stability of the enzyme. Experiments are in progress to elucidate the factors controlling ornithine decarboxylase turnover in these cells.

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Creatine Kinase Activity in Cultures of Differentiating Myoblasts

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Differentiation of myoblasts *in vitro* results in the fusion of single cells to form multinucleate syncytia and an increase in the activity of creatine kinase (Hauschka, 1968). The analogous increase in creatine kinase activity associated with muscle development *in vivo* is accompanied by a change in isoenzyme pattern (Eppenberger *et al.*, 1964), and preliminary findings suggest a similar change *in vitro* (G. Morris, A. Cooke & R. J. Cole, personal communication).

This abstract is concerned with the appearance of creatine kinase in primary cultures of chick myoblasts and the relationship between changes in the enzyme and cell fusion.

A burst of fusion (measured as the percentage of nuclei in multinucleate syncytia) began after 45h in culture, giving rise to 50% multinucleate syncytia at 69h. In contrast there was relatively little increase in creatine kinase activity/mg of DNA until 69h in culture, but by 96h the creatine kinase activity/ μ g of DNA was 20-fold higher than its initial value at 33h. Creatine kinase activity was assayed by measuring fluorimetrically the creatine produced by the back-reaction of the enzyme (Koedam, 1969).

The isoenzyme compositions of creatine kinase in cell culture were obtained from the ratios of enzyme activity at high and at low concentrations of creatine phosphate and ADP (Eppenberger *et al.*, 1967). Two forms of the enzyme from chick heart and breast muscle gave values of 2.1 and 5.0 respectively. In cell culture a constant ratio of approx. 2.4 was obtained at 33–58h and this then increased to 3.3 at 69h and to 4.0 at 96h. Thus in newly fused myoblasts the 'heart'-type isoenzyme characteristic of embryonic muscle is predominant, and a change in isoenzyme composition occurs coincidental with the

marked increase in creatine kinase activity rather than fusion of the myoblasts.

Cells grown with 0.1mM-dibutylryl cyclic AMP (6-*N*,2'-*O*-dibutylryladenine 3':5'-cyclic monophosphate) fused 10h later than their controls, but the increase in creatine kinase activity was not delayed. This result contrasts with that obtained by Shainberg *et al.* (1971), who found that the inhibition of fusion caused by low Ca^{2+} concentrations also prevented the increase in creatine kinase activity.

It therefore seems that, although cell fusion is necessary for the observed increase in creatine kinase activity and appearance of the new isoenzyme, the two processes are not as tightly linked as previously suggested (Shainberg *et al.*, 1971).

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The Effect of Aflatoxin B₁ on Ribonucleic Acid Synthesis in Control and Cortisol-Stimulated Rat Liver

By G. E. NEAL (*Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey, U.K.*)

The hepatocarcinogenic fungal toxin aflatoxin B₁ is a potent inhibitor of the induction by cortisol of tryptophan pyrrolase activity in rat liver (Clifford & Rees, 1967). The increased Mg^{2+} -stimulated RNA polymerase activity resulting from the administration of cortisol has been found to be strongly inhibited by aflatoxin B₁ *in vivo*, as is the increased incorporation *in vivo* of labelled orotate. The increase in the incorporation of labelled leucine *in vivo*, which results from the administration of cortisol, like the induction of tryptophan pyrrolase, is also strongly inhibited by aflatoxin B₁. The control tryptophan pyrrolase activity and leucine incorporation are not inhibited by aflatoxin B₁, whereas the control rate of RNA synthesis is strongly inhibited. Mg^{2+} -stimulated RNA polymerase activity in hepatic nuclei is decreased by prior adrenalectomy of the rats, but if these rats are also treated with aflatoxin B₁ the enzyme activity observed is no lower than that in intact animals treated with the toxin.

These findings suggest a close connexion between the stimulatory action of cortisol and the inhibitory

action of aflatoxin on hepatic RNA synthesis. Williams & Rabin (1969) have demonstrated that, at the level of the endoplasmic reticulum, a competition occurs between certain steroid hormones and aflatoxin B₁ for sites on the endoplasmic reticulum involved in the binding of polyribosomes. The possibility that a similar competition could take place between aflatoxin B₁ and nuclear cortisol-receptor substances was examined. No evidence was obtained indicating that the binding of labelled cortisol to macromolecules in the cytosol and nuclear fractions was affected by the administration of a dose of aflatoxin B₁ that gave maximal inhibition of RNA synthesis.

