in plasma containing 0·2 μg. of reserpine/ml. showed that the platelet 5HT content decreased slowly to a value, at 75min., of between 30 and 50% of the preincubation content. Approx. 20% of the 5HT is still retained by the cell after 24hr. of incubation.

Comparison of the gradient distribution of 5HT in homogenates from control and reserpine-treated platelets revealed that the granule-rich layer *C* was the most reserpine-sensitive subfraction. The 5HT content was decreased to zero after 60min. in most experiments. Other fractions showed less change in 5HT content.

Preincubation of the platelets with 5HT (2 μg./ml.), before reserpine treatment, resulted in essentially the same subcellular profiles. Between 70 and 90% of the reserpine in the cell was associated with the low-density membrane fraction *A*, and 40–70% of this was tightly bound to the membrane fragments. Incubation of isolated washed granules with reserpine showed a concentration-dependent 5HT release, which was optimum above 0·2 μg./ml.

A tentative explanation is presented for the mechanism of action of reserpine in the platelet.

Bartholini, G., Da Prada, M. & Pletscher, A. (1965). *Nature, Lond.*, **205**, 400.
Carlsson, A., Hillarp, N. Å. & Waldeck, A. (1963). *Acta physiol. scand.* **59**, Suppl. 215, 1.
Carlsson, A., Shore, P. A. & Brodie, B. B. (1957). *J. Pharmacol.* **120**, 334.
Crawford, N. (1965). *Clin. chim. Acta*, **12**, 274.
Glazko, A. J., Dill, W. A., Wolf, L. M. & Kazenko, A. (1956). *J. Pharmacol.* **118**, 377.
Hughes, F. B., Shore, P. A. & Brodie, B. B. (1958). *Experientia*, **14**, 178.
Minter, B. M. & Crawford, N. (1967). *Biochem. J.* **105**, 22P.

Characterization of the Ribonucleic Acid Product Synthesized by an Influenza A_2 Virus-Induced Ribonucleic Acid Polymerase *in vitro*

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The appearance of an RNA polymerase activity in the microsomes of chick chorioallantoic membranes infected *in vitro* by an influenza A virus (PR8 strain) has been described (Ho & Walters, 1966). A similar enzyme activity has now been identified in the chorioallantoic membranes of embryonated eggs infected with an influenza A_2 virus (Leningrad/62 strain). The induced RNA polymerase activity, as measured by the incorporation of [^{3}H]GMP into RNA from [^{3}H]GTP by the microsomal fraction, reached a maximum at 6–8hr. after infection (50–100 $\mu\mu$moles of GMP/mg. of protein), which was at least 10-fold greater than that of microsomes from uninfected cells. The influenza A_2-induced RNA polymerase was dependent upon the presence of Mg^{2+} and all four ribonucleoside triphosphates and was not inhibited by deoxyribonuclease or actinomycin D.

Influenza viruses have been reported to contain heterogeneous RNA components with sedimentation coefficients from 7s to 21s (Duesberg & Robinson, 1967; Nayak & Baluda, 1967; Barry & Davies, 1968). ^{32}P-labelled RNA components from the influenza virus used in these experiments sedimented on sucrose density gradients at 18s, 14s and 7s. The nucleotide composition of the 18s RNA was: CMP, 27·6; AMP, 15·0; GMP, 22·0; UMP 35·4; that of the 14s RNA was: CMP, 29·8; AMP, 13·8; GMP, 25·6; UMP, 30·8; and that of the 7s RNA was: CMP, 26·5; AMP, 21·2; GMP, 28·1; UMP, 24·2.

Microsomes from infected membranes incubated in the presence of [^{3}H]GTP, the other three ribonucleoside triphosphates and actinomycin synthesized RNA that sedimented in the 12–18s region. A ribonuclease-resistant component sedimenting at about 12s was also detected. 7s RNA was not detectable since degraded RNA, which arose during the *in vitro* incubation, was present in this region of the gradient. The 12–18s RNA extracted from microsomes incubated with all four ^{3}H-labelled ribonucleoside triphosphates had a nucleotide composition of: CMP, 20·4; AMP, 20·4; GMP, 24·9; UMP, 34·3. High-molecular-weight RNA was not detected in incubations of microsomes from uninfected cells.

The RNA polymerase of the microsome fraction of influenza A_2-infected cells therefore synthesized RNA *in vitro* with similar sedimentation properties and base composition to that found in the purified virus.

Barry, R. D. & Davies, P. (1968). *J. gen. Virol.* **2**, 59.
Duesberg, P. H. & Robinson, W. S. (1967). *J. molec. Biol.* **25**, 383.
Ho, P. K. & Walters, C. P. (1966). *Biochemistry*, **5**, 231.
Nayak, D. P. & Baluda, M. A. (1967). *J. Virol.* **1**, 1217.

The Chromogranin of the Adrenal Medulla: a High-Density Lipoprotein

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Gel chromatography of crude lysate of the chromaffin granules has shown that a phospholipid fraction, free of protein, precedes the main protein peak in the eluate when a tris–sodium succinate buffer of low ionic strength is employed. At higher ionic strength the phospholipid could not be separated from the protein moiety (Smith & Winkler, 1967).

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 μg.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 24hr. at 100000*g*.

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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Hanahan, D. J. & Brockerhoff, H. (1965). In *Comprehensive Biochemistry*, vol. 6, p. 85. Ed. by Florkin, M. & Stotz, E. H. New York: Academic Press Inc.

Smith, A. D. & Winkler, H. (1967). *Biochem. J.* **103**, 483.

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

By Q. Mehdi and M. D. Yudkin (*Microbiology Unit, Department of Biochemistry, University of Oxford*)

In a previous communication (Mehdi & Yudkin, 1967) we reported that in the presence of 500 μg. 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 μg. 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 5min. 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 μg./ml. was present during derepression, enzyme was synthesized for 5–6min. 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 μg./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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Magasanik, B. (1961). *Cold Spr. Harb. Symp. quant. Biol.* **26**, 249.

Mehdi, Q. & Yudkin, M. D. (1967). *Biochim. biophys. Acta*, **149**, 288.

Sercarz, E. E. & Gorini, L. (1964). *J. molec. Biol.* **8**, 254.

Preparative Polyacrylamide Gel Electrophoresis in the Study of Isoenzymes

By J. K. Smith and D. W. Moss (*University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh*)

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-