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The location of intracellular teichoic acids

Although teichoic acids were first found in the walls of a number of Gram-positive bacteria[1], it is now known that they occur much more frequently, but in smaller amounts, in an unspecified region of the cell contained by the wall[2]. These compounds have been called "intracellular" teichoic acids, and all are glycerol phosphate polymers bearing alanine ester residues and varying amounts of sugar attached to glycerol. They are isolated from disrupted bacteria after centrifugation of wall material, whereupon they may be removed from the supernatant either by extraction with trichloroacetic acid solution or by sedimentation at high speed as complexes with RNA[2-6].

The widespread and possibly universal occurrence of these compounds within the cells of Gram-positive bacteria indicates that they may be important in cell metabolism, and it has been suggested that they could be concerned in transport mechanisms[2]. Consequently, it was important to establish more precisely their location with respect to other cell structures. In this connection, the observation that nearly all the intracellular teichoic acid of an organism accompanies the ribosomes during differential centrifugation of disrupted cells does not imply that it is a ribosomal component; it is more likely that an artificial complex is formed as a result of agitation of soluble and particulate material in the disrupted cell[6].

In this study protoplasts have been prepared from *Bacillus megaterium* 7581 (N.C.I.B.) and a Group-D streptococcus strain 8191 by the action of muramidase (EC 3.2.1.17, formerly known as lysozyme), and fractions representing the cell contents, protoplast membranes and wall components have been examined with respect to teichoic acid. *B. megaterium* 7581 (N.C.I.B.) has no teichoic acid in its walls, but contains appreciable amounts of the intracellular compound (observation by A. L. DAVISON); the streptococcus contains an intracellular glycerol compound and a ribitol teichoic acid in its walls (observation by J. J. ARMSTRONG); thus, it would be possible to identify the origin of teichoic acid which might be found in the various fractions.

The bacillus was grown with aeration from a 1 % inoculum in 2-l batches at 37° for 3.5 h in a medium containing peptone (1 %), glucose (1 %) and NaCl (0.5 %); the harvested organisms, which had reached almost the end of their logarithmic growth phase, were washed in 0.03 M phosphate buffer (pH 7.0) and suspended (10 mg/ml, dry wt.) in 0.03 M phosphate buffer containing sucrose (10 %), and $MgCl_2$ (to 0.005 M). Protoplasts were prepared by the action of muramidase (1 mg/ml) for 1 h at room temperature, the procedure being similar to that used by WEIBULL[7]. These conditions were chosen after trial experiments with different concentrations of sucrose and muramidase. Phase-contrast microscopy indicated that 90–99 % of the resulting structures were protoplasts. The protoplasts were separated from the wall hydrolysate, disrupted osmotically, and cytoplasmic contents and membranes separated; this procedure is similar to that described by WEIBULL, but does not include treatment with DNAase. The RNA and DNA content of each fraction were determined[8] as an indication of the extent of lysis or leakage during the experiment, and in each case the remaining material was treated with 10 % trichloroacetic acid solution at 4° for 48 h in order to extract teichoic acid. This was recovered in an impure state by the addition of 5 vol. of ethanol followed by centrifugation. No simple reliable method for the quantitative determination of teichoic acid in mixtures has

yet been developed, but its presence was demonstrated in all fractions by hydrolysis in 2 N HCl at 100° for 2 h followed by paper chromatography and identification of hydrolysis products according to methods described previously[3,4]. That from the cytoplasmic contents was particularly impure, and it was necessary to purify all samples by chromatography on Sephadex G-75 (*cf.* refs. 3 and 6) before quantitative determination could be attempted. Semi-quantitative analysis of the amounts of teichoic acid in each fraction was achieved by comparison of intensities of colours given on paper chromatograms by the glycerol and its mono- and diphosphates in acid hydrolysates. Results are given in Table I.

TABLE I

DISTRIBUTION OF NUCLEIC ACIDS AND TEICHOIC ACID IN CELL FRACTIONS FROM *B. megaterium*

Washed cells (2.746 g, dry wt.) were converted to protoplasts and fractionated.

