Arvin on Amberlite IRC-50 resolves the fraction into two enzymes; one of these (IRC-50 Arvin) coagulates solutions of fibrinogen by catalysing the release of fibrinopeptides A, AP and AY from the α (A)-chain of fibrinogen (Ewart *et al.* 1970).

IRC-50 Arvin shows trypsin-like specificity towards ester substrates. The rates of hydrolysis of the methyl, ethyl and cyclohexyl esters of α -N-benzoyl-L-arginine are identical (k_0 is 85 s^{-1} at pH 8.0 at 25°C) and comparable with those reported for trypsin and thrombin. The enzyme fails to catalyse the hydrolysis of simple amides and the trypsin-susceptible bonds in casein, glucagon and insulin.

IRC-50 Arvin is inactivated by di-isopropyl phosphorofluoridate and phenylmethanesulphonyl fluoride; the second-order rate constants for the inactivation are $2.11\cdot\text{mol}^{-1}\cdot\text{min}^{-1}$ and $32.0\text{ l}\cdot\text{mol}^{-1}\cdot\text{min}^{-1}$ respectively (at pH 7.0 at 25°C). Studies with these inactivators in the absence and in the presence of active-site protectors show that the coagulant and esterase activities of IRC-50 Arvin are associated with the same active site.

The esterase activity and the rate of acylation of IRC-50 Arvin are pH-dependent, increasing initially owing to the ionization of a group on the protein having pK_{app} approx. 7.0. The rate of each of these reactions decreases sharply above pH 9, and the phenomenon cannot be described by an ionization curve for a single group.

From these results and by analogy with the serine proteinases it is likely that the catalytic activity of IRC-50 Arvin is associated with an active-site serine residue that becomes acylated during the hydrolysis of esters; deacylation of the acyl-enzyme intermediate is the rate-limiting step in the reaction. The reactivity of this serine residue is influenced by the ionization of another group on the protein that is probably a histidine residue.

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Rapid Stimulation of Nucleolar Ribonucleic Acid Synthesis by Growth Hormone

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Although growth hormone is known to stimulate liver cytoplasmic and nuclear RNA synthesis in the rat (Korner, 1965), very little is known about the nature of the species of RNA synthesized during the hormone action. A recent study in this laboratory using chromatography on methylated albumin-kieselguhr has demonstrated an effect of growth hormone within 4 h on the labelling of ribosomal-like RNA followed by a later rise in the labelling of DNA-like RNA (Oravec & Korner, 1971). A parallel study was undertaken to isolate rat liver nucleolar RNA and to investigate the earliest effects of growth hormone on incorporation of [^3H]orotic acid into this fraction *in vivo*.

Nucleoli were prepared by ultrasonication of purified nuclei (Muramatsu, Smetana & Busch, 1963) in the presence of 0.1% diethyl pyrocarbonate to inhibit ribonuclease activity, and the RNA was isolated and examined by electrophoresis on polyacrylamide gels. The profiles obtained closely resembled those obtained by Narayan & Birnstiel (1969), with E_{260} peaks corresponding to 45S, 36S, 32S, 28S and 21S, all of which were labelled in 30 min. Growth hormone (0.5 mg or 1 mg/rat intraperitoneally) stimulated incorporation into all these precursor species when given to normal male or female rats 30 min before the [^3H]orotic acid, the effect increasing with time up to 2 h. These changes were accompanied by a slight increase in the quantity of 45S and 32S precursors relative to 28S nucleolar RNA. Similar changes were observed in hypophysectomized rats 4 h after growth hormone, but the quantity of the larger precursors was much decreased in these animals. Uptake of [^3H]orotic acid into the acid-soluble pool was unaffected by growth hormone in 1 h, but nuclear acid-soluble specific radioactivity was slightly decreased.

Further information on the action of growth hormone was obtained by assay of RNA polymerase activity in isolated nuclei in the presence and in the absence of the selective inhibitor α -amanitin. Both enzyme activities were stimulated by growth hormone; the α -amanitin-insensitive (nucleolar) activity was increased by 90% after 1 h, declining to 45% by 3 h, while the α -amanitin-sensitive (extra-nucleolar) activity reached 75% stimulation at $1\frac{1}{2}$ h, returning to the control value by 3 h. These changes are considerably more rapid than those observed in hypophysectomized animals (Tata, 1966).

In an attempt at a 'pulse-chase' experiment *in vivo*, [^3H]orotic acid was injected intravenously at

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.

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Effects of Hormones on Glycerol Metabolism in the Isolated Perfused Liver

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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.

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Control of Protein Synthesis during Lymphocyte Stimulation

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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