siderably lower amplitude. The oscillations occurred more often in the light phase and began within 60 sec after addition of the valinomycin.

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The phosphorus content of cell walls of *Micrococcus lysodeikticus*

The cell wall of *Micrococcus lysodeikticus*, reported by SALTON[1] to contain 0.09% phosphorus (P), has more recently been stated to contain no P (refs. 2, 3). In this laboratory, preparations of the cell wall of *M. lysodeikticus* NCTC 2665 have always contained 0.11–0.13% P and in this note we present evidence for believing that the P is an integral component of the wall and not a contaminant.

ARCHIBALD *et al.* could not isolate teichoic acid from *M. lysodeikticus* cell walls[4]. Thus, the low amount of P found in the wall could be due to a contaminant which might originate from membrane or nucleic acid. Due to the presence of carotenes, the membranes (and thus cells) of *M. lysodeikticus* are highly coloured and the preparation of "clean" walls is facilitated. The morphological purity can be checked by staining and by electron microscopy. Walls used in our experiments were prepared from cells in the stationary phase by shaking with glass beads and were thoroughly washed with Tris buffer and water.

Extraction of dry walls with refluxing ether or with chloroform–methanol (2:1) did not remove any P (Table I). Chloroform–methanol is used widely to remove phospholipid from bacterial membranes, in particular that from membranes of *M. lysodeikticus*[6]. Thus, any contamination of the wall did not appear to be due to membrane. This possibility was also checked by establishing how much membrane would be expected to be present in the wall. Membranes were prepared by lysing protoplasts[6,7] and were then shaken with glass beads under the same conditions employed for wall preparation. 20–25% of the membrane (or membrane fragments) was sedimented under the conditions used to sediment walls. Moreover, with respect to colour and P content (0.5–0.6%), the material sedimented had properties identical

TABLE I

EXTRACTION OF *M. lysodeikticus* CELL WALLS

A number of extractions of the type described here were carried out. The table gives the results for a preparation of wall containing 0.11% P.

Extraction with	*Weight of wall (mg)*		*P content[5] of wall (μg)*	
	Before	*After*	*Before*	*After*
Chloroform–methanol*	21.56	20.80	23.1	22.6
Ether**	53.50	52.90	57.2	56.5
Trichloroacetic acid***	25.11	11.44	27.0	17.6

* Dry chloroform–methanol (2:1), two 5-h extractions at 25°, each with 5 ml. Evaporation of the combined extracts yielded 0.35 mg of a waxy solid. The whole of this was digested with H_2SO_4 but no P was detected. The method was capable of detecting 1 μg of P with ease.

** Dry ether, two 30-min extractions under reflux, each with 15 ml. No residue was obtained on evaporation of the extract.

*** 5% trichloroacetic acid, 5 ml, for 30 min at 90°. The extract was washed with ether to remove trichloroacetic acid. The solution did not absorb in the ultraviolet when examined in cells with 1-cm light path.

to those of intact membrane. The membrane sedimented with the wall was readily recognised as a thin, mobile, yellow layer on top of the firmly packed wall. It could be removed by gentle washing and the top portion of the wall was sacrificed to ensure a membrane-free preparation of wall.

Extraction of the wall with trichloroacetic acid solubilized some P (Table I). The extracts were apparently free of nucleic acid, however, since after removal of trichloroacetic acid with ether, the extract had no absorption in the ultraviolet. It is known[2] that trichloroacetic acid solubilizes part of the wall for such extracts contain all the components normally found in the wall. The results are compatible with the P being part of a normal component of the wall some of which is solubilized by trichloroacetic acid treatment.

One other experiment was performed to test the possibility that the P might belong to a wall contaminant. When actively growing cells of *M. lysodeikticus* are removed from a nutrient medium and transferred to a medium containing only the amino acids found in the wall (glutamic acid, lysine, alanine and glycine), glucose, phosphate and magnesium, cell division virtually ceases but wall synthesis continues (Dr. P. HATTON, personal communication). Chloramphenicol can be added to suppress protein synthesis. Under these conditions of wall thickening, the contribution of the wall to the dry weight of the organism approximately doubled and there was no observable change in the composition of the wall. The P content of the thickened wall was the same as walls prepared from normal cells. If the P in normal wall is part of a contaminant, it would be expected that the thickened wall would contain a lower amount of P since the wall then constitutes a larger percentage of the cell mass.

Thus, from the experimental data outlined, it did appear more likely that the P was an integral component of the wall rather than a contaminant. The ultimate proof would lie in the isolation of a fragment, recognized as originating from the wall and containing P. Further experiments have been designed with this aim in

view. Exhaustive dialysis of lysozyme digests of the wall removed 40% of the wall. However, 80% of the P remained non-diffusible. Further concentration of the P was achieved by chromatography on DEAE-cellulose which permitted the removal of a small amount of non-P-containing material. Hydrolysis (4 M HCl, 100°, 4 h) of the P-enriched material and examination of that portion of the hydrolysate not retained by Dowex 50 ion-exchange resin by paper electrophoresis at pH 6.3 revealed the presence of two major P-containing compounds in addition to P_i. Both compounds were ninhydrin positive and gave a positive amino sugar reaction. The fastest moving compound was homogeneous on paper electrophoresis over a wide pH range and always moved towards the anode. It was dephosphorylated with phosphatase from human prostate gland and yielded a compound behaving on paper chromatography in three different solvent systems as muramic acid. It thus appeared to be muramic acid phosphate, a compound which has been reported on a number of occasions but whose authenticity has yet to be fully established. The other compound was neutral and behaved very similarly on electrophoresis to glucosamine 6-phosphate. It did not yield glucosamine on dephosphorylation with phosphatase, however, and the amino sugar released did not correspond in mobility to any known amino sugar on paper chromatography.

Thus the presence of hexosamine phosphates in the hydrolysate indicates that that P is associated with components normally concerned in cell wall structure. It is therefore reasonable to suggest that P is a normal component of the isolated wall but the role that P might play in the wall's structure is a matter for conjecture at present.

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