placental phosphatases so that their effects on enzymes of the three categories could be compared. Enzyme samples were treated with 0.6M-cyanate at pH 10 and 37°C (Carey & Butterworth, 1969) or with 0.1M-acetic anhydride in half-saturated sodium acetate at pH 8 and 0°C (Brunel *et al.* 1969), followed by dialysis against tris-HCl buffer, pH 8.

Compared with control samples all three enzymes were decreased in activity by both reagents by up to 80%, the enzymes from the three tissues differing in the degree of inactivation produced. The inorganic pyrophosphatase activities of the phosphatases were depressed approximately in parallel with their orthophosphatase action. Loss of activity was not accompanied by a change in catalytic characteristics: e.g. K_m values for the hydrolysis of *p*-nitrophenyl phosphate were unaltered and the treated samples responded similarly to activation by Mg^{2+} ions.

Both acetylation and cyanate treatment increased the mobility of the phosphatases on starch-gel electrophoresis, but the three enzymes were affected differently. The mobilities of placental and intestinal phosphatases increased by approx. 20–30%, but that of the liver enzyme by only about 5% in parallel electrophoretic runs. When enzyme extracts in which minor active zones were present were treated these zones were also accelerated.

Cyanate and acetic anhydride probably act similarly, by decreasing the number of positive charges due to ionizable amino groups on the enzyme molecules, and the smaller effect on the mobility of liver phosphatase presumably indicates the existence of fewer of these groups in the molecule of this enzyme than in those from the other two tissues. The similar effects on the mobilities of intestinal and placental phosphatases show a further resemblance between these enzymes. The loss of activity on treatment without a change in catalytic characteristics suggests a general modification of the enzymes, rather than a specific action on groups in the active centres. An ϵ -amino group of lysine has been implicated in the activity of rat intestinal phosphatase (Fishman & Ghosh, 1967), but may not be reactive under these conditions.

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Hydrolysis of Indoxyl Acetate by Bovine Testicular Hyaluronidase Preparations

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Assay procedures generally employed for the assay of mammalian hyaluronidases have many disadvantages. It has been shown by Guilbault, Kramer & Hackley (1967) that crude bovine testicular hyaluronidase preparations can deacetylate indoxyl acetate (deacetylase activity) below pH 7.0 to yield the fluorescent Indigo White. These authors suggest that this property provides a basis for a convenient assay for hyaluronidase and have detailed the procedure to be followed.

In the present study deacetylase activity was examined for different crude commercial bovine testicular hyaluronidase preparations [Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K., 300 i.u./mg; Sigma (London) Chemical Co. Ltd., London S.W.6, U.K., 350 i.u./mg; BDH Chemicals Ltd., Poole, Dorset, U.K., 350 i.u./mg] and for a highly purified preparation kindly supplied by Dr B. Högberg of A. B. Leo, Helsingborg, Sweden (38000 i.u./mg). In spite of the fact that the Leo hyaluronidase is about 100 times as pure as the other preparations its deacetylase activity was of the same order as those preparations, suggesting that the two activities are not related. The Koch-Light and Leo preparations were then selected for more detailed studies. In each case concentrations of polyphloretin phosphate that were able to inhibit hyaluronidase activity completely (cf. Diczfalussy *et al.* 1953) were without effect on the deacetylase activity. Hyaluronidase was stable at pH 2.2 at 0°C, whereas deacetylase activity was lost under these conditions. At pH 6.0 hyaluronidase activity was lost between 60°C and 65°C, conditions under which deacetylase activity was retained, whereas diluted enzyme preparation (100 µg/ml) lost hyaluronidase activity but retained deacetylase activity when shaken mechanically at 37°C.

Gel chromatography on Sephadex gave different elution diagrams for the two activities. Similar elution diagrams on Sephadex G-100 and G-200 were obtained for the hyaluronidase activity of either the Koch-Light or Leo preparation. Elution diagrams for the deacetylase activity showed differences for each preparation; the Koch-Light preparation had one peak of activity, which was eluted behind the hyaluronidase peak, the Leo preparation showed two peaks of activity, which were eluted in advance of and after the hyaluronidase activity, although complete separation of the activities was not achieved.

