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Stimulation of Tryptophan Metabolism in the Rat by 4-Dimethylamino-3'-methylazobenzene and 2-Acetamidofluorene

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Tryptophan 2,3-dioxygenase may be induced in rat liver by cortisol or tryptophan, and the activity of the enzyme is measured by the conversion of tryptophan into kynurenine under suitable conditions. Altman & Greengard (1966) found that the administration of cortisol to patients caused increases in the activity of tryptophan 2,3-dioxygenase and a corresponding increase in urinary kynurenine. In the rat Tenconi & Pasquariello (1967) described increases in urinary kynurenine and other tryptophan metabolites after treatment with cortisol. It has since been reported that triamterene (2,4,7-triamino-6-phenylpteridine), a synthetic pteridine, increased the cortisol induction of tryptophan 2,3-dioxygenase and also the excretion of kynurenine in the rat (Duncan & Ghosh, 1969; Ghosh & Green, 1970).

A number of studies have been made of the induction of liver enzymes during hepatocarcinogenesis. In a feeding experiment in which rats were given 4-dimethylamino-3'-methylazobenzene for 5 weeks Poirier & Pitot (1969b) found no significant change in the dietary induction of tryptophan 2,3-dioxygenase. Poirier & Pitot (1969a) reported an inhibition of enzyme induction after the administration of 2-acetamidofluorene. Kizer *et al.* (1969) also recorded an inhibition of the cortisol induction of tryptophan 2,3-dioxygenase after the administration of 4-dimethylamino-3'-methylazobenzene.

In the present experiments male rats were fed on a diet containing a supplement of 4-dimethylamino-3'-methylazobenzene (0.06%) or 2-acetamidofluorene (0.03%). At intervals during the experiment the rats were injected with a combination of cortisol and tryptophan, and the urine was collected individually for 5h thereafter. Compared with controls it was found that there was an increased elimination of

kynurenine in the rats receiving prior treatment with the carcinogens.

In view of the findings by other workers that in feeding experiments with liver carcinogens the induction of tryptophan 2,3-dioxygenase is unchanged or inhibited, it was expected that the kynurenine elimination would also be unchanged or decreased. There were differences, however, in the experimental conditions, chiefly in the method of enzyme induction with cortisol and tryptophan but also in the duration of feeding.

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Lysine Biosynthesis in Mammals

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The synthesis of saccharopine [ϵ -N-(L-glutaryl-2)-L-lysine] from L-lysine and α -oxoglutarate is catalysed by the enzyme 'lysine-oxoglutarate reductase' [L-lysine-2-oxoglutarate reductase (saccharopine-synthesizing)], which has been partially purified from human liver (Hutzler & Dancis, 1968). Our studies on ox and human liver mitochondria have shown the presence of an enzyme capable of hydrolysing saccharopine to lysine and α -oxoglutarate. This enzyme, 'saccharopine oxidoreductase' [saccharopine-NADP⁺ oxidoreductase (hydrolysing)], has an absolute requirement for NADP⁺ and has a pH optimum about 9.0. 'Saccharopine oxidoreductase' preparations from human or ox liver are stimulated by NH₄Cl, which was found to inhibit 'lysine-oxoglutarate reductase'. The two enzymes exhibit different stabilities on incubation, 'saccharopine oxidoreductase' losing its stability after 90min incubation at 30°C and 'lysine-oxoglutarate reductase' being stable for a period of 4h. The two enzymes have been partially resolved by fractionation of mitochondrial extracts from human and ox livers.

Experiments with mammals have shown that lysine does not participate in a reversible deamination-reamination reaction (Schoenheimer & Rittenberg, 1940; Weissman & Schoenheimer, 1941) and is directly incorporated into proteins (Clark & Ritten-