Factors Influencing Premature Induction of Enzyme in Cultured Chick-Embryo Liver Cells

By B. R. SKEA and A. M. NEMETH (introduced by G. J. DUTTON). (*Departments of Biochemistry and of Anatomy, The University, Dundee, Scotland, and University of Pennsylvania, Philadelphia, Pa., U.S.A.*)

UDP-glucuronyltransferase (GT), present at low activity in chick-embryo liver, increases rapidly to adult activities on hatching at 21 days (Dutton & Ko, 1966). GT develops prematurely to adult activity in chick-embryo liver cultured with serum (Ko, Dutton & Nemeth, 1967). Chemically defined media have now allowed earlier and more rapid induction from zero activity, with healthier cells permitting histological monitoring.

Liver from 5-day-old embryos is cytologically immature and possesses no GT activity. Raft cultures of 5-day-old embryo liver in glutamine-reinforced Eagle's minimum essential medium developed adult GT activity within 3 days of culture, reaching five times the adult activity by 8 days. From electron microscopy, it appears that this unusual development of enzyme is associated with very active synthesis of rough endoplasmic reticulum. Cell division seems to be unnecessary for GT development, for X-irradiation preventing mitosis still permitted rapid induction in 5-day-old embryo liver cultures.

Neither is organ structure of embryo liver required for GT induction. Trypsin-dispersed 12-day-old embryo liver cells were plated at high density in the above medium plus dialysed horse serum (DHS); the resulting cell monolayer developed adult GT activities within 3 days. Replacement of DHS by embryo extract prevented monolayer formation and gave rise to cell aggregates; GT development remained unaffected. Addition of serum converted aggregates into monolayers, again without altering the rate of development of GT.

Ko, Dutton & Nemeth (1967) demonstrated GT development despite derangement of intracellular organization. However, the 'dead' cells of 5-day-old embryo liver osmotically shocked or frozen before culture do not develop GT. Eventual loss of GT on prolonged cell culture parallels cellular transformation from parenchymal to fibroblastic appearance; X-ray irradiation retards both transformation and GT decay.

Ko, Dutton and Nemeth (1967) suggested extrahepatic control of embryonic liver GT during development. In 5-day-old embryo liver grafted to chorioallantoic membrane, GT was not induced, cytological development proceeding normally. Subsequent trypsin treatment and culture of graft cells resulted in GT induction. If suppression of GT

induction in grafts mimics normal control in the egg, then adenosine 3'-phosphate 5'-sulphatophosphate sulphotransferase, which reaches high levels in embryonic liver, should not be suppressed in grafts. However, adenosine 3'-phosphate 5'-sulphatophosphate sulphotransferase did not develop in chorioallantoic grafts of 5-day-old embryo liver although appearing readily in raft cultures of liver.

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Tritium-Exchange Studies of the Transcription of Deoxyribonucleic Acid by Ribonucleic Acid Polymerase

By ROSEMARY HEWITT and B. H. NICHOLSON. (*Department of Physiology and Biochemistry, University of Reading*)

Early experiments on the transcription of double-stranded DNA *in vitro* with bacterial RNA polymerases, including that from *Escherichia coli* (Chamberlin & Berg, 1962) showed that the template was undenatured at the end of the reaction. However, during transcription, recognition of the incoming nucleoside triphosphates by the bases normally internal to the DNA molecule must occur. If recognition is by hydrogen-bonding of the Watson-Crick type and not by other hydrogen-bonding or a 'like-to-like' intercalation, some local denaturation of the template at or preceding the polymerization site and subsequent renaturation is necessary. The exposure of the bases to solvent that this mechanism implies should give rise to a facilitated hydrogen exchange which, if the experimental procedure can be carried out fast enough, could be followed with tritium Printz & von Hiffel (1965).

