measurements (Gryzbowski, Tate & Datta, 1970) suggest that even univalent anions such as Cl^- will complex with Mg^{2+} , and this reaches significant proportions when $[\text{Cl}^-]$ approaches 100mm. The extent to which $[\text{Mg}^{2+}]$ is less than the total $[\text{Mg}]$ in the types of cell-free systems widely employed and the amount of complexes formed by the various ions will be presented. Novelli (1967) has emphasized the variability of optimum ionic requirements for the charging of tRNA, and analysis of such values offers an interesting contrast with likely conditions *in vivo*.

Both Mg^{2+} and Mn^{2+} can activate RNA polymerase, but the precise role they play is not known. The situation in the presence of high concentrations of ammonium sulphate is particularly interesting. Not only does the high concentration of sulphate complex a large proportion of the bivalent metal ion (e.g. the free ion is a few per cent of the total present), but the amount of nucleotide triphosphates as their bivalent cation chelates also decreases, the ammonium complexes rising. Kinetic studies must clearly take these factors into account.

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Quantitative Analysis of Proteins by Displacement Electrophoresis

By D. PEEL*, J. O. N. HINCKLEY and A. J. P. MARTIN. (Abbotsbury Laboratories, Barnet Lane, Elstree, Herts., U.K.)

The method of electrophoresis in which a simple mixture is placed in a tube between a fast-moving ion and a slow ion was first described by Kendall & Crittenden (1923). It has been applied by various workers for the separation of isotopes, inorganic and small organic ions, in a variety of apparatus forms: see Kendall (1942), Longworth (1953), Konstantinov & Oshurkova (1967), Preetz (1967), Behne, Bilal, Freyer & Thiemann (1968) and Everaerts (1968).

The ions separate with the most mobile ion leading until each occupies a section of tube, with a more or less sharp boundary between neighbours. The concentration within each section is constant and all move at the same speed, the concentration automatically falling until the potential gradient is inversely proportional to the mobility (Kohlrausch, 1897), irrespective of the original concentration in the sample. With strong ions any counter-ion can be used, but weak ions require a buffering counter-

ion to avoid disturbance due to pH changes (Everaerts, 1968).

Because of the analogy with displacement chromatography, Martin & Everaerts (1967) named it 'displacement electrophoresis'. In their method a capillary tube is used that permits the use of high potential gradients, since cooling is efficient and radial temperature gradients are small. The sharpness of the boundaries is proportional to the potential gradient and inversely to the mobility difference. Flow of liquid, whether due to electroendosmosis or density difference, or deliberately introduced to keep the fronts stationary, makes the boundaries less sharp.

If the current is held constant, the heat output of each zone is proportional to the potential gradient. In thin-walled tubes the temperature is proportional to the heat output, so it is possible with a fixed thermocouple to record the passage of each front and the mobility of each ion. The recorder chart shows a curve of staircase form, the height of each step showing the mobility, and the length the length of tube occupied by each ion.

In this instance we have built an apparatus and found suitable conditions for the separation of proteins. Quantitative and qualitative analyses of mixtures of crystalline proteins will be shown.

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The Effect of Mechanical Stress on the Properties of Reconstituted Collagen

By D. A. HALL. (University of Leeds Department of Medicine, The General Infirmary, Leeds LS1 3EX, U.K.)

Collagen reconstituted by alterations of temperature or pH will redissolve to an extent that depends on the period during which the precipitating conditions are maintained (Gross, 1958). In the present study macromolecular ox achilles-tendon collagen

* Present address: Pye Unicam Ltd., York Street, Cambridge CBI 2PX, U.K.

(Steven, 1964) was precipitated from acetic acid dispersion by exposure to ammonia vapour. The gel was allowed to form in the interstices of a nylon mesh, either in the unstretched state or during 20 cycles (3h) of repeated extension (33.5%) and relaxation. Both forms of gel were maintained for a further 16h in the atmosphere of ammonia and the cyclical extension was continued in the one case for this period.

