measured after treatment of the protein with guanidine hydrochloride are reduced in the enzyme form 1 as compared with a mixture of forms 3, 4 and 5. Electrophoresis of alkylated enzyme forms on sodium dodecyl sulphate polyacrylamide gels led to a major protein band in each case with an approximate molecular weight of 15 000.

Our results indicate that stepwise oxidation of thiol groups results in the formation of multiple electrophoretically distinct forms of creatine kinase from this invertebrate source. Serious doubts must thus be raised on the use of crude extracts of invertebrates for the detection of 'isoenzymes' of creatine kinase.

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from the wall by trypsin or papain, and the cation-binding capacity of the wall was thereby decreased by 50%.

Yeast phosphomannan may have a similar role to teichoic acids of bacteria in cation-binding and transport. In fact the act of flocculation, phosphate groups on the surface of adjacent cells attract Ca^{2+} to form salt bridges.

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Cation-Binding by Yeast Cell Walls

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Brewer's-yeast cell walls were isolated from flocculent and non-flocculent strains. Samples (10mg) of cell wall were washed with 1mM-EDTA and suspended for 40 min at 25°C in 3.7mM-calcium chloride (0.4ml) containing 0.4mCi of ^{45}Ca at pH 4.5. The walls were sedimented at 2000g, washed with glass-distilled water (3 × 2ml) and dried, and the radioactivity was measured in a liquid-scintillation counter (Lutwak, 1959). Flocculent yeast walls bound on average 3.4mmol of Ca^{2+} /100g dry wt. of wall whereas non-flocculent walls bound 1.6mmol/100g dry wt. of wall.

Mg^{2+} and certain other cations replaced Ca^{2+} freely. The degree of binding with Ca^{2+} was affected by prior treatment with surface-acting agents, e.g. maltose and urea, or esterifying agents, e.g. 1,2-epoxypropane. Walls derived from yeast grown in an Mg^{2+} -deficient medium had a greater ability to bind cations and a higher phosphorus content than controls. [Growth of *Bacillus subtilis* in an Mg^{2+} -limited medium also led to an increase in cell-wall phosphorus (Tempest, Dicks & Ellwood, 1968).] Yeast grown in a phosphorus-deficient medium bound Ca^{2+} to a below-normal extent.

Flocculent cell walls fractionated with ethylenediamine (Korn & Northcote, 1960) had higher phosphorus contents in 'fraction A' than did non-flocculent walls (Lyons & Hough, 1970). Gel electrophoresis and gel electrofocussing revealed the presence in 'fraction A' of a phosphorylated manno-protein, involving phosphodiester linkages, with a low isoelectric point. This component was released

Some Properties of the Ion-Gating Polypeptide Alamethicin

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Physicochemical studies on solutions of the cyclic polypeptide alamethicin have been carried out (Chapman *et al.* 1969; A. I. McMullen & J. A. Stirrup, unpublished work) in an attempt to throw light on the relationship between its structure and its 'excitability-inducing' properties in phospholipid bilayers (Mueller & Rudin, 1968). Interfacial-tension studies have indicated that micelle formation occurs in aqueous solvents at a very low concentration, and ultracentrifuge experiments show that the aggregates increase in size as the concentration increases, at fixed ionic strength. The presence of 6M-urea decreases the size to about 5 monomers/micelle, and alcohols prevent aggregation. Increase in ionic strength increases the aggregation number, and the pH-titration curve indicates that the carboxyl group has a high pK_a value, exhibiting marked hysteresis in the alkaline region.

The 'pure' material can be separated by t.l.c. into three components of very similar, but not identical, amino acid contents (A. I. McMullen, J. H. R. Slade & J. A. Stirrup, unpublished work). Molecular models of the major component, which corresponds closely to the structure of alamethicin proposed by Payne, Jakes & Hartley (1970), indicate that no significant degree of parallel or antiparallel pleated sheet hydrogen-bonding is possible either inter- or intra-molecularly. This is due to the presence of the well-distributed methylalanine (and proline) residues. The presence of two methyl groups on the α -carbon of the former peptide prevents, by steric