cytoplasmic fraction. These changes in enzyme activity would favour an anti-ketogenic action of a glucocorticoid in the bovine liver.

Administration of 1 mg of dexamethasone 21-isonicotinate or 1 mg of betamethasone to healthy female rats fed on an adequate ration resulted in effects in liver, 24 and 48 h later, quite the opposite from those observed in bovine liver. Hepatic phosphopyruvate carboxylase activity was increased in the cytoplasmic fraction and hepatic citrate synthase activity was depressed by 30% in the particulate fraction. These enzyme changes would favour the diversion of oxaloacetate to phosphoenolpyruvate in rat liver.

A noteworthy observation was that in bovine liver there was a significant correlation between the citrate concentration and the citrate synthase activity. This correlation was not observed in rat liver experiments.

C. B. M. is a Placement Student from Huddersfield Polytechnic, Queensgate, Huddersfield HD1 3DH, Yorks., U.K.

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Single-Step Purification of Dihydrofolate Reductases on Pteridine Matrices

By J. G. DANN, N. G. L. HARDING, P. C. H. NEWBOLD and J. M. WHITELEY (*The Postgraduate Medical School, Hills Road, Cambridge CB2 2QH, U.K.*)

Intracellular dihydrofolate reductase maintains the tetrahydrofolate pool at the expense of NADPH. The enzyme uses folate or 7,8-dihydrofolate as substrate, and it is powerfully inhibited by their 2,4-diamino analogues, e.g. methotrexate. The basal dihydrofolate reductase activity of wild-type cells is often increased after therapy with methotrexate, following oncogenic transformation, or with the emergence of mutants resistant to methotrexate. In the latter case two forms of the intracellular enzyme were observed, and showed disparate increase in activity as resistance developed (Harding *et al.*, 1970).

Attempts to define the mechanism underlying these increases in reductase activity have been unrewarding, since poor yields of active protein are obtained by conventional chromatography. Since the 2,4-diamino substituents are largely responsible for binding of the inhibitors to the enzyme, a distal carboxyl group of these derivatives is coupled to aminoalkyl-agarose to produce insoluble matrices that will retard or retain the mammalian and bacterial enzyme according to the K_m or K_i of the compound (Newbold & Harding, 1971). Similar affinity matrices will also bind the enzyme from T4 bacteriophage (Erickson & Mathews, 1971) and chicken liver (Kaufman & Pierce, 1971), and will retain reductase-independent

protein (Newbold & Harding, 1971; Kaufman & Pierce, 1971).

We have sought to answer whether an increase in the intracellular lifetime or rate of synthesis of each form of the reductase would account for the previous observations (Harding *et al.*, 1970). Trial of the affinity columns with the enzyme from *Lactobacillus casei* showed that one of the two forms was lost during purification. This was due to substrate-dependent interconversion by loss of bound NADPH. Whether this occurs by a catalytic function of the enzyme or by elution of the bound NADPH is not clear.

Apart from the enzyme from *Plasmodium* (Ferone *et al.*, 1969), dihydrofolate reductases are relatively small proteins (mol.wt. approx. 20000). Although the pteridine moiety of the folate molecule primarily determines interaction with the active site, the *p*-aminobenzoylglutamate side chain contributes to the binding (Baker, 1967). For maximal interaction between the reductase and the matrix, there must be an extended linking agent between the agarose and the inhibitor. With compounds of K_i 10^{-8} – 10^{-9} M a spacer length of C_4 – C_6 is required for good recovery (>50%) of catalytic activity. If the γ -carboxyl group of the folate derivative is linked to the spacer, addition of one methylene group to a C_3 spacer changes the pteridine-agarose distance by about 6%. Since the enzyme structure is not resolved, we are unable to explain how this small change considerably alters retention and elution of the enzyme.

For high yields of active enzyme 5-formyltetrahydrofolate, 7,8-dihydrofolate and folate are particularly effective counter-substrates. Buffer composition is important, both for determining stability of the enzyme and for elution of reductase-independent proteins. Dissociation of the enzyme from the matrix can lead to inactivation, whereas binding to it does not. Active and inactive species of the eluted enzyme migrate identically on sodium dodecyl sulphate-polyacrylamide gel. Hence loss of activity is apparently due to irreversible deformation of the secondary or tertiary structure of the enzyme. Inactivation occurs particularly when folate and NADPH are both in the eluent, although two forms are eluted when NADPH is present. In contrast, NADPH stabilizes the reductase against intracellular degradation (N. G. L. Harding and G. Court, unpublished work).

