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The Binding of Polypeptides by Neurophysins

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COMMUNICATIONS

A Requirement for Zinc Ions for Deoxyribonucleic Acid Synthesis by Lymphocytes Stimulated by Phytohaemagglutinin

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Alford (1970) reported that the transformation of human peripheral-blood lymphocytes by phytohaemagglutinin required a bivalent cation in addition to Ca^{2+} . His results suggested that this requirement was most efficiently met by Zn^{2+} .

Lymphocytes from pig blood obtained at slaughter were used for the present investigations. The incorporation of [^3H]thymidine into the DNA of the lymphocytes was inhibited by 80–90% by the addition to the medium of 0.6mM-EDTA or 0.6mM-diethylenetriamine-*NNN'N'N'*-penta-acetate but not by 0.6mM-nitrilotriacetic acid. The addition of Zn^{2+} as ZnSO_4 reversed the inhibition by EDTA or diethylenetriamine-*NNN'N'N'*-penta-acetate. With 0.6mM-EDTA complete reversal of the inhibition required 0.05mM- Zn^{2+} ; concentrations less than this caused only partial reversal, whereas higher concentrations again decreased the incorporation. The initial pattern of reactivation with increasing concentrations of Zn^{2+} suggested a competition between EDTA and a cellular chelator that, complexed with Zn^{2+} , was directly or indirectly involved in [^3H]thymidine incorporation. The competition between chelators for the metal ions present in the system was investigated by using a computer simulation based on the program described by Perrin & Sayce (1967). The simulation suggested that the cellular chelator had to have a stability constant of the order of 10^{12} to account for the observed competition with EDTA for the activating Zn^{2+} . The validity of the simulation was tested by using this value for the stability constant of the active site to calculate the proportion of the sites that had accepted Zn^{2+} over a range of added Zn^{2+} concentrations. These proportions were found to agree well over a range of suboptimal Zn^{2+} concentrations with the experimentally determined rates of thymidine incorporation.

The time-course of the requirement for Zn^{2+} was investigated by adding EDTA at various times after stimulation of the cells with phytohaemagglutinin.

It was further studied by adding EDTA before phytohaemagglutinin and subsequently attempting to reverse the inhibition by adding an optimal amount of Zn^{2+} . At least half the maximum rate of incorporation was still obtained provided EDTA was added not earlier than 12h before the incorporation was measured. If EDTA was added initially, Zn^{2+} had to be supplied at least 17h before the incorporation was measured if at least half the maximum activity was to be obtained.

The specificity of the reversal by Zn^{2+} of the inhibition caused by EDTA will be discussed together with the implications of an apparently high affinity constant for Zn^{2+} at the active site within the cells.

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Electrophoretic Slowing of Sensitized Lymphocyte-Macrophage Mixtures: a Cellular Technique in Immunochemistry

By E. A. CASPARY and E. J. FIELD (*Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, U.K.*)

Immunochemical studies have generally been confined to the interaction of substances with specific antibody prepared against them. The study of specific cellular immunity, however, is more difficult and not readily made quantitative. A very specific change in immunologically sensitized cells may, however, be measured by using cell electrophoresis in the presence and in the absence of the sensitizing antigen, when the mobility of the cell will be decreased by the antigen, and a number of studies of this kind have been reported over the last 5 years (Sundaram *et al.*, 1967a,b; Hartveit *et al.*, 1968; Deingdoh & Turk, 1968). An initial study of guinea-pig peritoneal-macrophage exudates from sensitized animals showed that the antigen slowing response depended first on the presence of lymphocytes and secondly that it could be inhibited by puromycin or other inhibitors of protein synthesis (Caspary, 1971). It was further