from the assays resulted in variable and inconsistent reductions of enzyme activity, both in the control assays, and in those containing aflatoxin. The effect of β-mercaptoethanol on the spectrum of aflatoxin was found to be negligible.

We thank the Royal Society for an Equipment Grant, the Science Research Council for financial support, the Director, Toxicology Research Unit, Carshalton, for the aflatoxins, the Director, Microbiological Research Establishment, Porton, for the *E. coli* cells, and Dr G. E. Boxer, Mercke Institute, New Jersey for actinomycin D. One of us (A. M. Q. King) thanks Professor Tyler for a Research Demonstratorship.

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## Inhibition of Liver Glycerol Kinase by Adenosine Monophosphate and L-α-Glycerophosphate

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Glycerol kinase is a key enzyme in the metabolism of glycerol in animal tissues, and its activity is likely to be a factor controlling the metabolism of glycerol. Therefore the properties of this enzyme have been studied to determine which factors may be relevant to metabolic control. Glycerol kinase was purified from rat liver according to the method of Bublitz & Kennedy (1954): alternatively liver was extracted in 1% (w/v)-KCl–1mM-EDTA, centrifuged at 80000*g* for 1hr. and the supernatant used for activity measurements. In both preparations activity was assayed by the method of Newsholme, Robinson & Taylor (1967).

Bublitz & Kennedy (1954) reported that glycerol kinase was inhibited by ADP, and this has been confirmed. AMP also inhibits glycerol kinase: 0·4mM-AMP produced 50% inhibition ($Mg^{2+}$ was 2·0mM). As there was no adenylate kinase activity in the purified enzyme preparation the effect of AMP could not be explained by conversion to ADP. The inhibitions by ADP and AMP were increased by increasing the $Mg^{2+}$ concentration. Thus 1·0mM-ADP produced 26% inhibition at 0·2mM-$Mg^{2+}$ and 83% at 20·0mM-$Mg^{2+}$; 0·2mM-AMP produced 30% inhibition at 2·0mM-$Mg^{2+}$ and 70% at 10·0mM-$Mg^{2+}$. The concentration of ATP was always 1·0mM. The inhibitions by ADP and AMP were additive, provided excess $Mg^{2+}$ was present. The inhibitions were uncompetitive with glycerol. L-α-Glycerophosphate inhibited glycerol kinase competitively with glycerol: at 1mM-ATP and 2·0mM-$Mg^{2+}$ the $K_i$ was 0·6mM.

These observations suggest that the activity of glycerol kinase, and therefore the rate of glycerol utilization in liver may be regulated by the intracellular concentrations of AMP, ADP, $Mg^{2+}$ and L-α-glycerophosphate. The inhibition of glycerol kinase by its products, ADP and α-glycerophosphate, is similar to hexokinase, and, as with hexokinase, the enzymes controlling the rate of product (i.e. L-α-glycerophosphate) removal could regulate the activity of glycerol kinase.

J.R. wishes to thank the Medical Research Council for a Training Scholarship.

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## Some Properties of Pig Epididymal β-*N*-Acetyl-D-Glucosaminidase

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The enzyme (EC 3.2.1.30) was purified according to Findlay & Levvy (1960) and then, at 30° and pH4·3, catalysed the hydrolysis of 1·5μmoles of methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (I)/sec./mg. of protein with retention of configuration. The preparation had residual β-D-galactosidase, β-D-glucosidase and β-D-glucuronidase, but not detectable α-D-mannosidase, α-*N*-acetyl-D-glucosaminidase, 1-β-L-aspartamido-2-acetamido-2-deoxy-β-D-glucosylamine hydrolase (Murakami & Eylar (1965), neuraminidase (orosomucoid substrate) or proteinase (Ali & Lack, 1965).

Activities towards solutions (10mM) of 2-acetamido-, 2-fluoroacetamido-, 2-propionamido-, 2-formamido- and 2-amino-derivatives of phenyl 2-deoxy-β-D-glucopyranoside (II) were 100 : 8 : 4 : 1 : 0, respectively (cf. Greig, 1960; Greig, Leaback & Walker, 1961.

Activities towards I, were linear with time at pHs 3·3 to 9·1 for at least 60min., were maximal at pH4·3, and were unaffected by 0·01M-$MgCl_2$ and/or 0·05M-2-mercaptoethanol (cf. Reithel, Newton & Eagleson (1966). Using sodium carboxylate buffers activities were in formate > in citrate, isobutyrate, trimethylacetate > in propionate ≫ in acetate, and varied with pH as to suggest competition between the unionized acids and the substrate for the enzyme's 'acetamido'-receptor-site (cf. Pugh, Leaback & Walker, 1957).