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

hindrance, such coupling with any other residue. It is not justifiable, for instance, to attribute the aggregation in a 'pore' or stacked ring format (Mueller & Rudin, 1968) to significant hydrogen-bonding forces.

Extensive intramolecular hydrogen-bonding can occur, however, in α -helical format, and it is possible to construct a model wherein 60% of the residues are thus bound. Experiments suggest that this structure may obtain in an interfacial monolayer, where the area/molecule at high pressure corresponds well with the theoretical value, and also in other non-aqueous systems such as alcohol, wherein a considerable degree of helicity is indicated by circular-dichroic results (P. Bayley, personal communication). These results are discussed in connexion with the ion-gating properties of alamethicin.

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Chitin Synthesis in *Mucor rouxii*

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Evidence has been obtained that chitin, a cell-wall component of *Mucor rouxii*, is synthesized from UDP-*N*-acetylglucosamine. Cell-free extracts of *M. rouxii* were shown to catalyse the synthesis of UDP-*N*-acetyl[¹⁴C]glucosamine from [¹⁴C]glucosamine, through a succession of intermediary steps involving kinase, transacetylase, mutase and pyrophosphorylase activities. Cell-free extracts also catalysed the incorporation of *N*-acetyl[¹⁴C]glucosamine into a chromatographically immobile acid- and alkali-insoluble polymer. This polymer was identified as chitin, being composed entirely of *N*-acetylglucosamine units. No evidence was obtained for any substantial synthesis of chitosan, a polymer of glucosamine, which is also a component of the *M. rouxii* cell wall.

Fractionation of the cell-free extracts by differential centrifugation revealed that the chitin synthetase activity was distributed among pellets sedimented at 2000g, 10000g and 100000g. Isolated cell walls were particularly rich in chitin synthetase activity. For most of this investigation particles

sedimented at 100000g were used. It was shown that chitin synthesis was stimulated by the presence of *N*-acetylglucosamine, ATP, soluble chitodextrins and a lipid fraction isolated from the fungus. Mg²⁺ was a requirement for chitin synthesis, which proceeded at optimum rate at pH 6.2–6.5 and 22–24°C. The enzymic activity of 100000g particles was most stable to storage when the particles were frozen in phosphate buffer. Chitin synthetase activity of germinating spores of *M. rouxii* increased rapidly from a time in the growth cycle coincident with the emergence of the germ tubes.

Free Lipids and Carbohydrates of *Agaricus bisporus* Mycelium

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Mycelium of the cultivated mushroom, *Agaricus bisporus* (J. Lange) Pilat, grown in stationary culture at 25°C for about 3 months in 2% (w/v) malt extract buffered with 0.1% CaCO₃, was harvested by filtration (wet weight 57 g) and washed with 0.9% NaCl. An acetone-dried powder was ground in sand with chloroform-methanol (2:1, v/v) and chloroform-methanol (1:2, v/v) and filtered. The combined acetone and chloroform-methanol filtrates were dried and washed (Folch, Lees & Sloane-Stanley, 1957) to yield an aqueous fraction (1 mmol of glucose equivalent) and lipid fraction (300 mg).

Paper chromatography of the aqueous fraction in ethyl acetate-acetic acid-formic acid-water (18:3:1:4, by vol.) and detection with AgNO₃-NaOH showed three well-separated carbohydrates with *R_F* values identical with those of glucose, trehalose and mannitol. The identifications were confirmed by chromatography in other systems, by the fact that trehalose on hydrolysis produced only glucose and by the non-reducing reaction of mannitol to aniline phthalate. Moreover trehalose and mannitol have been found in the sporophores of many basidiomycetes, including *A. bisporus* (fruiting stage) (Hughes, Lynch & Somers, 1958; Rast, 1965).

The lipid fraction was applied to a silicic acid column, which was eluted with chloroform (40% of total lipid), chloroform-acetone (1:1, v/v) (20% of lipid), acetone (10% of lipid) and chloroform-methanol (1:1, v/v) (20% of lipid). T.l.c. of the chloroform eluate in light petroleum-diethyl ether-acetic acid (85:15:2, by vol.) show triglyceride, both 1,2- and 1,3-diglyceride, monoglyceride, free fatty

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