- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). *J. biol. Chem.* **226**, 497.
 Tierney, D. F., Clements, J. A. & Trahan, H. J. (1967). *Amer. J. Physiol.* **213**, 671.

Influence of Metal Ions on the Orthophosphatase and Inorganic Pyrophosphatase Activities of Human Alkaline Phosphatase

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It is now recognized that mammalian alkaline phosphatases (EC 3.1.3.1) are able to hydrolyse both pyrophosphate and orthophosphate substrates (Cox & Griffin, 1965; Moss, Eaton, Smith & Whitby, 1967; Fernley & Walker, 1967), and it seems probable that a single type of active centre is responsible for activity towards both classes of substrate (Eaton & Moss, 1967a). However, Mg^{2+} ions have a differential effect on the two activities of alkaline phosphatase, activating the hydrolysis of orthophosphates but inhibiting that of pyrophosphates (Fernley & Walker, 1967; Eaton & Moss, 1967a,b). The influence of Zn^{2+} and Mg^{2+} ions on the activities of purified human intestinal alkaline phosphatase (Moss *et al.* 1967) has now been examined in more detail, and the results indicate that the differential effects of these ions are consistent with the suggestion that both catalytic activities are properties of a single type of active centre.

Both the inorganic pyrophosphatase and orthophosphatase (substrate, *p*-nitrophenyl phosphate) activities of the enzyme were measured at pH 9.0 in tris-HCl buffer; at this pH both substrates are hydrolysed at measurable rates over a wide range of concentration. Addition of Zn^{2+} ions to the pyrophosphatase assay system almost completely inhibits release of inorganic phosphate when the concentration of Zn^{2+} is greater than that of the substrate, inorganic pyrophosphate. As the concentration of pyrophosphate is increased above that of Zn^{2+} , competitive inhibition of the pyrophosphatase activity is seen. These observations can be explained by assuming that, when Zn^{2+} ions are in excess, all the substrate is in the form of a Zn^{2+} -pyrophosphate complex which is not broken down by the enzyme, or is only slowly attacked. As the substrate concentration is increased free pyrophosphate appears and is hydrolysed, but the Zn^{2+} -pyrophosphate complex is a competitive inhibitor of this hydrolysis. The Zn^{2+} -pyrophosphate complex is also a competitive inhibitor of the orthophosphatase activity when Zn^{2+} and pyrophosphate are added to this assay system in proportions calculated to produce the complex.

A similar explanation seems to be valid for the effect of Mg^{2+} ions on the pyrophosphatase activity of alkaline phosphatase. However, the inhibition of pyrophosphate hydrolysis by the Mg^{2+} -pyrophosphate complex is less marked than that by the Zn^{2+} complex, and the stoichiometry of the interactions of the two metal ions with pyrophosphate appears to be different.

- Cox, R. P. & Griffin, M. J. (1965). *Lancet*, ii, 1018.
 Eaton, R. H. & Moss, D. W. (1967a). *Biochem. J.* **102**, 917.
 Eaton, R. H. & Moss, D. W. (1967b). *Biochem. J.* **105**, 1307.
 Fernley, H. N. & Walker, P. G. (1967). *Biochem. J.* **104**, 1011.
 Moss, D. W., Eaton, R. H., Smith, J. K. & Whitby, L. G. (1967). *Biochem. J.* **102**, 53.

The Role of Endogenous Cations in the Na^+ - K^+ - Mg^{2+} -Effected Adenosine Triphosphatase of Cerebral Microsomes

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Hydrolysis of [^{32}P]ATP by cerebral microsomes is associated with a Na^+ -dependent and K^+ -inhibited binding of ^{32}P to a protein component in the membrane (Rodnight, Hems & Lavin, 1966, and references cited therein). Assuming that the Na^+ - K^+ - Mg^{2+} -effected ATPase* proceeds by a multistage reaction, the action of K^+ can be considered as one of increasing the turnover of a phosphorylated intermediate, this resulting in a lower steady-state content of intermediate when ATP is limited. With $2.5\mu M$ -ATP and twice-washed microsomes, however, appreciable Na^+ -dependent hydrolysis of ATP occurs in the absence of added K^+ and Mg^{2+} , the steady-state content of bound ^{32}P declining to zero as hydrolysis approaches completion. This suggests that sufficient endogenous K^+ and Mg^{2+} are present to permit the Na^+ - K^+ - Mg^{2+} -effected ATPase to hydrolyse very low concentrations of ATP.

The above observations were made with a microsomal preparation prepared by a modification of an earlier method (Rodnight & Lavin, 1966) involving several centrifugal washes of the crude microsomes to remove free cations. The preparation contained ($m\mu$ moles/mg. of protein): Mg^{2+} , 20 ± 2 ; Na^+ , 90 ± 20 ; K^+ , 27 ± 3 . By further washing with EDTA-containing buffers these levels have now been decreased to: Mg^{2+} , 3 ± 1 ; Na^+ , 24 ± 12 ; K^+ , 7 ± 2 . The exhaustively washed preparations showed a definitive requirement for Mg^{2+} for full phosphorylation, but turnover of bound ^{32}P still continued, presumably due to the residual bound

* Abbreviation: ATPase, adenosine triphosphatase.