Speakman, 1966). These results may indicate that very large polyribosomes, or several polyribosomes, are involved in the biosynthesis of epidermal fibrous assemblies, as in collagen synthesis (Manner, Kretsinger, Gould & Rich, 1967; Speakman, 1968).

However, in experiments designed to discover whether there is a soluble precursor of epidermal protein, it has proved difficult to distinguish between incorporation of radioactive leucine into protein and non-specific adsorption of the label on protein. Trichloroacetic acid precipitation of epidermal protein gives a precipitate that cannot be redissolved. Isopycnic density-gradient centrifugation of a supernatant from the tissue homogenate, in a CsCl solution containing unlabelled leucine, shows an absorption peak at 280 nm at protein density. Much non-specific label is displaced from the peak, but some label, which may represent incorporated leucine, remains associated with it.

The incorporation of leucine into epidermal protein that is insoluble at neutrality but soluble at pH 2.5 is also being studied.

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The Site of the Inhibitory Action of Salicylate on Protein Biosynthesis *in vitro*

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Salicylate, in concentrations of 0.3 mM and above, inhibits the incorporation of radioactive leucine into the protein of cell-free systems prepared from rat liver (Dawkins, Gould & Smith, 1966; Reunanen, Hänninen & Hartiala, 1967). This action of the drug is independent of its uncoupling action on oxidative phosphorylation, since it occurred in microsomal preparations supplied with an external source of energy. We have therefore studied the effects of salicylate on the two main stages of protein biosynthesis *in vitro*.

The transfer of the radioactive amino acid from leucyl-tRNA to a polyribosome fraction prepared from rat liver is not affected by 10 mM-salicylate. Aminoacyl-tRNA synthetase activity was studied by the ATP-pyrophosphate exchange reaction and by the formation of aminoacyl-tRNA species from labelled amino acids. Salicylate, in concentrations of 0.6 mM and above, inhibits the ATP-pyrophosphate exchange reaction promoted by either an equimolar mixture of 20 amino acids or by individual

amino acids including leucine. However, the formation of the aminoacyl-tRNA species is differently affected by salicylate. The most sensitive amino acids are glutamate, aspartate and histidine. High concentrations of the drug (10 mM) inhibit the incorporation of cysteine, methionine, threonine and tryptophan, but not of leucine and the other amino acids.

These results show that salicylate interferes with the biosynthesis of proteins *in vitro* by preferentially inhibiting the formation of certain aminoacyl-tRNA species. They also suggest that the ATP-pyrophosphate exchange reaction does not accurately reflect the first stage in the incorporation of amino acids into proteins *in vitro* (Loftfield & Eigner, 1969).

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Protein and Ribonucleic Acid Synthesis *in vitro*: Calculation of the Bivalent Cation Requirement and the Extent of Complex-Formation in Incubation Media

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Staehelin (1969) has recently drawn attention to the possibility that the use of excess quantities of compounds such as phosphoenolpyruvate in protein-synthesizing systems *in vitro* can complex Mg^{2+} ions to such an extent that dissociation of polyribosomes occurs. It is a frequent practice in studies of amino acid incorporation by ribosomes *in vitro* to measure the optimum magnesium concentration of the system. This value is always the total [Mg] and as such gives little indication of the concentration of free Mg^{2+} , which is presumably the crucial entity affecting the activity of the preparation. Thus with 5 mM total magnesium, 1 mM-ATP and 10 mM-phosphoenolpyruvate, calculation suggests that $[Mg^{2+}]$ is likely to be about 0.8 mM. Knowledge of the concentration of free Mg^{2+} should enable more meaningful comparisons to be made of optimum magnesium requirements of systems developed by various workers (usually coming out at about 0.5–3 mM for translation of endogenous messenger), the actual concentration of Mg^{2+} at which use of synthetic messenger is most efficient (4–5 mM) and the $[Mg^{2+}]$ at which misreading of the code occurs (from 3.5 mM upwards). Recent

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

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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

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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

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