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The Metabolism of [³⁵S]Homosulphanilamide

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Homosulphanilamide (4-aminomethylbenzenesulphonamide) is a valuable antibacterial drug when used topically for burns (Lindberg, Moncrief, Switzer, Order & Mills, 1965). Systemically it is ineffective because it is rapidly converted into the inactive 4-carboxybenzenesulphonamide (Hartles & Williams, 1947). The renewed therapeutic interest in this drug caused us to reconsider its metabolism, in particular the possibility of its deamination by the intestinal tissues.

[³⁵S]Homosulphanilamide was administered orally (50mg/kg) to the rhesus monkey, rat, guinea pig and rabbit, and two metabolites apart from the unchanged drug were found in the urine. These were 4-carboxybenzenesulphonamide and 4-acetamidomethylbenzenesulphonamide, the latter being shown to be a metabolite for the first time. In the monkey, guinea pig and rabbit the major product was 4-carboxybenzenesulphonamide (70–90% of the 24h excretion) with smaller amounts of the free and the acetylated drug. In the rat, however, the outputs of total homosulphanilamide and 4-carboxybenzenesulphonamide were approximately the same. The drug was thus less readily deaminated in the rat than the other species.

Homosulphanilamide is a substrate for monoamine oxidase (Beyer & Govier, 1945; Blaschko & Duthie, 1945), which is present in both liver and intestine. Therefore the effect of route of administration on the deamination of the drug was examined. The amount of 4-carboxybenzenesulphonamide excreted in the 24h urine in the rabbit (50% of dose) and rat (30% of dose) was the same whether [³⁵S]homosulphanilamide (500mg/kg) was administered orally or intravenously. This suggests that the intestine may not play a major role in deaminizing the drug.

The metabolism of the ³⁵S-labelled drug was studied in rabbits and rats pretreated with the monoamine oxidase inhibitor iproniazid (1-is-

nicotiny-2-isopropylhydrazine; Marsilid). In the rabbit the rate of excretion of ³⁵S was increased by about 25% by pretreatment, but in the rat it decreased by 25%. In both species the excretion of 4-carboxybenzenesulphonamide was markedly depressed and there was a corresponding increase in the excretion of total homosulphanilamide. Various combinations of oral and intravenous administration of homosulphanilamide and iproniazid were tried in an attempt to obtain information about the role of the gut compared with the other tissues in deaminating the drug, but no definite conclusions could be drawn from the results. It appeared that the drug was deaminated mainly by the liver rather than by the intestine.

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Protection by Zinc Ions against the Toxicity of Cadmium Ions

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Although Zn²⁺ is known to protect against the selective injurious effect of Cd²⁺ on the rat testis (Pařízek, 1960; Kar, Das & Mukerji, 1960; Gunn, Gould & Anderson 1961; Mason & Young, 1967), the mechanism of this protection has not been established. It seems unlikely that this is due to inhibition of the displacement by Cd²⁺ of Zn²⁺ from metalloenzymes, a possibility considered by Pařízek (1960), since the primary action of Cd²⁺ appears to be on the vascular system (e.g. Clegg, Carr & Niemi, 1969). The amount of Cd²⁺ accumulated by the rat testis is small (e.g. Pařízek, 1960) and does not lead to displacement of Zn²⁺. Indeed, the testicular content of Zn²⁺ remains unaltered for 48h after injection of Cd²⁺ (2.2 μmol/100g body wt.) and then increases to a value 9–10-fold greater than normal at 30 weeks.

In contrast with the testes, appreciable amounts of the injected Cd²⁺ accumulate in the livers and kidneys, this accumulation being accompanied by increases in the contents of Zn²⁺. In both tissues most of the bound Cd²⁺ is found as a single fraction in the cell sap, which also contains Zn²⁺. These proteins, in which the cations are bound at thiol groups, are not identical, but have properties in

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common and resemble metallothionein, a Cd^{2+} -containing protein of unknown function isolated by Kāgi & Vallee (1960, 1961) from horse kidney. The subsequent isolation of 'metallothioneins' from human kidney (Pulido, Kāgi & Vallee, 1965), from the livers of Cd^{2+} -injected rabbits (Piscator, 1964) and from the livers and kidneys of rats after intravenous injection of $^{203}\text{HgCl}_2$ (Jakubowski, Piotrowski & Trojanowska, 1970) suggests that these proteins constitute detoxifying mechanisms against certain heavy metals.

At least in the male rat, the Cd^{2+} -binding protein does not appear to be a normal component of the liver, but is induced in response to Cd^{2+} . It is synthesized also in response to excess of Zn^{2+} and, in livers of animals pre-injected 24–48 h previously with Zn^{2+} , parenterally administered Cd^{2+} accumulates, and is thus immobilized, more rapidly than in the normal liver. This provides a possible explanation for the protection by Zn^{2+} against Cd^{2+} injury to the testes, particularly as it is known that Zn^{2+} has maximal activity when administered at least 24 h before Cd^{2+} (Mason & Young, 1967).

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Secretion of Sulphated Respiratory-Tract Mucins by Cultured Tracheal Explants

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Previous work (Stahl & Ellis, 1970) has shown canine trachea incubated *in vitro* is capable of actively synthesizing protein-carbohydrate complexes. Calf tracheal cultures have been similarly used in studies of epithelial secretions (Kent, Molineux, Haegle & Stevenson, 1969). Histochemical and radioautographic studies have indicated that sulphate is incorporated into the mucus-producing structures of the tracheobronchial tree

(Havez *et al.* 1967; Havez & Biserte, 1968; Lamb & Reid, 1969; Chakrin, Baker, DeSanctis, Wardell & Spicer, 1970). The present study is concerned with the characterization of sulphated mucins secreted by these structures.

Explants were cultured in $^{35}\text{SO}_4^{2-}$ -containing medium as previously described (Stahl & Ellis, 1970). Radioautography indicated extensive uptake of radioactivity into the submucosal glands and the goblet cells of the surface epithelium. Secreted macromolecules, isolated from the spent culture medium, were also heavily labelled. Electrophoresis in 1% agarose gels in the absence of urea or reducing agents indicated the presence of large mucin-type glycoproteins. Carbohydrate content was approx. 40% and consisted principally of *N*-acetylgalactosamine, galactose and *N*-acetylglucosamine with smaller amounts of fucose, mannose, glucose and sialic acids. Seventeen amino acids were identified, with aspartate, leucine, glutamic acid, glycine, threonine and serine present in the largest amounts.

Fractionation on 1% agarose columns in the presence of 6M-urea furnished two major components. The material appearing in the void volume contained 60–70% of the $^{35}\text{SO}_4^{2-}$ label. This material was subjected to chromatography on DEAE-cellulose with a citrate (pH 6.5)–sodium chloride gradient. Three major components, all labelled, were isolated. One fraction contained 60% of the recovered radioactive label. Agarose-gel electrophoresis of the same material in the presence of sodium dodecyl sulphate and dithioerythritol gave three bands staining positively for carbohydrate. Radioactivity was primarily concentrated in one band.

The concept of sulphated respiratory-tract mucins has been based on histochemical and radioautographic studies together with analysis of tissue extracts. The present work shows that, after uptake by the mucus-producing structures, sulphate is incorporated into macromolecules that are then secreted as mucin-type glycoproteins. To characterize these secretions further we are currently investigating possible differences between sulphated mucins from goblet cells and the submucosal glands.

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