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*

By I. McMURROUGH* and S. BARTNICKI-GARCIA.
(Department of Plant Pathology, University of California, Riverside, Calif. 92502, U.S.A.)

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[^{14}C]glucosamine from [^{14}C]glucosamine, through a succession of intermediary steps involving kinase, transacetylase, mutase and pyrophosphorylase activities. Cell-free extracts also catalysed the incorporation of *N*-acetyl[^{14}C]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. Mg^{2+} 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

By P. F. S. GRIFFIN, P. J. BRENNAN and D. M. LÖSEL (introduced by F. G. WINDER). *Departments of Biochemistry and Botany, Trinity College, Dublin 2, Irish Republic*

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% CaCO_3 , 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 AgNO_3 -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

* Present address: Arthur Guinness Son and Co. (Dublin) Ltd., St James's Gate, Dublin 8, Irish Republic.

acids and possibly a sterol ester. Similar classes of neutral lipids were found in the sporophore of *A. bisporus* (Hughes, 1962). The acetone eluate from the column contains the acylated form of the carbohydrates found in the aqueous fraction. The composition of this fraction will be described elsewhere.

Phospholipids were found only in the chloroform-methanol eluate. T.l.c. in chloroform-methanol-water (65:25:4, by vol.) and spraying with specific reagents showed principally phosphatidylinositol and phosphatidylethanolamine. Phosphatidylcholine was absent. These identifications were confirmed by acid hydrolysis and paper chromatography, which demonstrated glycerol, inositol, ethanolamine and traces of serine, but no choline. Mild alkaline hydrolysis and paper chromatography in propan-2-ol-aq. ammonia (sp.gr. 0.88)-water (7:1:2, by vol.) showed glycerylphosphorylinositol, glycerylphosphorylethanolamine and glycerylphosphorylserine.

This work is the early part of a study of the composition of *A. bisporus*, in the fruiting and vegetative stages, which may provide some insight into the metabolic differences underlying the initiation of sporophore development. It may also help to elucidate the role of lipids in stimulating spore germination (Lösel, 1967).

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Rennin Coagulation of Cow's Milk

By J. V. WHEELLOCK and J. P. PENNEY. (*School of Biological Sciences, University of Bradford, Yorks. BD7 1DP, U.K.*)

There are wide variations between milks of individual cows in the time taken to form a clot with rennin (EC 3.4.4.3). These variations may be due to factors such as Ca^{2+} concentration (Pyne, 1953) or pH (White & Davies, 1958) of the milk, which affect the coagulation phase, or to the amount and composition of the κ -casein, which could affect the primary phase of rennin action.

The primary phase may be conveniently studied by following the release of glycopeptides from κ -casein as determined by the increase in nitrogen soluble in the 12%-(w/v)-trichloroacetic acid filtrate

of the milk after the addition of the enzyme (Alais, Mocquot, Nitschmann & Zahler, 1953). Using the milk samples from individual cows, which were in mid-lactation and free of bacterial infections of the udder, we have observed that the clotting time of the milk decreased with increasing initial rate of the primary phase. This finding demonstrates that the primary phase is a critical factor in determining differences in clotting time between milks of individual animals. Further, we have observed that the initial rate of release of glycopeptides increased with the amount of glycopeptides released by rennin.

It is possible that at the concentrations used the enzyme may be present in excess, so that the initial rate of release is determined by substrate concentration. However, this explanation is unlikely because when the rennin concentration is varied there is a corresponding change in the initial rate of release of the glycopeptides and in the clotting time of the milk (Castle & Wheelock, 1970).

It has been postulated that the micellar κ -casein stabilizes the casein micelles and that the serum or 'rennin-accessible' κ -casein is responsible for coagulation of the milk (Parry & Carroll, 1969). When the serum κ -casein is hydrolysed the insoluble para- κ -casein becomes bound to the surface of several micelles and so joins them together into a clot. It follows from this that the amount of serum κ -casein will be directly related to efficiency of coagulation. Assuming that the total release of glycopeptides by rennin action can be used as a measure of the amount of serum κ -casein in the milk, then our results are in complete agreement with the ideas presented by Parry & Carroll (1969).

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Kinetics of the Primary Phase of Rennin Action on Whole Milk

By A. V. CASTLE and J. V. WHEELLOCK. (*School of Biological Sciences, University of Bradford, Yorks. BD7 1DP, U.K.*)

The kinetics of the primary phase of rennin (EC 3.4.4.3) action on whole milk have been studied by using the approach outlined in the previous