with a 10% decrease in ATP (γ -P) radioactivity. By contrast adenosine and AMP, added at 0.125mM concentration for 5 min, had no effect on the turnover of protein-bound phosphoserine, despite their more potent effect in increasing the cyclic AMP content of the tissue (Sattin & Rall, 1970). However, rather higher concentrations of adenosine inhibit protein kinase activity in preparations of membrane fragments from brain (Weller & Rodnight, 1971), and it is possible that the compound may exert a similar effect on protein phosphorylation in the intact tissue.

These observations support the hypothesis that some post-synaptic responses to neurotransmitter release may be mediated through cyclic-AMP-dependent protein kinase activity in the receptor cell. Whether these are events occurring at the post-synaptic membrane or intracellularly remains to be investigated.

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Partial Reactions of D-Glucose 6-phosphate-1L-myoInositol 1-Phosphate Cyclase from Rat Testis

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From a study of hydrogen movements during the cyclization, we have previously proposed (Barnett & Corina, 1968) that the sequence of events in the enzyme reaction is oxidation by NAD^+ of C-5 of D-glucose 6-phosphate to give 5-oxo-D-glucose (D-xylulo-5-hexosulose) 6-phosphate, an aldol condensation between C-6 and C-1 of this molecule to give 1L-myoinosose-2 1-phosphate, and, finally, reduction by the NADH first formed to give 1L-myoinositol 1-phosphate. The NADH and oxidized intermediates were tightly bound to the enzyme and could not be isolated.

We have found that the NAD^+ can be removed from the enzyme by using charcoal, and that on readdition of NAD^+ (K_m 5×10^{-5} M) the enzyme retains 75% of the original activity, but has modified

properties. By using the charcoal-treated enzyme lacking NAD^+ we have been able to incorporate hydrogen from $[^3\text{H}]\text{NADH}$ into 5-oxo-D-glucose 6-phosphate. The label was located in the D-[5- ^3H]-glucose 6-phosphate formed by the methods previously described (Barnett & Corina, 1966).

For kinetic measurements carrier glucose 6-phosphate was added, the reaction mixture was reduced with sodium borohydride, the D-glucitol 6-phosphate was hydrolysed, separated by paper chromatography, benzoylated and purified on t.l.c., and the D-glucitol hexabenzoylate was assayed by its E_{232} value and its radioactivity counted.

The incorporation of radioactivity was linear with time up to 2h, and after 4h about 7% of the label from the NADH was incorporated. The incorporation was abolished by pretreatment with trichloroacetic acid, and 90% inhibited by 0.2mM-2-deoxy-D-glucose 6-phosphate, which has K_i 2×10^{-5} M for the cyclization of glucose 6-phosphate. No label was incorporated if untreated enzyme containing bound NAD^+ was used.

Incubation of the substrate analogue 5-oxo-D-glucitol 6-phosphate (L-sorbose 1-phosphate) with the charcoal-treated enzyme and $[^3\text{H}]\text{NADH}$ gave D-[5- ^3H]-glucitol 6-phosphate.

The enzyme-catalysed reaction of the hypothetical intermediates of the enzyme to give substrate strongly suggests that the mechanism previously proposed for the enzyme is correct.

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Pullulanase Synthesis in *Klebsiella (Aerobacter) aerogenes*

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Bacterial pullulanase, an inducible enzyme, has proved valuable in the analysis of starch-type polysaccharides (Manners, 1966) and has potential industrial uses. Initially reported by Bender & Wallenfels (1961) in a strain of *Klebsiella aerogenes* that they had isolated, pullulanase has also been detected in *K. aerogenes* A.T.C.C. 9621 (N.C.I.B. 8021) grown in appropriate conditions (Brown *et al.*, 1965). We have investigated the precise conditions necessary for its optimum synthesis by *K. aerogenes* N.C.I.B. 418 grown in chemostat continuous culture. In these conditions the enzyme remains entirely cell-bound. When maltose, maltotriose or pullulan was used as sole carbon source in carbon-limited conditions the steady-state values of pullulanase specific