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Possible Role of a Thiol Group in Lactose Synthetase A Protein

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Lactose synthetase A protein (EC 2.4.1.22) catalyses the transfer of D-galactosyl units from UDP-D-galactose to N-acetyl-D-glucosamine, forming N-acetyl-lactosamine, and to D-glucose, forming lactose. α -Lactalbumin partially inhibits the first reaction and greatly enhances the second one (Brew, 1970). The enzyme requires Mn²⁺ for activity and is inhibited by *p*-chloromercuribenzenesulphonate (Babad & Hassid, 1966).

We found that 80% of the catalytic activity of the A protein extracted from human milk (Andrews, 1970) was lost when it was incubated in 0.1 mM-*p*-hydroxymercuribenzoate at 25°C for 30 min, but the activity was restored in 2.5 mM-2-mercaptoethanol. Similar incubation with *p*-hydroxymercuribenzoate in glucose (80 mM), N-acetylglucosamine (6 mM), Mn²⁺ (8 mM) and α -lactalbumin (150 μ g/ml), either separately or in various combinations, still resulted in 70–80% loss of activity. With inhibitor in 1 mM-UDP-galactose, however, only about 50% of the activity was lost, and with 8 mM-Mn²⁺ also present little if any inactivation occurred.

The enzyme reacted rapidly with 0.1 mM-5,5'-dithiobis-(2-nitrobenzoate) (Ellman's reagent) at pH 8 at 25°C in the presence of 7.5 M-urea to give 0.93 mol of 2-nitro-5-thiobenzoate/mol of enzyme of molecular weight 40000 (Andrews, 1970). Reaction in the absence of urea was much slower but reached the same end point after 3 h. Enzyme inhibited with *p*-hydroxymercuribenzoate did not react with Ellman's reagent.

These results indicate the presence of a partly

shielded thiol group at the active centre of the enzyme molecule that is apparently involved with Mn²⁺ in binding UDP-galactose. Since neither N-acetylglucosamine nor α -lactalbumin afforded much protection to the enzyme against inactivation by *p*-hydroxymercuribenzoate it seemed likely that the inactivated enzyme would still form a complex with these compounds. This was confirmed by experiments with a column of Sepharose- α -lactalbumin (Andrews, 1970). Enzyme inactivated with *p*-hydroxymercuribenzoate was retained on the column in the presence of N-acetylglucosamine, but it appeared in the effluent when the sugar was omitted from the eluting buffer.

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Active-Site-Directed Irreversible Inhibition of 4-Aminobutyrate Aminotransferase by Ethanolamine O-Sulphate

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Many pyridoxal phosphate enzymes have been shown to catalyse reactions differing from those that the 'normal' substrates undergo. For example, aspartate aminotransferase (EC 2.6.1.1), when presented with amino acids with a good leaving group in the β -position, such as β -chloro-L-glutamate, catalyses a β -elimination rather than a transamination reaction (Manning *et al.*, 1968). If L-serine O-sulphate (see Tudball *et al.*, 1969) is used as substrate for this enzyme, the β -elimination reaction is accompanied by progressive inactivation of the enzyme caused by irreversible binding of the C₃ moiety of this substrate to the enzyme (John & Fasella, 1969). These findings suggested a general approach to the specific inactivation and labelling of pyridoxal enzymes, through substrate analogues.

Ethanolamine O-sulphate is the O-sulphate analogue of 4-aminobutyrate, which is believed to be an inhibitory neurotransmitter in the mammalian brain (Krnjević, 1970). We have investigated the effect of this compound on 4-aminobutyrate aminotransferase (4-aminobutyrate-2-oxoglutarate aminotransferase, EC 2.6.1.19), the enzyme that catalyses the degradation of 4-aminobutyrate.

Time-dependent irreversible inhibition of enzymic