striking contrast with serum proteins. The specific radioactivity of the serum proteins was highest in the globulin fraction and decreased in correlation with the decrease of molecular weights of the serum proteins in the globulin and albumin fractions.

The results indicate that the proteins of tissues that display a rapid rate of cell proliferation and/or rapid synthesis of proteins, hormones and antibodies (gastrointestinal epithelium, pancreas, thymus, spleen, liver) show a high affinity for assimilation of glucose carbon, in sharp contrast with the relatively low rates of assimilation of glucose carbon by heart or skeletal muscles. However, the brain proteins had a higher specific radioactivity than did the liver proteins.

This work was supported by the Medical Research Council of Canada and the Canadian Heart Foundation.

Curtis-Prior, P. B., Trethewey, J., Stewart, G. A. & Hanley, T. (1969). *Diabetologia*, **5**, 384.

Vrba, R. (1966). *Biochem. J.* **99**, 367.

Vrba, R. (1967). *Biochem. J.* **105**, 927.

Vrba, R. & Cannon, W. (1970). *Biochem. J.* **116**, 745.

Vrba, R. & Winter, A. (1971). *Can. J. Biochem.* (in the Press).

Activation of Membrane Adenosine Triphosphatase by Chelating Reagents and Phospholipids

By K. P. WHEELER. (School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.)

Solubilization of a microsomal fraction from brain tissue with deoxycholate removes most or all of the ouabain-inhibited ATPase* activity and subsequent addition of certain phospholipid dispersions, notably phosphatidylserine, partly restores this activity, suggesting that the ATPase consists of a phospholipid-protein complex (Tanaka & Abood, 1964; Fenster & Copenhaver, 1967; Wheeler & Whittam, 1970). However, this interpretation was rendered questionable by the observation that phosphatidylserine also activates the ouabain-sensitive ATPase of the particulate enzyme, untreated with deoxycholate (Wheeler & Whittam, 1970). An alternative interpretation is that phosphatidylserine activates by virtue of its ability to chelate endogenous ions, such as Ca^{2+} or Fe^{2+} , that inhibit the ATPase. To test this possibility the effects of both EDTA and EGTA on similar preparations from rabbit kidney have been examined and compared with the effect of phosphatidylserine.

In the absence of added phospholipid the mean ouabain-sensitive ATPase activities (in μmol of P_i

liberated/min per g of protein) of the particulate and solubilized preparations were 172 and 31 respectively, and both of those rates increased, to 273 and 278, when 0.2mm-phosphatidylserine was added. However, addition of 0.2mm-EGTA to the particulate enzymes similarly stimulated the ATPase, to 314; and with both EGTA and phosphatidylserine the activation was virtually identical, giving a value of 295. Thus EGTA effectively mimicked the activating effect of the phospholipid. In contrast, 0.2mm-EGTA increased the ouabain-sensitive ATPase activity of the solubilized enzymes from 31 to only 58, whereas the further addition of the same concentration of phosphatidylserine raised the activity to 332. Almost identical results were obtained when EDTA was substituted for EGTA, and when these reagents were added at a final concentration of 2mm, compared with only 0.2mm-phosphatidylserine.

Phosphatidylserine therefore appears to activate the membrane ATPase both by its chelating ability and, presumably, by forming a phospholipid-protein complex. Further, the chelating action accounts completely for its activation of the particulate ATPase, but not of the solubilized enzyme, so that the phospholipid-protein-complex concept of the ATPase system remains tenable. This conclusion is not affected if the chelators and the phosphatidylserine produce their effects by some direct action on the enzyme, rather than by chelating endogenous metal ions.

This work was supported by the Science Research Council.

Fenster, L. J. & Copenhaver, J. H. (1967). *Biochim. biophys. Acta*, **137**, 406.

Tanaka, R. & Abood, L. G. (1964). *Archs Biochem. Biophys.* **108**, 47.

Wheeler, K. P. & Whittam, R. (1970). *J. Physiol., Lond.*, **207**, 303.

Some Catalytic Properties of IRC-50 Arvin

By J. P. COLLINS, J. M. BASFORD and J. G. JONES. (Department of Biochemistry, University College, P.O. Box 78, Cardiff CF1 1XL, U.K.)

Arvin, an enzyme fraction isolated from the venom of *Agkistrodon rhodostoma* (Esnouf & Tunnah, 1967), is useful for producing controlled defibrination in patients requiring anticoagulant therapy. Arvin catalyses a limited proteolysis of fibrinogen to yield a readily dispersible fibrin clot (Ewart, Hatton, Basford & Dodgson, 1970); it has no effect on other clotting factors (Bell, Pitney & Goodwin, 1968; Sharp, Warren, Paxton & Allington, 1968; Bell, Bolton & Pitney, 1968). Chromatography of

* Abbreviations: ATPase, adenosine triphosphatase; EGTA, ethanedioxybis(ethylamine) - NNN'N' - tetraacetate.

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.

J. P. C. is grateful to the Science Research Council for a CAPS award. We thank Professor F. A. Robinson of Twyford Laboratories Ltd. for gifts of Arvin.

Bell, W. R., Bolton, G. & Pitney, W. R. (1968). *Br. J. Haemat.* **15**, 589.

Bell, W. R., Pitney, W. R. & Goodwin, J. F. (1968). *Lancet*, **i**, 490.

Esnouf, M. P. & Tunnah, G. W. (1967). *Br. J. Haemat.* **13**, 581.

Ewart, M. R., Hatton, M. W. C., Basford, J. M. & Dodgson, K. S. (1970). *Biochem. J.* **118**, 603.

Sharp, A. A., Warren, B. A., Paxton, A. M. & Allington, M. J. (1968). *Lancet*, **i**, 493.

Rapid Stimulation of Nucleolar Ribonucleic Acid Synthesis by Growth Hormone

By D. F. SALAMAN and A. KORNER. (*School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, U.K.*)

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