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.

We thank the Australian Dairy Produce Board for the award of a Postgraduate Studentship to B. J. K.

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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

activity occurred when ethanolamine *O*-sulphate was incubated with various preparations of 4-aminobutyrate aminotransferase. With an extract of *Pseudomonas fluorescens* prepared as described by Jakoby & Scott (1959) $t_{\frac{1}{2}}$ for the reaction at 25°C was 22 min at an inhibitor concentration of 3.6 mM. With an acetone-dried powder of mouse brain (Waksman & Roberts, 1965) as source of enzyme activity, $t_{\frac{1}{2}}$ for the reaction at 37°C was 27 min at an inhibitor concentration of 1.0 mM. Partially purified preparations of enzyme from rat brain (Vasil'ev & Eremin, 1968) were also inhibited by ethanolamine *O*-sulphate (at 37°C and at 0.5 mM $t_{\frac{1}{2}}$ was 30 min). In all cases the inhibition was shown to follow pseudo-first-order kinetics. Loss of enzyme activity was slowed by the addition of 4-aminobutyrate to the reaction mixture, suggesting that the inhibitor exerts its action at the 4-aminobutyrate-binding site of the enzyme.

Ethanolamine *O*-sulphate was tested as an inhibitor of glutamate decarboxylase (EC 4.1.1.15), aspartate aminotransferase and alanine aminotransferase. With preincubation times of up to 90 min and 1.0–100 mM concentrations of ethanolamine *O*-sulphate it was not possible to demonstrate any inhibition of these pyridoxal phosphate enzymes.

We conclude that ethanolamine *O*-sulphate is a specific active-site-directed irreversible inhibitor of 4-aminobutyrate aminotransferase.

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Metabolism of Sulphated Insulin in the Rat

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Sulphated insulin is less neutralizable by antibodies than is unmodified insulin and has been shown to provide better control in insulin-resistant diabetes (Moloney *et al.*, 1964). The sulphate ester groups are introduced into the insulin molecule by the action of conc. H_2SO_4 at low temperature (Reitz *et al.*, 1946). Insulin, however, contains a number of potential sites for sulphation, and the reaction product repre-

sents a mixture of sulphated insulins carrying from one to eight sulphate groups/molecule (Thomas, 1971).

A sulphated bovine insulin preparation containing eight sulphate groups/molecule (completely sulphated insulin) was isolated by ion-exchange chromatography on QAE (quaternary aminoethyl)-Sephadex. This material had a biological activity of 7.1 units/mg as measured by the mouse convulsion test, and was virtually non-neutralizable by anti-insulin serum.

Free-ranging rats received completely sulphated insulin (120 µg/kg body wt.) labelled with ^{35}S either by intravenous or by subcutaneous injection, and urine and faeces were collected. With both routes of administration over 70% of the injected radioactivity was recovered in the urine within 48 h.

After intravenous administration of the completely sulphated insulin preparation to wholly anaesthetized rats with bile-duct and ureter cannulae 43–53% of the injected radioactivity was excreted in the urine over 6 h; relatively small amounts (less than 2.5%) of the dose appeared in the bile. Less than 5% of the radioactivity excreted in the urine was precipitable by trichloroacetic acid.

It has been established that both the liver and the kidney play significant parts in the metabolism of insulin (Rubenstein & Spitz, 1968). In subsequent experiments when either an isolated rat liver or a rat kidney was perfused with blood containing completely sulphated insulin an increase in the trichloroacetic acid-soluble ^{35}S was observed, thereby demonstrating that appreciable degradation had taken place.

The distribution of completely sulphated insulin in the tissues was examined by whole-body radioautography by the method of Powell *et al.* (1967). It was observed that within 1 min ^{35}S accumulated in the kidneys of young male rats receiving the labelled preparation intravenously. The radioactivity appeared to be localized in the outer part of the cortex of the kidney. A series of radioautograms prepared up to 2 h after injection showed that only traces of radioactivity were associated with the liver. The effect of the sulphation of insulin on its metabolism was discussed.

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