glucokinase is much higher than for the other two and is comparable in magnitude to the K_m for glucose (Table I) and mannose[4].

The present results thus extend the earlier observations and emphasize the need to use not only high glucose concentrations for the measurement of the maximum rate of processes depending upon the phosphorylation of glucose as the rate-determining step but also high concentrations of 2-deoxy-D-glucose if this analogue is to have a marked direct effect upon hepatic carbohydrate metabolism[7]. SPIRO[8] demonstrated that D-glucosamine and *N*-acetyl-D-glucosamine strongly inhibited the synthesis of glycogen in rat-liver slices in a competitive manner. In spite of the complexity of the system, it was possible to implicate competitive inhibition of a "non-specific hexokinase" by the glucose analogues as the operative mechanism[8]. KONO AND QUASTEL[9] studied the effects of the three analogues at concentrations up to 50 mM on "liver hexokinase" but the substrate concentration used was only 5 mM glucose. While their conclusion[9] that these analogues elevate hepatic phosphorylase activity thereby increasing glycogen breakdown remains valid, the present results show that the analogues can also have a marked effect upon glycogen synthesis by inhibiting the phosphorylation stage. These facts, together with the changes in the relative contributions of the two glucose-phosphorylating enzymes to the total activity which occur with changing glucose concentration[10], make the problem of assessing the effect of the inhibitors on complex systems such as found in tissue slices and *in vivo* extremely difficult.

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[1] D. L. DIPIETRO, C. SHARMA AND S. WEINHOUSE, *Biochemistry*, 1 (1962) 455.
[2] D. G. WALKER, *Biochem. J.*, 84 (1962) 118P.
[3] D. G. WALKER, *Biochim. Biophys. Acta*, 77 (1963) 209.
[4] E. VIÑUELA, M. SALAS AND A. SOLS, *J. Biol. Chem.*, 238 (1963) PC 1175.
[5] H. L. LINEWEAVER AND D. BURK, *J. Am. Chem. Soc.*, 56 (1934) 658.
[6] M. DIXON, *Biochem. J.*, 55 (1953) 170.
[7] J. BROWN, *Metabolism*, 11 (1962) 1098.
[8] R. G. SPIRO, *J. Biol. Chem.*, 233 (1958) 546.
[9] M. KONO AND J. H. QUASTEL, *Biochem. J.*, 85 (1962) 24.
[10] D. G. WALKER AND S. RAO, *Biochem. J.*, in the press.

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SC 11097

Separation of cellulases on Sephadex G-100

Cellulolytic culture filtrates from fungi and bacteria have been resolved into several cellulase components by ion-exchange chromatography and electrophoresis[1]. Both of these methods separate proteins mainly according to charge. The gel-filtration method newly adopted for the purification of cellulases from the basidiomycete *Polyporus*

versicolor[2] separates mainly according to an entirely different principle namely that of the molecular size. The gel-filtration method has now proved to be useful for the fractionation of cellulases from the two moulds *Aspergillus niger** and *Penicillius notatum***. The experiments were carried out as follows.

Sephadex G-100*** (3 g) was allowed to swell for two days in 0.1 M pyridine–acetic acid buffer (pH 5.0). The swollen de-aerated gel was poured into a chromatographic tube 1.5 × 40 cm. After sedimentation, a gel bed (38 cm high) was formed.

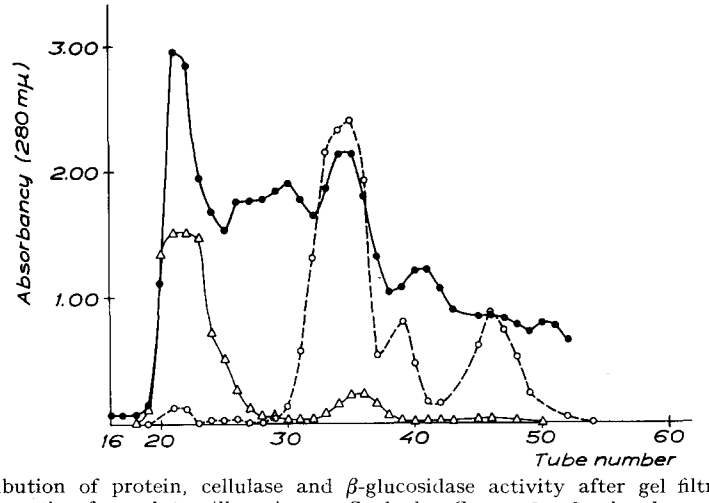

Fig. 1. Distribution of protein, cellulase and β-glucosidase activity after gel filtration of an enzymic preparation from *Aspergillus niger* on Sephadex G-100. ●—●, absorbancy at 280 mμ; △—△, β-glucosidase activity measured against *p*-nitrophenyl-β-D-glucoside; ○- - - -○, cellulase activity measured against CM-cellulose.