Calf thymus DNA (0.6 ml.) (1.1 mg./ml. in 0.1 M-NaCl-0.01 M-potassium phosphate buffer, pH 7.6), ultrasonically treated at 2° for 1 min., was equilibrated with 0.05 ml. of $^3\text{H}_2\text{O}$ (5.5 c./ml.) for 3 hr. at room temperature. Subsequent operations were at 2° to decrease the rate of exchange. [^3H]DNA was separated by rapid passage through Sephadex G-25. To the [^3H]DNA, now in a volume of 2.5 ml., was added 0.14 ml. of 1.2 M-sodium phosphate buffer, pH 7.6, and 0.5 ml. samples were pipetted into four thin-walled tubes containing 0.01 ml. of 0.9 M- β -mercaptoethanol and 0.12 ml. of a solution of all

four nucleoside triphosphates (each 2.5mM); 0.01ml. of 0.15M-MnCl₂ was added to each tube followed by 0.1ml. of *E. coli* RNA polymerase (1.56mg./ml.) to two of the tubes and bovine serum albumin (1.56mg./ml.) to the other two. The amount of polymerase added was, on the basis of previous experiments, sufficient to transcribe the DNA present under these conditions of low divalent cation concentration and low temperature. Incubation at 20° was for 1 min., followed by rapid transfer to four columns (2.5cm. × 8cm.) of Sephadex and automatic collection of 1–2ml. fractions. Samples of fractions were assayed for DNA by the diphenylamine reaction and for tritium by dilution with Bray's fluid and scintillation counting.

The elution profiles show that the radioactivity present when DNA is incubated with bovine serum albumin is several times that when it is incubated with RNA polymerase. The results therefore support the hypothesis that local denaturation of the DNA template occurs during transcription.

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Determination of the Molecular Weight of Ribonucleic Acid Polymerase by Gel Filtration

By A. M. Q. KING and B. H. NICHOLSON. (*Department of Physiology and Biochemistry, University of Reading*)

Andrews (1965) described the use of Sephadex-gel columns for the determination of the molecular weights of proteins. If the elution volume of the protein is plotted against the logarithm of the molecular weight a smooth curve is obtained. The exclusion limit of Sephadex G-200 lies between 2×10^5 and 8×10^5 daltons. It is therefore unsuitable for measuring molecular weights in this range. We report here an investigation of the use of Sepharose 4B (exclusion limit 3×10^6 daltons) for the deter-

mination of high molecular weights, where Sephadex G-200 loses its power of separation.

A column (70cm. × 2.5cm.) was packed with Sepharose 4B and run at 0–2°; 0.01M-tris buffer, pH 8.0, containing MgCl₂ (5mM), EDTA (0.1mM) and KCl (0.05–0.4M) was pumped through the column in a descending direction, at 20ml./hr. Protein (2mg.) was applied in 1.7ml. at the beginning of each run, and the eluate was collected in fractions (3ml.). Fractions were assayed spectrophotometrically at an appropriate wavelength (usually 225nm.), and in the case of enzymes were also assayed for their biological activity.

The following proteins were used to calibrate the column: aldolase (1.45×10^5 daltons); γ -globulin (Cohn fraction II) (1.6×10^5); catalase (2.4×10^5); fibrinogen (3.3×10^5); urease (4.8×10^5); thyroglobulin (6.7×10^5); ferritin (7.5×10^5). In the plots of elution volume versus log(molecular weight), fibrinogen and ferritin appeared to be anomalous: they behaved as though their molecular weights were approximately reversed. The anomalous behaviour of fibrinogen can be explained on the basis of its highly asymmetric shape, and of ferritin by its very low partial specific volume. These results suggest that, for Sepharose, the elution volume provides a measure of molecular radius rather than molecular weight, just as it does for Sephadex (Siegel & Monty, 1965). The method can, however, yield molecular weights for globular proteins that approximate to spheres, and have conventional degrees of hydration and partial specific volumes.

On these assumptions molecular weights have tentatively been assigned to RNA polymerase from *Escherichia coli* of 7.1×10^5 daltons at I 0.07, and 4.6×10^5 daltons at I 0.22.

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