into protein was determined in a system containing limiting quantities of lymphocyte ribosomes, excess of rat liver cell sap and an energy-generating system. Other constituents were as described by Munro, Jackson & Korner (1964). Ribosomes were pre-incubated for 15 min at 5 mM-Mg²⁺. This procedure releases ribosomes from endogenous mRNA. They were then incubated with poly(U) at 12.5 mM-Mg²⁺, conditions in which initiation factors are not required. Ribosomes from lymphocytes incubated with phytohaemagglutinin for 24 h showed a 1.3–3.5-fold increase in [¹⁴C]phenylalanine incorporation per ribosome. Thus either the ribosomes from unstimulated cells translate poly(U) less efficiently, or a proportion of them are blocked in some way that prevents them acting in this system.

We thank the Medical Research Council for support and a studentship to T. A.

Munro, A. J., Jackson, R. J. & Korner, A. (1964). *Biochem. J.* **92**, 289.

Nowell, P. C. (1960). *Cancer Res.* **20**, 462.

Zylber, E. A. & Penman, S. (1970). *Biochim. biophys. Acta*, **204**, 221.

Ribonucleic Acid Polymerase Activity as a Measure of Ribonucleic Acid Synthesis

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Addition of phytohaemagglutinin to human lymphocyte cultures results in a 20-fold increase in the rate of incorporation of [³H]uridine into RNA after 24 h. The increase is a function of uridine uptake and phosphorylation rather than RNA synthesis (Hausen & Stein, 1968; Kay & Handmaker, 1970). The incorporation of other precursors into RNA is less stimulated, but the dramatic effects of phytohaemagglutinin on membrane permeability render such measurements suspect as indicators of RNA synthesis.

The rate of RNA synthesis can be determined accurately by using sedimentation studies to estimate the percentage of RNA synthesis attributable to rRNA (Cooper, 1969a) and [³H]methionine incorporation as a measure of rRNA synthesis. The saturation rates of the methionine pools in 24 h-stimulated and resting lymphocytes are very similar. By this method total RNA synthesis increases two- to four-fold in 24 h. The proportion of RNA synthesis devoted to rRNA increases from 8–16% in resting lymphocytes to 20–38% at 24 h, if wastage (Cooper, 1969b) and processing of rRNA precursors are taken into consideration.

We have studied RNA polymerase activity in nuclei from resting and 24 h-phytohaemagglutinin-stimulated lymphocytes (Handmaker & Graef, 1970). The incorporation of [³H]UTP into RNA was determined in the presence of 0.2 M-ammonium sulphate and with and without α -amanitin (1 μ g/ml). α -Amanitin at this concentration totally inhibits RNA polymerase II without affecting rRNA synthesis by RNA polymerase I (Novello & Stirpe, 1970). The total nuclear RNA polymerase activity is stimulated twofold. At 24 h after phytohaemagglutinin addition the α -amanitin-resistant polymerase activity is increased four- to five-fold, the percentage resistant increasing from 17 to 41% of the total activity. These values correspond closely to those obtained by using the more rigorous method outlined above.

Thus estimation of RNA polymerase activity in nuclei provides an accurate, simple and rapid means for determining alterations in RNA synthesis in growing and non-growing cells. It is especially applicable when the rate of alteration is too rapid for the more rigorous method to be used.

We thank the Medical Research Council for support and the Science Research Council for a postdoctoral fellowship to A. C.

Cooper, H. L. (1969a). In *The Biochemistry of Cell Division*, p. 91. Ed. by Baserga, R. Springfield, Ill: Charles C. Thomas.

Cooper, H. L. (1969b). *J. biol. Chem.* **244**, 5590.

Handmaker, S. D. & Graef, J. W. (1970). *Biochim. biophys. Acta*, **199**, 95.

Hausen, P. & Stein, H. (1968). *Eur. J. Biochem.* **4**, 401.

Kay, J. E. & Handmaker, S. D. (1970). *Exptl Cell Res.* **63**, 411.

Novello, F. & Stirpe, F. (1970). *FEBS Lett.* **8**, 57.

An Assay Method for the Initiation Factors for Protein Synthesis from Rat Liver

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Poly(U) can be translated in a cell-free system derived from *Escherichia coli* and reticulocytes at high Mg²⁺ concentrations, but at low Mg²⁺ concentrations the translation of poly(U) requires initiation factors (Nakamoto & Kolakofsky, 1967; Miller & Schweet, 1968). Lucas-Lenard & Lipmann (1967) showed that *E. coli* initiation factors f₁ and f₂ were required for the translation of poly(U) at low Mg²⁺ concentrations, and Prichard, Gilbert, Shafritz & Anderson (1970) and Shafritz & Anderson (1970) have shown a requirement for the initiation factors M₁ and M₂ for the translation of poly(U) in a reticulocyte cell-free system at low Mg²⁺