Therefore it cannot be ruled out that the phospholipid constitutes an integral part of the storage complex of chromogranin. Phospholipids are believed to be bound to proteins by ionic interaction between the phosphatidyl phosphate group and the amino group of lysine (Hanahan & Brockerhoff, 1965). DL-Lysine at 10mM concentration added to the lysate before gel filtration on Sephadex G-200 in 0.1M-phosphate buffer, pH 6.5, caused a complete dissociation of the phospholipid from the protein component that was not observed when lysine was omitted. Thus it is not unlikely that the dissociating effect observed with tris buffer of low ionic strength may be explained as a competitive binding of the tris ion with the phosphate group of the phospholipid.

In granular lysates, freed of catecholamines and ATP by gel exclusion on Sephadex or extensive dialysis, the ratios of phosphate bound to protein were found to be 0.2–0.4 $\mu\text{g. atom of P/mg. of protein}$ at solvent concentrations in the range 0.015–4M-NaCl.

Free phospholipids and lipoproteins are most frequently characterized by their low molecular densities, as determined by flotation techniques. Samples of granular lysates showed a sedimentation pattern at a density of 1.24g./ml. (NaBr) in the analytical ultracentrifuge, and flotation was observed with a density of 1.50g./ml. In sucrose of density 1.34g./ml. the lysate protein appeared to be close to equilibrium even after 24 hr. at 100 000g.

These observations are consistent with the presence of chromogranin as a lipoprotein complex of very high density, reflecting its rather low concentration of bound phospholipid.

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Coupling of Transcription to Translation in the Synthesis of Enzymes by *Escherichia coli*

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In a previous communication (Mehdi & Yudkin, 1967) we reported that in the presence of 500 $\mu\text{g. of chloramphenicol/ml.}$, which blocks the movement of ribosomes, transcription of the β -galactosidase cistron is prevented. If, however, the movement of ribosomes is permitted (e.g. in the presence of puromycin or 50 $\mu\text{g. of chloramphenicol/ml.}$) transcription can continue without the concomitant synthesis of protein.

The synthesis of β -galactosidase by wild-type *Escherichia coli* is known to be subject to catabolite repression (Magasanik, 1961). In the studies just described, we used strain LA-12G, which was believed to be free of catabolite repression, to avoid difficulties in interpreting experiments that involved inhibitors. However, even this strain shows catabolite repression under some conditions (V. Moses & M. D. Yudkin, unpublished work). It could therefore be argued that our previous conclusion that transcription is coupled to translation in the induced synthesis of β -galactosidase is unsound.

We therefore did analogous experiments with ornithine transcarbamylase (OTCase), a biosynthetic enzyme. We confirmed that the synthesis of this enzyme is insensitive to catabolite repression.

In *E. coli* BC28, the synthesis of OTCase is fully derepressed in minimal medium in the absence of arginine but completely repressed in the presence of arginine (Sercarz & Gorini, 1964). Cells grown in the presence of arginine were filtered and then incubated in arginine-free minimal medium for 5 min. to derepress OTCase (derepressing phase). They were then transferred back to medium with arginine, and enzyme formation was measured in the ensuing growth phase. If puromycin or chloramphenicol at 50 $\mu\text{g./ml.}$ was present during derepression, enzyme was synthesized for 5–6 min. in the growth phase. However, as in our work with β -galactosidase (Mehdi & Yudkin, 1967), no enzyme was produced in the growth phase when chloramphenicol was present at 500 $\mu\text{g./ml.}$ during derepression.

These results lend support to the conclusion that in bacteria growing in the absence of inhibitors, transcription normally depends on the translation of messenger into protein.

