## On the probable absence of "mucocomplex" from *Mycoplasma mycoides*

It is now well established that the cell walls of all typical bacteria, whether gram-positive or gram-negative, contain a mucopolysaccharide-like material, "mucocomplex", which is probably responsible for their structural rigidity (see review by WORK[1]). The amino-sugars glucosamine and muramic acid appear to be essential components of these structures. $\alpha,\varepsilon$-Diaminopimelic acid is also present, except in some gram-positive bacteria where it is replaced by lysine. The pleuropneumonia-like organisms appear to lack a rigid cell wall. KANDLER AND ZEHENDER[2], who examined three strains of pleuropneumonia-like organisms of diverse origin, were unable to detect hexosamines or diaminopimelic acid in any of them. I wish to report analyses of the pleuropneumonia organism, *Mycoplasma mycoides*.

Two strains of *Mycoplasma mycoides*, strains V5 and GY, were examined. Strain V5 is used in this laboratory for the preparation of a vaccine against bovine contagious pleuropneumonia. Strain GY was isolated from a case of peritonitis in a goat[3]. The organisms were grown at 37° for 24–28 h in rotated flasks of BVF-OS medium[4] inoculated with 0.8 % (v/v) of a 4-day culture in the same medium. They were harvested by centrifugation and washed in cold 0.2 *M* NaCl followed by distilled water. For comparison, a gram-negative bacillus, *Actinobacillus lignieresi* strain S40, was analysed by the same procedures. The cells were grown in veal-infusion broth at 37° for 24 h, harvested by centrifugation and washed in distilled water.

Samples of the washed suspensions were hydrolysed in 6 *N* HCl at 100° for 20 h. The hydrolysates were filtered through sintered glass, concentrated *in vacuo*, and dried in an evacuated desiccator over NaOH pellets. The residues were dissolved in water and chromatographed on Dowex 50 ($H^+$-form, 8 % cross-linked Analytical grade resin, 200–400 mesh) columns 10 cm × 0.6 cm. The sample chromatographed corresponded to 51 mg dry weight of washed organisms in the case of *M. mycoides* strain V5 and *A. lignieresi*. The hydrolysate of *M. mycoides* strain GY was first chromatographed on paper with the solvent of DOUGHERTY, GORDON AND ALLEN[5] to remove some of the faster-moving amino acids. The region containing the slower-moving acids was eluted with water. A sample of the concentrated eluate, corresponding to 140 mg dry weight of washed cells, was placed on the Dowex column. This preparation was not analysed for hexosamines.

After sorption of the sample, the resin was washed with 8 ml distilled water and eluted with 40 ml 0.26 *N* HCl followed by 60 ml 1.25 *N* HCl. Portions of the effluent fractions were analysed for hexosamines by the method of RONDLE AND MORGAN[6], using glucosamine as the standard, and for diaminopimelic acid with ninhydrin reagent (b) of WORK[7]. 0.4-ml samples of the 1.25 *N* HCl effluent were heated with 0.4 ml glacial acetic acid and 0.4 ml of the ninhydrin reagent at 100° for 5 min. Diaminopimelic acid standards (in 1.25 *N* HCl) were run simultaneously. After cooling, the absorption of the undiluted solution was measured at 440 mμ and 500 mμ in a Beckman model DU spectrophotometer using micro cuvettes of 1-cm light path. Under these conditions diaminopimelic acid yields a spectrum with peaks at about 425 mμ and below 380 mμ. This is similar to that obtained by WORK[7] using a reagent at pH 0.3 (reagent (a)). The difference $\varepsilon_{440}-\varepsilon_{500}$ was proportional to diaminopimelic acid concentration. Tyrosine and tryptophan interfered at high concentrations, giving

spectra with peaks below 380 mμ. 320 μg tyrosine or 110 μg tryptophan gave approximately the same value for $\varepsilon_{440}$–$\varepsilon_{500}$ as did 10 μg diaminopimelic acid. Interference from lysine, cystine and histidine was negligible. The formation of a coloured product from lysine was almost completely suppressed by the HCl present.

Fig. 1 shows the chromatograms obtained from the hydrolysate of strain V5, alone and in admixture with glucosamine and diaminopimelic acid. The small peak emerging immediately before diaminopimelic acid was due mainly to tyrosine. There was no significant absorption at the point corresponding to the diaminopimelic acid peak. The appropriate fractions were concentrated *in vacuo* and chromatographed on paper using the solvent of RHULAND, WORK, DENMAN AND HOARE[8]. By comparison with the spots given by known amounts of diaminopimelic acid, it was shown that the washed cells contained less than 0.02 % diaminopimelic acid. Similar results were obtained with strain GY of *M. mycoides*.