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Promotion of Deoxyribonucleic Acid-Dependent Ribonucleic Acid Synthesis by Protein Isolated on a Plant Hormone Affinity Column

By M. A. VENIS* (introduced by R. B. BEECHY)
 (Research Institute, Canada Agriculture, London 72, Ont., Canada)

Although it has long been considered that there may be specific proteins mediating the primary action of plant hormones, activity in this area has been relatively unproductive. Recently preliminary reports have indicated that, in the presence of certain protein fractions, auxins (Matthyse & Phillips, 1969) and cytokinins (Matthyse & Abrams, 1970) can stimulate RNA synthesis directed by isolated pea chromatin or DNA. Similarly it appears that both gibberellic acid (Johri & Varner, 1968) and abscisic acid (Pearson & Wareing, 1969) effects on RNA synthesis *in vitro* may require the presence of factors that are rapidly lost from nuclei or chromatin during the isolation procedures.

An attempt has been made to obtain possible protein mediators of this kind by the affinity-chromatography principle, by using an agarose column to which a derivative of the synthetic auxin 2,4-dichlorophenoxyacetic acid is attached by the method of Cuatrecasas *et al.* (1968). Such a column retains small amounts of protein from crude extracts of pea or maize shoots. Elution with 2mM-KOH gives a protein factor that enhances DNA-dependent RNA synthesis (supported by *Escherichia coli* polymerase) by 50–300% in different preparations. Activity is not due to inhibition of adenosine triphosphatase or ribonuclease action, nor is it an endonuclease effect. Time-course experiments involving rifampicin suggest the factor acts, partially at least, on RNA chain initiation.

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* Present address: Biochemistry Division, Shell Research Ltd., Sittingbourne, Kent, U.K.

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Purification of Tobacco Ribonuclease by Affinity Chromatography

By L. JERVIS (Department of Biochemistry, Rothamsted Experimental Station, Harpenden, Herts., U.K.)

The number of plant ribonucleases that have been studied in detail is small in comparison with ribonucleases from other sources (Barnard, 1969). Those plant ribonucleases that have been studied exhibit no absolute base-specificity, but do show a marked preference for purine bases. They will hydrolyse purine cyclic nucleotides and are inhibited by purine mononucleotides (Wye *et al.*, 1969; L. Jervis, unpublished work). Wilchek & Gorecki (1969) have purified bovine pancreatic ribonuclease on an affinity medium based on the inhibitory properties of uridine 2'(3')-monophosphate. Several affinity media have been developed that will purify tobacco ribonuclease. These media make use of the inhibition of tobacco ribonuclease by guanosine 2'(3')-monophosphate.

Adsorbent 1 was prepared by coupling 5'-(4-aminophenylphosphoryl)guanosine 2'(3')-monophosphate to Sepharose 2B by the CNBr procedure (Axén *et al.*, 1967). Adsorbent 2 was obtained by binding guanosine 2'(3')-monophosphate to Sepharose 2B-aminohexanoic acid by the water-soluble carbodi-imide method (Cuatrecasas, 1970). Adsorbent 3 was made by coupling guanosine 2'(3')-monophosphate to CNBr-activated Sepharose 2B.

Adsorbents 1 and 2 bind tobacco ribonuclease strongly. This can be released either by substrate elution or by changing the buffer conditions. Adsorbent 3 does not bind ribonuclease, presumably owing to steric hindrance. It does, however, bind other proteins that are bound by adsorbents 1 and 2, and is consequently useful when crude enzyme preparations are being used. Adsorbent 2 exhibits ion-exchange properties in addition to specific adsorption. This has been shown to be largely due to free carboxyl groups remaining on the Sepharose-aminohexanoic acid after reaction with GMP. This ion-exchange behaviour can be avoided by careful control over the coupling conditions or by blocking the free carboxyl groups after reaction with GMP. In addition to binding ribonuclease, the affinity media described also adsorb some phosphomonoesterase activity and phosphodiesterase from crude preparations. These enzymes can be selectively removed by substrate elution, leaving the ribonuclease still bound to the column.

Use of these affinity media has given tobacco ribonuclease preparations of substantially greater