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Preparative Polyacrylamide Gel Electrophoresis in the Study of Isoenzymes

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Analytical electrophoresis in gel media (originally starch gel and more recently polyacrylamide) has proved of considerable value in the investigation of enzyme heterogeneity. The limitations of the methods available for the identification, quanti-

fication and characterization of the separated isoenzymes in analytical gels have, however, stimulated interest in the possibility of obtaining corresponding resolutions on a preparative scale. Several types of apparatus for preparative gel electrophoresis have been described (e.g. Jovin, Chrambach & Naughton, 1964; Gordon & Louis, 1967) and that used in the present study has certain features in common with them in making use of downward migration of protein zones in a vertical, annular gel column with a central condenser for the cooling water and a stream of elution buffer flowing across the lower face of the gel. The elution chamber is of a new pattern, however, which is designed to provide complete elution of protein zones entering the chamber in a small volume of buffer, thus preserving the high resolution of the separation process during collection. The performance of this apparatus has been assessed by the fractionation of alkaline phosphatases from several human tissues and of lactate dehydrogenases from rat tissues in 7.5% polyacrylamide gel in tris-borate buffer, pH 8.6.

For both types of enzyme recoveries of nearly 100% of the added activity were obtained. The relative proportions of the lactate dehydrogenase isoenzymes of rat heart extract determined by preparative electrophoresis were in close agreement with those derived by Wieland & Pfeleiderer (1961) from ion-exchange chromatograms. By contrast, staining and scanning the zones after separation on analytical agar gel electrophoresis underestimated isoenzyme 1. Human small-intestinal and liver alkaline phosphatases were separated by preparative gel electrophoresis, and the heterogeneity within the zones of each enzyme that had previously been demonstrated on analytical gels was preserved in the fractions collected from the preparative column. The closely migrating bone and liver phosphatase isoenzymes could not be resolved completely, but the heterogeneity of a mixture of the two isoenzymes was demonstrated.

The advantages of the apparatus in studies of isoenzymes are thus in providing quantitative recoveries of enzyme fractions with a degree of resolution comparable with that of analytical gel electrophoresis.

The apparatus (patent applied for) is obtainable from Wright Scientific Ltd., 3 Lower Road, Kenley, Surrey.

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The Stoichiometry of Electron Transfer by Bacterial and Plant Ferredoxins

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The number of electrons accepted by spinach ferredoxin on reduction by illuminated chloroplasts was determined by Whatley, Tagawa & Arnon (1963) and by Horio & San Pietro (1964) by measuring the stoichiometry of the reoxidation of the ferredoxin by NADP⁺. Both groups found that spinach ferredoxin accepted one electron/molecule on reduction. Sobel & Lovenberg (1966) measured the number of electrons accepted by bacterial ferredoxin from *Clostridium pasteurianum* by using the method of Whatley *et al.* (1963) and several other methods. They found that clostridial ferredoxin accepted two electrons/molecule on reduction. Tagawa & Arnon (1968) redetermined the number of electrons accepted by clostridial ferredoxin by using the stoichiometry of ferredoxin reoxidation by NADP⁺ or benzyl viologen, and obtained a value for one electron/molecule of ferredoxin.

In the course of our work on the physical properties of ferredoxins we have determined the number of electrons accepted by two bacterial ferredoxins and two plant ferredoxins. The bacterial ferredoxins were from the photosynthetic bacterium *Chromatium* and from the non-photosynthetic anaerobe *Clostridium welchii*. The plant ferredoxins were from spinach and from the blue-green alga *Anacystis nidulans*. By both the methods described by Tagawa & Arnon (1968), we find that both bacterial ferredoxins accept two electrons/molecule on reduction, and that spinach and *Anacystis* ferredoxins each accept one electron/molecule.

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Flavoprotein Properties of D-2-Hydroxyacid Dehydrogenase Purified from Rabbit Kidney

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D-2-Hydroxyacid dehydrogenase (EC 1.1.99.6) is a widely distributed enzyme which catalyses the oxidation of the D-stereoisomer of a number of 2-hydroxyacids to the corresponding keto-acids, using a variety of artificial acceptors (Tubbs &