formed membranes do not participate in the transfer of compounds from liver to bile.

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The Effect of a Structural Modification on the Metabolism and Mode of Excretion of Naphthyl 2[³⁵S]-sulphate

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Previous investigations (Hearse, Powell, Olavesen & Dodgson, 1967, 1968) showed that after the administration of the potassium salts of biphenyl 4[³⁵S]-sulphate or cyclohexylphenyl 4[³⁵S]-sulphate to the rat the injected radioactivity is rapidly recovered in the urine. Hawkins & Young (1954) obtained similar results after the administration of naphthyl 2[³⁵S]-sulphate. Further investigations with biphenyl 4[³⁵S]-sulphate and cyclohexylphenyl 4[³⁵S]-sulphate showed that, in both cases, the ultimate urinary elimination is preceded by significant biliary circulation of radioactivity. However, the latter compound undergoes a much greater biliary circulation than the former. This difference in the metabolic behaviour of these two structurally very similar compounds is explicable only on the basis of the absence of aromatic character in the ring remote from the sulphate moiety of cyclohexylphenyl 4[³⁵S]-sulphate. It was decided to determine whether any biliary circulation of radioactivity could be demonstrated after the administration of naphthyl 2[³⁵S]-sulphate and, further, to investigate how the biliary excretion was effected by saturation of the ring remote from the sulphate moiety. Consequently, the metabolic fate and mode of excretion of the potassium salts of naphthyl 2[³⁵S]-sulphate and 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate were compared.

In preliminary experiments, free-ranging rats (male and female) received intraperitoneal injections (1mg./100g. body wt.) of either naphthyl 2[³⁵S]-sulphate or 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate, and urine and faeces were collected over 48hr. In all experiments up to 100% of the

injected radioactivity appeared in the urine and small amounts (5-13%) of the radioactivity appeared as inorganic [³⁵S]sulphate.

In subsequent experiments, the ³⁵S-labelled esters were administered (1mg./100g. body wt.) to male and female rats with bile-duct and ureter cannulae, and bile and urine were collected over 6hr. Over the experimental period up to 1.5% of the injected radioactivity appeared in the bile of rats receiving naphthyl 2[³⁵S]-sulphate and up to 4.7% in that of rats receiving 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate; up to 87% of the dose was recovered in the urine in both cases.

Chromatographic analysis of urine samples obtained from animals receiving naphthyl 2[³⁵S]-sulphate revealed the presence of only one major radioactive component, which was identified by isotope-dilution analysis (Dodgson, Powell, Rose & Tudball, 1961) as unchanged parent ester. Similar chromatographic analysis of urine obtained from rats receiving 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate revealed the presence of four major radioactive components, one of which has been identified as unchanged parent ester.

It seemed possible that rapid clearance of the injected radioactivity by the kidneys might account for the low biliary excretion observed with both esters. This possibility was explored by measuring the biliary radioactivity in rats in which renal function had been eliminated by ligation of the renal pedicles. Under these conditions, although the extent of biliary excretion was increased, it still represented less than 14% of the administered dose.

In experiments with biphenyl 4[³⁵S]-sulphate and cyclohexylphenyl 4[³⁵S]-sulphate, the presence of a saturated ring in the latter compound resulted in increased biliary elimination. In contrast, the present findings indicate that in the analogous situation with naphthyl 2[³⁵S]-sulphate and 5,6,7,8-tetrahydronaphthyl 2[³⁵S]-sulphate, the presence of a saturated ring does not facilitate biliary excretion. It does, however, appear to render the molecule far more susceptible to metabolic degradation than was recorded with naphthyl 2[³⁵S]-sulphate. The results further indicate that the absence of any significant biliary elimination of the esters cannot be explained by their rapid renal clearance but may reflect the influence of certain physicochemical characteristics of the molecules on their ability to penetrate liver membranes or utilize hepatic excretory mechanisms.

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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