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Induction of δ -Aminolaevulate Synthetase Activity in Cultured Chick-Embryo Liver Cells by Ribonucleic Acid

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AIA* is porphyrinogenic in experimental animals (Goldberg & Rimington, 1954), and specifically induces δ -aminolaevulate synthetase, the initial and rate-limiting enzyme of porphyrin biosynthesis (Granick, 1966).

Hickman, Saunders, Dowdle & Eales (1967) found that high-molecular-weight RNA isolated from the livers of rats rendered porphyric by administration of AIA increased the porphyrin content of segments of chick-embryo liver grown in organ culture. Repetition of these experiments in this laboratory has given inconclusive results. In the present study, however, we have measured aminolaevulate synthetase activity by the method of Dowdle, Mustard & Eales (1967) in cultured chick-embryo liver cell monolayers (Skea & Nemeth, 1969).

Rats were rendered porphyric by intraperitoneal injection of AIA and RNA was prepared from their livers and from the livers of normal rats by the method of Parish & Kirby (1966). Incubation of cell monolayer cultures of 14–15-day-old chick embryo liver with a solution of RNA from porphyric rat liver (0.2mg of RNA/ml of Eagle's minimum essential medium) caused a two- to threefold increase in aminolaevulate synthetase activity within 4h, the enzyme activity returning to the control value by 8h. No increase of aminolaevulate synthetase activity was found in cultures incubated with normal rat liver RNA. This effect is apparently RNA-dependent, since prior incubation of porphyric rat liver RNA with ribonuclease abolished it. Since RNA isolated from normal rat liver homogenate deliberately contaminated with AIA did not increase aminolaevulate synthetase activity in the cultures, the effect was presumably not due to contaminating traces of AIA.

Preliminary fractionation of porphyric-rat liver RNA has been carried out on Sephadex G-100 by the method of Kay & Cooper (1969). By this method 90% of the RNA was excluded from the column, the rest being eluted later in two shallow peaks. The RNA from the three fractions was reprecipi-

tated with ethanol and tested separately at a concentration of 0.2mg of RNA/ml for ability to increase aminolaevulate synthetase activity in chick-embryo liver cultures. All three fractions showed significant inducing activity, but the two lower-molecular-weight fractions were two to three times as active as the Sephadex-excluded fraction. Similar fractions of normal rat liver RNA showed no significant inducing activity.

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Binding of Polylysine and Toluidine Blue to Deoxyribonucleoprotein

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The accessibility of the DNA component of dNP* to molecules of various sizes was studied by measuring its binding to the dye Toluidine Blue, and to poly-L-lysine hydrobromide of three different molecular weights. Mixtures with dNP were made at concentrations such that all the dNP precipitated. Estimates were then made of the excess of unbound dye or polylysine over a range of dNP concentrations.

With Toluidine Blue, about 60% of the DNA phosphate groups in dNP were available for binding. This high value was not attributable to dissociation of the DNA from the protein, nor to any substantial binding to the protein acidic groups.

With polylysine, estimates were made of both completely unused polylysine molecules and of lysyl residues in molecules only partly bound to dNP, i.e. of unused ('wasted') residues. 'Titration' values of available DNA phosphate groups were 46, 38 and approx. 44% respectively for polylysine preparations of mol.wt. 1250, 7790 and 62000 (PL1, PL2 and PL3). In DNA, 100% of the phosphate groups were available. Near the point of mutual charge neutralization with dNP and DNA no lysyl residues were 'wasted', at least for preparations PL1 and PL2. This result and the

* Abbreviation: AIA, allylisopropylacetamide.

* Abbreviation: dNP, deoxyribonucleoprotein.