After this period of 'aging' the acetic acid-solubility of the unstretched gel fell from $93.2 \pm 3.6\%$ to $46.6 \pm 8.2\%$ and that of the stretched gel to $15.0 \pm 2.3\%$ ($P = 0.005$). Stretching also induced a 25% decrease in the susceptibility of the gel to collagenase but increased its susceptibility to elastase by 117%.

A decrease in solubility and in susceptibility to collagenase such as is observed could indicate the formation of an increased number of cross-linkages during the stretching process. However, since Hall, Tunbridge & Wood (1953) and Banga (1953) have demonstrated that denatured collagen is more susceptible to elastase, the results with this enzyme would not appear to be in agreement with the hypothesis.

This apparent paradox can, however, be resolved if one considers the groups that are essential for binding elastase to its substrate (Hall & Czerkawski, 1961; Tolnay & Bagdy, 1959). Both carboxyl and hydroxyl groups are required in a free state on the substrate, but the blocking of the latter permits increased adsorption of enzyme without subsequent elastolysis. It may therefore be that elastase is bound on to unstretched collagen in such a fashion that the hydroxyl groups are too far distant from the carboxyl groups for active complex-formation, but that on stretching realignment places these essential groups in the desired spatial relationship to one another and proteolysis ensues.

Factors such as these may account for the extra-cellular production of an elastase-susceptible material (pseudo-elastin; Hall, 1969) in those aging tissues such as skin and vascular wall that during life have been subjected to marked cyclical stress.

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Isolation of Ovomucoid by using an Insoluble Trypsin Derivative

By J. G. BEELEY. (*Department of Biochemistry, University of Glasgow, Glasgow W.2, U.K.*)

In a study of the biosynthesis of the egg-white glycoprotein ovomucoid it was necessary to isolate small quantities of completed protein from tissue extracts. To meet this need the interaction of ovomucoid with an insoluble trypsin derivative has been examined as a micropreparative procedure. Chicken ovomucoid is known to be an inhibitor of trypsin but not of chymotrypsin (Rhodes, Bennett & Feeney, 1960).

Trypsin, in the presence of 50mM-benzamidine to minimize autolysis, was linked covalently to Sephadex G-200 that had been activated by reaction with cyanogen bromide (Axén, Porath & Ernback, 1967). Proteolytic activity was measured with casein as substrate (Northrop, Kunitz & Herriott, 1948) in stirred suspensions. The activity of Sephadex G-200-trypsin was equivalent to $12.5 \mu\text{g}$ of soluble trypsin/mg dry wt. Of this activity 71% was inhibited by ovomucoid at pH 7.6. The residual activity was not affected by the presence of a large excess of ovomucoid and apparently arises from enzyme molecules that are not accessible to inhibitor.

The insoluble trypsin-ovomucoid complex was stable at pH 7.6, but could be dissociated at pH 1.5 with the release of ovomucoid into solution and the restoration of 100% of the initial tryptic activity. Isoelectric focusing on polyacrylamide gel of the released product did not reveal any new components, indicating that the procedure did not degrade ovomucoid. On isoelectric focusing ovomucoid is separated into five closely related components (Beeley, 1969).

The Sephadex G-200-trypsin has been used to isolate inhibitors directly from egg white and from homogenates prepared from the washed oviducts of laying hens. In each case isoelectric focusing of the isolated material showed it to contain the characteristic pattern of three major and two minor ovomucoid components. However, an additional component of higher isoelectric point was also present. This substance also bound to Sephadex G-200-chymotrypsin and may therefore be both a chymotrypsin inhibitor and a trypsin inhibitor. The isolation procedure was made specific for ovomucoid by including a preliminary precipitation of the crude extract with 1 vol. of 10% (w/v) trichloroacetic acid adjusted to pH 3.5 with sodium hydroxide (Fredericq & Deutsch, 1949).

The experiments reported indicate that very small quantities of ovomucoid can be prepared rapidly and with a high degree of purity by a method