These and other results suggest that hyaluroni-

dase and deacetylase activities cannot be associated with the same enzyme and certainly not with a single active site. Moreover, it is clear that indoxyl acetate cannot be used as a substrate for the assay of hyaluronidase activity. Other results obtained during Sephadex gel chromatography of hyaluronidase activity suggest that the enzyme exists as a monomer (mol. wt. 70 000) in dilute solution and as a dimer (mol. wt. 120 000) at higher concentrations.

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Effect of Bivalent Cations on some Enzymes of Gluconeogenesis

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Three of the enzymes important in gluconeogenesis, pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase, are all activated by both Mn^{2+} and Mg^{2+} . Surprisingly little information is available on the behaviour of pyruvate carboxylase and fructose 1,6-diphosphatase of rat liver towards these ions or towards Ca^{2+} , whose presence variously in the mitochondrion or cytoplasm has been suggested (Gevers & Krebs, 1966) as a possible mechanism for control of glucose metabolism through alternate inhibition of pyruvate carboxylase and pyruvate kinase. The effects of these ions on the three rodent hepatic enzymes important in the reverse of glycolysis were investigated.

Mn^{2+} is an activator of all three enzymes at concentrations much lower than are found for Mg^{2+} , a phenomenon in common with several other enzymes (e.g. RNA polymerase; Roeder & Rutter, 1969). In each case inhibition by Mn^{2+} is seen at concentrations at which Mg^{2+} concentration is optimal. When Mn^{2+} is added in addition to optimum concentrations of Mg^{2+} differing responses are seen. With pyruvate carboxylase addition of up to 2 mM- Mn^{2+} in the presence of 8 mM- Mg^{2+} has no effect on activity (Harris, Berent & Wimbhurst, 1970). With fructose 1,6-diphosphatase, which Tate (1966) found to be activated by EDTA saturated with Mg^{2+} , addition of Mn^{2+} at concentrations in excess of 0.05 mM-EDTA produced inhibition of the enzyme despite the presence of excess of Mg^{2+} . By contrast, Mn^{2+} in the range 0.01–0.1 mM in the presence of 6 mM- Mg^{2+} produced a marked

increase in the activity of phosphoenolpyruvate carboxykinase over that with Mg^{2+} alone, results similar to those of Foster, Lardy, Ray & Johnston (1967).

Pyruvate carboxylase, phosphoenolpyruvate carboxykinase and fructose 1,6-diphosphatase were all sensitive to inhibition by Ca^{2+} ; with the first two enzymes the loss of activity in the Mn^{2+} -activated system was less than when Mg^{2+} -activated but for fructose 1,6-diphosphatase the inhibition produced by Ca^{2+} was similar at optimum concentration of either Mn^{2+} or Mg^{2+} . Ca^{2+} inhibition of pyruvate carboxylase was noted by Kimmich & Rasmussen (1969), who also observed inhibition of its activity in mitochondria, and similar observations were made for phosphoenolpyruvate carboxykinase by Bartley & Dean (1969). Since key enzymes in gluconeogenesis, both those in the mitochondrion and in the cytoplasm, are subject to inhibition by Ca^{2+} it seems unlikely that extrusion of the cation will stimulate glucose formation. On the other hand exit of Ca^{2+} in conjunction with citrate (Harris & Berent, 1969) will tend towards inhibition of phosphofructokinase, but evidence is against enhanced citrate concentrations in liver under conditions favouring gluconeogenesis (Start & Newsholme, 1968).

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Acylation of a Cerebral Sodium-plus-Potassium Ion-Stimulated Adenosine Triphosphatase Preparation

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Conversion of amino groups in a particulate Na,K-ATPase* preparation into acidic functions by reaction with 2-methyl- and 2,3-dimethylmaleic anhydride has been examined as a procedure

* Abbreviation: Na,K-ATPase, $Na^{+} + K^{+}$ -stimulated adenosine triphosphatase.