The samples, in portions of 100 mg, were dissolved in 0.6 ml of 0.1 M pyridine–acetic acid buffer (pH 5.0) and then transferred to the top of the column. Elution was carried out at a rate of 12 ml/h. Fractions of ml each were collected and analyzed for protein (absorbancy at 280 mμ) and for enzymic activity. Cellulase (EC 3.2.1.4) and β-glucosidase (EC 3.2.1.21) activities were assayed as described earlier[2].

The elution profile for the Aspergillus preparation, shown in Fig. 1, reveals the presence of at least 4 components active enzymically on CM-cellulose and *p*-nitrophenyl-β-D-glucoside. The question whether the same enzyme components are active against both substrates can not yet be satisfactorily answered. The components which have the highest enzyme activity on *p*-nitrophenyl-β-D-glucoside (the low molecular weight substrate) are least retarded and therefore should have the highest molecular weight if the general principles of gel filtration are obeyed. A similar relationship between specificity and molecular size was observed upon gel filtration of culture filtrates from the basidiomycete *P. versicolor*[2].

Gel filtration of the Penicillium enzyme (Fig. 2) gives three well-separated components active on CM-cellulose. No activity on *p*-nitrophenyl-β-D-glucoside could

* Commercially available from Worthington Biochemical Corporation Freehold, N.J. (U.S.A.).

** Kindly supplied by Dr. F. Eriksson, AB Astra Södertälje (Sweden).

*** From AB Pharmacia, Uppsala (Sweden).

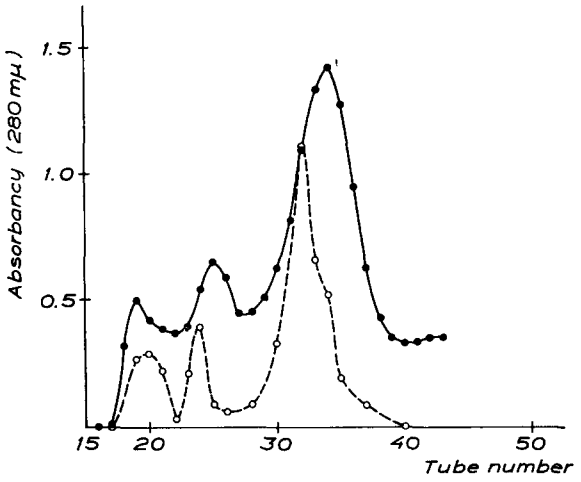

Fig. 2. Distribution of protein and of cellulase activity after gel filtration of an enzymic preparation from *Penicillium notatum* on Sephadex G–100. ●—●, absorbancy at 280 mμ; ○- - - -○, cellulase activity measured against CM-cellulose.

be found. Behind the last active peak a brown coloured material migrated, not shown in the diagram.

From the experiments made so far using gel filtration it appears that fungi produce multiple forms of cellulase and that the components may vary widely in molecular size. However, as will be published later, it seems that the multiple cellulase system of *P. notatum* consists of a common low molecular weight enzymic component associated with different inactive substances.

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[1] J. A. GAISCOIGNE AND M. M. GAISCOIGNE, *Biological Degradation of Cellulose*, Butterworth, London, 1960, p. 72.
[2] G. PETTERSSON, E. B. COWLING AND J. PORATH, *Biochim. Biophys. Acta*, 67 (1963) 1.

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Studies on lipolytic enzymes
II. The effects of *n*-butyl carbamic acid methyl ester on hydroxamic acid synthesis catalyzed by canine-liver lipase

It has been reported previously by us[1] that BCME is a remarkably potent species-specific inhibitor of canine liver and kidney lipase (EC 3.1.1.3) both *in vitro* and *in vivo*.

Abbreviation: BCME, *n*-butylcarbamic acid methyl ester.