intervals of 5–40 min and growth hormone was given 2½ h before death. There was a 100% stimulation of peak incorporation into 45S RNA, which was reached in 10 min, but little evidence of an alteration in the rate of processing or first appearance of intermediate RNA species.

The stimulation of ribosomal precursor RNA synthesis in the nucleolus within 1 h and the smaller more transient stimulation of extranucleolar (heterodisperse) RNA synthesis are among the earliest observed actions of growth hormone *in vivo*, and may be important in the subsequent changes in cytoplasmic RNA and protein metabolism.

This work was supported by a fellowship to D. F. S. from CIBA Ltd., Horsham, Sussex, U.K.

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Effects of Hormones on Glycerol Metabolism in the Isolated Perfused Liver

By J. S. CHAHL and J. H. OTTAWAY. (*Department of Biochemistry, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG, U.K.*)

Female rats weighing 180–220 g were starved for 18–20 h and their livers were perfused through the portal vein with haemoglobin-free Krebs–Ringer bicarbonate solution to study the metabolism of exogenous glycerol. The glycerol concentration in the perfusate was maintained at 10 mM by constant infusion. Just before the experiment was commenced 10 μ Ci of [2-¹⁴C]glycerol was added to the perfusate.

At the end of 60 min 30% of the label remained in glycerol and up to 90% of the rest of the label was found in glucose, lipid and CO₂. Glucose accounted for up to 70% of this label. A significant fraction was in CO₂, up to 10%, which indicated that some glycerol was completely oxidized. In livers that were perfused with insulin in the medium the proportion of radioactivity found in lipids was significantly increased, but there was no radioactivity in the CO₂ of the system. When growth hormone was added the radioactivity in lipids was not significantly different from that in control rats, but no labelled CO₂ could be detected.

About 2% of the glycerol that disappeared accumulated as lactate and this was not affected

by the hormones. The accumulation of acetoacetate was enhanced when livers were perfused with either insulin or growth hormone.

Thus gluconeogenesis from exogenous glycerol appears not to be affected by either insulin or growth hormone in the isolated perfused liver. Insulin stimulates lipogenesis, but growth hormone does not affect this process when compared with the control rat. Both these hormones prevent the complete oxidation of glycerol to CO₂.

This work was supported by funds from the Science Research Council.

Control of Protein Synthesis during Lymphocyte Stimulation

By THERESA AHERN and J. E. KAY. (*School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.*)

Human lymphocytes maintained *in vitro* do not grow, but can be induced to do so by the bean protein phytohaemagglutinin. DNA synthesis begins 30 h and cell division 2 days after the addition of phytohaemagglutinin (Nowell, 1960).

Incorporation of ¹⁴C-labelled amino acids into protein represents accurately the rate of protein synthesis in this system. The rate of incorporation first increases 2–3 h after the addition of phytohaemagglutinin, and continues to rise to 5–15 times the initial rate after 24 h and to 10–40 times the initial rate after 48 h. Determination of the amount of RNA in lymphocyte cultures showed that it increased only slightly by 24 h and to 1.5–2.5 times the initial value after 48 h. The rate of protein synthesis per ribosome thus increases an average of sevenfold in the first 24 h and tenfold by 48 h after the addition of phytohaemagglutinin.

Analysis on sucrose density gradients containing 0.5 M-sodium chloride (Zylber & Penman, 1970) showed that the percentage of ribosomes active in protein synthesis is increased from 22–34% in unstimulated lymphocytes to 55–85% after 24 h and 44–78% after 48 h. Part of the increase in the rate of protein synthesis could thus be attributed to an increase in the numbers of ribosomes involved, indicating an increase in the rate of initiation of polypeptide-chain synthesis relative to the rate of translation. However, the rate of protein synthesis per active ribosome also increased, showing that there must also be an increase in the rate at which ribosomes translate mRNA.

The abilities of ribosomes from resting and from stimulated lymphocytes to translate the synthetic mRNA poly(U) in a cell-free system were therefore compared. Incorporation of [¹⁴C]phenylalanine

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.

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Ribonucleic Acid Polymerase Activity as a Measure of Ribonucleic Acid Synthesis

By ANNE COOKE,* J. E. KAY* and H. L. COOPER.†
(*School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K., and †National Institute of Dental Research, National Institutes of Health, Bethesda, Md. 20014, U.S.A.)

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.

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

By J. SAMPSON, A. F. BORGHETTI and A. KORNER.
(School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.)

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⁺