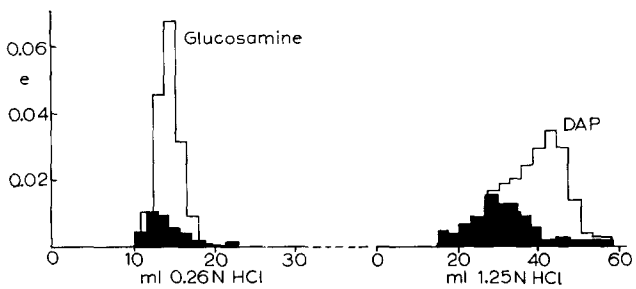


Fig. 1. Chromatography of *M. mycoides* (strain V5) hydrolysate on Dowex 50. Hydrolysate from 51 mg dry weight of washed cells chromatographed alone (shaded areas) and with added glucosamine-HCl (60 μg) and diaminopimelic acid (51 μg) (clear areas). Effluent fractions analysed as described in the text. DAP = diaminopimelic acid.

The recoveries of added glucosamine and DAP calculated from the data of Fig. 1 were 98 % and 107 % respectively. These values are necessarily approximate only, since the absorbancies were very low. The results are summarised in Table I. The significance of the small amount of material reacting as hexosamine in *M. mycoides* strain V5 is not known. The possibility of contamination with traces of medium constituents cannot be ruled out.

The absence of "mucocomplex" is consistent with the resistance of the organisms to high concentrations of penicillin, and with the observation that the turbidity of

TABLE I

HEXOSAMINE AND DIAMINOPIMELIC ACID CONTENT OF *Mycoplasma mycoides* AND *Actinobacillus lignieresi*

| *Organism* | *% of dry weight* | |
|---|---|---|
| | *Hexosamine** | *diaminopimelic acid* |
| *M. mycoides* strain V5 | 0.03 | < 0.02 |
| strain GY | — | < 0.02 |
| *A. lignieresi* strain S40 | 1.5 | 0.48 |

* In terms of glucosamine standard.

suspensions is unaffected by incubation with lysozyme and ethylenediaminetetraacetate[9]. Viability of strain V5 is well preserved in 0.4 *M* sucrose, buffered to pH 7.2 with 0.01 *M* phosphate[9], but the cells are easily killed by exposure to distilled water. When grown under the conditions used here they may be washed in distilled water with the loss of only a small amount of acid-precipitable nucleic acid (less than 2 %), although acid-soluble nucleotides are lost and at least 99.9 % of the cells are killed. Moreover, suspensions in 0.4 *M* sucrose —0.01 *M* phosphate may be diluted 10-fold into distilled water or 1 % (w/v) aqueous ethylene diaminetetraacetic acid without undergoing visible lysis. It may be noted that a small organism such as *M. mycoides* (diameter 0.2 $\mu$ or less) immersed in a hypotonic medium will be subject to a much smaller disruptive force than will the protoplast of a typical bacterium under similar conditions. Other factors which may contribute to the osmotic stability of the pleuropneumonia-like organisms are increased permeability to small molecules, and pliability of the surface membrane, as suggested by SMITH AND SASAKI[10], who found that several strains of human and avian origin were not killed by osmotic shock.

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## Optical-rotatory changes associated with the dimerization of bovine mercaptalbumin with mercurials

The dimerization of both human and bovine mercaptalbumin with mercurials is characterized by a large positive entropy of activation, $\Delta S^{\neq}$ (EDELHOCH *et al.*[1]; KAY AND EDSALL[2]). This is in contrast to the values of the order of —50 cal/deg./mole which are commonly found in reactions between simpler substances when two molecules unite to form one. EDSALL *et al.*[3] have previously suggested that the positive terms in the entropy of activation may be due to a preliminary unfolding of the albumin molecule in the vicinity of the sulfhydryl group which is necessary if dimerization is to occur. This hypothesis received experimental support when it was observed that $\Delta S^{\neq}$ for bovine mercaptalbumin in 8 *M* urea (—18 cal/deg./mole) was much lower than any of the values for the dimerization reaction in water[2]. Presumably, when the albumin molecule is dissolved in urea, the requisite folding has,