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Identification of the Amino Acid Residues that Bind Azo-dye in the Soluble Azo-Dye Carcinogen-Binding Proteins of the Rat Liver

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Metabolites of azo-dye carcinogens bind covalently to specific soluble proteins in the rat liver (Miller & Miller, 1947; Sorof, Young, McCue & Fetterman, 1963). A basic azo-dye-binding protein has been isolated in apparently pure form and a small-molecular azo-dye-binding protein fraction has been substantially purified (Ketterer, Ross-Mansell & Whitehead, 1967). Considerable interest attaches to the identity of the amino acid(s) involved in carcinogen binding. A sulphur amino acid has been implicated by Andersen (1964) and evidence has been published that this is methionine (Scribner, Miller & Miller, 1965; Higashinakagawa, Matsumoto & Terayama, 1966).

Previous workers have used whole homogenate as starting material. The present authors have studied azo-dye-bound protein fragments obtained from purified azo-dye-binding proteins into which label has become incorporated from either [³⁵S]-methionine or [³⁵S]-cysteine.

Fifty mg. of 4-dimethylamino-3'-methylazo-benzene in 2ml. of corn oil and 60 μ C of labelled amino acid were injected into rats 40hr. and 16hr. respectively before they were killed. Basic and small-molecular azo-dye-binding proteins were isolated and digested with Pronase. Azo-dye-bound peptides were extracted and degraded further by alkaline hydrolysis. These fragments were purified by 'fingerprinting', eluted with formic acid, estimated spectrophotometrically and their specific radioactivities determined. The specific radioactivity of methionine and cysteine obtained from the same protein fractions was also determined.

After the administration of [³⁵S]-methionine the specific radioactivity of azo-dye-bound protein fragments from basic azo-dye-binding protein, small-molecular azo-dye-binding protein, cysteine and methionine were all of the same order. After the administration of [³⁵S]-cysteine, however, there was radioactivity in the azo-dye-bound fragments from the basic protein and cysteine only.

Thus, although the azo-dye binds to methionine in the small-molecular azo-dye-binding protein fraction, in the case of the basic azo-dye-binding protein, which is the major soluble dye-binding protein, it binds to cysteine.

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Blood Concentrations of Ketone Bodies and Non-Esterified Fatty Acids in Starved and Re-Fed Rats

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Fritz (1961) suggested that the rate of ketone-body formation by the liver was dependent on the plasma concentration of non-esterified fatty acids; experimental support for this idea was obtained by Ontko & Zilversmit (1966). However, Foster (1967) has recently proposed that ketone-body formation may be independent of the fatty acid concentration. This problem has been investigated by force-feeding 48hr.-starved rats (males weighing 250g.) with solutions of glucose, fructose, galactose or casein (0.5-2g./rat) under light ether anaesthesia; at various times (10-240min.) after re-feeding, blood was collected by aortic cannulation under ether anaesthesia. Blood fatty acids and ketone bodies were estimated by titration and by enzymic analysis respectively.

Glucose, fructose or casein feeding resulted in a rapid decrease in both fatty acids and ketone bodies; in a systematic time-course study the decrease in blood ketone bodies was always accompanied by a decrease in the plasma concentration of fatty acids. Re-feeding with galactose also caused a decrease in ketone bodies and fatty acids, but this occurred only after several hours.

These results support the hypothesis that the rate of ketone body production by the liver *in vivo* is dependent on the plasma concentration of fatty acids; it does not, however, preclude the possibility that the liver can regulate the rate of ketone body production independently of the fatty acid concentration.

The rapid fall in the fatty acid concentration after re-feeding with fructose and casein may be explained by the observations that these compounds can stimulate insulin release (Berger, Joondeph & Rachmeler, 1964; Floyd, Fajans, Conn, Knopf & Rull, 1966; Grodsky *et al.*, 1963). However, galactose does not cause insulin release (Grodsky