid.

The finding that teichoic acids from two strains of *S. aureus* were each mixtures of an α- and a β-linked *N*-acetylglucosaminyl-ribitol-phosphate polymer raises important questions as to the role of teichoic acids in cell wall structure and also of the biosynthesis of teichoic acids. For example, do individual staphylococci synthesize two types of teichoic acid or are the present strains mixtures of two mutants one synthesizing an α- and the other a β-teichoic acid? This question might be resolved by isolation of teichoic acid from single clones of the various strains. If there are two teichoic acids in a single cell wall, what is the function of each and how are they associated with the mucopeptide moiety? What is the synthetic pathway for biosynthesis of the α- and β-polymer? Since the two enzymes present (40) synthesize separate polymers, what determines whether an α-enzyme or a β-enzyme will function? It would appear that α- or

β-*N*-acetylglucosaminylation of a ribitol phosphate would not readily give rise to separate polymers. Nathenson and Strominger reported that a polymer from which β-*N*-acetylglucosaminyl linkages had been split, accepted both α- and β-*N*-acetylglucosaminyl linkages. In terms of the present findings they probably began their incorporation studies with an α–teichoic acid and a polyribitol phosphate. Whether incorporation of α- and β-*N*-acetylglucosaminyl linkages occurred on separate molecules of polyribitol phosphate or whether polyribitol phosphate as an acceptor can incorporate both types of linkages on a single molecule deserves further study. In any event the synthesis of separate α– and β–teichoic acids by the staphylococcus might not proceed by direct *N*-acetylglucosaminylation of a polyribitol phosphate.

SUMMARY

Human sera were found to contain antibodies precipitating with each of two samples of teichoic acid of *Staphylococcus aureus* prior to immunization; these antibodies were probably formed as a result of contact or infection with this microorganism. Injection of teichoic acid into two individuals resulted in a rise in circulating antibody to teichoic acid; a third subject probably had a primary response to α–teichoic acid. Quantitative precipitin and agar diffusion studies revealed the presence of two distinct antibodies in the sera and showed that each specimen of teichoic acid was a mixture of two polymers an α-linked *N*-acetylglucosaminyl-ribitol polymer and a β-linked *N*-acetylglucosaminyl-ribitol polymer, termed α– and β–teichoic acids respectively. The α–teichoic acid anti–α-teichoic acid system was inhibited best by α-linked glucosaminides and the β-anti-β-teichoic acid system was inhibited best by a β-linked glucosaminide. The α– and β–teichoic acids could be separated from each other by specific precipitation under appropriate conditions and recovered from the washed specific precipitates. The existence of two distinct teichoic acid polymers raises important questions as to cell wall structure and the biosynthesis of the teichoic acids.

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