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