	Teichoic acid (% of total)	*DNA (% of total)*	*RNA (% of total)*
Wall hydrolysate	85	8.4	17.5
Protoplast membranes	12	10.5	15.5
Cytoplasmic contents	3	71.0	60.0
Residual cells and granules	2	11.5	8.5

The streptococcus was grown in a 6-l batch at 37° for 18 h on a medium containing glucose (1 %), tryptone (1 %), yeast extract (0.5 %) and K_2HPO_4 (1 %) from a 1 % inoculum grown for 18 h. This organism is known to be sensitive to muramidase, and protoplasts were prepared by a procedure similar to that described by ABRAMS[9]; the optimum concentration of muramidase (1.6 mg/ml containing 4.5 mg dry wt. of cells for 2 h at 37°) was determined experimentally. The wall hydrolysate was removed and the protoplasts were disrupted by suspending in water (4.5 mg/ml) for 10 min and fractionated; the supernatant from the muramidase digestion was clarified by centrifugation at 5000 × *g* for 30 min. About 75 % of the cells in the preparation were protoplasts, and no attempt was made to separate the whole cells from membrane material during fractionation. Thus, although the wall hydrolysate was essentially uncontaminated with other fractions, the cell contents and whole cell fractions were probably not homogeneous.

The wall hydrolysate was dialysed for 24 h, and teichoic acids were extracted from all fractions by the method used for the bacillus. Nevertheless, much difficulty was experienced through the presence of large amounts of ribitol teichoic acid in the walls. Some purification of the various fractions was achieved by chromatography on Sephadex G-75[10], followed by passage through columns of Dowex-50 (H^+ form) and Dowex-1 (Cl^- form) resins (elution with 0.5 M LiCl). Although manipulative losses were probably considerable and the paper-chromatographic method for quantitative determination was more difficult through the presence of the wall teichoic acid, it was shown that at least 85 % of the glycerol teichoic acid was present in the wall hydrolysate. About 7.5 % of the total DNA and RNA was in the wall hydrolysate.

These experiments show that protoplasts from both organisms contain only a small proportion of the so-called intracellular teichoic acid. On the other hand, isolated walls in both cases contain no trace of these compounds. We conclude that they are probably located in a region between the wall and the protoplast membrane, perhaps in weak association with the latter structure. The possibility that they are

truly intracellular in the intact organisms, but have diffused through the membrane after the protoplasts were prepared, seems unlikely; although these compounds possess lower molecular weights than the nucleic acids (that from the streptococcus[6] has a mol. wt. of 14000), they do not dialyse through cellophane membranes and their shape and polyionic structure confer on them characteristics towards Sephadex gels normally associated with much larger molecules[4,6,10]. The presence of only small amounts of nucleic acid in the wall hydrolysate indicates that the membrane surrounding the protoplasts is an effective osmotic barrier.

The presence of small amounts of glycerol teichoic acid in the cytoplasmic contents could arise from incomplete separation of fractions, or could suggest either that these compounds are synthesised there or that certain cytoplasmic structures contain teichoic acid. The presence of traces of ribitol derivatives in the cytoplasmic contents of the streptococcus may be explained similarly.

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Microestimation of zinc in human blood serum

For the estimation of zinc in biological materials spectrography[1], polarography[2] or colorimetry of zinc dithizonate[3] is usually employed. For routine serial estimations the relatively simple colorimetric method appears to be the most suitable. However, dithizone yields coloured compounds with a number of other metals, so that the method is to be considered less specific and masking reagents must be employed. The purification of these reagents which usually contain considerable amounts of zinc causes difficulties.

The new method as proposed circumvents these difficulties by combining the colorimetry with chromatographic separation of zinc so that the masking reagents need not be used.

1 ml of blood serum was digested by the Kjeldahl method according to WOLFF[3]. The digest was then dried, dissolved in 1 ml three-times distilled water and transferred